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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
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EDITORIAL

Opportunities and challenges of clinical trials in cardiology using composite primary endpoints

Geraldine Rauch, Bernhard Rauch, Svenja Schüler, Meinhard Kieser

Geraldine Rauch, Svenja Schüler, Meinhard Kieser, Institute of Medical Biometry and Informatics, University of Heidelberg, D-69120 Heidelberg, Germany
Bernhard Rauch, Institut für Herzinfarktforschung, D-67063 Ludwigshafen, Germany
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Correspondence to: Dr. Geraldine Rauch, Institute of Medical Biometry and Informatics, University of Heidelberg, Im Neuenheimer Feld 305, D-69120 Heidelberg, Germany. rauch@imbi.uni-heidelberg.de
Telephone: +49-6221-561932
Fax: +49-6221-561932
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Abstract

In clinical trials, the primary efficacy endpoint often corresponds to a so-called "composite endpoint". Composite endpoints combine several events of interest within a single outcome variable. Thereby it is intended to enlarge the expected effect size and thereby increase the power of the study. However, composite endpoints also come along with serious challenges and problems. On the one hand, composite endpoints may lead to difficulties during the planning phase of a trial with respect to the sample size calculation, as the expected clinical effect of an intervention on the composite endpoint depends on the effects on its single components and their correlations. This may lead to wrong assumptions on the sample size needed. Too optimistic assumptions on the expected effect may lead to an underpowered of the trial, whereas a too conservatively estimated effect results in an unnecessarily high sample size. On the other hand, the interpretation of composite endpoints may be difficult, as the observed effect of the composite does not necessarily reflect the effects of the single components. Therefore the demonstration of the clinical efficacy of a new intervention by exclusively evaluating the composite endpoint may be misleading. The present paper summarizes results and recommendations of the latest research addressing the above mentioned problems in the planning, analysis and interpretation of clinical trials with composite endpoints, thereby providing a practical guidance for users.

Key words: Composite endpoint; Competing risks; Multiple testing; Time-to-event; Adaptive designs

Core tip: When planning a clinical trial with a composite primary endpoint: (1) Be aware of planning uncertainties when calculating the sample size and incorporate them in an adequate way; (2) Include a multiple testing strategy for an improved interpretation of the study results; (3) Take into account competing risks when analyzing the individual components of a composite endpoint; and (4) Analyze subsequent events in an adequate multi-stage model.

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RATIONALE FOR USING COMPOSITE ENDPOINTS

Clinical trials often focus on event variables as primary efficacy endpoints. In cardiology, "death" is often considered as the outcome of primary interest. However, clinically most relevant event types like "death" may be rare in many clinical conditions under investigation[1]. For example, due to the beneficial effects of modern treatments, patients with cardiovascular events like acute myocardial infarction experience a low mortality in the following years. Therefore, the assessment of differences in the survival curves of several treatment options may be difficult[2]. Using a rare event as primary endpoint results in the need of large sample sizes, a prolonged follow-up, and consequently an increased financial support, which often is not available. Thus, a "relevant and important treatment benefit" as claimed by the ICH E9 Guideline[3] cannot always be achieved by evaluating a single event endpoint, especially if this event type occurs with a low frequency[4]. By combining several types of events in a composite endpoint, the number of expected events is increased thereby intending an enlarged overall treatment effect. In the field of cardiovascular research, apart from death, clinical events like "non-fatal myocardial infarction", "non-fatal stroke", or "cardiovascular hospital admissions" also are of clinical interest and thus included into composite endpoints.

Most often, the composite endpoint is defined as a time-to-first-event variable, where different event types are counted as target events. In some applications, where the time period until the occurrence of an event is not of interest, composite endpoints can also be defined as binary event variables.

In summary, by using composite endpoints the required sample size is usually reduced and the study duration is shortened. Thereby, the use of composite endpoints very often is the only way to realize clinical trials investigating special interventions of interest.

Another important reason to use composite endpoints is when the effect of a new intervention may only be adequately assessed by considering several event variables. For example, atherosclerosis may result in a variety of clinical complications, and a single event endpoint therefore might not be sufficient for an adequate clinical evaluation[5]. Instead of formulating a multiple testing problem for several primary event endpoints, which always results in a loss of power, the ICH E9 Guideline[3] states that a composite outcome "addresses the multiplicity problem without requiring adjustment to the type I error".

CHALLENGES OF USING COMPOSITE ENDPOINTS

Planning and interpreting clinical trials with composite endpoints

Apart from the advantages of composite endpoints as outlined above, there also exist some serious problems and challenges.

In the planning stage of a clinical trial with a composite primary endpoint, calculation of the power may be particularly difficult as the assumed effect of the intervention depends on the effect sizes of the single components and their correlations. However, the level of evidence for these quantities may be low in many applications, as good historical data do not always exist. This complicates the choice of valid parameter assumptions in the planning phase of a study.

Analyzing and interpreting clinical trials with composite endpoints can be challenging as the composite effect as a "net measure" does not necessarily reflect the influence of the new intervention on the individual components[6,7]. Even in case a statistically significant and clinically relevant effect in the composite endpoint is observed, it may happen that the effects for some components are of very different magnitude or even point in opposite directions. As the efficacy of a treatment is usually judged on the composite effect alone, these situations may result in serious misinterpretations. This especially is a problem in case the composite endpoint consists of components of different clinical relevance and the less relevant endpoints refer to the larger effect sizes. The CPMP Guideline "Points to Consider on Multiplicity Issues"[8] therefore recommends to combine only components, which are expected to show effects of similar magnitude and with the same direction. This recommendation, however, may not be realistic in clinical practice. Even in thoroughly planned clinical studies, the initial assumptions about the underlying effect sizes can be wrong. Furthermore, the choice of the components must primarily be guided by their clinical relevance, and similar effects for all relevant event types cannot be expected in many cases.

Competing risks as a source of bias

The individual components of a composite endpoint usually define competing risks. In the presence of competing risks, the event rate of a specific event type also depends on the rates of all competing events[9]. For this reason, the event rates cannot be interpreted without simultaneously reporting all competing event rates. To illustrate this concept, assume that a novel therapeutic intervention in patients with cardiovascular disease is associated with a "one year mortality" of 0.3 as compared to 0.5 in the control group. Within the same group of patients, the rate for a "non-fatal myocardial infarction" might be 0.4 in the treatment group but only 0.2 in the control. If the death rates would not have been reported, one might come to the wrong conclusion that the control is superior to the treatment group with respect to "non-fatal myocardial infarction". When looking at the death rates, however, it becomes evident that the lower rate of "non-fatal myocardial infarction" in the control could exclusively be due to the fact that many patients had died before experiencing a (non-fatal) myocardial infarction. Ignoring the competing event scenario therefore may lead to a serious misinterpretation of treatment efficacy.

Therefore, methods taking into account competing events must be applied whenever the components of a composite endpoint are separately analyzed[5,8].

Follow-up beyond the first event

Composite time-to-first-event variables only take into account the first occurring event. This of course does not imply that there are no other subsequent events of interest occurring later. However, in the time to first event analysis these later events are not investigated, thereby leading to a loss of information.

On the other hand, an adequate and meaningful analysis of subsequent events may be a complex and difficult task, as-once a primary event has occurred the risk for all following events usually changes. For the latter reason models only focusing on a certain type of event, but not taking into account whether other events have occurred before, will yield biased results.

An unbiased approach to evaluate subsequent events would be to use more complex multistate models, which investigate all transition hazards between different subsequent event types[9]. The complexity of these models may be very high, and in order to get estimates with reasonable accuracy of all transition probabilities, the required sample size soon becomes unrealistically large. Therefore, for the confirmatory analysis of the composite and its components the time-to-first-event approach should usually be preferred. However, a descriptive presentation of the absolute numbers of all observed events should be provided in addition, keeping in mind that a correct interpretation of these results may be difficult.

NEW BIOMETRICAL METHODS FOR THE INTERPRETATION OF COMPOSITE ENDPOINTS

Overcoming uncertainties in sample size calculation

The standard approach to take account of planning uncertainties is the use of group-sequential or adaptive study designs. These designs allow stopping a trial at an interim stage due to an early demonstration of efficacy or due to futility. Whereas for group-sequential designs the number of interim analyses and the corresponding time points must be strictly planned in advance, adaptive designs additionally allow to change design parameters within an ongoing trial while still controlling the type I error rate.

A standard group-sequential design with one interim analysis (*e.g.*, after inclusion of 50% of the total study population) only offers two options-either to stop the study at interim or to continue the study until the full number of patients specified in the planning stage has been recruited[10-12].

In contrast, when using an adaptive design with one interim analysis, the sample size for the second stage can be recalculated based on the observed treatment effect at interim. If the observed effect at interim is large but not yet significant, only a small sample size for the second stage is needed, whereas the additionally required sample size is large, if the effect observed at interim is small. Moreover, it is possible to incorporate predefined stopping-for-futility rules in such designs, allowing to stop the study early with the acceptance of the null hypothesis whenever, on the basis of the interim data, the primary study goal becomes unrealistic. Thereby the number of patients being exposed to an ineffective treatment can be limited, and time and financial resources can be saved.

Another way to deal with uncertainties in the study planning assumptions is to use a more flexible power approach for sample size calculation. While the classical power is defined as the probability to reject the null hypothesis under a fixed parameter constellation of the alternative hypothesis, Rauch *et al*[13] proposed a so-called "expected power", which is defined as a weighted average over the classical power for different parameter constellations. Thereby, parameter constellations assumed to be more realistic in the planning stage of a study are assigned a higher weight, whereas other, less realistic assumptions are down-weighted. If there is no preexisting evidence available at all, equal weights for all possible parameter constellations might be assigned. The weights, which are defined by prior distributions, thus reflect the level of evidence or uncertainty in the planning stage. Calculating the sample size based on the "expected power" therefore defines a more robust approach in the common case of uncertain planning assumptions. The "expected power" can also be interpreted as a semi-Bayesian power approach[14].

Improving the interpretation of study results

The interpretation of study results may become difficult as the effect of the intervention under investigation on the composite endpoint does not necessarily reflect its effects on the single components. A possible solution of this problem would be to incorporate the (most important) components within the confirmatory test strategy by a multiple testing problem. However, this approach might seem to be contradictory as one main rationale for the use of a composite endpoint was to avoid multiplicity. A multiple testing problem always comes along with a certain loss in power resulting in an increase in sample size. The aim therefore is to create an adequate compromise by a multiple testing procedure, which mainly focuses on the composite endpoint but additionally gives some confirmatory evidence (at least) on the most important components.

In the literature, there exist a variety of either simple but also of more sophisticated multiple testing procedures, which can be applied to evaluate composite endpoints and their components. Simple applicable multiple testing strategies include the Bonferroni-Holm approach[15] or the sequential testing approach for hierarchically ordered hypotheses[16]. The application of at least a simple multiple testing strategy, which allow to address the components in a confirmatory way, is generally recommended. Even, if the trial is powered to

assess only the composite endpoint, these methods often allow a gain in information without increasing the sample size[17].

There also exist a variety of more sophisticated multiple testing procedures, which can be applied to provide sufficient power for the composite as well as for the (most relevant) components. So called "sequentially-rejective methods" represent extensions of the simple approaches outlined above. The underlying idea is to use an optimal splitting of the global significance level to test the individual hypotheses corresponding to the composite and the components. By "recovering" local levels of rejected hypotheses, the power loss due to multiplicity can be limited. Moreover, the test hypotheses for the components may be formulated less strictly than for the composite. For example, if the treatment under investigation already exhibits a significant and relevant effect on the composite, it might be sufficient to demonstrate in addition that the most relevant component is not adversely affected.

The application of sequentially-rejective multiple testing strategies in the evaluation of composite endpoints and their components has to be combined with the methodology for competing risks in order to provide an unbiased analysis and to prevent misinterpretations[18,19].

These methods can be further improved, if the correlation between the test statistics is taken into account. As an event referring to a single component always corresponds to an event in the composite endpoint, the test statistic of the composite and its components are usually highly correlated. By incorporating the information of the underlying correlation, the local significance levels of a multiple testing problem can be chosen less stringent, and the power loss often can be markedly decreased. These two approaches have been investigated recently by Rauch *et al*[20,21].

A completely different approach to improve the interpretation of clinical trials with composite endpoints is to use a weighted combined effect measure, which assigns higher weights to the more important components with the intention that an opposite effect in a relevant component (*e.g.*, "death") is less likely to be masked by a large effect in a component of secondary importance (*e.g.*, "cardiovascular hospital admission"). Recently, Pocock *et al*[22] and Buyse[23] proposed two similar combined effect measures, referred as the "win ratio" and the "proportion in favor of treatment", respectively. Both approaches are based on the same idea: All components are ordered with respect to their clinical relevance. The individual patients are compared between the groups. Based on the component of primary importance, for each comparison the patient with the "better" outcome is determined. In case no unique "winner" can be determined with respect to the most relevant component (*e.g.*, due to censoring, missing values or due to equal performance of both patients), the comparison will be based on the component of secondary importance and so on. This approach intends a higher weighting of the more relevant components, but also allows incorporating subsequent events.

Although this approach appears to be attractive in general, it also has some deficiencies. On the one hand, it can be shown that the weights, which are assigned to the single components, depend on the follow-up and the censoring distribution[24]. Moreover the weights are not standardized, that means they do not sum up to 1. As a consequence, the combined effect measure is not comparable between various studies as required-for example-within the context of meta-analyses. A small effect in the combined measure might thus be due to small effects in the components, but also could be explained by an unfavorable censoring distribution. Therefore, it cannot generally be deduced that these two approaches provide a gain in interpretation.

CONCLUSION

The use of a composite endpoint as primary efficacy variable can provide major advantages compared to a single event endpoint, if the event of primary interest is rare. However, care has to be taken when planning, analyzing and interpreting clinical trials with a composite endpoint as the primary efficacy outcome. The current statistical literature provides a variety of methods to overcome typical challenges arising from the use of composite endpoints thereby strengthening the interpretation of the results of clinical trials and avoiding serious misinterpretations. Now, the time has come to routinely incorporate these new methods into clinical trial applications.

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Cardiac magnetic resonance in clinical cardiology

Andreas Kumar, Rodrigo Bagur

Andreas Kumar, Rodrigo Bagur, Division of Cardiology, Department of Medicine, Quebec University Hospital Centre, G1R 2J6 Quebec, Canada
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Correspondence to: Rodrigo Bagur, MD, PhD, FAHA, Attending Cardiologist and Interventional Cardiologist, Division of Cardiology, Department of Medicine, Quebec University Hospital Centre, 11 Côte du Palais, L'Hôtel-Dieu de Québec, G1R 2J6 Quebec, Canada. rodrigobagur@yahoo.com
Telephone: +1-418-6915022
Fax: +1-418-6915714
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Abstract

Over the last decades, cardiac magnetic resonance (CMR) has transformed from a research tool to a widely used diagnostic method in clinical cardiology. This method can now make useful, unique contributions to the work-up of patients with ischemic and non-ischemic heart disease. Advantages of CMR, compared to other imaging methods, include very high resolution imaging with a spatial resolution up to 0.5 mm × 0.5 mm in plane, a large array of different imaging sequences to provide *in vivo* tissue characterization, and radiation-free imaging. The present manuscript highlights the relevance of CMR in the current clinical practice and new perspectives in cardiology.

Key words: Cardiac magnetic resonance; Gadolinium enhancement; Myocarditis; Myocardial; Cardiomyopathy

Core tip: The present manuscript highlights the relevance of cardiac magnetic resonance in the current clinical practice and new perspectives in cardiology.

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INTRODUCTION

Over the last decades, cardiac magnetic resonance (CMR) has transformed from a research tool to a widely used diagnostic method in clinical practice. While other imaging modalities like echocardiography and cardiac computed tomography depend solely on tissue density, the most important feature that CMR affords to the diagnostic toolset of the clinic cardiologist, is its ability to provide with a very-high spatial resolution, up to 0.5 mm × 0.5 mm in plane, a large array of different imaging sequences in order to assess *in-vivo* tissue characterization, in addition to radiation-free imaging. These imaging sequences investigate the presence of protons in different chemical environments, thereby allowing conclusions on the presence of fat, water (edema), blood or myocardium among other tissues. The addition of a contrast agent enhances the diagnostic capabilities to assess perfusion, fibrosis and necrosis as well as identify thrombus. Exploiting these different imaging sequences, in addition to the capability of performing high spatial resolution imaging in any desired imaging plane in 3-dimention (3D)

space, CMR provides what could be also called "*in-vivo* pathology". Therefore, this has led to substantial progress in the assessment of patients with ischemic and non-ischemic heart disease[1].

ISCHEMIC HEART DISEASE

Acute ischemic disease

After the development of electrocardiographic-triggered fast CMR imaging using gradient-echo sequences, the late gadolinium enhancement (LGE) imaging technique opened new horizons for CMR at the beginning of the century[2-4]. The method exploits the fact that gadolinium-based contrast agents have a much higher volume of distribution in necrotic and fibrotic tissue, when the cardiomyocytes have lost their cell wall integrity or have been replaced by collagen. Late enhancement imaging therefore allows for an assessment of viability with unprecedented image contrast and very-high spatial resolution. Clinical applications included the detection, when the diagnosis is unclear, the differences between acute myocardial infarction (AMI) and chronic ischemic cardiomyopathy[5,6]. The assessment of viability predicts functional recovery in acute myocardial infarction based on the transmural extent of the necrosis[7].

The use of CMR in this setting was subsequently enhanced by the development of water-sensitive T2-STIR sequences, allows the assessment of tissue edema. Of note, since only acute infarction has edema, the combination with LGE imaging, T2-STIR helps to differentiate acute from chronic myocardial infarction[6,8,9]. The edematous tissue in AMI is thought to reflect the area-at-risk, allowing for quantitative assessment of salvaged myocardium after reperfusion therapy[10-12]. This can be measured as the difference between edematous tissue minus necrotic tissue, where the latter is seen on LGE.

Microvascular obstruction (MVO) as a consequence of ischemia and reperfusion injury in AMI is reliably detected with first-pass perfusion imaging or the LGE sequence applied early after contrast injection. The presence of MVO is an independent predictor of adverse outcome, independent of infarct size and left ventricular systolic function[13,14]. Severe microvascular injury can be complicated by reperfusion hemorrhage, which again can be visualized and also quantified with a specific CMR sequence (T2*-weighted imaging)[15]. It is currently unclear whether hemorrhage has independent prognostic effects beyond MVO, since insufficient sample size and flaws in study design, limited most of the clinical studies trying to address this question.

Chronic ischemic disease

Newer imaging approaches are emerging to fine-tune risk assessment in chronic ischemic heart disease, and help, for example, with patient's selection for implantable cardioverter-defibrillators (ICD) implantation. Several authors have shown that the peri-infarct zone between chronic infarction tissue and healthy myocardium displays an intermediate contrast signal. The extent of this "grey-zone" has been associated with ventricular arrhythmia and major adverse cardiac events, probably due to electrical re-entry circuits being located in this area[16]. Prospective studies are under way to assess, whether advanced tissue characteristics such as the LGE grey-zone would be helpful to better select patients for ICD implantation, thereby switching selection criteria from the current left ventricular systolic function to a tissue characteristic. Hence, an improved patient's selection could be of tremendous help, allowing for better selection of patients at higher risk, and also avoiding potentially unnecessary ICD implantations.

In stable coronary artery disease, CMR perfusion imaging with and without stress agents (predominantly adenosine) can detect myocardial ischemia with high accuracy. Depending on the reference standard, it has been reported a sensitivity and specificity of about 90% and 70%-90%, respectively, for the detection of myocardial ischemia[17,18]. Advantages of CMR in this setting include a higher spatial resolution than nuclear imaging methods, allowing the diagnosis of sub-endocardial perfusion defects and microvascular disease[19]. Research efforts are under way to detect ischemia without using contrast agents. Indeed, blood-oxygen-level-dependent (BOLD) sequences are able to create image contrast-based on the tissue's oxygen content in the brain, and initial reports have suggested that modified BOLD sequences could also be applied in the heart[20,21]. This approach, once developed to a clinically applicable tool, promises to revolutionize the ischemia detection field by measuring myocardial oxygen directly, and moving away from perfusion as a surrogate marker.

NON-ISCHEMIC HEART DISEASE

Cardiac magnetic resonance has allowed significant progress in understanding of non-ischemic cardiomyopathies. Beyond accurate assessment of ventricular volume and function, tissue characterization using T1, T2, T2*, perfusion and contrast-enhanced sequences allows for comprehensive tissue characterization as a non-invasive pathology[22-24]. This further contributes to identify the etiology of heart failure, and initial studies have started to identify CMR-based tissue characteristics as prognostic markers[25-28]. In fact, CMR is now the reference diagnostic tool to diagnose myocarditis, as recommended by the Lake Louise consensus criteria[29]. Importantly, T2-weighted imaging identifies edema as a marker of inflammation in acute/active disease, and late gadolinium enhancement is typically present in a "patchy", thus, a non-ischemic pattern. Of note, the combined imaging sequences yield a diagnostic power to assess myocarditis with a sensitivity of 76% and specificity of 96%[30]. Noteworthy, patients with LGE in myocarditis have a worse prognosis than patients

without LGE[31]. Moreover, infiltrative cardiomyopathies such as amyloidosis are reliably diagnosed based on their typical pattern of signal change on T2 and LGE, usually involving the entire myocardium as an organ[32]. The diagnostic power of CMR is especially well exploited in iron deposition disease like thalassemia and hemochromatosis. In fact, CMR can semi-quantitatively assess iron deposition by measuring the T2* value of myocardium. The latter highly correlates with myocardial iron content[33,34]. Furthermore, it is of prognostic value as can be used to monitor the effect of iron chelation therapy, let's say, to start, titrate or finish iron chelation therapy.

THE FUTURE OF CMR

Cardiac magnetic resonance is still a relatively "young" imaging technique, and new technical developments are continuously entering the clinical arena. While current imaging sequences mostly provide a contrast suited for visual analysis, imaging methods that quantitatively map T1, T2 and T2* characteristics are under evaluation[35]. Moving away from qualitative assessment to semi-quantitative or quantitative image analysis will allow increased diagnostic accuracy and reduced observer bias, as well as improve inter-study variability. Normal values will have to be established for different field strengths, and differences in sequence programming between different CMR vendors as a source of variability of normal values will have to be addressed. Eventually, advanced tissue characterization with mapping sequences could reduce (but probably not eliminate) the dependence on gadolinium-based contrast agent. New imaging sequences that apply self-triggering may eliminate the need for electrocardiographic tracing and breath-hold maneuvers[36], further increasing patient comfort and reduce scan time.

CONCLUSION

Cardiac magnetic resonance has become a basic diagnostic tool in cardiovascular medicine. The next decade will be marked by clinical trials investigating the prognostic value of the detailed imaging findings that can be obtained today, and may guide therapy and improve patient prognosis.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (2): Coronary artery disease

Coronary artery disease in women: From the yentl syndrome to contemporary treatment

Sofia Vaina, Anastasios Milkas, Christina Crysohoou, Christodoulos Stefanadis

Sofia Vaina, Anastasios Milkas, Christina Crysohoou, Christodoulos Stefanadis, First Cardiology Clinic, Hippokration Hospital, School of Medicine, University of Athens, 15127 Athens, Greece
Anastasios Milkas, Athens Naval Hospital First Cardiology Department, Athens, 15127 Athens, Greece
Author contributions: All authors have contributed equally to the development of this review.
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Correspondence to: Anastasios Milkas, MD, MSc, First Cardiology Clinic, Hippokration Hospital, School of Medicine, University of Athens, Petropoulou 1 Melissia, 15127 Athens, Greece. tmhlkas@otenet.gr
Telephone: +30-69-77224581
Fax: +30-21-07261585
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Abstract

In recent years attention has been raised to the fact of increased morbidity and mortality between women who suffer from coronary disease. The identification of the so called Yentl Syndrome has emerged the deeper investigation of the true incidence of coronary disease in women and its outcomes. In this review an effort has been undertaken to understand the interaction of coronary disease and female gender after the implementation of newer therapeutic interventional and pharmaceutics' approaches of the modern era.

Key words: Yentl syndrome; Women coronary disease; Acute coronary syndromes; Female gender; Invasive treatment

Core tip: Coronary disease although remains a leading cause of morbidity and mortality in women, is however underestimated mainly because of the protective role of estrogens that results in lower rates of the disease until the age of mid-fifty. In this review detailed information about the prevalence and the consequences of the disease in women are quoted as well as evidence concerning the results of invasive treatment and use of modern drug therapy.

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INTRODUCTION

Coronary artery disease (CAD) constitutes a form of modern epidemic, as it still remains the leading cause of mortality and morbidity in the ageing western societies[1]. The prevalence of CAD in the general population varies, depending on age and sex. In terms of age, there is a trend of more incidents in older ages. Concerning sex, until the age of 60 years old, the predicted probability of having an acute myocardial infarction (AMI) is by

Table 1 Remaining lifetime risks for cardiovascular disease and other diseases among men and women free of disease at 40 and 70 years of age remaining life[57]

Diseases	Remaining lifetime risk at the age of 40 yr		Remaining lifetime risk at the age of 70 yr	
	Men	Women	Men	Women
Any CVD	2 in 3[1]	1 in 2[1]	2 in 3[2]	1 in 2
CHD	1 in 2	1 in 3	1 in 3	1 in 4
AF	1 in 4	1 in 4	1 in 4	1 in 4
CHF	1 in 5	1 in 5	1 in 5	1 in 5
Stroke	1 in 6[3]	1 in 5[3]	1 in 6	1 in 5
Dementia	…	…	1 in 7	1 in 5
Hip fracture	1 in 20	1 in 6	…	…
Breast cancer	…	1 in 8	…	1 in 15
Prostate cancer	1 in 6	…	1 in 9	…
Lung cancer	1 in 13	1 in 16	1 in 15	1 in 20
Colon cancer	1 in 19	1 in 21	1 in 25	1 in 27
DM	1 in 3	1 in 3	1 in 9	1 in 7
Hypertension	9 in 10[3]	9 in 10[3]	9 in 10	9 in 10
Obesity	1 in 3	1 in 3	…	…

[1]Age 45 yr; [2]Age 65 yr; [3]Age 55 yr. AF: Atrial fibrillation; CHD: Coronary heart disease; CHF: Congestive heart failure; CVD: Cardiovascular disease; DM: Diabetes mellitus; …: Not estimated.

far higher in men than in women (60.6% *vs* 33.0%, respectively)[1]. Therefore, CAD is widely believed to be a man's disease, although it accounts for more deaths in women at the age of 35 years than breast cancer (Table 1)[2]. This has been mainly attributed to the protective role of estrogens in the cardiovascular system as they enhance vascular function, reduce the inflammatory response, increase metabolism and insulin sensitivity and finally promote cardiac myocyte and stem cell survival[3]. As a consequence, female hormones may partially account for women's longevity observed in randomized control trials, where women with CAD are older than men and have more co-morbidities such as diabetes, hypertension and chronic kidney disease[4,5]. At menopause, the lack of the protective effect of estrogens leads to a 10-fold increase in the prevalence of CAD in women compared to a 4.6 fold increase in men of the same age[6]. Finally, by the 7th decade of life the increasing rate of CAD among women results in similar rates of the disease among the two genders, although lifestyle factors seem to have a different impact on clinical outcome between gender[1,7,8].

Due to the above mentioned characteristics of the female gender, and the fact that the majority of trials more often enroll younger patients, the representation of women in clinical trials was until recently relatively low, approximately 30% (Table 2)[9,10]. Even in one of the largest contemporary trials designed to compare outcomes between invasive and conservative pharmacological treatment in patients with stable CAD, the COURAGE trial (Clinical Outcomes Utilizing Revascularization and AGgressive Drug Evaluation), men encountered for approximately 85% of the study group[11]. As a result, women suffering from CAD are handled diagnostically and therapeutically based on conclusions drawn mainly from male populations. This observation underscores the need for a more gender focused approach both in every day clinical practice but also in large scale trials. The purpose of the present review is to explore the available data depicting the best strategies to recognise and treat CAD in women.

WOMEN AND SYMPTOM PRESENTATION

It has been demonstrated that women tend to present for chest pain evaluation in the emergency room at a greater rate compared to men (4.0 million visits for women *vs* 2.4 million visits for men). However, women tend to present with less typical symptoms, such as fatigue (70.7%), sleep disturbance (47.8%) and shortness of breath (42.1%)[12], back pain, indigestion, weakness, nausea/vomiting, dyspnoea and weakness[13,14]. At an older age, with more co-morbidities including diabetes, peripheral vascular disease, chronic kidney disease and hypertension[15]. A great amount of literature explored this phenomenon giving rise to large scale clinical trials which resulted in the identification of three paradoxes with regard to female sex and CAD manifestation[13]. (1) Women have disproportionately lower burden of atherosclerosis and obstructive CAD compared with the extent of angina they complain for; (2) Compared to men, women have less severe CAD despite the fact that they are older with a greater risk factor burden; and (3) Even though CAD is less evident in women, as illustrated by invasive diagnostic imaging modalities, females still have a more adverse prognosis compared to men.

Another parameter of great importance is the increasing prevalence of the cardiac X syndrome or coronary microvascular dysfunction among women of post-menopausal age. It is evident that almost 40% of the operated coronary angiographies reveal non obstructive atherosclerosis although patients present with anginal symptoms and positive exercise training results[16]. A large proportion out of 30% of these findings is attributed to coronary microvascular dysfunction as no other identifiable

Table 2 Women are considerably underrepresented in clinical trials[9]

Study	Sample MT.PCI	Enrollment *n*	Mean age (yr)	Male (%)	DM (%)	% Prior MI	1/2/3-Vessel CAD	% No symptoms	% Mean EF	% Follow up, yr
RITA-2	514/504	1992-1996	58 (Median)	82	9	47	60/33/7	20	ND[1]	7
ACME-1	115/112	1987-1990	60	100	18	31	100/0/0	9	68	2.4-5[2]
ACME-2	50/51	1987-1990	60	100	18	41	0/100/0	18	67	2.4-5[2]
AVERT	164/177	1995-1996	59	84	16	42	56/44/0	16	61	1.5
Dakik *et al*[58]	22/19	1995-1996	53	59	ND	100	44/41/15	0	46	1
MASS	72/72	1988-1991	56	58	18	0	100/0/0	0	76	5
MASS II	203/205	1995-2000	60	68	30	41	0/42/58	ND	67	1
ALKK	151/149	1994-1997	58	87	16	100	100/0/0	0	ND[3]	4.7
Sievers *et al*[59]	44/44	ND	56	ND	0	55	100/0/0	ND	ND	2
Hambrecht *et al*[60]	51/50	1997-2001	61	100	23	46	58/27/15	0	63	1
Bech *et al*[61]	91/90	ND	61	64	12	25	66/28/6	0	65	2

[1]Ninety-three percent of patients had very good or excellent wall motion score; [2]Ninety-seven percent of patients were in New York Heart Association class 1; [3]Follow-up was 2.4 years (mean) to follow-up interview, 3 years (mean) to follow-up exercise test, and 5 years (median) for ascertainment of deaths and MI events. MT: Medical treatment; DM: Diabetes mellitus; EF: Ejection fraction; ND: No data available.

cause can be found. The most interesting aspect of this group of patients is the fact that it is consisted in its great proportion (almost 70%) of post-menopausal women[17]. In contrast to the findings of earlier studies that microvascular angina does not affect long term prognosis[18], it is evident nowadays from a large retrospective analysis of 11223 patients referred for coronary angiography with stable angina, that patients with non-obstructive CAD consolidate a further increase in the risk of coronary events and of all-cause mortality (HR = 1.85; 95%CI: 1.51-2.28; and 1.52; 95%CI: 1.24-1.88, respectively)[19].

Thus, women are frequently a clinical challenge for the cardiologist and their symptom misinterpretation may lead to the wrong diagnosis and treatment with potentially unfavourable consequences. Symptom evaluation and recognition in women is a matter of great importance, since it has been shown that when typical symptoms accompany an acute coronary syndrome (ACS) there is no difference in the disease diagnosis between women and men[20]. Moreover, when prodromal symptoms are recognised in women before an ACS, women have better survival in comparison to men[21].

WOMEN AND TREATMENT STRATEGIES

Not only diagnostic evaluation of women may be misleading, but also the appropriate treatment selection can be difficult. It was already recognized in 1991 that women suffering from CAD had less chances to be introduced either in coronary angiography or percutaneous coronary intervention (15.4% of women *vs* 27.3% of men, $P < 0.001$)[22]. This approach was demonstrated even in cases were admission symptoms were more prominent in women than in men[23]. Unlike men, women were submitted less frequently to any diagnostic or therapeutic intervention creating in this way dissimilarity on curing procedures. This alarming fact was described by Bernadine Healy, the first woman director of National Health Institute in United States, as the Yentl syndrome named after the Jewish heroine of Isaac Singer, who was masqueraded as a boy in order to be educated in the Talmud philosophy. Healy concluded that when a woman has been shown to have extensive CAD, like men, only then she gets the appropriate treatment[24]. Since, a plethora of studies examined gender differences in order to provide the best treatment options for women.

WOMEN AND PERCUTANEOUS CORONARY INTERVENTION

One of the first studies comparing the impact of percutaneous coronary interventions (PCI) with bare metal stent (BMS) implantation between females and males, revealed that women had 50% more chance of death in comparison to men after adjustment for age, co-morbidities, and extend of coronary atherosclerosis[25]. However, in the same analysis, after final adjustment for Body Surface Area, mortality rates were similar between the two genders, although a slighter increased rate of stroke, vascular complications and repeat in-hospital revascularization was observed in women[25]. Similar results were reported in a retrospective analysis from Mayo Clinic investigating 18885 consecutive, patients who underwent PCI between 1979 and 1995 (early group) and between 1996 and 2004 (late group)[26]. The results indicated no difference in terms of 30-d mortality, while after adjustment for baseline risk factors, again there was no difference observed in short or long term mortality between the two genders (Figures 1 and 2)[26]. The study indicated that between the two groups a decrease in 30 d mortality was observed in both genders during the 25-year follow up period.

A retrospective study from Rotterdam investigated the outcomes of Sirolimus Eluting Stents, Pacliataxel Eluting Stents and BMS in women[27]. In this study, even though women had worse baseline characteristics compared to men, no differences in 3-year outcomes were detected between males and females.

A recent meta-analysis, which included 43904 patients (26.3% women) in 26 trials, assessed the safety and

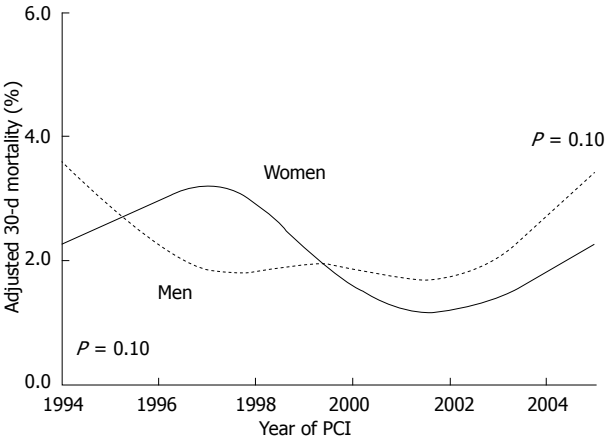

Figure 1 Mayo clinic percutaneous coronary interventions registry: 12798 pts adjusted 30-d mortality after percutaneous coronary interventions[26].

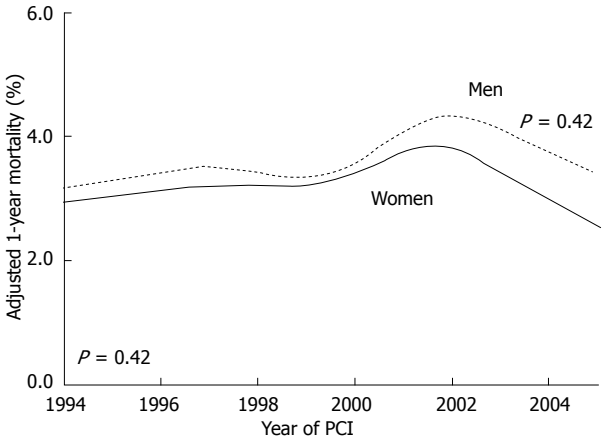

Figure 2 Mayo clinic percutaneous coronary interventions registry: 12798 pts Adjusted 1-year mortality after percutaneous coronary interventions[26].

efficacy of DES in women[28]. The study showed that DES implantation in women was more effective and safe than BMS implantation. Furthermore, it was observed that 2nd and 3rd generation DES, such as everolimus-eluting Xience and Promus stents, zotarolimus-eluting Endeavor and Resolute stents, biolimus-eluting Biomatrix and Nobori stents, and sirolimus-eluting Yukon stents, were associated with an improved safety profile compared with early-generation DES[28]. These results suggest that women undergoing PCI may benefit more when DES and moreover newer generation DES are used.

An interesting meta-analysis was designed in order to evaluate whether female gender is an independent risk factor for repeated coronary revascularization after PCI. The results indicated that although female sex increases the short term rate of repeated revascularization after PCI the long term rate was the same between the two genders clarifying the fact that even for this parameter female gender is not an independent risk factor[29].

Bleeding complications at the point of vascular puncture, hematomas and retroperitoneal bleedings are decreased in the current era. This is mainly due to introduction of less aggressive anticoagulant regimens, adjustment of heparin dose according to body mass index and smaller size catheters. However, women still continue to be at 1.5 to 4 times greater risk for bleeding in comparison to men (Table 3)[30]. Reduced Body Surface Area, altered pharmacokinetics and diminished drug metabolism are the main aspects of female gender that attribute mostly in these higher bleeding rates.

Newer anticoagulant agents were recently introduced in clinical practice raising expectations for further bleeding risk reduction. In order to evaluate this hypothesis, novel studies were contacted to evaluate the action of direct thrombin inhibitor bivalirudin (ANGIOX®) in both genders who suffered from moderate and high-risk ACS[31]. Out of 7789 patients submitted to PCI, 2561 received heparin and Ⅱb-Ⅲa glycoprotein inhibitor, 2609 received bivalirudin in combination to Ⅱb-Ⅲa glycoprotein inhibitor and 2619 received bivalirudin solely. The group of patient to receive bivalirudin in comparison to the group of patient receiving heparin in combination to Ⅱb-Ⅲa glycoprotein inhibitor displayed the same degree of ischemic events but with a lesser degree of major bleeding (4% *vs* 7%, $P < 0.0001$)[31]. Interestingly, bivalirudin alone decreased the variance of bleeding between the two genders, but it did not completely eliminate it.

In order to achieve decreased bleeding rates in women, the idea of radial approach during intervention was implemented into clinical practice. However, due to women's smaller vessel size and lower pain threshold it was revealed that 14% of women were finally switched to femoral approach in contrast to 1.7% of men[32]. In the same study however, during 299 radial interventions in women no major bleeding was observed, whereas in 601 femoral intervention, 25 major bleedings where recorded ($P = 0.0008$). In addition, radial approach was related with a lower rate of minor bleeding (6.4% *vs* 39.4% $P = 0.00001$). These favourable results indicate that radial approach during PCI in women is safer in terms of bleeding, even though there are more difficulties to initiate the procedure through the particular access site.

WOMEN AND CORONARY ARTERY BY-PASS GRAFTING

In the past, women submitted to CABG were shown to have higher perioperative morbidity and mortality compared to men[33-35]. These first results raised the issue of CABG safety in women and initiated the conduction of several newer trials. Indeed, over 20 trials investigated the impact of CABG in women compared to men. A recent meta-analysis confirmed that women experience higher mortality rates in comparison to men in terms of short-, mid- and long-term follow-up with the higher mortality recorded in the short-term period[36]. Several explanations for this observation have been proposed such as the delayed reference of women to CABG when

Table 3 Women have higher rate of vascular complications after percutaneous coronary interventions[30]

Study	*n*	Vascular complications		
		Women	Men	*P*-value
Alfonso	981	11/157 (7.0%)	16/824 (2.0%)	< 0.01
Antoniucci	1019	14/234 (6.0)	24/785 (3.0%)	0.01
BOAT	989	6/237 (2.5%)	6/752 (0.8%)	0.05
CAVEAT	512	14/128 (10.8%)	22/384 (5.8%)	0.003
NACI	2855	39/971 (4.0%)	28/1884 (1.5%)	< 0.05
NCN	109708	1955/36204 (5.4%)	1985/73504 (2.7%)	< 0.001
NHLBI	2136	24/555 (4.4%)	36/1581(2.3%)	< 0.05
NHLBI	2524	44/883 (5.0%)	43/1641(2.6%)	< 0.01
STARS	1965	44/570 (7.8%)	39/1395 (2.8%)	< 0.01
Trabatoni	1100	15/165 (9.3%)	33/935 (3.5%)	0.004
Welty	5989	34/2096 (1.6%)	23/3893 (0.6%)	< 0.001
WHC	7372	78/2064 (3.8%)	125/5308 (2.4%)	< 0.001
Combined	137150	2278/44264 (5.1%)	2380/92886 (2.6%)	

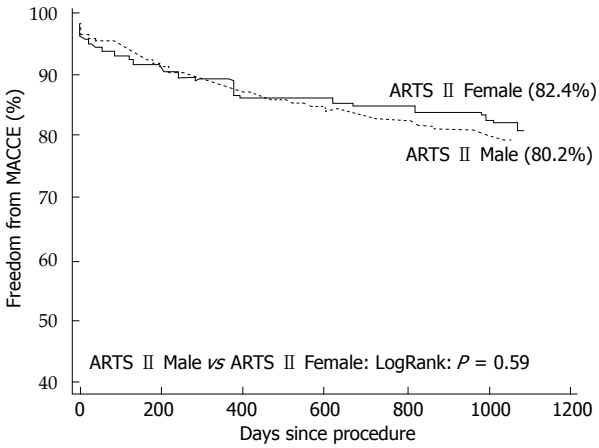

Figure 3 Freedom from major adverse cardiac and cerebrovascular events after percutaneous coronary intervention in Arterial Revascularization Therapies Study-Part Ⅱ[41].

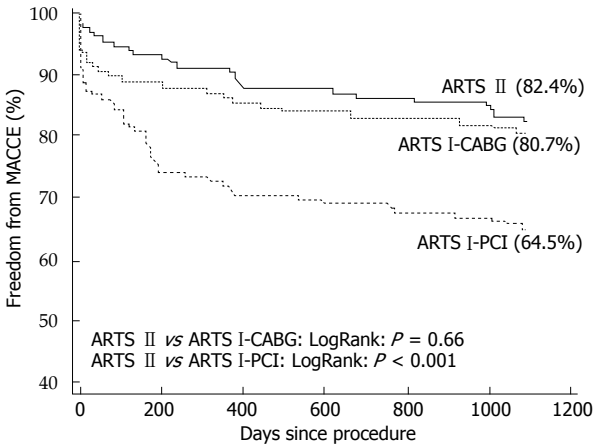

Figure 4 Freedom from major adverse cardiac and cerebrovascular events after coronary artery by-pass grafting and percutaneous coronary intervention with bare metal stent and sirolimus eluting stent implantation in women[41].

CAD extends to a greater degree, the smaller size of women coronary vessels that creates technical issues to the surgeon or finally the limited use of left internal mammary artery in women[37-39].

Arterial Revascularization Therapies Study Part I (ARTS I) was one of the first studies to compare CABG and PCI in women. The study demonstrated that for a total of 1205 patients there was no significant difference in terms of death, stroke, or myocardial infarction between the two genders. However, stenting was ascossiated to a greater need for repeated revascularization[40,41].

Newer studies in the Drug Eluting Stent (DES) era sought to further investigate the effect of gender on PCI and CABG outcomes. The multicenter randomized study Arterial Revascularization Therapies Study-Part Ⅱ (ARTS Ⅱ) was designed to evaluate the outcomes of Sirolimus Eluting Stent implantation in comparison to BMS implantation and Coronary Artery Bypass Grafting (CABG) in patients with multivessel CAD[41]. In ARTS Ⅱ, although women tended to have more risk factors compared to men, they experienced the same rate of adverse events with men at 30 d, one year and three years after Sirolimus Eluting Stent implantation (Figure 3)[37]. Additionally, it was observed that both genders had a more favorable clinical outcome with Sirolimus Eluting Stents compared with BMS but similar to CABG (Figure 4)[41].

These results could potentially institute PCI as the first choice treatment in women with multivessel disease.

WOMEN AND ACUTE CORONARY SYNDROMES

The vast majority of women, about 60%, experience an acute coronary syndrome (ACS) or sudden cardiac death as the first manifestation of the disease[42]. The initial results comparing gender differences in patients with ACS were presented in the pre-thrombolytic era, where a 28% mortality rate was demonstrated in women compared to a 16% mortality among men[43]. Women also experienced a 3 fold higher rate of reinfarction. In the following years, the introduction of thrombolysis decreased the total mortality rates in the general population. However,

a discrepancy was still evident between the two genders (30 d unadjusted mortality rate was 13.1% in women and 4.8% in men)[44]. Newer, large scale studies were undertaken in order to re-evaluate these results in the modern era of invasive approach to ACS. One of the largest trials investigating these aspects enrolled 78254 patients (39% women) with AMI in 420 United States hospitals from 2001 to 2006[45]. The results reconfirmed the data observed in previous trials. The study showed that women with ACS are older, with more comorbidities such as hypertension, diabetes, and metabolic syndrome and tend to present less often with ST-elevation AMI[46-48]. Adjusted analysis revealed no differences in terms of mortality between the two genders for ACS, but in the subgroup of ST Elevation Myocardial Infarction (STEMI) there was a statistically significant and almost double proportion of mortality in women (10.2% women *vs* 5.5% men, $P < 0.0001$). An important conclusion from this trial was the fact that women received less often aspirin and b-blockers and were less often treated in an invasive manner with percutaneous transluminal coronary angioplasty.

This approach was also noticed in an earlier contacted study in Minnesota (46% less chances of invasive approach) as well as in a in a Swiss national registry where the rate of women introduced in PCI was significantly lower than men (OR = 0.70; 95%CI: 0.64 to 0.76)[15,48].

These observations raised the question of whether physicians prefer more conservative strategies because women have higher mortality rates with invasive procedures or whether women are less willing to undergo such a procedure. PRimary Angioplasty in patients transferred from General community hospitals to specialized PTCA Units with or without Emergency thrombolysis 1 and 2 studies evaluated 520 patients with STEMI treated with thrombolytics and 530 patients treated with primary PCI. Women treated with thrombolytics had almost two fold higher mortality than women treated with primary PCI ($P = 0.043$)[49]. Therefore, although patient demographic data were not adjusted to body mass index, which could have an effect on the unadjusted doses of streptokinase used, it can be concluded that primary PCI to treat women with AMI is superior to a more conservative approach. Similar results were demonstrated in the Global Utilization of Streptokinase and TPA for Occluded Coronary Arteries Ⅱ-B and in a sub-analysis of the Primary Angioplasty in Myocardial Infarction trial comparing newer thrombolytic agents *vs* PCI[50,51]. More recent studies showed that mortality and major adverse cardiac events, though higher among women with primary PCI in unadjusted analyses, are comparable in both genders after adjusting for age, hypertension, smoking, diabetes mellitus, stent diameter, and time between symptoms onset and ambulance arrival[52-55]. A meta-analysis that included 8 trials and almost 10115 patients, demonstrated that low-risk women with ACS may benefit from a more conservative approach. However, males and high-risk females with ACS treated with an invasive strategy have similar clinical outcome in terms of death, MI, or rehospitalisation for ACS[56].

CONCLUSION

It has been consistently shown that women who are suffering from CAD usually present with less typical symptoms, at an older ager and with more co-morbidities compared with men. Therefore, they constitute a high risk group that potentially poses a diagnostic and therapeutic challenge. However, it seems that in the modern era, where sophisticated interventional and surgical techniques have emerged, women significantly benefit from an early invasive approach provided an intense medical monitoring is implemented.

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REVIEW

Helicobacter pylori: Does it add to risk of coronary artery disease

Vishal Sharma, Amitesh Aggarwal

Vishal Sharma, Department of Gastroenterology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India
Amitesh Aggarwal, Department of Medicine, University College of Medical Sciences and GTB Hospital, Delhi 110095, India
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Correspondence to: Amitesh Aggarwal, Assistant Professor, Department of Medicine, University College of Medical Sciences and GTB Hospital, Dilshad Garden, New Delhi, Delhi 110095 India. dramitesh@gmail.com
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Abstract

Helicobacter pylori (*H. pylori*) is a known pathogen implicated in genesis of gastritis, peptic ulcer disease, gastric carcinoma and gastric lymphoma. Beyond the stomach, the organism has also been implicated in the causation of immune thrombocytopenia and iron deficiency anemia. Although an area of active clinical research, the role of this gram negative organism in causation of atherosclerosis and coronary artery disease (CAD) remains enigmatic. CAD is a multifactorial disease which results from the atherosclerosis involving coronary arteries. The major risk factors include age, diabetes mellitus, smoking, hypertension and dyslipidemia. The risk of coronary artery disease is believed to increase with chronic inflammation. Various organisms like Chlamydia and Helicobacter have been suspected to have a role in genesis of atherosclerosis *via* causation of chronic inflammation. This paper focuses on available evidence to ascertain if the role of *H. pylori* in CAD causation has been proven beyond doubt and if eradication may reduce the risk of CAD or improve outcomes in these patients.

Key words: Extra gastric; Coronary artery disease; *Helicobacter pylori*; Atherosclerosis; Inflammation

Core tip: Coronary artery disease (CAD) is a multifactorial disease and inflammation plays an important role in Atherogenesis. *Helicobacter pylori* (*H. pylori*) is speculated to be one organism which may incite the inflammatory response thereby predisposing infected individuals to CAD. This paper looks at clinical evidence in relation to *H. pylori* infection and CAD and also examines the evidence of effects of eradication of *H. pylori* on CAD and its risk factors.

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INTRODUCTION

Helicobacter pylori (H. pylori), first identified by Marshall and Warren in 1982, is a ubiquitous gram negative bacterium. A mixture of serendipity and diligent research lifted the veil off this enigmatic organism which was first

thought to be Campylobacter like. The Easter holidays of 1982 had ensured that the culture plates were not destroyed after 48 h of absence of growth and led on to the discovery of *H. pylori*[1]. However, it was after much perusal that the scientific community accepted the bacterium-ulcer-cancer dogma eventually culminating in the 2005 Nobel Prize[2]. Over years it has become clear that this bacterium is responsible for many disease other than the gastric diseases. In the stomach *H. pylori* is implicated in the causation of chronic gastritis, peptic ulcer (gastro-duodenal), gastric MALTOMA (mucosa associated lymphoid tissue lymphoma) and gastric adenocarcinoma. It is also associated with certain extra-gastric disorders like immune thrombocytopenia and iron deficiency anaemia[3,4]. Although the role of these in causation of gastric injury has emerged in recent times, the role of *H. pylori* and its virulence factors in causation of atherosclerosis and coronary artery disease is not entirely clear as yet. The present review will focus on the relationship between this bacterium and the coronary artery disease

THE BACTERIUM

H. pylori have an inherent ability to survive in the gastric epithelium where they reside in the mucous layer and remain protected from the gastric acid. Urease, an enzyme abundantly present in this flagellate organism, helps create an alkaline environment to help in survival in the otherwise acidic environment. While most infected individuals remain unaffected, others develop a myriad of clinical manifestations ranging from gastritis to gastric cancer. What fuels and drives the pathogenesis of these varied clinical spectrums is not completely understood. While it is estimated that around half the world's population harbours infection with *H. pylori*, only a fraction of the infected manifest with the implicated diseases. The various factors implicated in disease causation following infection by *H. pylori* include both the bacterial virulence factors and the host response to the infection. The bacterial virulence factors include BabA (bacterial binding and inflammation), lipopolysaccharide (interaction with toll-like receptors and mediation of inflammation), Cag pathogenicity island (heightened inflammatory response to infection) and vacA toxin (impaired host responses). The host responses which affect the outcome of infection include interleukin (IL)-1β (certain polymorphisms associated with carcinogenesis), activation of nuclear factor (NF)-κB, IL-8 levels, recruitment of neutrophils, macrophages and oxidative injury and TH1 cell response may all mediate tissue injury and reaction to *H. pylori* infection.

CORONARY ARTERY DISEASE: A MULTIFACTORIAL DISEASE

Coronary artery disease (CAD) is a multifactorial disease manifesting in a number of clinical presentations including angina, myocardial infarction and heart failure. The CAD is primarily a result of coronary atherosclerosis for which a multitude of risk factors are implicated including hyperlipidemia, smoking, diabetes mellitus, lack of physical activity, male gender, increasing age, obesity amongst others[5]. There is a growing acknowledgement of inflammatory factors including C-reactive protein in prediction of increased risk of CAD[6]. *H. pylori* has also been implicated by some to have a role in predisposition to cardiac risk and causation of CAD. Indeed, in a polymerase chain reaction (PCR) based study for detection of *H. pylori* in the coronary plaques of patients who underwent coronary artery bypass grafting (CABG), 29.5% patients had a detectable *H. pylori* on PCR. Also there was serological evidence of infection in 53.3% of these 105 patients[7]. Therefore the infection by *H. pylori* may play a role in plaque rupture and causation of ischemic heart disease. Interestingly, cytotoxin asso-ciated gene A (Cag-A) may also play a role in the path-ogenesis of CAD as results of one study suggest that anti-Cag-A antibody titres were higher in patients with CAD vis-à-vis normal subjects. Also patients with anti-Cag-A positivity had more severe lesions of CAD[8]. It is believed that the chronic inflammation associated with chronic infections may result in progressive atherosclerotic disease eventually manifesting as CAD[9].

CAD AND *H. PYLORI*

Epidemiological evidence

A number of reports have evaluated the role of *H. pylori* in causation of CAD. In a report on 120 patients who underwent coronary angiography, the prevalence of serologically detectable evidence of *H. pylori* infection was more in patients with angiographically documented CAD (> 50% stenosis in at least one coronary artery). The evidence of infection was found in 70% patients with single vessel disease, 76.3% patients with double vessel disease but only in 50% individuals with no CAD[10]. Coronary artery calcium is believed to be a marker of atherosclerosis and its progression a predictor of CAD events. The correlation of coronary artery calcium (CAC) with various pathogens is conflicting. In a report on 201 asymptomatic subjects, the antibodies to heat shock protein 65 correlated with CAC score as also with evidence of *H. pylori* infection[11]. Another large study from South Korea which evaluated 2029 individuals for *H. pylori* antibody and coronary artery calcification score found that *H. pylori* seropositivity was different amongst those with and those without CAC[12]. This association was more evident in patients with early coronary atherosclerosis[12]. However another report about presence of *H. pylori* infection in a large cohort of individuals who underwent repeat CAC assessment, the presence of *H. pylori* infection (IgG to *H. pylori*) did not correlate with development or progression of CAC[13]. In a report comparing patients with CAD and healthy controls, sero-positivity for *H. pylori* infection was significantly higher

Table 1 Recent reports on association of *Helicobacter pylori* infection with coronary artery disease

Ref.	Population (number of subjects)	Diagnosis of CAD	Association between *H. pylori* infection and CAD
Shmuely *et al*[23]	CAD (173) *vs* Controls (123)	Myocardial Perfusion imaging	Yes No association with Cag-A
Vafaeimanesh *et al*[10]	CAD (62) *vs* Controls (58)	Angiographic	Yes
Laek *et al*[13]	5744 individuals, Age 45-84 yr, average follow-up of 2.4 yr	Newly detectable coronary artery calcium (CAC)	No correlation with CAC development
Mundkur *et al*[18]	CAD and controls (433 each) from South Asians	Angiography	None
Padmavati *et al*[24]	Acute myocardial infarction *vs* Controls	ECG, enzymes	None
Tewari *et al*[15]	200 CAD cases and controls	ECG, treatment records	Yes
Grdanoska *et al*[25]	Acute coronary syndrome (64), CAD (53), controls (35)	ECG, enzymes	Yes
Grub *et al*[26]	Controls (30), CAD (52) and CAD with rheumatic diseases (67)	Patients referred for CABG	None
Park *et al*[12]	2029 subjects	CAC	Yes
Al-Ghamdi *et al*[27]	CAD (50) and controls (15)	ECG, angiography	Yes
Azarkar *et al*[28]	Controls (78) and myocardial infarction (73)	ECG, enzymes	Yes
Khodaii *et al*[29]	Myocardial infarction (500) and controls (500)	ECG, enzymes	Yes Cag-A positivity also correlates with CAD

CAD: Coronary artery disease; Cag-A: Cytotoxin associated gene A; ECG: Electrocardiography; CAC: Coronary artery calcium; CABG: Coronary artery by-pass grafting; *H. pylori*: *Helicobacter pylori*.

in patients of CAD (59%) vis-à-vis the healthy controls (39%)[14]. Similar reports from India also corroborate that *H. pylori* sero-positivity was much higher in patients with CAD when compared with asymptomatic controls[15-17]. Few reports have indicated, to the contrary, that there is no significant association between *H. pylori* infection and CAD. In a report from Asian Indian families which evaluated role of multiple pathogens in causation of CAD, while CMV infection appeared to elevate the risk of CAD infection with *H. pylori* did not increase the risk[18]. In a large Japanese study to assess seroprevalence of *H. pylori* in CAD and asymptomatic controls no significant differences were detected between the two groups[19]. However when a subgroup of patients younger than 55 years was analysed the seroprevalence of *H. pylori* antibody was higher in cases than controls (58.7% and 43.3%, respectively)[19]. Another report about incidence of CAD in elderly individuals who were assessed for *H. pylori* infection at baseline and followed up for 10 years indicated that *H. pylori* positivity was not associated with increased incidence of CAD[20]. As described previously, PCR based studies of the coronary plaque have been done and have detected *H. pylori* DNA in them. In a controlled study of atheromatous plaques of 46 patients who underwent CABG, 22 (47.8%) showed *H. pylori* DNA while none of the controls who underwent coronary artery biopsy had PCR detectable *H. pylori*[21]. Aortic biopsies from areas free of atheromatous plaque have also been reported to be positive in a significant number of patients with CAD but none of the controls[22]. Table 1 summarises the recent studies reporting about association of *H. pylori* with CAD.

CAG-A AND CAD

As previously mentioned, role of Cag-A has also been evaluated as a predisposing factor for occurrence of coronary artery disease[8]. In a study of cardiac peptides including Brain Natriuretic Peptide in 103 patients with non-ST elevation myocardial infarction and their relation with *H. pylori* infection, it was found that individuals infected with Cag-A positive strains of *H. pylori* had higher levels of BNP in the serum[30]. BNP is a marker of heart failure and may predict a more serious course of the disease thereby suggesting that *H. pylori* infection with Cag-A positive strains may lead to an adverse outcome. Interestingly, IL-6 levels were also found to correlate with the Cag-A status. This suggests that the inflammatory response to Cag-A positive *H. pylori* may mediate atherogeneis in a subgroup of patients with CAD[30]. However other reports indicate that Cag-A positivity does not vary significantly between angiographically positive and negative group of individuals. In a report of 112 consecutive individuals who underwent coronary angiography, the Cag-A positivity did not affect the severity of CAD[31]. In a large study including 505 patients with CAD and 1025 matched controls, neither the prevalence of *H. pylori* infection was increased in the diseased subjects nor did the presence of Cag-A positive strains predict higher likelihood of CAD[32]. In a large population based report on 685 individuals, merely the presence of infection by *H. pylori* did not correlate with serum markers of inflammation. However those seropositive for Cag-A positive strains had increased values of common carotid artery intima-media thickness and the risk of atherosclerosis was enhanced by CRP positivity[33]. Another report also indicated that Cag-A positive strains appeared to raise the risk of CAD while merely the presence of *H. pylori* infection was not significantly different between cases and controls[34]. An

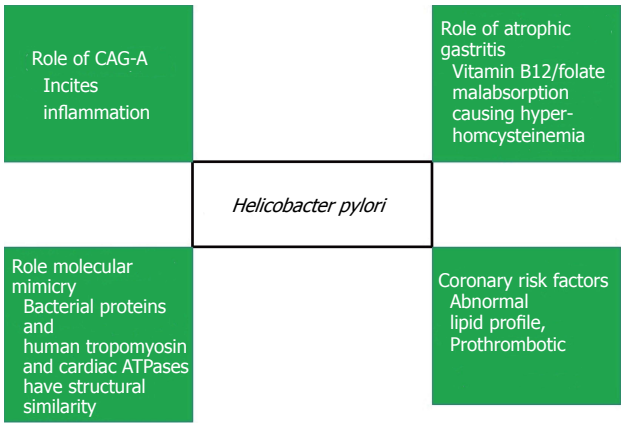

Figure 1 Postulated mechanisms of Atherogenesis in *Helicobacter pylori* infection.

interesting study reported about sero-prevalence of anti-Cag-A antibodies across a spectrum of presentations which included controls, stable and unstable angina and found that anti-Cag A titres were significantly higher in patients with unstable angina[35].

MECHANISMS BEHIND ATHEROGENESIS

One report has studied the association of atrophic gastritis with CAD. Atrophic gastritis is believed to be the end result of chronic gastric inflammation including that related to *H. pylori* infection. Decrease in serum pepsinogen I and a low Pepsinogen I / Ⅱ ratio points to the diagnosis of atrophic gastritis. In this intriguing report based on a population based study, Senmaru *et al*[36] reported that prevalence of CAD was higher in the patients having atrophic gastritis (5.8%) when compared with individuals not having atrophic gastritis (2.8%). Atrophic gastritis may result in malabsorption of Vitamin B12 and Folate and result in increased homocysteine levels. Hyper-homocysteinemia is a recognised risk factor for CAD[37]. One report has also suggested structural homology between bacterial proteins and human tropomyosin and cardiac ATPases thereby providing insight into molecular mechanism involved in the cardiac injury due to anti-*H. pylori* inflammatory response[30]. *H. pylori* has also been associated with dyslipidemia. In a Japanese study on 6289 subjects, infection with *H. pylori* was associated with low HDL and elevated LDL levels[38]. Other reports have also provided similar evidence[39]. Cag-A positive strains also exhibit elevated levels of highly sensitive CRP, oxidized LDL and apolipoprotein B all of which may participate in the pathogenesis of atherosclerosis[40]. There is also a suggestion that *H. pylori* may have a prothrombotic role which may also increase the associated risk of atherosclerotic diseases. The bacterium may promote aggregation of platelets by binding to the von-Willibrand factor[41]. Infection with *H. pylori* may stimulate an inflammatory response against heat shock protein (hsp60) which may drive a helper T cell (TH1) response and increase the risk of atherosclerosis[42]. The high degree of homology between bacterial and eukaryotic HSP may result in molecular mimicry and collateral immune damage from immune response primarily directed against infectious agents[43]. The host reaction to the *H. pylori* lipopolysaccharide (LPS) may also be a risk factor for atherosclerosis[44]. Figure 1 depicts the predominant mechanisms purported to play a role in genesis of *H. pylori*-related CAD.

EFFECT OF ERADICATION

The prognostic role of *H. pylori* infection has also been assessed in acute CAD. In 433 patients of acute coronary syndrome (ACS) the seroprevalence of *H. pylori* infection was determined using IgG and IgA serology. Those infected with *H. pylori* had an increased risk of short term adverse outcomes during the first month of follow-up[45]. Another report which evaluated role of eight pathogens on occurrence future events in patients diagnosed to have angiographic evidence of CAD. Serological evidence of *H. pylori* infection predicted an increased risk of future events and mortality in these 1018 patients and increase in pathogen burden also affected long term outcome[46]. An interesting study evaluated the role of *H. pylori* eradication on coronary artery lumen reduction in patients who underwent percutaneous intervention for CAD. A higher loss of coronary lumen was noted in those patients who had serological evidence of *H. pylori* infection. Also, eradication of *H. pylori* attenuated this reduction in lumen of the coronary artery vis-à-vis the placebo group[47]. Another report by the same group provides similar findings but it is not clear if the report was based on different patients[48]. This small but elegant study opens debate about possible benefit of *H. pylori* eradication in attenuating further atherosclerotic process which is driven primarily by inflammatory mediators. In a study assessing the effect of *H. pylori* eradication on coronary risk factors in 48 patients, no differences were observed in pre and post-treatment fasting sugars, lipid profile and levels of tissue-plasminogen activator, fibrinogen, plasminogen activator inhibitor-1 and D-dimer levels[49]. However a larger study of 496 patients and reporting about pre and post- *H. pylori* eradication profile, the eradication of *H. pylori* seemed to increase HDL levels and reduce the levels of C reactive protein and those of fibrinogen. This suggests that attenuation of inflammatory response is likely to occur after *H. pylori* eradication[50]. In a report documenting the effects of *H. pylori* eradication on insulin resistance in 159 patients using homeostasis model assessment of insulin resistance, the insulin resistance measured six weeks post-eradication was lower than the baseline. The study also reported changes in lipid profile including an increase in HDL levels and a fall in LDL levels with *H. pylori* eradication[51]. Another report also indicates that the *H. pylori* eradication may increase HDL levels and lead to reduction of CRP levels[52]. Table 2 depicts various studies reporting about the effects of *H. pylori* eradication on CAD and its risk

Table 2 Effect of *Helicobacter pylori* eradication on coronary artery disease

Ref.	Population	Intervention	Results
Kowalski *et al*[47,48]	40 patient with single vessel CAD and *H. pylori* infection	All underwent PTCA and 20 each received eradication or placebo	Attenuated reduction mean coronary artery lumen at 6 mo in those undergoing eradication
Lu *et al*[49]	*H. pylori* positive individuals	Testing of coronary risk factors before and after *H. pylori* eradication	No change in sugar, lipid and fibrinolytic parameters with eradication
Pellicano *et al*[50]	*H. pylori* positive individuals	Testing of coronary risk factors before and after *H. pylori* eradication	Improvement in HDL-C, reduction in CRP and fibrinogen levels. Elevation in BMI and diastolic blood pressure
Gen *et al*[51]	*H. pylori* positive individuals	Testing for insulin resistance, lipid profile and CRP before and after eradication	Improvement in insulin resistance, lipid abnormalities and CRP levels
Kanbay *et al*[52]	*H. pylori* positive individuals	Testing for lipid profile and CRP before and after eradication	Increase in HDL and reduction in CRP with successful eradication

CAD: Coronary artery disease; CRP: C-reactive protein; HDL: High density lipoprotein; PTCA: Percutaneous transluminal coronary angioplasty; *H. pylori*: *Helicobacter pylori*.

factors.

ATHEROGENESIS BEYOND CORONARY ARTERIES

In contrast to CAD, data is scarce on the relation between *H. pylori* infection and stroke. A meta-analysis found that Cag-A-positive *H. pylori* increases the risk of both ischemic stroke and coronary heart disease[53].

A case-control study of 150 patients by Yang *et al*[54] in 2011 does not reveal any strong association between chronic *H. pylori* infection and ischemic stroke. However, another study by Pan[55] suggested lowering of inflammatory markers and decrease in cerebral infarction readmission rates in patients of stroke with positive urease test treated with (conventional therapy + anti- *H. pylori* therapy. Wu *et al*[56] suggested role of increased expression of CD62p on platelets and increase in clotting indexes in pathogenesis of stroke in *H. pylori* positive patients.

A meta-analysis of 13 studies including 4041 participants indicated that positive anti-*H. pylori* IgG, anti-Cag-A IgG and (13)C-urea breath test were significantly associated with increased risk of IS, respectively, and positive anti-Cag-A IgG was more effective for predication of IS risk[57].

But a formal meta-analysis of ten prospective observational studies indicated no strong association between *H. pylori* infection and stroke, neither in those with cytotoxin-associated gene-A-positive infection[58].

All in all, the evidence supporting the role of *H. pylori* in causation of CAD is equivocal and interventions aimed at *H. pylori* eradication have not shown conclusive evidence of benefit in eradicating the organism vis-à-vis cardiovascular outcomes. Perhaps multicentre randomised trials comparing eradication of *H. pylori* in large populations at risk of CAD and then follow-up to deterimine risk of CAD may answer this question.

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MINIREVIEWS

Relationship between vascular endothelium and periodontal disease in atherosclerotic lesions: Review article

Marco Aurélio Lumertz Saffi, Mariana Vargas Furtado, Carisi Anne Polanczyk, Márlon Munhoz Montenegro, Ingrid Webb Josephson Ribeiro, Cassio Kampits, Alex Nogueira Haas, Cassiano Kuchenbecker Rösing, Eneida Rejane Rabelo-Silva

Marco Aurélio Lumertz Saffi, Mariana Vargas Furtado, Carisi Anne Polanczyk, Cardiovascular Division, Hospital de Clínicas de Porto Alegre, Porto Alegre 90035-003, Brazil
Márlon Munhoz Montenegro, Ingrid Webb Josephson Ribeiro, Cassio Kampits, Alex Nogueira Haas, Cassiano Kuchenbecker Rösing, Federal University of Rio Grande do Sul, Faculty of Dentistry, Periodontology, Porto Alegre 90030-035, Brazil
Eneida Rejane Rabelo-Silva, School of Nursing, Federal University of Rio Grande do Sul, Porto Alegre 90620-110, Brazil
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Correspondence to: Eneida Rejane Rabelo-Silva, RN, ScD, School of Nursing, Federal University of Rio Grande do Sul, Escola de Enfermagem da UFRGS, Rua São Manoel, 963-Santa Cecília, Porto Alegre 90620-110, Brasil. eneidarabelo@gmail.com
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Abstract

Inflammation and endothelial dysfunction are linked to the pathogenesis of atherosclerotic disease. Recent studies suggest that periodontal infection and the ensuing increase in the levels of inflammatory markers may be associated with myocardial infarction, peripheral vascular disease and cerebrovascular disease. The present article aimed at reviewing contemporary data on the pathophysiology of vascular endothelium and its association with periodontitis in the scenario of cardiovascular disease.

Key words: Endothelium; Vascular; Atherosclerosis; Periodontal diseases; Nitric oxide; Cardiovascular diseases

Core tip: Recent studies underscore the importance of endothelial dysfunction and inflammatory markers for the development of atherosclerotic disease. In addition, the literature suggests a direct association between periodontal and cardiovascular diseases. Nevertheless, more robust intervention studies are required to clarify specific gaps, especially in relation to the biological and clinical effects of periodontal disease on the genesis and progression of atherosclerotic disease.

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INTRODUCTION

Cardiovascular disease is still the leading cause of morbidity and mortality worldwide. Nevertheless, as a

result of new and effective strategies to prevent and treat atherosclerosis, the number of deaths associated with cardiovascular events has not increased, and seems to have stabilized in some countries[1].

Because it has regulatory, secretory, metabolic, immunological, and synthesizing properties, the vascular endothelium may be regarded as a heterogeneous and dynamic organ. An imbalance of these properties is linked to the onset of endothelial dysfunction and atherogenesis, and to increased risk of cardiovascular events[2]. Added to that, in the past years, the role of inflammation in the development of atherosclerosis has also been explored. Data from epidemiologic studies confirm the association between high levels of inflammatory markers and the progression of cardiovascular disease[3,4].

Emerging evidence suggests that periodontal infection may be an independent risk factor for myocardial infarction, peripheral vascular disease, and cerebrovascular disease[5,6]. A metanalysis has shown increased incidence of coronary heart disease [relative risk (RR) = 1.14; 95%CI: 1.07-1.21; $P < 0.001$] in patients with periodontal disease even after adjustment for confounding factors such as smoking, diabetes, alcohol intake, obesity, and arterial hypertension, also suggesting a positive correlation between dental loss and coronary artery disease[6]. It should be noted that much of this evidence was generated by observational studies. In this sense, additional studies with more robust designs should be carried out to provide answers regarding the association between periodontal and cardiovascular diseases.

With the aim of furthering the understanding of the relationship between vascular endothelium, periodontal disease, and the process of atherosclerosis, this article will review contemporary data about endothelial pathophysiology and its association with periodontitis in cardiovascular disease. For that, the MEDLINE-PubMed database was searched to retrieve articles published between 1980 and 2014, using the following DeCS terms: "endothelium, vascular"; "atherosclerosis"; "periodontal diseases"; "nitric oxide"; "cardiovascular diseases".

VASCULAR ENDOTHELIUM AND ATHEROSCLEROSIS

Endothelial cells (ECs) form an organ weighing approximately 1 kg; they are distributed along the body (total estimated area: 7000 m^2), and are characterized by heterogeneous structure and function, with phenotypic variation according to their location in different organs, tissues, or blood vessel type[7]. Located at the interface between blood and tissues, the vascular endothelium plays an important role in the cardiovascular system, including regulation of vascular tone (smooth muscle), synthesis and secretion of molecules, and control of homeostasis, coagulation, and inflammatory and atherogenic responses[8].

Atherosclerosis is a progressive disease, characterized by accumulation of lipid particles and fibrous elements on the arterial wall. A more recent concept has introduced the notion that, in addition to the thrombotic process, inflammation and endothelial dysfunction are also directly related to all stages of atherosclerosis. In the undamaged endothelium, ECs resist leukocyte adhesion and aggregation, in addition to promoting fibrinolysis. However, when associated with inflammatory factors, such as periodontal disease, cardiovascular risk factors (smoking, obesity, sedentary lifestyle, dyslipidemia, diabetes) promote changes in endothelial permeability and hence endothelial function[9]. At this initial stage, ECs express adhesion molecules that selectively recruit various leukocyte classes into the tunica intima[10]. Monocytes mature into macrophages, forming foam cells that release cytokines and factors that affect ECs. This process induces migration of smooth muscle cells from the media to the intima and affects metabolism of the arterial extracellular matrix (metalloproteinase), synthesis and release of procoagulant factors, and the bioavailability of nitric oxide (NO)[9]. NO, initially defined by Furchgott *et al*[11] as an "endothelium-derived relaxing factor", is synthesized by the action of an enzyme, endothelial nitric oxide synthase (eNOS), from the amino acid L-arginine. NO plays a fundamental part in endothelial function, promoting smooth muscle relaxation and consequently vasodilatation. In addition, NO supports inhibition of platelet aggregation, smooth muscle cell proliferation, and maintenance of anti-sclerotic effect[12].

The inflammatory process may also contribute to atherosclerotic plaque rupture and thrombosis. Inflammation regulates the fragility of the fibrous cap and the thrombogenicity of the atherosclerotic plaque, influencing collagen metabolism, which provides strength and stability to the cap[13]. Pro-inflammatory cytokines such as C-reactive protein (CRP), fibrinogen, tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and IL-6 reduce the endothelial expression of NOS[14], increasing endothelial synthesis of NADPH oxidases and promoting endothelial expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin and P-selectin[14,15]. As a result, the absence of anti-atherogenic properties in the endothelium increases leukocyte migration and platelet activation to form the atherosclerotic plaque[4].

In this sense, endothelial function and inflammatory markers are important predictors of future cardiovascular events in individuals at risk for atherosclerotic disease.

CELL ADHESION MOLECULES

Cell adhesion molecules (CAMs) are glycoproteins expressed on the cellular surface. CAMs are involved in cell-cell and cell-extracellular matrix binding and encompass immunoglobulins such as ICAM-1 and VCAM-1, as well as the selectin family, including leukocyte-endothelial adhesion molecules (E-selectin), P-selectin and leukocyte-lymphocyte adhesion molecules (L-selectin), integrins, and cadherins[16].

Selectins are expressed on the surface of endothelial

Table 1 Summary of the involvement of pro-inflammatory cytokines and adhesion molecules in atherosclerosis

Pro-inflammatory cytokines and adhesion molecules	Cells involved	Atherogenic effect
C-reactive protein	Adhesion molecules and endothelial cells	Stimulates production of adhesion molecules and chemokines by endothelial cells[14]
Fibrinogen	Platelet, adhesion molecules and smooth muscle	Activates platelet aggregation and promotes the migration and proliferation of smooth muscle[14]
Tumor necrosis factor-alpha	Monocytes, neutrophils and endothelial cells	Activates monocytes, neutrophils and endothelial cells to express adhesion molecules[14]
Interleukin-6	Epithelial cells, fibroblasts and macrophages/monocytes	Is involved in promoting coagulation, which result in the development of atherosclerosis[14]
Interleukin-1β	Macrophages/monocytes	Impedes fibrinolysis, facilitates coagulation and thrombosis[14]
Vascular cell adhesion molecule-1	Endothelial cells	Suggested as potential candidate markers of endothelial dysfunction[19]
Intercellular adhesion molecule-1	Endothelial cells	Implicated in leukocyte recruitment and migration into the vessel wall[19]
Leukocyte-endothelial adhesion molecules	Endothelial cells	Migration of monocytes down into the subendothelial space[16]

cells, leukocytes, and platelets, and their expression in the endothelium is induced by various inflammatory cytokines. In the first phase of leukocyte migration, selectins mediate the capture and transport of circulating leukocytes into the vascular endothelium. In the second phase, leukocytes adhere to the endothelium through the action of ICAM-1 and VCAM-1, migrating into the interstitial tissue space[17].

Soluble forms of CAMs are found in plasma and correlate to endothelial dysfunction[18]. Thus, these markers are associated with biological mechanisms that promote thrombus formation, plaque rupture, and subsequently acute coronary events[19]. The summary of the involvement of pro-inflammatory cytokines and adhesion molecules in atherosclerosis is described in Table 1.

SHEAR STRESS

Even if the multifactorial pathophysiologic nature of atherosclerosis is recognized, special attention should be paid to a specific component in this scenario-shear stress. Shear stress is a biomechanical force determined by blood flow, vessel geometry, and fluid viscosity, aspects modulating the structure and function of the vascular endothelium. The presence of "disturbed" flow-that is, nonlaminar flow-favors atherosclerotic plaque formation. Atherosclerotic plaque development is favored by a combination of cardiovascular risk factors and altered arterial hemodynamics around curvatures, arterial branch ostia and bifurcations[20].

Studies have shown that different types of shear stress correlate with "resistant" or "susceptible" regions in the endothelium during atherogenesis[21]. Pulsatile blood flow triggers many types of hemodynamic, hydrostatic, and cyclic forces that have the ability to influence vascular endothelial physiology[22]. The most susceptible atherosclerotic lesions are associated with certain sites in the proximal branches, bifurcations, and in areas of greater curvature. However, regions with uniform laminar flow are typically more resistant to atherogenic plaque formation[23].

PERIODONTAL DISEASE, INFLAMMATORY MARKERS, AND ENDOTHELIAL DYSFUNCTION

Periodontal disease encompasses two large groups of gum diseases. Gingivitis, which is characterized by inflammation of the gingival margin, is easily reversed with adequate oral hygiene. Periodontitis entails a chronic infectious/inflammatory process involving the supporting tissues of the tooth, including periodontal ligament and alveolar bone. The main consequence of periodontitis is the loss of tooth support structures and tooth loss[24]. Data from different countries show a prevalence of periodontitis reaching up to 50%[25-27]; however, progression is usually slow[28].

Epidemiologic studies provide evidence of an association between periodontitis and cardiovascular disease[6,29]. The biological plausibility for this association is based mainly on the fact that patients with periodontitis present increased levels of CRP, TNF-α, interleukins, and other inflammatory markers, which are associated with endothelial dysfunction and cardiovascular events[30,31]. Most studies employ different inflammatory and endothelial biomarkers, with secondary outcomes, whereas primary outcomes such as death or brain stroke have not yet been evaluated[32-34].

A recent systematic review and metanalysis analyzed the effect of periodontal treatment on cardiovascular risk profile in patients with established periodontitis. The main findings show a significant reduction in CRP (-0.50 mg/dL), IL-6 (-0.48 ng/L), TNF-α (-0.75 pg/mL), fibrinogen (-0.47 g/L) and total cholesterol (-0.11 mmol/L) in the intervention group. In addition, there was improvement of endothelial function and an additional benefit regarding inflammatory markers in patients with traditional cardiovascular risk factors[24]. Investigating the same outcome in a different scenario, another study compared patients with coronary heart disease with or without periodontitis. The results indicate that treatment of periodontal disease promoted a reduction in serum concentrations of CRP, from 2.7 ± 1.9 mg/L to 1.8 ± 0.9 mg/L ($P < 0.05$), and of IL-6, from 2.6 ± 3.4 mg/L to 1.6

Table 2 Summary of the effects of periodontal disease on pro-inflammatory cytokines and adhesion molecules

Pro-inflammatory cytokines and adhesion molecules	Effect of periodontal disease
C-reactive protein	Increased[24]
Fibrinogen	Increased[24]
Tumor necrosis factor-alpha	Increased[24]
Interleukin-6	Increased[24]
Interleukin-1β	Increased[24]
Vascular cell adhesion molecule-1	Increased[30]
Intercellular adhesion molecule-1	Increased[30]
Leukocyte-endothelial adhesion molecules	Increased[30]

± 2.6 mg/L ($P < 0.05$) in patients with periodontitis[32].

In addition to inflammatory markers and adhesion molecules, the measurement of brachial artery flow-mediated dilation (FMD), a technique developed initially in 1992, is also useful to assess the endothelium[35]. This non-invasive technique evaluates the diameter of the brachial artery before and after induced forearm ischemia. A blood pressure cuff is inflated at the distal or proximal section of the arm, and FMD is expressed as the percent change in brachial artery diameter at the end of ischemia. This dilatation is mediated by endothelial release of NO in response to shear stress at the arterial wall[36].

FMD is decreased in individuals with cardiovascular risk factors (diabetes, hypertension, obesity, and smoking, among others) and established atherosclerosis[37]. A study published in 2005 evaluated endothelial function in patients with a diagnosis of severe periodontitis. The main findings following periodontal treatment show significant improvement in FMD, of 9.8% ± 5.7% ($P = 0.003$) as compared to baselines measures, accompanied by a reduction in the levels of CRP from 1.1 ± 0.9 to 0.8 ± 0.8 ($P = 0.026$)[34]. In this sense, evaluation of FMD and cardiovascular disease biomarkers have recently been studied and associated with endothelial dysfunction and occurrence of cardiovascular events[38,39]. The summary of the effects of periodontal disease on pro-inflammatory cytokines and adhesion molecules is depicted in Table 2.

CONCLUSION

The present literature review suggests that periodontal treatment reduces the risk of cardiovascular disease by improving plasma levels of inflammatory markers (CRP, TNF-α, IL), thrombotic markers (fibrinogen) and adhesion molecules (VCAM-1, ICAM-1, P-selectin), in addition to improving endothelial function as assessed by FMD. Future intervention studies are required to further elucidate the association between periodontal and cardiovascular disease, especially in terms of the biological effects of periodontal disease on the atherogenic cascade affecting the vascular endothelium.

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ORIGINAL ARTICLE

Case Control Study

End-organ protection in hypertension by the novel and selective Rho-kinase inhibitor, SAR407899

Matthias Löhn, Oliver Plettenburg, Aimo Kannt, Markus Kohlmann, Armin Hofmeister, Dieter Kadereit, Peter Monecke, Alexander Schiffer, Anke Schulte, Hartmut Ruetten, Yuri Ivashchenko

Matthias Löhn, Oliver Plettenburg, Aimo Kannt, Markus Kohlmann, Armin Hofmeister, Dieter Kadereit, Peter Monecke, Alexander Schiffer, Anke Schulte, Hartmut Ruetten, Yuri Ivashchenko, Translational Medicine, Sanofi-Aventis, Industriepark Hoechst, 65926 Frankfurt am Main, Germany

Author contributions: Löhn M performed the majority of experiments, designed the study and wrote the manuscript; Plettenburg O, Hofmeister A and Kadereit D designed and synthetized SAR407899; Kannt A performed an initial compound screening; Kohlmann M performed the pharmacokinetics of SAR407899; Monecke P and Schiffer A were involved in structural biology and modeling required for the compound optimization; Schulte A performed the histology; Ruetten H and Ivashchenko Y were also involved in writing and editing the manuscript.

Ethics approval: All animal experiments were performed in accordance with the German law for the protection of animals and current Aventis Laboratory Animal Science and Welfare (LASW) guidelines.

Informed consent: Not applicable.

Conflict-of-interest: There is no conflict-of-interest to disclose. All authors are employees of Sanofi-Aventis Deutschland GmbH.

Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at Matthias.loehn@sanofi.com. Participants gave informed consent for data sharing. No additional data are available.

Correspondence to: Dr. Matthias Löhn, Translational Medicine, Sanofi-Aventis, Industriepark Hoechst, 65926 Frankfurt am Main, Germany. matthias.loehn@sanofi.com
Telephone: +49-69-30517118

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Abstract

AIM: To compare the therapeutic efficacy of SAR407899 with the current standard treatment for hypertension [an angiotensin converting enzyme (ACE)-inhibitor and a calcium channel blocker] and compare the frequency and severity of the hypertension-related end-organ damage.

METHODS: Long-term pharmacological characterization of SAR407899 has been performed in two animal models of hypertension, of which one is sensitive to ACE-inhibition and the other is insensitive [deoxycorticosterone acetate (DOCA)]. SAR407899 efficiently lowered high blood pressure and significantly reduced late-stage end organ damage as indicated by improved heart, kidney and endothelial function and reduced heart and kidney fibrosis in both models of chronic hypertension.

RESULTS: Long term treatment with SAR407899 has been well tolerated and dose-dependently reduced elevated blood pressure in both models with no signs of tachyphylaxia. Blood pressure lowering effects and protective effects on hypertension related end organ damage of SAR407899 were superior to ramipril and amlodipine in the DOCA rat. Typical end-organ damage was significantly reduced in the SAR407899-treated animals. Chronic administration of SAR407899 significantly reduced albuminuria in both models. The beneficial effect of SAR407899 was associated with a reduction in leukocyte/macrophage tissue infiltration. The overall protective effect of SAR407899 was superior or comparable to that of ACE-inhibition or calcium

channel blockade. Chronic application of SAR407899 protects against hypertension and hypertension-induced end organ damage, regardless of the pathophysiological mechanism of hypertension.

CONCLUSION: Rho-kinases-inhibition by the SAR407899 represents a new therapeutic option for the treatment of hypertension and its complications.

Key words: Hypertension; End organ damage; Rho-kinase; Angiotensin converting enzyme-inhibition

Core tip: Rho-kinase (ROCK) is considered an important ta-rget in cardiovascular diseases, *e.g.*, hypertension and nephropathy. Currently available treatment only moderately alleviates the progression of chronic kidney diseases. We have identified a novel, potent and selective inhibitor of ROCK (SAR407899) and characterized its effects *in vivo*. The therapeutic efficacy of SAR407899 has been compared to the current standard treatment for hypertension in two animal models of hypertension, one of which is sensitive and the other insensitive (deoxycorticosterone acetate) to angiotensin converting enzyme-inhibition. ROCK-inhibition by SAR407899 may represent a new therapeutic option either as a monotherapy or in combination with existing modern therapeutics for the treatment of hypertension and its complications.

Löhn M, Plettenburg O, Kannt A, Kohlmann M, Hofmeister A, Kadereit D, Monecke P, Schiffer A, Schulte A, Ruetten H, Ivashchenko Y. End-organ protection in hypertension by the novel and selective Rho-kinase inhibitor, SAR407899. *World J Cardiol* 2015; 7(1): 31-42 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i1/31.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i1.31

INTRODUCTION

Hypertension increases the risk of target organ damage, including heart hypertrophy, heart ischemia, kidney dysfunction or failure, cerebrovascular events and malfunction of the endothelial tissue[1-5]. The contractile response of smooth muscle tissue is controlled at different levels by a tight balance of pro-contractile and relaxing mechanisms. In particular, it has been persuasively shown that Rho-kinases (ROCK1 and ROCK2) are intimately involved in the transmission of contractile signaling within smooth muscle tissue[6-13]. Upon activation of the small GTPase RhoA by ligand-bound specific GPCRs, ROCKs, the downstream effectors of RhoA, phosphorylate the myosin light chain phosphatase and the myosin regulatory light-chain itself, resulting in a net increase in activated myosin. This promotes smooth muscle contraction and actin cytoskeleton re-organization. Inhibition of ROCKs leads to relaxation of vascular smooth muscle cells and, consequently, to a decrease in blood pressure[7,9,14-20]. Several inhibitors of ROCK (in particular, fasudil and Y27632) have been extensively used to evaluate the role of ROCK in cardiovascular physiology and pathology. In addition, fasudil is used in Japan to treat cerebral vasospasm after focal cerebral ischemia or aneurysmal subarachnoid hemorrhage. However, both inhibitors have a moderate specificity, moderate potency and short duration of action *in vivo* that limit their clinical use. Therefore, the development of a more potent and specific inhibitor with a better pharmacokinetic profile is needed to explore the potential of ROCK inhibition in the therapy of hypertension and its complications. We have identified a novel ROCK-inhibitor, SAR407899, and previously characterized its acute effects *in vitro* and *in vivo*[21]. Here, we describe the long-term effects of SAR407899 treatment in two animal models of hypertension, one being sensitive (Nω-Nitro-L-arginine methyl ester hydrochloride, LNAME) and the other being insensitive [deoxycorticosterone acetate (DOCA)] to angiotensin converting enzyme (ACE)-inhibition. The DOCA-induced hypertension model is characterized by a hypervolemic and low plasma renin status, which promotes resistance to ACE-inhibition, whereas the LNAME model is normovolemic and displays a high renin activity in plasma[22-24]. The results of large clinical trials (IDNT, RENAAL and IRMA-2) revealed that the current standard treatment only modestly (approximately 20%) reduces the progression of chronic kidney diseases. From these data it can be concluded that a simple decrease of blood pressure is not sufficient for kidney protection. Our results indicate that chronic inhibition of the ROCK kinases efficiently controls blood pressure and significantly reduces the frequency and severity of the hypertension-related end organ damage.

MATERIALS AND METHODS

DOCA and LNAME-induced hypertension

Adult male Sprague Dawley rats (190 to 210 g, Harlan Winkelmann, Borchen, Germany), were treated with DOCA-salt or Nω-Nitro-L-arginine methyl ester hydrochloride (LNAME) to induce hypertension. To compare the pharma-cological potency of ROCK-inhibition with the current anti-hypertension drugs, the individual blood pressure lowering effect of the respective compounds was measured in spontaneous hypertensive rats (SHR). Oral application of SAR407899 at 3 mg/kg lowered blood pressure in conscious telemetred SHR by 26 ± 4 mmHg (n = 10), which was comparable to the action of amlodipine at 3 mg/kg (blood pressure reduction by 33 ± 8 mmHg, n = 10) and ramipril at 1 mg/kg (blood pressure reduction by 21 ± 7 mmHg, n = 10). Therefore, the animals were divided into the following groups: (1) Control; (2) DOCA or LNAME; (3) DOCA or LNAME + SAR407899 at 3 mg/kg; (4) DOCA or LNAME + SAR407899 at 10 mg/kg;

(5) DOCA or LNAME + ramipril at 1 mg/kg; and (6) DOCA or LNAME + amlodipine at 3 mg/kg. All DOCA-salt treated animals underwent a unilateral nephrectomy, received a subcutaneous injection of DOCA (30 mg/kg; Sigma Chemical, St. Louis, MO, United States) dissolved in sesame oil once a week and 1% NaCl in the drinking water ad libitum. All LNAME groups received 40 mg/kg per day LNAME in the drinking water *ad libitum*. After a 4-wk treatment, the animals were placed into metabolic cages and 24 h-urine samples and blood samples were taken to analyze kidney function. Urinary and plasma creatinine levels were determined using standard kits (Roche diagnostics, Basel, Switzerland) on a Hitachi 912 E analyzer (Hitachi, Mountain View, Calif., United States). Urinary albumin was measured using a standard kit from Progen (Mikroflural, Progen, Heidelberg, Germany) and was normalized to urinary creatinine concentrations. After 5 wk of treatment, measurements of: (1) blood pressure (invasively, two hours after the last treatment); (2) organ weights; and (3) endothelial function were taken. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication no. 85-23, revised 1996).

Invasive blood pressure measurement

The animals were anaesthetized with an intraperitoneal injection of thiopental (0.1 g/mL per 100 g body weight). The common carotid artery was catheterized with a heparinized microcatheter (diameter 1.6 mm, Vycon, France). Blood pressure was measured with a Hugo Sachs hemodynamic system (March-Hugstetten, Germany) using the software Hemodyn.

In vitro vascular and heart function

Assessment of *in vitro* vascular function was performed as described earlier[21,25,26]. Heart function was determined using a Langendorff-setup in the working heart perfusion mode. This technique allows the heart to perform its physiological pumping action, *i.e.*, it performs pressure/volume work. The working heart technique therefore provides a complete analysis of heart function. Heart power is an integrative parameter and is calculated by formula (1):

$$HW\ (J) = 133.3\ [(N/m^2)/mmHg] \times (ALPmean\text{-}PLPmean)\ (mmHg) \times SV\ (m^3) + 0.5 \times 1.004\ (kg/m^3) \times SV\ (m^3) \times \{SV\ (m^3)/[\pi \times r^2\ (m^2) \times ET\ (s)]\}^2 \quad (1)$$

where HW-heart power, ALPmean-afterload pressure, PLPmean-preload pressure, SV-stroke volume, and ET-Ejection time. If not otherwise indicated, chemicals were obtained from Sigma (Deisenhofen, Germany).

RT PCR

Real-time quantitative PCR was performed using the QuantiTect Probe RT-PCR Kit (Qiagen, Hilden, Germany). Each sample was assayed in quadruplicate. For relative quantification of gene expression, the ΔCt method was used with GAPDH as a control. Amplification of the target and housekeeping genes was detected simultaneously using differently fluorescent-labeled Taq Man probes (Col1A1: Rn00801665_g1, CD3: Rn01417941_g1 and CD68: Rn01495634_g1), which were obtained from Applera/Applied Biosystems (Foster City, United States). Amplification linearity of the target and housekeeping genes within the multiplex RT-PCR was assessed by performing RT-PCR reactions with dilutions of the templates. RT-PCR reactions and data acquisition was performed using the iCycler-iQ-Thermocycler (Bio-Rad Laboratories GmbH, Munich, Germany). Relative gene expression was calculated as fold induction *vs* control samples.

Histology

Heart and kidneys underwent a standard fixation procedure and standard haematoxylin-eosin and sirius red staining. The hearts and kidneys were analyzed with regard to incidence and extent of fibrosis, inflammatory events, glomerulosclerosis and tubular atrophy. A semi-quantitative score was assigned to each specimen by an experienced pathologist ranging from 1 (minimal changes) to 4 (marked alterations) at a standard magnification of 4 to 20-fold. All histopathological analyses were performed in a blinded fashion. Anti-podocin staining was performed using anti-podocin antibodies (Sigma-Aldrich, United States).

Statistical analysis

All values are given as the mean and standard error of mean. Normality of the distribution and the homogeneity of variance were checked using the Levene test. For group comparisons, one-way analysis of variance (*ANOVA*) or two-way ANOVA was performed followed by Dunnett's post-hoc test using the SAS version 8.2 software. Differences between groups were considered significant if $P < 0.05$; n represents the number of specimens or animals tested.

The statistical methods of this study were reviewed by and complies to the standard of Sanofi-Aventis GmbH Deutschland.

RESULTS

Effect of SAR407899 on body weight, blood pressure and kidney function

The long-term effects of SAR407899 in DOCA- and LNAME-induced hypertension were compared to those of the current standard anti-hypertensive drugs, namely ramipril (ACE-inhibitor) and amlodipine (calcium channel blocker, CCB). Figure 1 depicts the effects of SAR407899 on body weight of the DOCA- and LNAME-hypertensive animals. Treatment with SAR407899 was well tolerated and showed a significant protective effect on body weight in both hypertensive animal models (Figure 1A and C). Factors involved in the continuous body weight loss are not known and most likely depend on hypertension related end-organ damage,

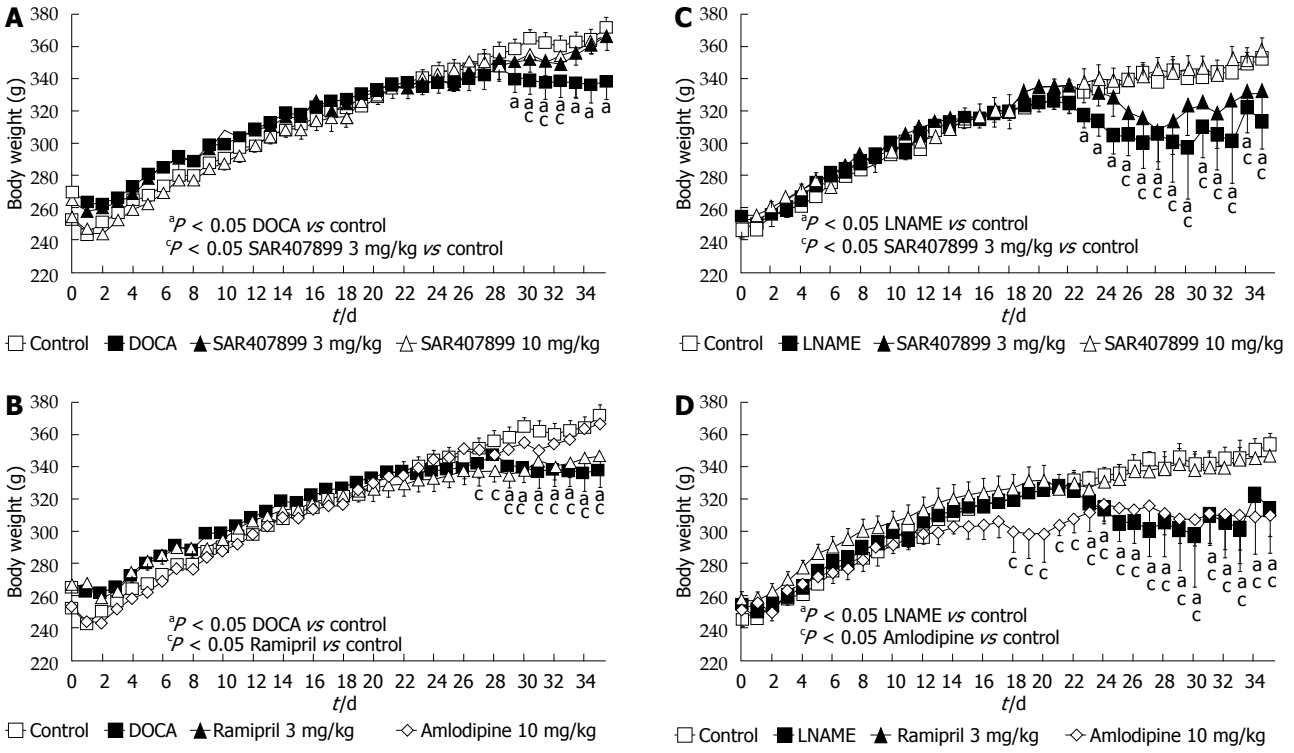

Figure 1 Effect of SAR407899 on body weight in deoxycorticosterone acetate and Nω-Nitro-L-arginine methyl ester hydrochloride hypertensive animals. A and B: Body weight of deoxycorticosterone acetate (DOCA) rats. A significant decrease in body weight was observed after 28 d; A: Effect of SAR407899 at 3 mg/kg and 10 mg/kg on body weight. Both doses protected DOCA rats against body weight loss; B: Effect of ramipril at 1 mg/kg and amlodipine at 3 mg/kg on body weight. Only amlodipine showed protective effects on body weight loss; C and D: Body weight of Nω-Nitro-L-arginine methyl ester hydrochloride (LNAME) rats. A significant decrease in body weight was observed after 22 d. C: SAR407899 at 10 mg/kg significantly protected LNAME rats from body weight loss; D: Effect of ramipril at 1 mg/kg and amlodipine at 3 mg/kg on body weight. Only ramipril showed significant protective effects on body weight loss.

including proteinuria. Ramipril showed protective effects on body weight only in the LNAME model (Figure 1D), whereas amlodipine significantly protected the DOCA hypertensive animals from body weight loss (Figure 1B).

Figure 2 demonstrates the effects of SAR407899 (3 mg/kg and 10 mg/kg), ramipril (1 mg/kg) and amlodipine (3 mg/kg) on blood pressure in the DOCA- (Figure 2A) and LNAME-treated rats (Figure 2B). SAR407899 effectively reduced blood pressure in both hypertensive models. Because the DOCA-induced hypertensive model is characterized by hypervolemia and resistance to ACE-inhibition, it was not surprising that ramipril had no significant effect on blood pressure. At the dose employed, amlodipine non-significantly lowered blood pressure in the DOCA rats. In the LNAME rats, all treatments significantly lowered blood pressure. In comparison to amlodipine and ramipril, SAR407899 showed superior blood pressure lowering effects at both doses in the LNAME rats. Thus, only SAR407899 was able to control blood pressure efficiently in both models and was therefore superior to the reference substances.

Kidney function (Figure 2C and D) was assessed by the measurement of albuminuria. In both models, SAR407899 dose-dependently reduced albuminuria. Ramipril significantly reduced albuminuria in only the LNAME model but not in the DOCA model. At the dose administered, amlodipine did not significantly reduce albuminuria in the DOCA or LNAME models. The lack of efficacy of amlodipine has been linked to the inability of CCB's to dilate renal efferent vessels, which is critical for improvement of the renal microcirculation.

Long term treatment effects of SAR407899 on heart and endothelial function

Figure 3 illustrates the effect of SAR407899 and of reference substances on heart function (A and B), measured in isolated Langendorff perfused hearts in the working heart mode and on vessel function *in vitro* (C and D). Hearts of hypertensive the DOCA rats and the LNAME rats were functionally compromised. At an afterload pressure of 40 mmHg (basal afterload), a similar heart performance was found in all groups; however, at higher afterload pressures (60 and 80 mmHg), a significant reduction was found in heart function from the DOCA- and LNAME-treated rats. Long-term treatment with SAR407899 restored heart function in both groups. In contrast, treatment with either amlodipine or ramipril had no significant effect. Endothelial function was found to be severely compromised in the DOCA rats. Figure 3C and D show the effect of SAR407899 and the reference substances on endothelial function. Long-term treatment with SAR407899 improved endothelial function in a dose-dependent manner (Figure 3C). As expected, ramipril had no effect on endothelial function of arteries from the DOCA-induced hypertensive rats (Figure 3D). Amlodipine had similar protective effects

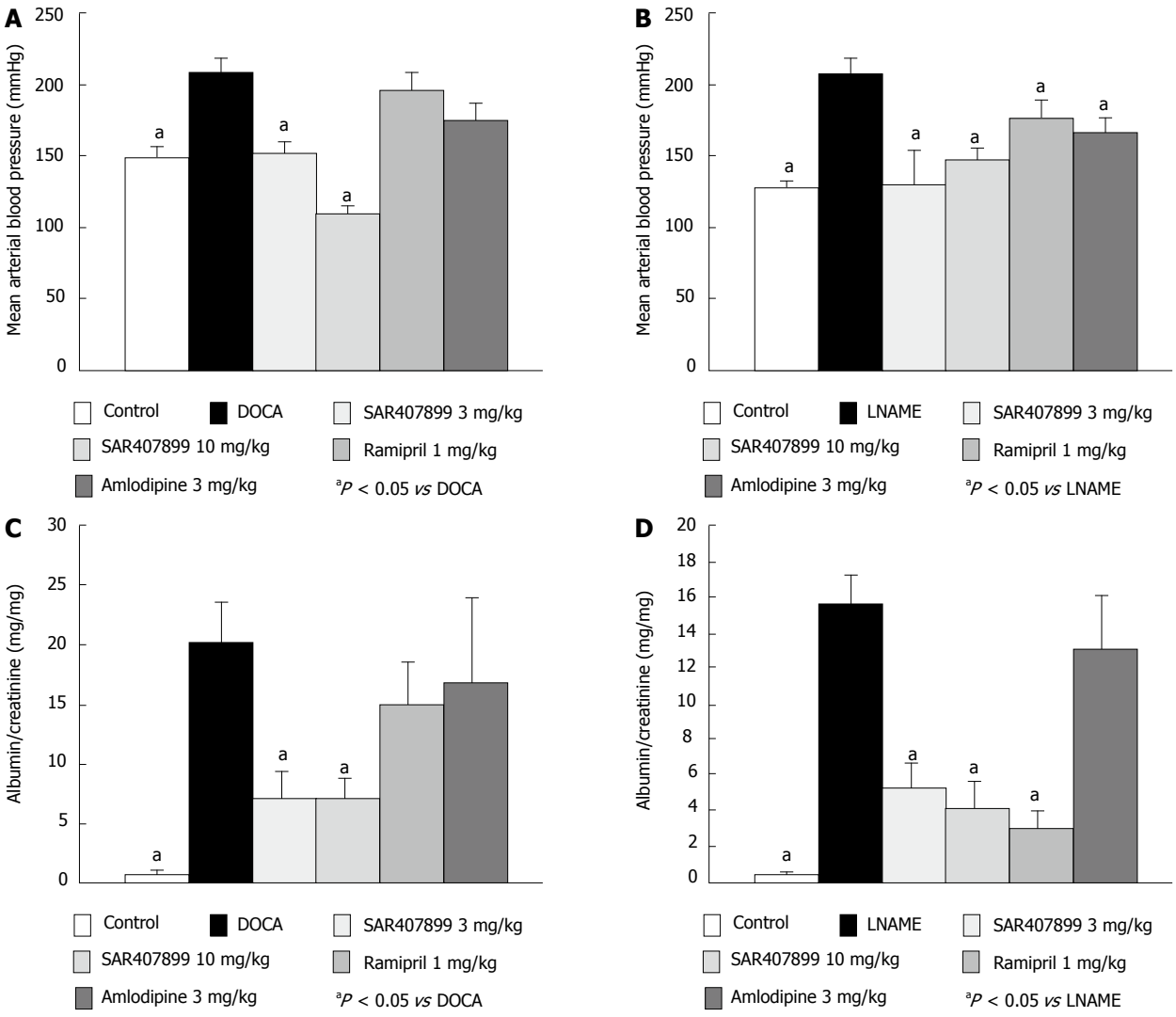

Figure 2 SAR407899 efficiently lowers blood pressure, proteinuria and mortality in hypertensive rats. Invasive measurement of blood pressure in deoxycorticosterone acetate (DOCA) (A) and in Nω-Nitro-L-arginine methyl ester hydrochloride (LNAME) (B) rats. At the given dose, SAR407899 showed superior blood pressure lowering effect in comparison to ramipril and amlodipine in both animal models; C and D: Assessment of kidney function in DOCA and LNAME rats. SAR407899 showed significant protective effects on the kidneys in both models, whereas ramipril reduced albuminuria only in the LNAME model. Amlodipine had no therapeutic effect on kidney in either model.

on endothelial function in the DOCA hypertensive rats (Figure 3D). The endothelial function of arteries of the LNAME-treated rats has not been assessed because LNAME exerts a potent and long lasting inhibition of the endothelial nitric oxide synthase.

Effect of SAR407899 on DOCA- and LNAME-induced heart and renal pathology

Histological examination of the hearts of the DOCA- (Figure 4A-F) and LNAME-treated (Figure 4G-L) rats revealed a prominent perivascular fibrosis, massive infiltration of leukocytes into the interstitium and sclerotic changes. Figure 4A-C and 4G-I shows sirius red staining of DOCA- (Figure 4B) and LNAME-(Figure 4H) induced heart fibrosis in comparison to control (Figure 4A and G) and SAR407899 treatment (Figure 4C and I). Figure 4D-F and 4J-L shows haematoxylin eosin staining of DOCA- (Figure 4E) and LNAME-(Figure 4K) induced heart fibrosis in comparison to control (Figure 4D and J) and SAR407899 treatment (Figure 4F and L). In the hearts of the DOCA and LNAME rats, SAR407899 treatment at 10 mg/kg abolished the development of fibrosis (protection with SAR407899 at 3 mg/kg was less pronounced and data not shown). Chronic treatment of the LNAME hypertensive rats with SAR407899 attenuated leukocyte infiltration and perivascular fibrosis. Figure 4M and N summarize heart lesions according to the scoring procedure. The heart lesions of the DOCA rats were significantly reduced by SAR407899 at 10 mg/kg. Both reference compounds showed only marginal non-significant effects on heart lesions (Figure 4M). SAR407899 (at 3 and 10 mg/kg) and ramipril significantly diminished heart lesions in the LNAME-treated rats (Figure 4N). Histological analysis of kidneys from the DOCA- (Figure 5A-F) and LNAME- (Figure 5G-L) treated rats revealed severe

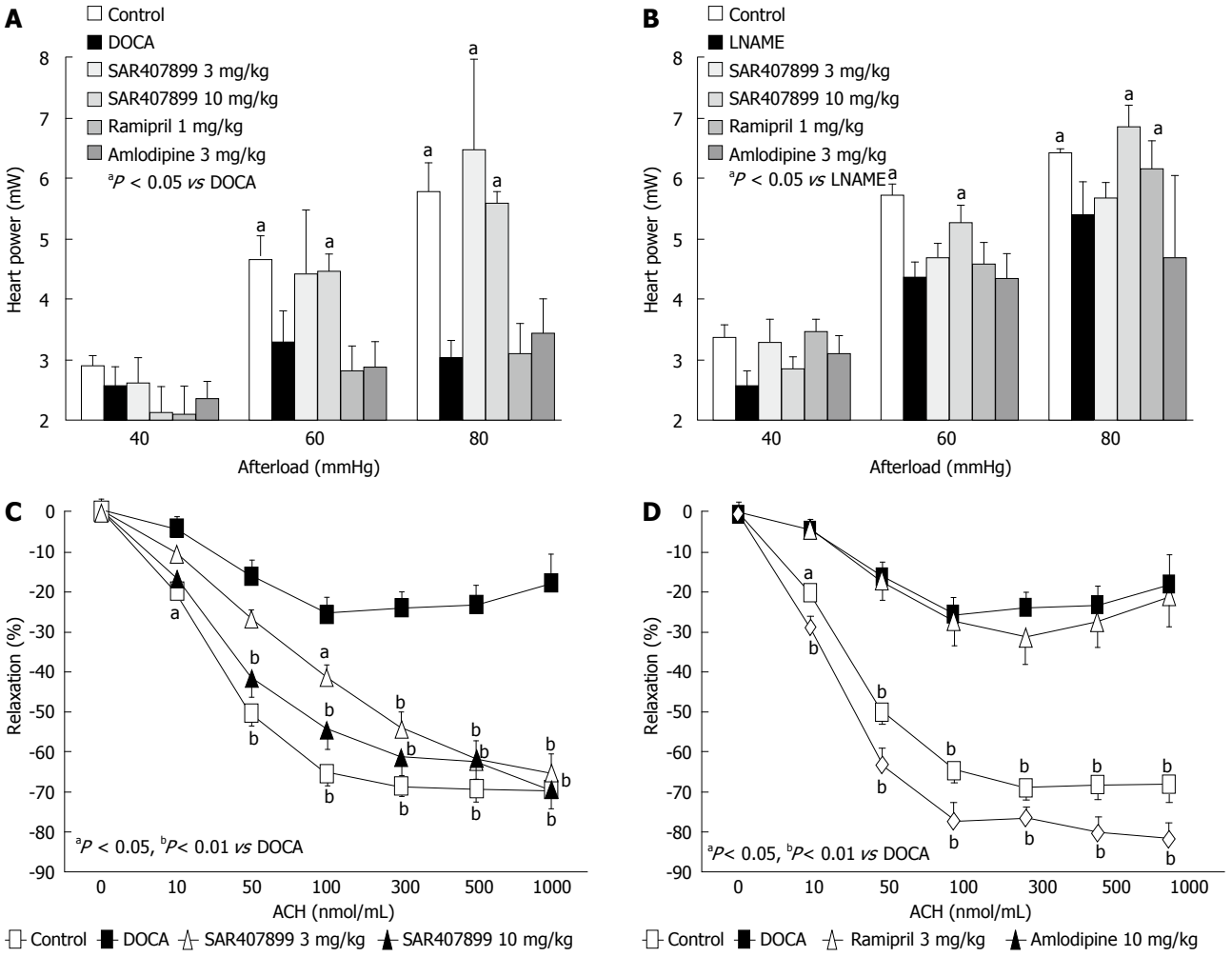

Figure 3 Effect of SAR407899 on heart and endothelial function. Measurement of heart function of isolated hearts of deoxycorticosterone acetate (DOCA) (A) and Nω-Nitro-L-arginine methyl ester hydrochloride (LNAME)-treated rats (B). SAR407899 showed a significant improvement of heart function compared to reference substances at both doses and in both models; C and D: Measurement of endothelial function of aortas of DOCA rats treated with SAR407899 (C) or with reference substances (D). Ramipril treatment had no effect on endothelial function. Long-term treatment with SAR407899 improved endothelial function in a dose-dependent fashion. Amlodipine had similar protective effects as SAR407899.

lesions of glomeruli and tubuli. Figure 5A-C shows haematoxylin eosin staining in kidneys of DOCA- and of LNAME-treated rats (Figure 5G-I). Figure 5D-F shows podocin staining in kidneys of DOCA- and of LNAME-treated rats (Figure 5J-L). In kidneys from the DOCA rats (Figure 5B), severe destruction and sclerotic changes of glomeruli were associated with fibrinous discharges into the capsule, swelling of lobuli and multiple fibrotic events. In comparison to control (Figure 5A), a strong hypertrophy of glomeruli was detectable in the kidneys of the DOCA- treated animals (Figure 5B). Podocin staining (Figure 5D-F)of the DOCA rat kidneys revealed a drastic loss of podocin positive cells (podocytes) upon DOCA treatment (Figure 5E) in comparison to control (Figure 5D). Chronic treatment with SAR407899 attenuated these glomerular (much stronger staining of podocytes), vascular, and interstitial changes (Figure 5F). In contrast to kidneys of control animals (Figure 5G), in the kidneys from the LNAME hypertensive animals (Figure 5H), many changes were detected, including fibrotic changes, infiltration of leukocytes, hypertrophy of glomeruli, marked thickening and inflammation of the vascular wall, hyperplasia and degeneration of tubuli, and tubulointerstitial changes with inflammatory cell infiltration. Again, chronic treatment with SAR407899 attenuated these pathophysiological changes (Figure 5I).

Figure 5K shows the fading of podocin staining upon LNAME treatment indicating that podocin positive cells are damaged. Figure 5J demonstrates podocin staining in control animals. Long-term treatment with SAR407899 attenuated glomerular, vascular, and interstitial changes (Figure 5L). Figure 5M and N summarize kidney lesions according to the scoring procedure. The kidney lesions of the DOCA rats were significantly reduced by SAR407899 at 3 and 10 mg/ kg. Both reference compounds showed only marginal non-significant effects on kidney lesions (Figure 5M). SAR407899 (at 3 and 10 mg/kg) and ramipril significantly diminished kidney lesions in the LNAME-treated rats (Figure 5N). Expression of genes characteristic of fibrosis and leukocyte infiltration of the kidneys of the DOCA- and LNAME-treated rats was measured using RT PCR (Figure 6A-F). The expression of collagen

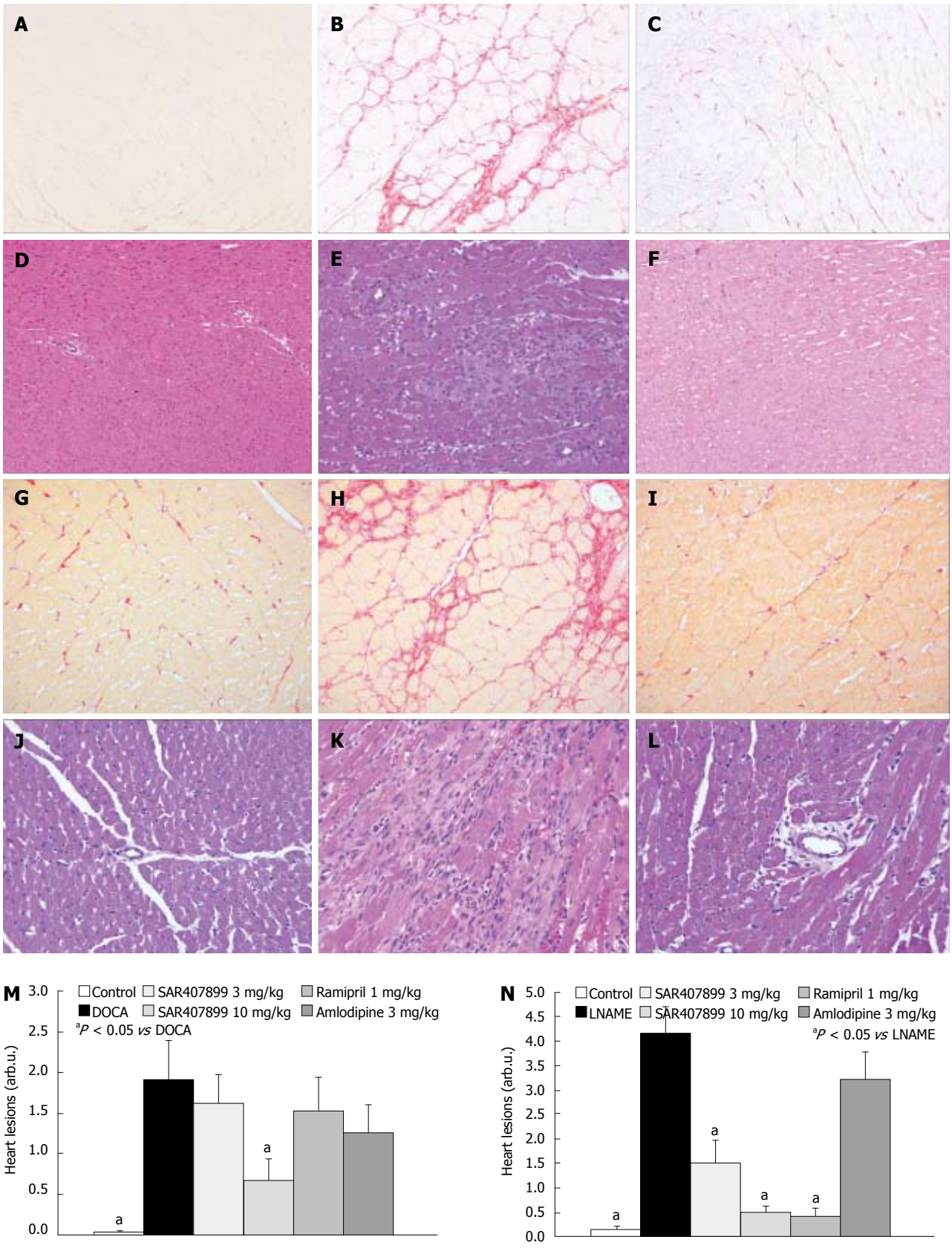

Figure 4 Histological examination of the effect of SAR407899 on the heart. Sirius red staining of hearts of deoxycorticosterone acetate (DOCA) (A-C) and Nω-Nitro-L-arginine methyl ester hydrochloride (LNAME) rats (G-I) showed a strong induction of myocardial fibrosis. Upon treatment with SAR407899 at 10 mg/kg, significant protective effects were observed. Haematoxylin eosin staining of hearts of DOCA (D-F) and LNAME rats (J-L) showed perivascular fibrosis, massive infiltration of leukocytes into the interstitium and sclerotic changes. SAR407899 treatment at 10 mg/kg attenuated multifocal fibrosis, perivascular fibrosis and leukocyte infiltration. M and N: Summary of heart lesions and the effect of SAR407899 and reference substances in DOCA (M) and LNAME rats (N). A, D, G, J: Control; B, E: DOCA; H, K: LNAME; C, F, I, L: SAR407899 10 mg/kg.

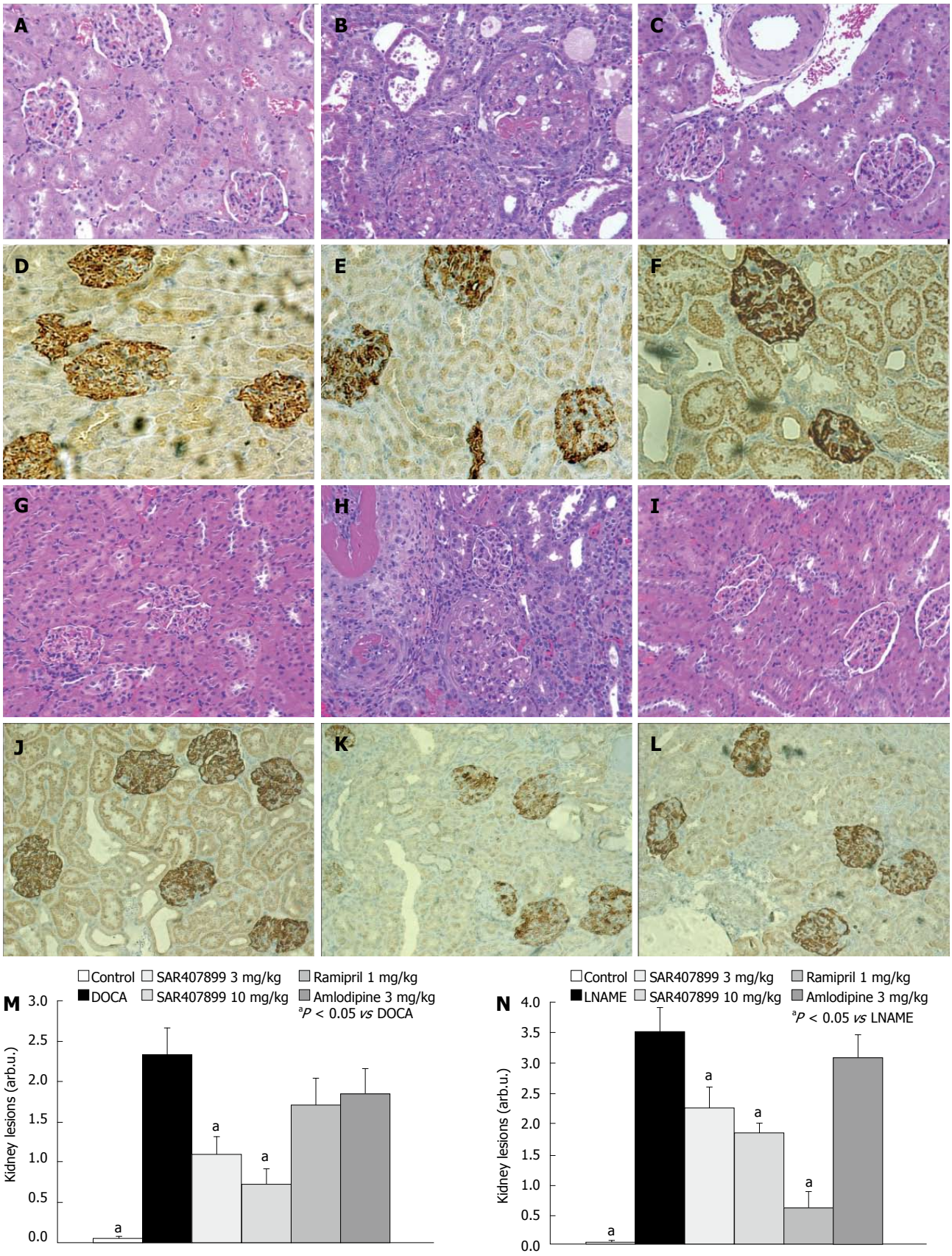

Figure 5 Histological examination of the effect of SAR407899 on the kidney. A-C: Haematoxylin eosin staining of normal glomeruli in control rats (left), sclerotic changes, dilation and hypertrophy of glomeruli of deoxycorticosterone acetate (DOCA) rats (center) and protective effects of SAR407899 at 10 mg/kg (right); D-F: Podocin staining in kidneys. Upon DOCA treatment, massive loss of podocytes can be detected. SAR407899 at 10 mg/kg exerts a protective effect in the kidneys of DOCA rats and rescues podocytes; G-I: Haematoxylin eosin staining of normal glomeruli in control rats (left), severe fibrotic changes, infiltration of leukocytes, and hypertrophy of glomeruli of Nω-Nitro-L-arginine methyl ester hydrochloride (LNAME) rats (center), protective effects of SAR407899 at 10 mg/kg (right); J-L: Loss of podocytes upon LNAME treatment. SAR407899 at 10 mg/kg protected against loss of podocytes. Summary of kidney lesions and effect of SAR407899 and reference substances in DOCA (M) and LNAME rats (N). A, D, G, J: Control; B, E: DOCA; H, K: LNAME; C, F, I, L: SAR407899 10 mg/kg.

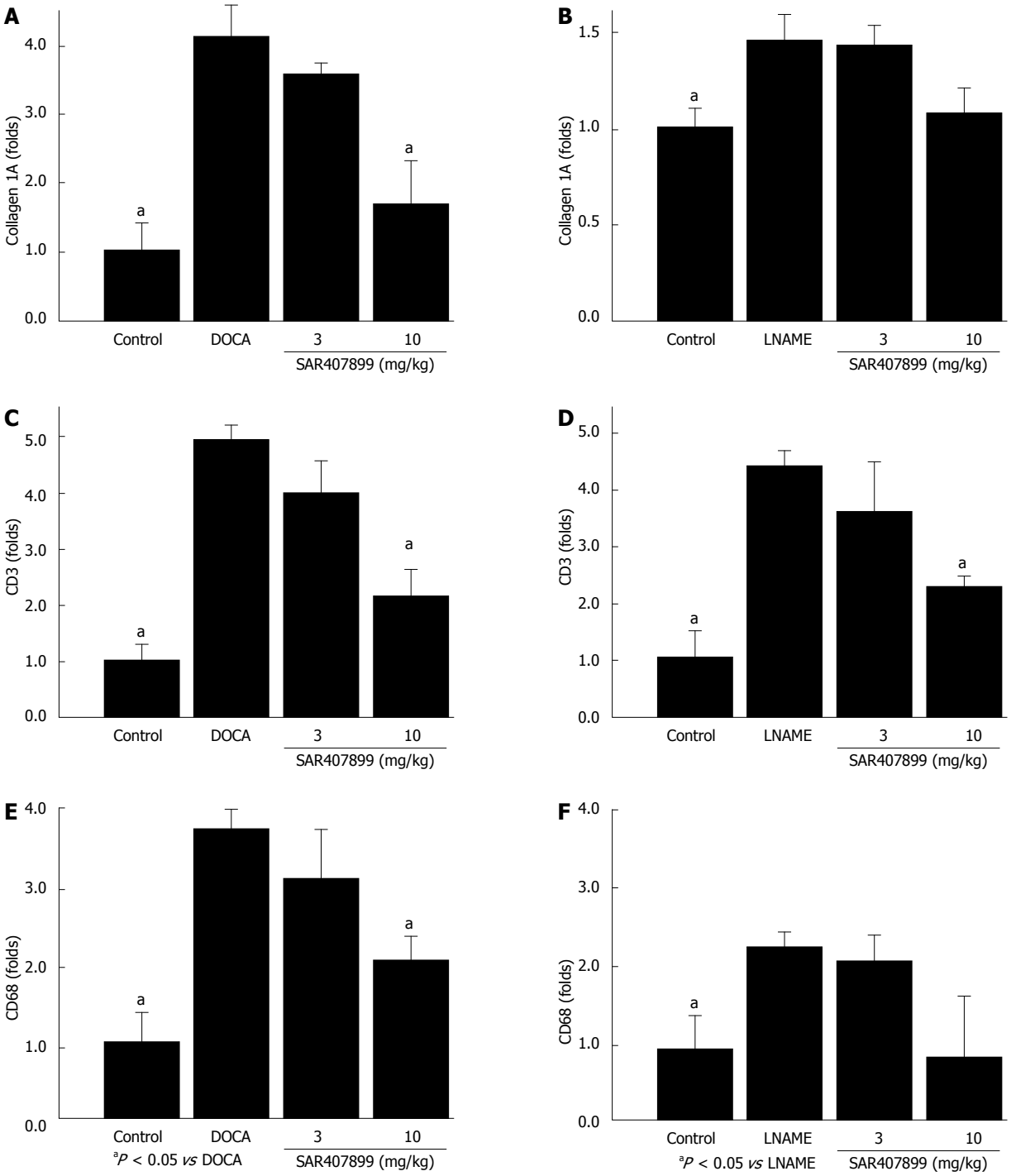

Figure 6 Effect of SAR407899 on the expression of fibrotic and leukocyte genes in the kidney. Determination of collagen, T cell (CD3) and macrophage (CD68) expression in the kidneys of deoxycorticosterone acetate (DOCA) (A, C and E) and Nω-Nitro-L-arginine methyl ester hydrochloride (LNAME) (B, D and F) rats. Expression of collagen was significantly increased upon DOCA and LNAME treatment. Upon DOCA or LNAME treatment, CD3 and CD68 abundance was induced, indicating that leukocytes infiltrated into the kidneys. SAR407899 treatment reduced expression of collagen and leukocyte genes in both models.

was significantly increased upon DOCA and LNAME treatment. SAR407899 at 10 mg/kg significantly reduced collagen expression in the kidneys of the DOCA rats and non-significantly in the kidneys of the LNAME rats (Figure 6A and B). Leukocyte infiltration into kidney tissue was quantified by measuring CD3 (T lymphocytes) and CD68 (macrophages) mRNA abundance in the kidneys of the DOCA and LNAME rats. Upon DOCA or LNAME treatment, both parameters were significantly induced, thus indicating significant leukocyte infiltration. SAR407899 at 10 mg/kg efficiently reduced leukocyte infiltration in the kidneys of the DOCA rats and non-significantly in the kidneys of the LNAME rats (Figure 6E and F), presumably by inhibition of leukocyte migration.

DISCUSSION

It has been generally accepted that RhoA-associated

kinases (ROCK1 and ROCK2) have important functions not only in vascular smooth muscle contractility but also in actin cytoskeleton rearrangement, cell adhesion, cytokinesis and motility in various cell types[7-12,27-31]. Two commercially available ROCK-inhibitors, fasudil and Y27632 were used in the majority of the published *in vitro* and *in vivo* studies. Both compounds were identified in the mid-1980s and were used to elucidate the pathophysiological role of ROCKs in several pathologies. Y27632 was first claimed in a patent in 1988[32,33] as a calcium channel blocker, and fasudil was patented in 1986[34,35], also as a novel calcium channel blocker. Later, discovery of ROCKs[36] led to the identification of these kinases as a direct target of both compounds. However, fasudil and Y27632 possess some unfavorable properties, including limited selectivity and importantly, a short duration of action. The latter might be a reason why fasudil was often administered at high doses, *e.g.*, 30 mg/kg per day or 100 mg/kg per day[37] in *in vivo* animal studies, which increases the probability of non-specific and toxic effects in different organs. In long-term animal studies of hypertension in which fasudil was used at low doses, *e.g.*, 10 mg/kg, no significant blood pressure lowering effect and only a partial renoprotective effect were found[38,39].

SAR407899 is a selective and potent inhibitor of ROCK that has been extensively characterized *in vitro*[21]. Acute treatment of conscious hypertensive animals (SHR, SHR-sp, LNAME or DOCA-salt treated rats) with SAR407899 resulted in a strong and sustained fall in blood pressure[21]. To evaluate the long-term effects of SAR407899, the compound was tested in two models of hypertension, one non-sensitive (DOCA) and the other sensitive (LNAME) to ACE-inhibition. The treatment effects of SAR407899 were compared to two current standard medications for high blood pressure, namely the inhibition of ACE (ramipril) or calcium channels blockade (amlodipine). As expected, treatment with ramipril demonstrated potent blood pressure lowering and end organ protective effects in the LNAME but not in the DOCA hypertension model[24,40,41]. Because the DOCA hypertensive model is characterized by hypervolemia and low plasma renin levels, this model is insensitive to ACE-inhibition[23]. The treatment effects of amlodipine at the given dose were small and non-significant in both models. Our data are in good agreement with several other studies in which amlodipine was found to be ineffective in protecting kidney function and structure in the DOCA rat or less effective than ACE-inhibitors in the LNAME rat[22,42,43]. Even when amlodipine was dosed three times higher, *e.g.*, at 10 mg/kg, no reduction in proteinuria or glomerular damage was found in the DOCA rats, although amlodipine efficiently lowered blood pressure. However, in other models, *e.g.*, in the LNAME rats, treatment with amlodipine showed pronounced protective effects on end organ damage and myocardial protection combined with the efficient blood pressure lowering effect in different rat strains. The lack of efficacy of amlodipine has been further linked to the inability of CCBs to dilate renal efferent vessels to improve renal microcirculation[22,43-47]. Long-term treatment with SAR407899 showed an efficient dose-dependent blood pressure reduction in both models with no signs of tachyphylaxia over the course of 35 d. Blood pressure lowering effects and protective effects on hypertension related end organ damage of SAR407899 were superior to ramipril and amlodipine in the DOCA rat. Typical end-organ damage observed upon chronic LNAME administration (hypertrophy and fibrosis of the heart, fibrosis of the kidney, glomerulosclerosis, tubular degeneration and leukocyte-infiltration) was significantly reduced in the SAR407899-treated animals as supported by histopathological and gene expression analyses. Albuminuria, which is considered the key parameter of kidney damage in general and of glomerular damage in particular, were strongly induced in the DOCA- and LNAME-challenged rats. Chronic administration of SAR407899 significantly reduced albuminuria in both models. Histologically visible improvements should translate into functional benefits (endothelial function and heart contractility) observed upon SAR407899 administration. Indeed, hearts of hypertensive the DOCA or LNAME animals treated with SAR407899 showed a significantly improved heart function (measured as heart power *in vitro*). Moreover, endothelial-dependent relaxation (*in vitro*) was significantly and dose-dependently improved after long-term treatment with SAR407899. Histological scoring and quantitative real time PCR analysis revealed a significant reduction in interstitial fibrosis, as measured by sirius red staining and collagen expression, respectively. SAR407899 treatment significantly reduced tissue expression of CD3 and CD68 (markers of infiltrating leukocytes and macrophages) in both models, which can be explained by the role of ROCKs in cellular migration and cytokinesis through modulation of the cytoskeleton. Similar data were reported by several groups studying the effect of ROCK inhibition on macrophage migration in atherosclerotic plaques[48-50]. Therefore, the beneficial effects of SAR407899 do not depend on only blood pressure control but also the suppression of inflammatory and fibrotic events in the target organs. These data suggest that inhibition of ROCKs could bring clear therapeutic benefit through different mechanisms and is not limited to vasorelaxation only. Recently, other authors stated that potential benefits of ROCK-inhibition, direct or indirect, could be more far-reaching than first thought[13]. Further studies using highly selective, potent, and safe ROCK inhibitors could open new perspectives for ROCK-based therapies in several different clinical indications.

COMMENTS

Background

Large clinical trials have demonstrated that current treatment with angiotensin converting enzyme-inhibitors, Angiotensinreceptor-blockers or calcium channel blockers only modestly reduces the progression of chronic kidney diseases. Hypertension increases the risk of target organ damage, including heart hypertrophy, heart ischemia, kidney dysfunction or failure, cerebrovascular events and malfunction of the endothelial tissue.

Research frontiers

Rho-kinase (ROCK) is considered an important target for a variety of cardiovascular diseases, including hypertension and hypertension-related end-organ damage. Several inhibitors of ROCK have been extensively used to evaluate the role of ROCK in cardiovascular physiology and pathology. However, these inhibitors have a moderate specificity, moderate potency and short duration of action *in vivo* that limit their clinical use. Therefore, the development of a more potent and specific inhibitor with a better pharmacokinetic profile is needed to explore the potential of ROCK inhibition in the therapy of hypertension and its complications.

Innovations and breakthroughs

The authors have identified a novel, potent and selective inhibitor of ROCK (SAR407899) and characterized its long-term effects in two animal models of hypertension.

Applications

ROCK-inhibition by the SAR407899 represents a new therapeutic option for the treatment of hypertension and its complications.

Terminology

Rho-kinases (ROCK1 and ROCK2) are intimately involved in the transmission of contractile signaling within smooth muscle tissue. Upon activation of the small GTPase RhoA by ligand-bound specific GPCRs, ROCKs, the downstream effectors of RhoA, phosphorylate the myosin light chain phosphatase and the myosin regulatory light-chain itself, resulting in a net increase in activated myosin. This promotes smooth muscle contraction and actin cytoskeleton re-organization. Inhibition of ROCKs leads to relaxation of vascular smooth muscle cells and, consequently, to a decrease in blood pressure.

Peer review

This article is particular meaningful for hypertension-related end-organ damage.

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CASE REPORT

Permanent transvenous pacemaker implantation in a patient with Cor triatriatum dextrum

Kun Xiang, George V Moukarbel, Blair Grubb

Kun Xiang, George V Moukarbel, Blair Grubb, Division of Cardiovascular Medicine, University of Toledo Medical Center, Toledo, OH 43614, United States

Author contributions: Xiang K, Moukarbel GV and Grubb B designed and wrote the report; Xiang K and Moukarbel GV performed the transesophageal echocardiogram; Xiang K and Grubb B performed the dual-chamber pacemaker placement.

Ethics approval: This is a clinical case report. All patients related identification information have been avoided according to the policy of University of Toledo Medical Center and the Health Insurance Portability and Accountability Act (HIPPA) by the United State of America.

Informed consent: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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Correspondence to: Blair Grubb, MD, Division of Cardiovascular Medicine, University of Toledo Medical Center, 3000 Arlington Avenue, Toledo, OH 43614,
United States. blair.grubb@utoledo.edu
Telephone: +1-419-3833963
Fax: +1-419-3836167

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Abstract

Cor triatriatum dextrum is an extremely rare congenital heart abnormality in which the right atrium is separated into two chambers by a persistent fibrous membrane. A transvenous approach to place a dual-chamber pacemaker in such patients is technically challenging. We report the first case of a transvenous permanent pacemaker placement in a patient with cor triatriatum dextrum. An 87-year-old woman was diagnosed with paroxysmal atrial fibrillation. She was accidentally found to have cor triatriatum dextrum during the transesophageal echocardiography (TEE) prior to cardioversion. Later during her hospital stay, it was indicated to place a permanant pacemaker due to high grade atrioventricular block. After thorough reviewing TEE imagings, a transvenous catheter-based approach was decided feasible. Patient successfully received a dual chamber pacemaker through left subclavian venous approach. Furthermore in our case, using specially designed pacemaker leads and cautious intra-procedural maneuvering under fluoroscopic guidance ensured procedural success. In summary, a thorough pre-operative evaluation with transesophageal echocardiography is critical for the planning and eventual success of the transvenous placement of right-sided leads.

Key words: Congenital heart defect; Complete heart block; Inter-atrial membrane; Dual-chamber pacemaker

Core tip: Cor triatriatum dextrum is an extremely rare congenital heart abnormality in which the right atrium is separated by a persistent fibrous membrane. This membrane poses a technical challenge for dual-chamber pacemaker placement through the transvenous approach. Here we report the first transvenous pacemaker placement in a patient with cor triatriatum dextrum. A thorough pre-operative evaluation by transesophageal echocardiogram was critical for the planning of transvenous catheter-based right-sided leads placement. Using specially designed pacemaker leads and cautious

intra-procedural maneuvering under fluoroscopic guidance ensured procedural success.

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INTRODUCTION

Cardiovascular disease is the leading cause of mortality and mobility according to recent statistics[1]. Adult congenital heart disease has become an important entity in the current cardiology practice due to the advances in pediatric cardiac care and improved survival[2]. Commonly seen congenital heart conditions include septal and valvular defects. Cor triatriatum dextrum is an extremely rare congenital heart anomaly in which the right atrium is separated into two chambers by the persistence of the right sinus venosus valve[3]. It is uncommon to encounter such patients requiring placement of a permanent pacemaker. We report the placement of a dual-chamber pacemaker through the transvenous approach in a patient with cor triatriatum dextrum. To our knowledge, there are no similar reports in the published literature.

CASE REPORT

An 87-year-old woman presented to the hospital with complaints of dyspnea on exertion. She has no significant medical history other than prior cigarette smoking. On her arrival to the emergency room she was found to be in atrial fibrillation with rapid ventricular response. She was treated with heart rate control medications and anticoagulation. A transesophageal echocardiogram (TEE) was performed prior to cardioversion to normal sinus rhythm given poor rate control. This revealed the presence of a well-defined transverse membrane dividing the right atrium (Figure 1) consistent with the diagnosis of cor triatriatum dextrum. Color Doppler evaluation indicated separation of blood flow in the two divisions of the right atrium. However the division of the right atrium by this membrane was not complete. Color Doppler confirmed partial obstruction in the superior portion (Figure 2). The right atrium was enlarged without significant tricuspid regurgitation. An enlarged coronary sinus was noted with a measured diameter of 15.8 mm. Agitated saline injections *via* the left arm showed no saline contrast in the coronary sinus, indicating the absence of a persistent left superior vena cava. In addition, a small atrial septal defect (ASD) (measured diameter 3 mm) with a left to right shunt was demonstrated by color flow Doppler (Figure 3). No thrombus was noted in the left atrium and left atrial appendage. Subsequently the patient was cardioverted to normal sinus rhythm without complication.

During her hospital course, she was found to have Morbitz type Ⅱ AV block along with periods of complete heart block by remote cardiac monitoring station. Considering patient's daily functional capacity predicting reasonable life expectancy, the decision was made to implant a permanent dual-chamber pacemaker. The presence of cor triatriatum dextrum with a membrane partially obstructing the cavity of the right atrium, presented a technical challenge in regards to adequate placement of the right ventricular and right atrial leads *via* the transvenous approach. After obtaining left subclavian venous access, a 6-French St. Jude Medical pacing electrode was inserted *via* a breakaway introducer sheath. The lead was gently guided to the area near the opening of the interatrial membrane. The area was gently probed with the pacing lead until it was seen to dip into the lower atrial area. Care was taken not to cross the ASD with the lead. Once the inner atrial membrane had been crossed, the lead was then advanced and fluoroscopically guided into the right ventricular apex. Once adequate positioning had been determined, the active fixation coil was deployed. *Via* a second breakaway introducer sheath, a Boston Scientific Dextrus active fixation lead was fluoroscopically guided into an area just below the membrane within the right atrial appendage, and the active fixation coil deployed (Figure 4). Each lead was then connected to a Boston Scientific Ingenio dual-chamber pulse generator. *Via* off field telemetry, adequate sensing and pacing levels of the leads were determined. The patient was discharged to home the next day.

DISCUSSION

The prevalence of adult congenital heart disease has increased in the past 10 years and its management has proposed new challenges to current cardiology practice[2]. We presented a case of successful pacemaker lead placement in a patient with non-obstructive cor triatriatum dextrum. Cor triatriatum dextrum is an extremely rare congenital heart abnormality in which the right atrium is separated into two chambers by the persistence of the right sinus venosus valve[3]. The superior chamber receives the venous blood from both vena cava and the inferior chamber is in contact with the tricuspid valve and the right atrial appendage. The size of the communicating orifice between the superior and inferior atrial chambers determines the natural course of cor triatriatum dextrum. If the communicating orifice is small, the patient shows symptoms of congestive heart failure during infancy or childhood and usually requires surgical intervention for survival. If the connection is large and non-obstructive, patient may remain asymptomatic for many years, as in our case. The clinical presentation therefore is somewhat variable. Patients with cor triatriatum dextrum may present with recurrent supraventricular tachycardia, right-side heart failure, or cyanosis in the presence of ASD with right-to-left shunt. In our case, patient presented initially with atrial fibrillation with rapid ventricular response. The disorder can be treated surgically in symptomatic patients by

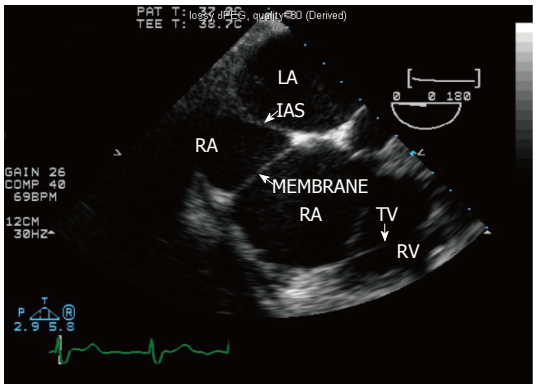

Figure 1 Four-chamber transesophageal echocardiogram view showing the division of the left atrium into two chambers by a transverse membrane. IAS: Interatrial septum; LA: Left atrium; RA: Right atrium; RV: Right ventricle; TV: Tricuspid valve.

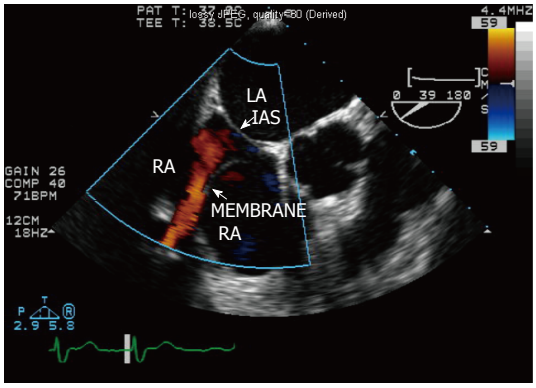

Figure 2 Color flow Doppler demonstrating the partially obstructive nature of the membrane, with blood flow through a small connection in the superior portion of the right atrium between the two chambers. IAS: Interatrial septum; LA: Left atrium; RA: Right atrium.

removing the membrane dividing the atrium.

During normal embryogenesis, the right atrium is formed by two different portions joining together: the right horn of the sinus venosus that forms the smooth posterior portion, and the original embryologic right atrium that forms the trabeculated anterior portion. The connection between these two portions is called sinoatrial orifice. The sinoatrial orifice is sided by two valvular folds that are called the right and left venous valves. During the development of right atrium, the right valve of the right horn of the sinus venosus forms a membranous valve that divides the right atrium in two parts. This valve directs oxygenated venous return from the inferior vena cava across the foramen ovale to the left side of the heart. This membranous valve normally regresses by the 12th week of gestation. Incomplete regression of the superior portion of right venosus valve forms membranes attached to the crista terminalis, while remnant of the inferior portion results in the Eustachian valve of the inferior vena cava, web-like remnant as Chiari network or the Thebesian valve of the coronary sinus. Failure of regression of this membrane causing persistent partition between the venous (smooth) and trabeculated portions of the right atrium

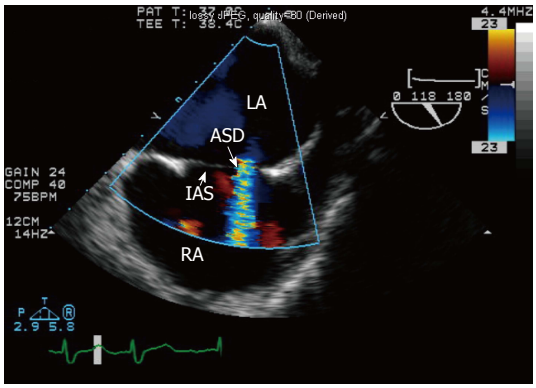

Figure 3 Color flow Doppler demonstrating a small atrial septal defect with left-to-right shunt. ASD: Atrial septal defect; IAS: Interatrial septum; LA: Left atrium; RA: Right atrium.

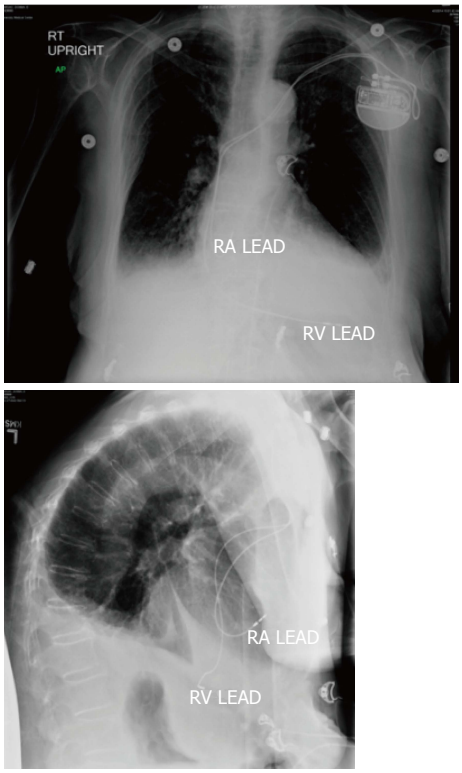

Figure 4 Chest X-ray showing proper placement of right ventricular and right atrial leads. RA: Right atrium; RV: Right ventricle.

leads to the formation of cor triatriatum dextrum[4].

Cor triatriatum dextrum has been associated with other congenital abnormalities, including ASD, patent foramen ovale, ventricular septal defect, hypoplastic right ventricle, hypoplastic tricuspid valve, and pulmonary atresia[3]. In our case, the presence of a small ASD posed the risk of crossing into the left atrium during manipulation of the lead across the atrial membrane. We also found an enlarged coronary sinus, measured diameter 15.8 mm (normal range 6.6 ± 1.5 mm)[5]. This is likely related to the elevated right atrial pressure. A rare but important congenital vascular anomaly associated with an enlarged coronary sinus is a persistent

left superior vena cava draining into the coronary sinus[6]. To investigate this possibility, an agitated saline injection *via* the left arm was performed during TEE and showed no saline contrast in the coronary sinus, indicating the absence of a persistent left superior vena cava.

Considering the rarity of cor triatriatum dextrum, a patient with such a congenital abnormality who requires a permanent pacemaker is unique. To our knowledge, this is the first reported case of a permanent pacemaker placement through a transvenous approach in a patient with cor triatriatum dextrum. Although it is technically challenging, transvenous catheter-based approach is feasible if the membrane in the right atrium is non-obstructive and caution exercised during the procedure. A thorough pre-operative evaluation by transesophageal echocardiogram was critical for the planning of the transvenous catheter-based right-sided leads placement. Using specially designed pacemaker leads and cautious intra-procedural maneuvering under fluoroscopic guidance ensured procedural success.

COMMENTS

Case characteristics

A 87-year-old woman presented with paroxysmal atrial fibrillation.

Clinical diagnosis

Atrial fibrillation with raid ventricular response, later was also diagnosed with high grade AV block.

Differential diagnosis

Any cause for atrial fibrillation and/or AV block such as hypertension, heart failure *etc*,.

Laboratory diagnosis

Lab test result was unremarkable.

Imaging diagnosis

A transesophageal echocardiogram revealed a rare congenital condition called cor triatriatum dextrum.

Treatment

A permanent pacemaker placement through a transvenous approach.

Related reports

To the knowledge, this is the first reported case of a permanent pacemaker placement through a transvenous approach in a patient with cor triatriatum dextrum.

Term explanation

Cor triatriatum dextrum is an extremely rare congenital heart abnormality in which the right atrium is separated into two chambers by the persistence of the right sinus venosus valve.

Experiences and lessons

Although it is technically challenging, transvenous catheter-based approach for a permanent pacemaker placement is feasible in a patient with cor triatriatum dextrum.

Peer review

It is interesting.

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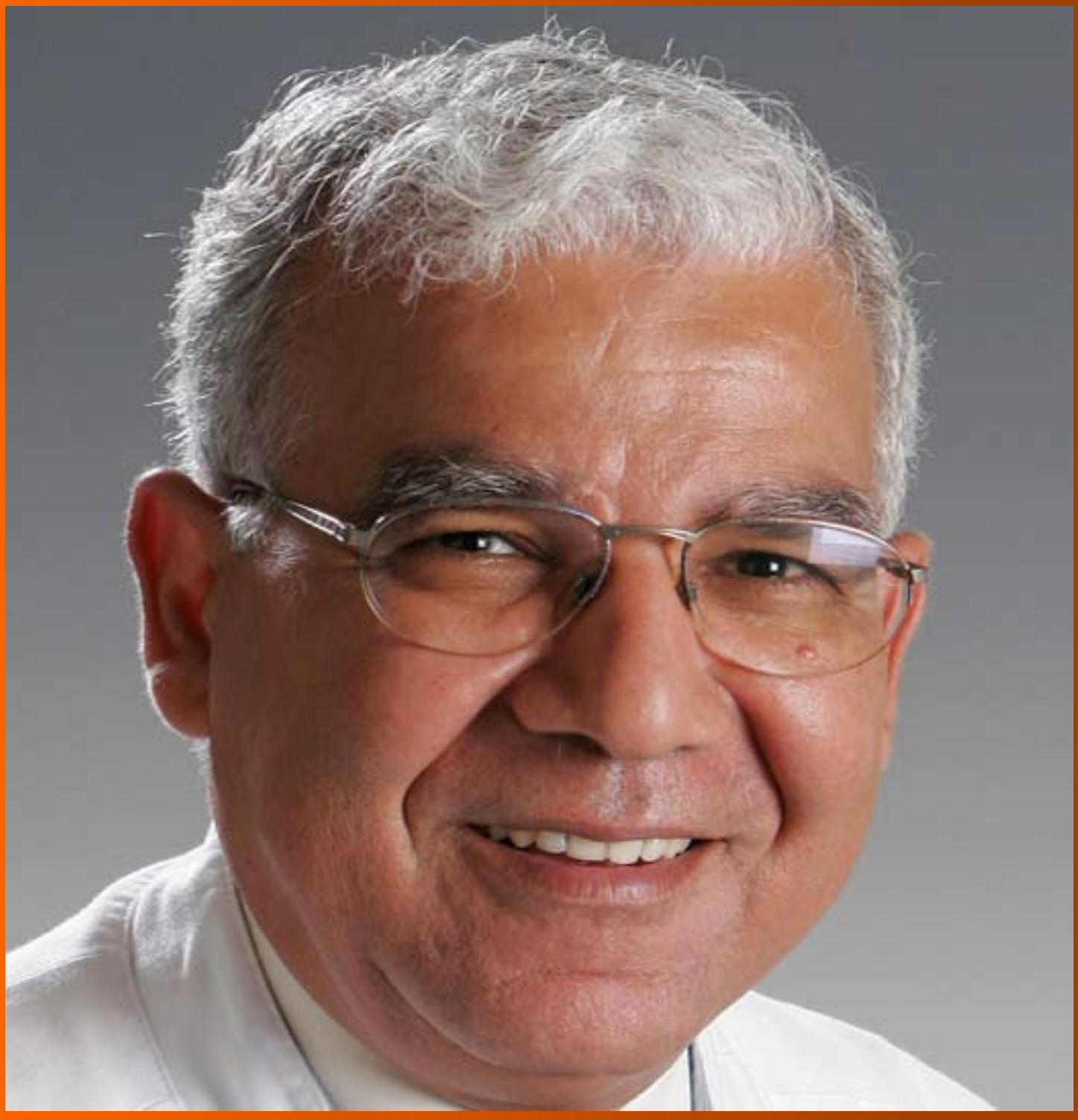

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Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
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EDITORIAL

Exercise training in the management of patients with resistant hypertension

Fernando Ribeiro, Rui Costa, José Mesquita-Bastos

Fernando Ribeiro, Rui Costa, School of Health Sciences, University of Aveiro, Portugal and CINTESIS.UA, 3810-193 Aveiro, Portugal
José Mesquita-Bastos, School of Health Sciences, University of Aveiro, 3810-193 Aveiro, Portuga
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Correspondence to: Fernando Ribeiro, PhD, School of Health Sciences, University of Aveiro, Building 30, Agras do Crasto, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal. fernando.ribeiro@ua.pt
Telephone: +351-23-4372455
Fax: +351-23-4401597
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Abstract

Hypertension is a very prevalent risk factor for cardiovascular disease. The prevalence of resistant hypertension, *i.e.*, uncontrolled hypertension with 3 or more antihypertensive agents including 1 diuretic, is between 5% and 30% in the hypertensive population. The causes of resistant hypertension are multifactorial and include behavioral and biological factors, such as non-adherence to pharmacological treatment. All current treatment guidelines highlight the positive role of physical exercise as a non-pharmacological tool in the treatment of hypertension. This paper draws attention to the possible role of physical exercise as an adjunct non-pharmacological tool in the management of resistant hypertension. A few studies have investigated it, employing different methodologies, and taken together they have shown promising results. In summary, the available evidence suggests that aerobic physical exercise could be a valuable addition to the optimal pharmacological treatment of patients with resistant hypertension.

Key words: Exercise training; Resistant hypertension; Blood pressure; Non-pharmacological; Cardiovascular disease

Core tip: Taken together, the available evidence indicates that, unless there is a contraindication to performing physical exercise, patients with resistant hypertension should be encouraged to engage in regular aerobic physical exercise in addition to the optimal pharmacological treatment.

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RESISTANT HYPERTENSION

Arterial hypertension (defined as blood pressure > 140/90 mmHg) is the most important risk factor for cardiovascular events and end-stage renal disease[1]. In the general population, arterial hypertension has a prevalence of 30%-45%[1]. The control of blood

pressure is essential to avoid cardiovascular events in primary and secondary prevention[1]. In an important percentage of subjects, arterial hypertension is not controlled (< 140 and 90 mmHg) with a strategy to correct lifestyle behavior and three antihypertensive drugs in high doses, including a diuretic[1,2], and is defined as resistant hypertension. Some authors suggest that resistant hypertension should be diagnosed in those who meet the criteria of blood pressure control with the use of four antihypertensive drugs[2]. Several studies found that the prevalence of resistant hypertension in the hypertensive population is between 5% and 30%[1]. So, it is essential that the diagnosis of resistant hypertension is well defined in order to exclude false resistant hypertension[3]. Normally, arterial hypertension is defined by office blood pressure obtained by an electronic oscillometric device, called casual blood pressure. Ambulatory blood pressure is another way to measure blood pressure. Ambulatory blood pressure is superior to casual blood pressure in the diagnosis[1] and the cardiovascular events prognosis[4,5].

Blood pressure shows circadian rhythm; it is higher in the morning after waking (morning surge), declines during the day and in a more pronounced way during the night with sleeping (nighttime dipping)[6]. In relation to cardiovascular events prognosis, several studies have shown that nighttime blood pressure is superior to daytime blood pressure[4,7]. In patients with resistant hypertension, the absence of nighttime dipping is more prevalent than in patients with nonresistant hypertension and it is associated with higher cardiovascular events[6]. Ambulatory blood pressure also makes the exclusion of the alert reaction (difference between casual blood pressure and day blood pressure determined by ambulatory blood pressure) possible, which is one of the causes of false resistant hypertension or pseudo resistant hypertension[3]. In pseudo resistant hypertension, blood pressure is not controlled in office behavior but has normal values in ambulatory blood pressure. Nonadherence to the prescribed treatment is another cause of false resistant hypertension[5].

Resistant hypertension is associated with higher organ damage and cardiovascular events and a worse renal prognosis[1]. Resistant hypertension can be caused by obesity, excessive alcohol ingestion, high salt intake and obstructive sleep apnea[5]. Secondary hypertension can be the cause of resistant hypertension[8]. Secondary causes of hypertension include hyperaldosteronism, obstructive sleep apnea, renal artery stenosis and pheochromocytoma[9]. Hyperaldosteronism is the most common secondary cause of hypertension; nonetheless, many newly diagnosed hypertensive patients[10] and resistant hypertension patients[11] can have undetected primary aldosteronism. So, in studies looking for the impact of lifestyle changes on blood pressure of patients with resistant hypertension, it is recommended to assess at least the plasma aldosterone-renin ratio even if the serum potassium level is normal[9]. The treatment of arterial hypertension implies changes in lifestyle attitudes, namely regarding exercise habits[8]. Recently, a prospective, blinded, randomized, sham-controlled trial assessing the effect of renal denervation or a sham procedure on ambulatory blood pressure monitoring measurements 6 mo post-randomization failed to demonstrate a benefit of renal artery denervation on reduction in ambulatory blood pressure[12]. However, recently several predictors of blood pressure response in the SYMPLICITY HTN-3 trial were identified, which could at least partially explain the results of the trial[13]; among the predictors are the total number of ablation attempts, baseline office SBP $\geqslant$ 180 mmHg, prescription of an aldosterone antagonist at baseline, and age < 65 years in age[13]. This information is significant for designing future studies in this field.

EXERCISE TRAINING AND RESISTANT HYPERTENSION

The cardioprotective benefits of exercise training in those with cardiovascular diseases include the modification of traditional cardiovascular risk factors, the improvement of exercise tolerance, myocardial and peripheral perfusion, cardiac function, arterial stiffness, autonomic function, endothelial repair, as well as the mitigation of endothelial dysfunction and low-grade vascular wall inflammation, and, most importantly, the reduction of morbidity and mortality[14-25]. Indeed, it is widely accepted that exercise training is a polypill with several beneficial effects, including antihypertensive effects. Indeed, exercise is able to induce a decrease of 5-7 mmHg in systolic blood pressure in patients with hypertension[26]. Nonetheless, the antihypertensive effects of exercise in patients with cardiovascular disease are often underestimated because the analysis is frequently made without assessing the influence of the baseline blood pressure on the effects of exercise training. We showed[17,27] in previous studies that if cardiovascular disease patients with high and low blood pressure at baseline are considered together, an exercise training intervention has no effect on blood pressure. But, if the analysis is conducted dividing patients into two subgroups on the basis of baseline blood pressure (pre-hypertension/hypertension *vs* normotension), exercise training significantly decreases systolic blood pressure[17,27]. An interesting aspect of exercise for patients with hypertension is that they could benefit from the antihypertensive effect of aerobic exercise after just three exercise sessions. Additionally, the duration of the exercise sessions can be as short as 10 min and the intensity of exercise can be relatively low (40% to < 60% VO_2 peak)[26]. The exercise prescription recommendation of the American College of Sports Medicine for those with high blood pressure

is to perform 30 min of continuous or accumulated aerobic exercise of moderate intensity (40%-60% of VO_2 Reserve) per day, on most, preferably all, days of the week[28]. The aerobic exercise could be supplemented by resistance exercise[28].

The management of resistant hypertension includes lifestyle interventions that aim to reduce sodium intake and increase the levels of daily physical activity[29]. Nonetheless, there are few studies evaluating the effects of lifestyle interventions, including physical exercise in patients with resistant hypertension. The potential of aerobic physical exercise as an adjunct non-pharmacological therapeutic tool to manage resistant hypertension was recently addressed in three studies[30-32].

Dimeo *et al*[30] first showed that patients with a reduced responsiveness to medication do not necessarily have reduced responsiveness to non-pharmacological therapies, *i.e.,* aerobic physical exercise, to lower blood pressure. The authors conducted a randomized trial encompassing fifty patients with resistant hypertension on an exercise training program, consisting of walking on a treadmill 3 times per week for 8 to 12 wk. Initially, the duration of the sessions was 30 min (interval training until the fifth week) and then was gradually increased to 30, 32 and 36 min of continuous training. Dimeo *et al*[30] observed a 6 ± 12 and 3 ± 7 mmHg reduction in ambulatory systolic and diastolic daytime ambulatory blood pressure, respectively. More recently, Guimaraes *et al*[31] confirmed these positive results using a different exercise approach. They enrolled 32 patients in a heated water exercise program or to a control group. The heated water exercise program was performed three times per week for 12 wk and consisted of callisthenic exercises (*i.e.*, exercises performed in a rhythmic, systematic way using the body weight for resistance) against water resistance and walking inside a pool with controlled temperature (30℃-32℃). After 12 wk, the exercise program group showed a decrease in 24 h systolic (from 137 ± 23 to 120 ± 12 mmHg) and diastolic blood pressure (from 81 ± 13 to 72 ± 10 mmHg), daytime systolic (from 141 ± 24 to 120 ± 13 mmHg) and diastolic blood pressure (from 84 ± 14 to 73 ± 11 mmHg), and nighttime systolic (from 129 ± 22 to 114 ± 12 mmHg) and diastolic blood pressure (from 74 ± 11 to 66 ± 10 mmHg). This reduction in blood pressure is of great importance as higher ambulatory blood pressure predicts cardiovascular morbidity and mortality in resistant hypertensive patients[33].

Concern for safety must come first in all that prescribe or supervise exercise. Thus, patients with resistant hypertension should consult a physician prior to engagement in exercise training, particularly vigorous intensity exercise[34]. The progression of intensity of aerobic exercise should be gradual to enhance compliance; slow progression of frequency and intensity of resistance exercise is also encouraged to avoid injuries. Isometric exercise is not recommended. In patients with poorly controlled blood pressure, vigorous intensity exercise should be discouraged or postponed until appropriate drug treatment has been instituted and blood pressure lowered[34]. It seems prudent to keep systolic blood pressures at ⩽ 220 mmHg and/or diastolic blood pressures ⩽ 105 mmHg during exercise[35]. It is also important to know that in some patients, β-blockers and diuretics have an adverse impact on thermoregulatory function and could cause hypoglycemia[35,36]. Additionally, patients treated with calcium channel blockers, β-blockers and vasodilators should stop exercise gradually as they have an increased likelihood of hypotension post exercise[35,36]. Hence, it is important to monitor the room temperature during exercise, use the Borg scale as an adjunct to heart rate to monitor exercise intensity, and extend the cool down period.

FUTURE PERSPECTIVES

The above-mentioned results are promising and provide good perspectives for the future. Nonetheless, more studies enrolling a large number of patients are clearly needed to reinforce the role of physical exercise associated with antihypertensive medication in the control of blood pressure in patients with resistant hypertension. Future studies are also warranted to disclose the mechanisms responsible for the positive effects of exercise. Several mechanisms, none of them definitive, have been proposed to explain the benefits of exercise training in these patients, including the decrease of sympathetic and the increase of vagal nerve activity, the improvement of the sensitivity of the baroreceptor reflex, the improvement of endothelial function and arterial stiffness, the decrease in the concentration of rennin, angiotensin Ⅱ and aldosterone, and the reduction of renal sympathetic outflow. These aspects seem to merit close attention in future studies.

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REVIEW

Cardiac remodeling and physical training post myocardial infarction

Michael A Garza, Emily A Wason, John Q Zhang

Michael A Garza, Emily A Wason, John Q Zhang, Laboratory of Cardiovascular Research, Department of Health, Kinesiology, and Nutrition, University of Texas at San Antonio, San Antonio, TX 78249, United States

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Correspondence to: John Q Zhang, PhD, Professor, Director of Laboratory of Cardiovascular Research, Department of Health, Kinesiology, and Nutrition, University of Texas at San Antonio, 1 UTSA Circle, San Antonio, TX 78249, United States. john.zhang@utsa.edu
Telephone: +1-210-4587390
Fax: +1-210-4585873

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Abstract

After myocardial infarction (MI), the heart undergoes extensive myocardial remodeling through the accumulation of fibrous tissue in both the infarcted and noninfarcted myocardium, which distorts tissue structure, increases tissue stiffness, and accounts for ventricular dysfunction. There is growing clinical consensus that exercise training may beneficially alter the course of post-MI myocardial remodeling and improve cardiac function. This review summarizes the present state of knowledge regarding the effect of post-MI exercise training on infarcted hearts. Due to the degree of difficulty to study a viable human heart at both protein and molecular levels, most of the detailed studies have been performed by using animal models. Although there are some negative reports indicating that post-MI exercise may further cause deterioration of the wounded hearts, a growing body of research from both human and animal experiments demonstrates that post-MI exercise may beneficially alter the course of wound healing and improve cardiac function. Furthermore, the improved function is likely due to exercise training-induced mitigation of renin-angiotensin-aldosterone system, improved balance between matrix metalloproteinase-1 and tissue inhibitor of matrix metalloproteinase-1, favorable myosin heavy chain isoform switch, diminished oxidative stress, enhanced antioxidant capacity, improved mitochondrial calcium handling, and boosted myocardial angiogenesis. Additionally, meta-analyses revealed that exercise-based cardiac rehabilitation has proven to be effective, and remains one of the least expensive therapies for both the prevention and treatment of cardiovascular disease, and prevents re-infarction.

Key words: Post-myocardial infarction; Exercise training; Myocardial remodeling; Angiotensin Ⅱ; Fibrosis

Core tip: After myocardial infarction, the heart undergoes extensive myocardial remodeling through the accumulation of fibrous tissue in both the infarcted and noninfarcted myocardium, which distorts tissue structure, increases tissue stiffness, and accounts for ventricular dysfunction. There is growing clinical consensus that exercise training may beneficially alter the course of post-myocardial infarction (MI) myocardial remodeling and improve cardiac function. This review

summarizes the present state of knowledge regarding the effect of post-MI exercise training on infarcted hearts.

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INTRODUCTION

Myocardial infarction (MI) is the major cause of heart failure in the adult American population[1]. Annually, 1.5 million Americans suffer from MI, with just over one-third of all cases inflicting serious heart disease and death. Because of this, post-MI treatments have become the major focus of research. There is growing clinical consensus that exercise training may beneficially alter the clinical course of post-MI myocardial remodeling and improve cardiac function[2,3]. Exercise training in post-MI patients with left ventricle (LV) systolic dysfunction has been recommended as a useful adjunct to the existing medical therapy, not only to attain symptomatic and functional improvement but also to prevent the progression of LV dysfunction and its attendant morbidity and mortality[4,5]. Significant improvements in exercise capacity were noted with no major complications in patients with moderate or severe LV dysfunction[4,6,7]. Post-MI training reverses skeletal muscle metabolic derangements[8,9], increases maximal cardiac output[6,10,11] and improves the quality of life in these patients. Exercise training also improves in myocardial perfusion, independent of regressive changes in coronary lesions[12]. The improvement in myocardial blood flow of the infarcted area, even late after acute infarction, may lead to a consistent recovery of both regional and global LV function. Patients with MI experienced an exercise training-induced improvement in myocardial oxygenation and LV function[13].

In recent years, cardiac rehabilitation (CR) has become a multi-disciplinary and multi-faceted intervention aimed at restoring well-being and impeding disease progression in patients with heart disease[14]. This complex intervention involves a variety of therapies, including risk factor education, psychological input, and drug therapy. Nevertheless, international clinical guidelines have consistently identified exercise-based CR as an essential element of therapy.

Despite guidelines recommending the use of CR programs for patients with MI, participation in these programs continues to be low; in fact, it has been reported that only 10% to 20% of patients who survive an acute MI participate in an exercise-based secondary prevention CR program[15]. Indeed, the reason for such low participation is likely multifactorial; additionally, conflicting results regarding the efficacy of experimental research and the absence of large randomized controlled trials with respect to re-infarction likely serve as additional barriers[3]. Therefore, we reviewed the evidence and the mechanisms by which post-MI exercise improves morbidity and mortality, as obtained by means of experimental and clinical studies.

POST-MI LV REMODELING

LV remodeling is the process by which ventricular size, shape, and function are regulated by mechanical, neurohormonal, and genetic factors[16,17]. After acute MI, the abrupt increase in volume overload induces a unique pattern of remodeling in the infarct zone and bordering non-infarct myocardium. The oxygen deprived myocardium experiences a localized inflammatory response *via* neurohormonal activation mediated in part by the migration of neutrophils, monocytes and macrophages[16]. Hypotension and the subsequent decrease in cardiac output stimulate temporary circulatory hemodynamic compensatory mechanisms including increased sympathetic nervous system, renin-angiotensin-aldosterone system (RAAS), and natriuretic peptide activity[18].

The induction of cardiomyocyte hypertrophy is a key process during post-MI remodeling that offsets increased volume over load, attenuates progressive dilation, and stabilizes contractile function; thus, post-MI myocyte hypertrophy initially serves as an adaptive, cardiac-preserving response[7,17]. However, over time, chronic neurohormonal activation, myocardial stretch, RAAS activity, and various paracrine and autocrine factors continue to promote eccentric, pathological hypertrophy, progressively deteriorating LV function to the point of failure. Interestingly, compelling evidence has shown that post-MI exercise favorably influences the course of LV remodeling, which accordingly, has attracted much attention[19].

EFFECT OF POST-MI EXERCISE TRAINING ON RAAS AND MYOCARDIAL REMODELING

Circulating angiotensin Ⅱ (Ang Ⅱ) is markedly increased following MI. Ang Ⅱ is a potent stimulant in pathologic myocardial remodeling both as a circulating hormone and as an autocrine/paracrine mediator produced in response to hemodynamic overload[20]. Ang Ⅱ plays a major role in vasoconstriction and aldosterone release. This peptide also serves as a growth factor and stimulates fibrous tissue formation in various[21-23]. Ang Ⅱ is also generated in the infarcted heart and regulates tissue structure in an autocrine and paracrine manner. All the components for Ang

Ⅱ generation including angiotensinogen, renin, and angiotensin converting enzyme (ACE), are present in the infarcted heart[24,25]. Locally generated AngⅡ stimulates transforming growth factor-β1 (TGF-β1) synthesis, which, in turn, enhances proliferation and collagen generation of myofibroblast, and leads to cardiac fibrosis[26]. Pharmacological intervention with ACE inhibitor or AngII receptor antagonist significantly attenuates cardiac fibrosis, and improves cardiac function and survival[27,28].

Acute physical exercise stimulates renin release and activates renin-angiotensin system[29,30] with an elevation of aldosterone[31], whereas chronic exercise training attenuates renin-angiotensin system at resting condition[32]. A study on patients with MI has demonstrated that the resting plasma AngⅡ reduced by 26% after 4 mo of exercise training[32]. The reduction in plasma AngⅡ was accompanied with 32% reduction in aldosterone, 30% reduction in vasopressin, and 27% reduction in atrial natriuretic peptide. An animal study using a pacing-induced heart failure in rabbits also revealed exercise training-induced attenuation of resting plasma AngⅡ[33].

In a previous study[34], we systematically examined the effect of exercise training on RAAS using a rat-MI model. Rats performed a moderate intensity exercise training on a rodent treadmill 1 wk after MI 5 d/wk for 8 wk at 16 m/min, 50 min per session. Our results showed that exercise training significantly attenuated circulating renin, ACE, AngⅡ, and aldosterone compared with sedentary rats with MI. Rats in exercise groups had similar LV end-diastolic diameters (LVEDd) compared with their sedentary counterparts and tended to have smaller LV end-systolic diameters (LVESd), and percent fractional shortening in exercise rats was significantly higher than in sedentary rats. These findings suggest that exercise training normalizes the circulating RAAS and improves LV function without compromising LV dilation.

In a similar study[35], we further evaluated the effect of post-MI exercise training on myocardial fibrosis, cardiac function, and factors inducing adverse remodeling. For the first time, changes caused by exercise training were investigated in type Ⅰ and Ⅲ collagen, matrix metalloproteinase (MMP-1), tissue inhibitor matrix metalloproteinase (TIMP-1), TGF-β1), AngⅡ receptor type 1 (AT1), and ACE at both gene and protein levels after MI. Our results indicated exercise training significantly attenuated the expression of TIMP-1 at both gene and protein level and improved balance between MMP-1 and TIMP-1 (imbalance between the two appear to be responsible for the increased MMP activity observed in congestive heart failure). Training also lowered expression of AT1 receptor protein and reduced ACE mRNA expression as well as ACE binding. In addition, training significantly decreased collagen content, thereby resulting in attenuated cardiac fibrosis. Lastly, exercise training preserved cardiac function.

AngⅡ receptor blockade has been widely used to alleviate detrimental effects associated with elevated RAAS[36,37]. In a subsequent study[38], we investigated the effect of combined exercise training along with AngII receptor blockade on post-MI ventricular remodeling in rats. Losartan (an AngⅡ receptor antagonist) treatment (20 mg/kg per day) was initiated 1-wk post-MI, and administered *via* gastric gavage for 8 wk. The results indicated significantly decreased levels of TIMP-1 in mRNA and protein expression in both trained and losartan treated groups. Exercise trained groups exhibited attenuated expression of AT1 receptor protein, and decreased ACE binding. These findings revealed that exercise training after MI provided beneficial effects on post-MI cardiac function and LV remodeling by the alteration of specific gene and protein expressions that regulate myocardial fibrosis, whereas the combination of both exercise training and losartan treatment improved the effects[35,38]. Tables 1 and 2 summarize both human and animal studies on post-MI physical training.

EARLY *VS* LATE PHASE POST-MI EXERCISE

Post-MI remodeling has been arbitrarily divided into two phases: the early phase, which lasts up to 72 h, and the late phase, lasting beyond 72 h[17]. Generally, adaptive responses that preserve stroke volume are invoked during the early stage, whereas late remodeling primarily involves hypertrophy and alterations in LV architecture in an attempt to distribute increased wall stresses more evenly. Differences in function between adjacent and remote non-infarcted regions are greatest at one week after anterior MI, and persist for a minimum of six months post-MI[39]; it is during this six-month period that systolic function decreases drastically, as the LV undergoes progressive dilatation, eccentric hypertrophy, and the lengthening of non-infarcted segments[17]. Thus, the question of when to begin exercise and at what intensity has proven elusive. Nevertheless, recent evidence offers novel insights and indeed provides an answer to some questions, although, as quality research often does, asks several more.

To date, several studies in humans reported contradictory effects of training on LV remodeling after MI[4,6,7,40-45]. However, careful inspection of these studies indicate that after small MI, exercise has no detrimental effect[7,41], or even improves[4,43,44,46] LV geometry and function, independent and irrespective of whether exercise was started late (1 year)[4,44] or early (< 2 mo)[7,41,43] after MI. Conversely, in patients with large MI (encompassing 35% to 50% of LV mass), exercise had either no[42], or a beneficial[4]

Table 1 Summary of physical training protocols and outcomes in selected human studies

Ref.	Type of exercise	Exercise intensity	Exercise duration	Exercise frequency	Training period	Assessment	Outcome
Braith *et al*[32] 1999	Treadmill walk	40%-70% of peak oxygen uptake (VO_2)	Started with 10-20 min as tolerated and increased to 30-45 min by the 10th wk	3 times/wk	4 mo	Plasma RAAS	Reduced Resting AngII, Aldosterone, vasopressin, and atrial natriuretic peptide
Myers *et al*[143] 2001	Outdoor walking at an elevation of 3500 ft, in addition to cycling	60%-70% of peak VO_2	Two 1-h sessions of walking, 45 min of cycling	5 times/wk	2 mo	Post-exercise oxygen uptake kinetics	High-intensity training did not result in a faster recovery of oxygen debt
La Rovere *et al*[144] 2002[1]	Graded exercise (cycling, calisthenics)	Adjusted to 75% of the heart rate at peak VO_2	30 min	5 times/wk	1 mo	BRS, LVEF	BRS improved by 26%, while LVEF remained unchanged
Marchionni *et al*[145] 2003[2]	Cycling	70%-85% of max heart rate	1 h	3 times/wk	6 mo	Total work capacity, health-related quality of life	Improved total work capacity and health-related quality of life
Zheng *et al*[146] 2008[1]	Bicycle ergometer	75% of peak heart rate	30 min	3 time/wk	6 mo	HR recovery, time to reach anaerobic threshold, left ventricular end-diastolic	Exercise training prevented ventricular remodeling to a certain extent
Giallauria *et al*[46] 2013	Bicycle ergometer	60%-70% of peak VO_2	30 min	3 times/wk	6 mo	diameter, left ventricular ejection fraction dipyridamole rest gated myocardial perfusion single photon emission computed tomography	Improved peak oxygen consumption, myocardial perfusion and LV function

[1]Exercise was part of a comprehensive secondary prevention program; [2]Combination study consisting of Home and Hospital/group participants. RAAS: Renin-angiotensin-aldosterone system; LVEF: Left ventricular ejection fraction; BRS: Baroreflex sensitivity.

effect on ejection fraction (EF) and LV volumes but only when started late after MI. However, when exercise after large MI is initiated at a time when LV remodeling is still ongoing (3 to 4 mo after MI), the majority of studies reported that exercise has either no[6,7,41], or even a detrimental[40,47] effect on LV volume and EF.

Similarly, experimental research using rat models of MI suggests that exercise initiated late (> 3 wk) after moderate to large MI does not aggravate[45,48], or even blunts[49-51] LV dilation and hypertrophy. Contrarily, exercise started < 1 wk after moderate to large MI resulted in variable outcomes with beneficial[52], no[53,54], or detrimental[55,56] effects on LV remodeling. Therefore, these rodent studies further evidence the concern that early exercise after MI may further exacerbate LV remodeling. Importantly, there are a number of concerns with the methodology of these studies. First, exercise experimental studies conducted late after MI predominately used treadmill running[45,48-50], whereas early exercise studies used swimming[51-55]. Since swimming is not a habitual activity for rats, this type exercise mode may markedly elicit both psychological and physiological stress to the animals, which potentially offsetting the beneficial effects of exercise compared to treadmill running[57,58].

Amazingly, in a recent study of evaluating 8-wk of volunteer exercise, de Waard *et al*[59] reported remarkable data addressing the question of exercise training 24 h after MI. As opposed to most humans, mice like to run, and will do so seemingly endlessly when presented the opportunity. During the first week after induction of MI, recovering mice slowly titrated up their daily running activity, reaching distances similar to their sham-operated counterparts towards the end of the study, thus, suggesting that early post-MI exercise training may have positive effect in post-MI recovery and myocardial remodeling. Authors reported that exercise had no effect on survival, MI size, or LV dimensions, but improved LV fractional shortening from 8% ± 1% to 12% ± 1%, LV dP/dt_{P30} from 5295 ± 207 to 5794 ± 207 mmHg/s, and reduced pulmonary congestion. Additionally, this study also provided novel information regarding myocardial Ca^{2+} handling after MI, debunking the previously held notion that exercise sensitizes myofilaments to the effects of Ca^{2+}[59]. A study from our group[34] systematically examined the timing effect of post-MI exercise training. Rats started exercise training at either 1 wk or 6 wk after MI on a treadmill for 8 wk. Rats in exercise groups had similar LVEDd compared with their sedentary counterparts and tended to have smaller LVESd, and percent fractional shortening (%FS) in exercise rats was significantly higher than

Table 2 Summary of physical training protocols and outcomes in selected animal studies

Ref.	Type of exercise	Exercise intensity	Exercise duration	Exercise frequency	Training period	Assessment	Outcome
Hashimoto *et al*[76] 2004	Treadmill running	10 m/min	60 min	5 d/wk	6 wk	Myosin heavy chain isoforms, cardiac wall measurements	Exercise training resulted in a significant increase of α-MHC expression in both anterior and posterior wall, ensuring a beneficial role in the remodeling of the heart
Xu *et al*[35] 2008	Treadmill running	16 m/min @ 5% grade	50 min	5 d/wk	8 wk	TIMP-1, AT1, ACE, collagen volume fraction, MMP	Early exercise training after MI reduces TIMP-1 expression, improves the balance between MMPs and TIMPs, and mitigates the expressions of ACE and AT1 receptor, thus attenuating myocardial fibrosis and preserving cardiac function
De Waard *et al*[59] 2007	Voluntary treadmill exercise training	N/A	N/A	5 d/wk	8 wk	LV fractional shortening, Ca^{2+} sensitivity, PLB, SERCA	Voluntary exercise improved LV and cardiomyocyte shortening, attenuates global LV dysfunction
Wan *et al*[34] 2007	Treadmill running	16 m/min @ 5% grade	50 min	5 d/wk	8 wk	Echo and RAAS	Exercise training improved cardiac function and attenuated RAAS. Early and late exercise training had similar beneficial results
Xu *et al*[106] 2010	Treadmill running	16 m/min @ 5% grade	50 min	5 d/wk	8 wk	SOD, GPx, MnSOD	Exercise training combined with Ang Ⅱ receptor blockade reduced oxidative stress
Yengo *et al*[147] 2012	Treadmill running	15% grade, speed increased from 13 to 24 m/min	Progressively increased to 60 min	6 d/wk	10 wk	Collagen concentration, non-reducible collagen cross-linking in the RV	Exercise training normalized the observed increase in cross-linking, and favorably modifies heart extracellular matrix

RAAS: Renin-angiotensin-aldosterone system; MHC: Myosin heavy chain; TIMP-1: Tissue inhibitor matrix metalloproteinase; AT1: AngII receptor type 1; ACE: Angiotensin converting enzyme; MMP: Matrix metalloproteinase; SOD: Superoxide dismutase; GPX: Glutathione peroxidase.

in sedentary rats. These finding suggest that exercise training does not cause LV dilation and preserves LV function.

POST-MI EXERCISE AND MYOCARDIAL CONTRACTION

Ca^{2+} handling abnormalities can largely explain depressed myocyte contractility in the remodeled myocardium, whereas abnormalities in myofilament function are less well understood. Previously, it was reported in pigs that impaired pump function three weeks after MI could also be attributed to decreased maximal isometric tension in skinned cardiomyocytes in areas remote from the ischemic border zone; as it turns out, the impairment occurred in the context of increased Ca^{2+} sensitivity of the myofilaments[60]. As a result, the authors attributed the increased post-MI Ca^{2+} sensitivity to reduced protein kinase A-mediated troponin I (TnI) phosphorylation[60]. Similarly, increased myofilament Ca^{2+} sensitivity has also been reported in end-stage human heart failure, mediated by decreased TnI phosphorylation.

Although experimentally challenging, investigators from the de Waard study were able to construct a full pCa-force relationships in isometrically contracting myocytes[59], which differs from previous studies relying on simultaneous measurements of FS% and Ca^{2+} fluorescence in unloaded myocytes to estimate myofilament Ca^{2+} sensitivity. Although a much simpler experimental approach, there are various problems associated with this method. First, maximal developed tension cannot be assessed in unloaded myocytes, and any changes in developed tension are ignored when estimating Ca^{2+} sensitivity. Secondly, basal sarcomere length is much shorter in unloaded myocytes (1.8 *vs* 2.2), and cannot be controlled; therefore, even a slight change in basal sarcomere length would confound the result, which in turn, has prompted investigators to wrongly conclude that exercise increases myofilament sensitivity[51]. Thus, data from de Waard *et al*[59] reveals that voluntary exercise training in mice early after MI normalizes myofilament dysfunction, which likely occurred in response to the exercise-induced improvement in unloaded shortening of isolated intact cardiomyocytes, as the Ca^{2+} transient amplitude was not found to be altered by exercise. Furthermore, basal Ca^{2+} was reduced by exercise, altogether suggesting that exercise decreases myofilament Ca^{2+} sensitivity.

Dysregulation of cardiac β-adrenergic receptor (β-AR) signaling represents another important factor leading to the pathological LV remodeling and the progression to heart failure. In the failing myocardium, adverse changes in β-AR signaling are mainly attributed to β_1-AR downregulation and desensitization/uncoupling of both β_1 and β_2-AR's. It has been reported that exercise after MI increases

β_1-AR, as evidenced by a 48% increase in β_1-AR protein, and a 36% increase in cAMP levels, and improves β-AR signaling[59,61], which in turn, may also contribute to improvement in myocardial contractility in patients with MI.

Myosin heavy chain (MHC) acts as the chemical-mechanical transducer of motion in muscle fibers by converting energy from ATP into the sliding myofilaments[62]. The isoform α-MHC elicits two to three times faster actin-activated ATPase activity and actin filament sliding velocity than the isoform MHC-β[63,64]. Thyroid hormone (TH) has profound effects on the cardiovascular system, and is known to critically regulate the expression of MHC isoforms in the myocardium[65]; in fact, in the absence of TH, the *α-MHC* gene is not transcribed[62]. Triiodothyronine (T_3), the active cellular form of TH, mediates its actions upon binding to thyroid hormone receptors (TRs)[66,67].

After MI, T_3 levels are significantly reduced in patients[68]; similarly, decreased serum concentrations of TH have also been observed in patients with chronic heart failure (CHF), which, in part, attributes to impaired cardiac function[69]. In experimental post-MI rat models, following the decrease of serum T_3, significant downregulation of α-MHC and the concomitant upregulation of MHC-β are observed in the LV non-infarcted myocardium, along with changes in TR isoforms at the mRNA level[68,70,71]. These, in addition to other MI-induced alterations in cardiac phenotype, are thought to further contribute to the progressive nature of LV systolic dysfunction, and have been associated with poor prognosis[62,63,72,73]. Interestingly, endurance exercise has been reported to favorably reverse MHC α- to β-cardiac isoform shifts after MI at both gene and protein levels[74,75], which in turn, may be associated with preserved cardiac functioning, attenuated LV remodeling, and increased myofibril function[76]. Recent evidence by our group[75] indicated that post-MI exercise training significantly increase cardiac expression of α-MHC and decrease cardiac expression of MHC-β without changing serum T_3 levels. Similarly, unpublished data from our group recently revealed that moderate-intensity treadmill exercise training markedly increased TRα-1 and TRβ-1 nine weeks after MI. Thus, it is likely that favorable changes in TH target gene transcription may be due to exercise-dependent upregulation of TR isoforms. Nevertheless, studies with experimental models of LV dysfunction and preliminary clinical investigation of patients with CHF reported that the TH analog 3,5-diiodothyroproionic acid elicits improvements in both systolic and diastolic LV function, accompanied by an increase in cardiac output and improved lipid profile[77]. Thus, it is conceivable that the combination of exercise combined with TH treatment could potentiate beneficial results, and warrants further investigation.

POST-MI OXIDATIVE STRESS AND EXERCISE TRAINING

Reactive oxygen species (ROS) including superoxide (O_2^-), hydroxyl (OH^-), and peroxynitrite ($ONOO^-$), have an unpaired electron[78]. These ROS serve as signaling molecules when in low concentrations; however, they elicit harmful oxidative stress when produced in excess[79]. ROS can directly damage the lipids of cell membranes, proteins and both nuclear and mitochondrial DNA resulting in serious or mortal cellular injury[80]. However, the toxicity associated with the excessive ROS can be prevented by antioxidant defense systems that provide a healthy cellular environment. Living cells have both enzymatic and non-enzymatic defense mechanisms to balance the multitude of oxidative challenges presented to them. The enzymatic antioxidant system includes superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX)[81]. SOD catalyzes the dismutation of superoxide (O_2^-) to hydrogen peroxide (H_2O_2). Catalase and GPX further metabolize H_2O_2 to water and oxygen. The non-enzymatic group includes a variety of biologic molecules, such as vitamins E and C[81,82]. Oxidative stress is enhanced by an unbalance between elevated ROS production and diminished antioxidant system.

Excessive oxidative stress has been observed in the myocardium of patients with CHF[83,84]. Heart failure subsequent to myocardial infarction is associated with oxidative stress in both infarcted and noninfarcted myocardium[83,85,86]. Researchers have identified a membrane-based NAD(P)H oxidase as a major source of O_2^- in the heart[87]. An elevated NAD(P)H oxidase expression has been observed in the infarcted rat heart and the extent of NAD(P)H oxidase elevation is negatively correlated with the deteriorated hemodynamic function and ventricular remodeling of the heart[88]. Furthermore, progressive decrease in antioxidant enzymes, SOD, catalase[89], and glutathione (an antioxidant)[90] has also been observed in the infarcted rat heart. These observations suggest that the impaired antioxidant system and/or augmented ROS promote oxidative stress, contributing to the adverse remodeling and dysfunction of the infarcted heart[91].

There is growing evidence that chronic exercise training adaptively bolsters the activity of protective antioxidant enzymes such as catalase, SOD, GPX[92], glutathione reductase (GR)[93], and antioxidant glutathione content[94,95] in skeletal muscles of healthy animals. Nine-weeks of treadmill training markedly elevated manganese-SOD (Mn-SOD, an isozyme of SOD) activity and its protein content both at rest and after an acute exercise bout in the soleus muscle of rats[96]. In contrast, the muscle Mn-SOD gene expression of untrained rats was significantly decreased after an acute bout of exercise[96]. Exercise

training also resulted in significant increase in SOD activity in the LV of normal rats[97,98]. These findings suggest that muscles have the capacity of responding to training in such a manner as to enhance antioxidant system and reduce the accumulation of ROS resulting from enhanced metabolic activity.

In patients with CHF, exercise training enhanced GPX and catalase activities, and mitigated lipid peroxidation in skeletal muscles[99]. Exercise training also downregulated both gene expression and activity of pro-oxidant NAD(P)H oxidase, and decreased vascular generation of ROS in human arterial tissue[100].

Inconsistent findings have been reported on the effect of post-MI exercise training on ROS and antioxidants. Yamashita *et al*[101] and Brown *et al*[102] reported that exercise training resulted in an increase in myocardial SOD content along with improved recovery from ischemia-reperfusion injury. Others, however, reported that exercise training increased cardioprotection without amplifying myocardial SOD content[103,104] and only certain cardiac antioxidant enzyme activities (*i.e.*, SOD) were enhanced in the exercise trained animals[97,101,105]. The variation in the findings of these studies may be due to the differences in the intensity and duration of exercise regimens. A study from our group[106] demonstrated that exercise training increased MnSOD gene expression after MI regardless of losartan treatment. In addition, exercise training together with losartan treatment remarkably enhanced the enzymatic activity of catalase, suggesting an additive effect of exercise training and Ang Ⅱ receptor blockade treatment. But exercise training did not enhance myocardial glutathione peroxidase activity. Our data also revealed that post-MI exercise training notably attenuated MI-induced elevation of plasma thiobarbituric acid reactive substances (TBARS, a marker of lipid oxidation) although cardiac TBARS was not altered.

It has been documented that Ang Ⅱ stimulates NAD(P)H oxidase activity, which promotes ROS production[107,108]. Thus, exercise training may improve antioxidant capacity and attenuate oxidative stress by attenuating RAAS[35,38,106].

MYOCARDIAL APOPTOSIS AND EXERCISE TRAINING

Loss of cardiomyocytes is an important mechanism in the development of myocardial remodeling and cardiac failure[109]. After MI, apoptotic cardiomyocyte death occurs in the infarcted myocardium as well as the surviving portions of the heart[110,111]. Myocyte apoptosis not only occurs at early phase (7 d) of MI[112,113], but also progresses to late phase (up to 6 mo) in myocardium remote from the area of ischemic damage[114,115], contributing to CHF[116]. ROS have proven to be powerful mediators of myocyte apoptosis[117,118]. Treatment of cardiac myocytes with O_2^- or H_2O_2 induces apoptosis, suggesting a mechanism of ROS as an initial pathogenic event[119]. Enhanced pro-apoptotic Bax expression coexists with oxidative stress and apoptosis in the infarcted heart[120], whereas oxidative stress activates pro-apoptotic enzymes, caspase-9 and caspase-3, resulting in cardiac apoptosis and ventricular dysfunction[117]. *In vivo* studies have demonstrated that long-term treatment with the antioxidants, probucol or pyrrolidine dithiocarbamate, attenuates oxidative stress and myocyte apoptosis within noninfarcted myocardium in rats[121,122].

Siu *et al*[98] demonstrated that endurance training downregulated the expression of caspase and Bax, and upregulated Bcl-2 (an anti-apoptotic gene product) in both skeletal and cardiac muscles of healthy rats. These anti-apoptotic effects were associated with elevated protein content of Mn-SOD. A clinical study also revealed that exercise training attenuated skeletal muscle apoptosis along with improved antioxidant capacity in patients with CHF[99]. Accordingly, the data are consistent with the idea that an increased antioxidant capacity and attenuated oxidative stress from exercise training may be involved in reducing pro-apoptotic genes, suggesting that exercise training may attenuate the extent of apoptosis in muscles. However, the influence of post-MI exercise training in myocardial apoptosis remains to be elucidated.

POST-MI EXERCISE AND CARDIAC ANGIOGENESIS

After myocardial infarction (MI), the adequate growth of new capillaries and arterioles, or angiogenesis, represents a critical process in the development of compensatory hypertrophy in the remaining non-infarcted myocardium[123]. Although compensatory angiogenesis can be observed in both the ischemic and infarcted heart, previous studies have demonstrated that angiogenesis may be inadequate[124,125]; in fact, recent evidence suggests that impaired angiogenesis may lead to maladaptive LV remodeling, promoting the transition from adaptive cardiac hypertrophy to LV dilation and dysfunction[61,126].

Exercise, through increased vascular shear stress, potentiates a powerful angiogenic stimulus[127]. The pro-angiogenic effect of exercise has previously been demonstrated in healthy swine hearts[128]. A study conducted by Leosco *et al*[61] reported that exercise induced a significant increase of capillary density in lateral border and remote zones to the infarct site, but not in the area close to the infarcted site. One of our recent studies (unpublished data) confirms that post-MI exercise training induced about 1.5-fold increase in capillary density in the septum

and left ventricle compared to non-exercised heart, suggesting that exercise promotes capillary growth in non-infarcted areas of severely decompensated hearts.

A number of studies clearly demonstrate that exercise activates vascular endothelial growth factor (VEGF) dependent angiogenic pathways[129-131], which represent critical molecular mechanisms by which exercise triggers angiogenesis[130]. In addition, exercise-induced upregulation of VEGF in patients with heart failure has also been documented[132]. Recently, experimental studies have revealed that exercise reactivates angiogenic signaling by increasing VEGF and eNOS phosphorylation by Akt in the heart, increases coronary vascular network and density, and enhances myocardial blood perfusion. Evidence of endothelial dysfunction in peripheral resistance arteries post-MI has also been observed in both experimental and clinical studies[133,134], which likely contributes to arterial dysfunction[135,136]; in this regard, post-MI exercise has been shown to reverse arterial dysfunction by virtue of restored production of nitric oxide (NO) in the endothelial vessel wall mediated by adaptive changes in eNOS, its activation by Akt, and by reduced NAD(P)H oxidase-generated ROS scavenging of NO[137].

EXERCISE-BASED CR IN PATIENTS WITH HEART DISEASE

Previously, four meta-analyses[138-141] of the effects of exercise-based interventions in patients with coronary heart disease reported a statistically significant benefit in patients receiving exercise therapy compared with usual medical care, with a reduction in total and cardiac morality ranging from 20% to 32%. However, randomized controlled trials (RCT) have generally been small and often of questionable methodological quality, raising concerns that the effect of exercise-based CR may be overestimated. In 2004, Taylor *et al*[142] aimed to update the systematic review of the effects of exercise-based CR in patients with coronary heart disease, addressing previous concerns regarding the applicability of this evidence to routine practice.

For the analysis, over 5000 articles were retrieved from a number of search sources, and only 425 full papers were considered for possible inclusion. Studies were excluded for various reasons including nonrandomized design, inappropriate patient groups, inappropriate intervention, the control group received an exercise intervention, inappropriate outcomes, in adequate follow-up, and preliminary results only available in abstract form. After identification of duplicate publications, only 48 eligible studies remained, and were still of poor methodological quality.

Although exercise-based CR was associated with a significant reduction in all-cause mortality and total cardiac mortality, there was no significant difference with respect to re-infarction[142]. Conversely, a recent meta-analyses conducted in 2011 consisting of 34 RCTs (n = 6111) found that patients randomized to exercise-based CR had a significantly lower risk of re-infarction, cardiac mortality, and all-cause mortality[3]. In a stratified analysis, treatment effects were consistent regardless of study periods, duration of CR, or time beyond the active intervention[3]. Additionally, Exercise-based CR had favorable effects on cardiovascular risk factors, including smoking, blood pressure, body weight, and lipid profile[143].

CONCLUSION

Most of the human and animal studies demonstrated that post-MI physical exercise training results in positive effect on myocardial remodeling. These beneficial effects include improved cardiac function, mitigated interstitial myocardial fibrosis, and enhanced physical capacity. As a result, physical exercise training provides good prognosis and improves the quality of life of MI patients. The current literature revealed the mechanism of physical training-induced improvement in post-MI cardiac remodeling. Physical training attenuates renin[29,30], ACE, Ang Ⅱ, and aldosterone[31,34]. The attenuation of Ang Ⅱ, in turn, reduces cardiac fibrosis[34] and aldosterone secretion[32,34], which may ease MI-induced plasma expansion. Physical training also improves the balance between MMP-1 and TIMP-1, which, in turn, reduces cardiac stiffness *via* regulation of collagen accumulation[38]. Studies show that physical training significantly improves ß-adrenergic receptor, cAMP[59,61], and favorably reverses MHC α- to β-cardiac isoform shifts[74,75], attributing to improvement in myocardial contractility. In addition, post-MI physical training may enhance antioxidant enzyme capacity and attenuate oxidative stress[97,101,105]. It is important to note that the existing studies have only investigated the effects of *endurance* exercise on post-MI remodeling; therefore, the effects of post-MI resistance training have yet to be systematically examined to identify a better exercise mode. Furthermore, although majority of the research has shown that post-MI exercise training improves cardiac remodeling and function, the suitable exercise intensity, duration, and the time to start training are yet to be optimized to provide clinically relevant information regarding the pathophysiology of post-MI recovery through physical training.

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Percutaneous left atrial appendage closure: Technical aspects and prevention of periprocedural complications with the watchman device

Sven Möbius-Winkler, Nicolas Majunke, Marcus Sandri, Norman Mangner, Axel Linke, Gregg W Stone, Ingo Dähnert, Gerhard Schuler, Peter B Sick

Sven Möbius-Winkler, Nicolas Majunke, Marcus Sandri, Norman Mangner, Axel Linke, Gerhard Schuler, Department of Internal Medicine/Cardiology, University of Leipzig, Heart Center, 04251 Leipzig, Germany
Gregg W Stone, Columbia University Medical Center, New York, NY 10032, United States
Ingo Dähnert, Department of Pediatric Cardiology, University of Leipzig, Heart Center, 04251 Leipzig, Germany
Peter B Sick, Clinic for Cardiology, Hospital Barmherzige Brueder, 93049 Regensburg, Germany
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Correspondence to: Peter B Sick, MD, Clinic for Cardiology, Hospital Barmherzige Brueder, Pruefeningerstrasse 86, 93049 Regensburg, Germany. peter.sick@barmherzige-regensburg.de
Telephone: +49-941-3692101
Fax: +49-941-3692107
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Abstract

Transcatheter closure of the left atrial appendage has been developed as an alternative to chronic oral anticoagulation for stroke prevention in patients with atrial fibrillation, and as a primary therapy for patients with contraindications to chronic oral anticoagulation. The promise of this new intervention compared with warfarin has been supported by several, small studies and two pivotal randomized trial with the Watchman Device. The results regarding risk reduction for stroke have been favourable although acute complications were not infrequent. Procedural complications, which are mainly related to transseptal puncture and device implantation, include air embolism, pericardial effusions/tamponade and device embolization. Knowledge of nature, management and prevention of complications should minimize the risk of complications and allow transcatheter left atrial appendage closure to emerge as a therapeutic option for patients with atrial fibrillation at risk for cardioembolic stroke.

Key words: Atrial fibrillation; Stroke prevention; Left atrial appendage; WATCHMAN® device; Complications

Core tip: Left atrial appendage (LAA)-Occlusion was developed as an alternative to chronic anticoagulation therapy in patients with nonvalvular atrial fibrillation. In two large randomized trials the principal concept of LAA-occlusion has been demonstrated to be noninferior to coumadine therapy, in longterm follow up being even superior to oral anticoagulation in terms of efficacy

and some safety issues like bleeding complications. However the procedure is complex and knowledge of nature, management and prevention of complications should minimize the risk of the procedure and allow transcatheter left atrial appendage closure to emerge as a therapeutic option for patients with atrial fibrillation at risk for cardioembolic stroke.

Möbius-Winkler S, Majunke N, Sandri M, Mangner N, Linke A, Stone GW, Dähnert I, Schuler G, Sick PB. Percutaneous left atrial appendage closure: Technical aspects and prevention of periprocedural complications with the watchman device. *World J Cardiol* 2015; 7(2): 65-75 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i2/65.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i2.65

INTRODUCTION

Stroke is one of the leading causes of death and disability worldwide. Approximately 25% of all strokes have a cardioembolic origin. More than 90% of all left atrial thrombi in patients with non-rheumatic atrial fibrillation (AF) originate in the left atrial appendage (LAA)[1,2]. Oral anticoagulation therapy with the vitamin K antagonist warfarin including all its well-known limitations is standard for the prevention[3]. Only about 55% of patients with AF, who are indicated for warfarin therapy, are really treated, however these patients are only about 67% of the time in the therapeutic range[4,5]. Several major recent trials have demonstrated the superiority of the new oral anticoagulants Dabigatran, Rivaroxaban and Apixaban as compared to standard therapy with warfarin[6-8]. Nevertheless, even with these new agents bleeding complications were substantial and in part comparable to that observed with warfarin except for intracranial bleedings being less. A certain amount of patients discontinued drug therapy prematurely for adverse events, mainly gastrointestinal reasons for example in dabigatran patients.

Several studies have been conducted focusing on the development of novel therapeutic tools to prevent AF-related strokes as an alternative to medical treatment. The technique which has arguably shown the most promise is the percutaneous (transcatheter) exclusion of the LAA from systemic circulation.

Three different devices specifically designed for occlusion of the LAA have been clinically evaluated: the Percutaneous LAA Transcatheter Occlusion system (PLAATO®), (ev3, Plymouth, MN), the WATCHMAN® system (Boston Scientific Corp., Natick, MA, United States), and the AMPLATZER® Cardiac Plug (St. Jude Medical, Inc., St. Paul, MN, United States) (Figure 1). Although safety and feasibility of the PLAATO device was demonstrated in several small non-randomized studies, the device was withdrawn from the market for commercial reasons. But even with the PLAATO device we have learned that there are specific complications associated with the implantation procedure such as device embolizations and cardiac tamponade[9-11]. Initial experience with the AMPLATZER® cardiac plug (ACP) has been published recently from a registry containing 132 successfully implanted devices[12]. To date, only the WATCHMAN® device has demonstrated superiority in long term follow up compared with chronic warfarin therapy in randomized, controlled trials[12-14]. In Europe, the latter two systems are approved for implantation, whereas in the United States both systems are under FDA investigation for potential approval. Despite being a lesser invasive procedure (than surgical ligation of the LAA), transcatheter LAA closure has been associated with potentially serious complications due to the necessity of transseptal puncture, manipulation of stiff wires and guide catheters in the left atrium and the release of the device in the LAA.

The following review highlights the potential procedural complications of transcatheter LAA closure with the Watchman-Device and discusses their nature, management and prevention.

IMPLANTATION PROCEDURE

Before starting the procedure transesophageal echocardiography (TEE) has to be performed as the gold standard for thrombus detection within the LAA (Figure 2). Thrombi have been reported to be present in the LAA in 8%-15% of patients with AF lasting greater than 48 h[15]. Understanding the anatomy of the LAA is also critical for procedural safety. To facilitate successful implantation, width and depth of the LAA as well as number and position of different lobes are important to know. Studies have shown that the width of the LAA orifice can vary from 15-35 mm and length from 20-45 mm[16] with various anatomical configurations.

Patient preparation

Implantation of the LAA closure devices should be usually performed under conscious sedation or even general anaesthesia. This is necessary to reduce unintended movement of the patient to avoid perforation of the LA/LAA and for tolerance of TEE, which is crucial for guidance of the procedure with the Watchman-Device. Usually a combination of midazolam and propofol is used, although alternative regimens may be possible. Implantation of the device is performed *via* the right femoral vein through a transseptal puncture with a small sheath in the femoral artery for pressure control or even management of air embolism as outlined below, which might not be mandatory.

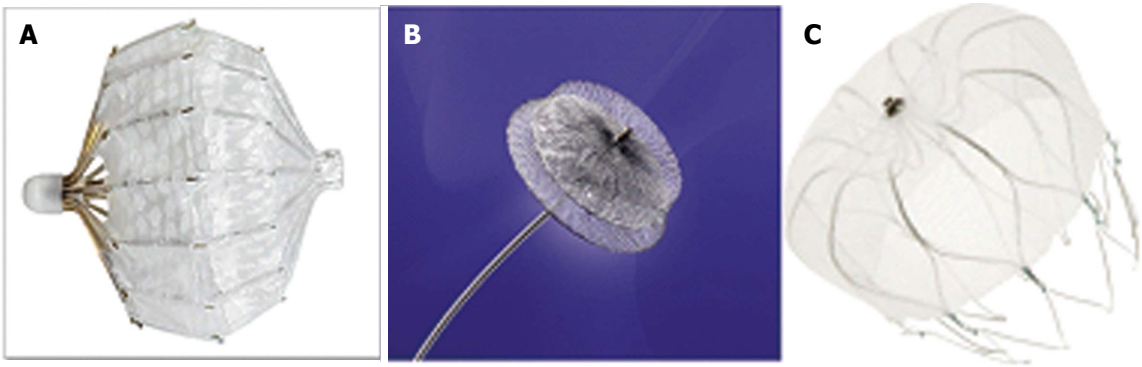

Figure 1 Devices for percutaneous occlusion of the left atrial appendage. A: PIAATO® device; B: Amplatzer® cardiac plug; C: Watchman® device.

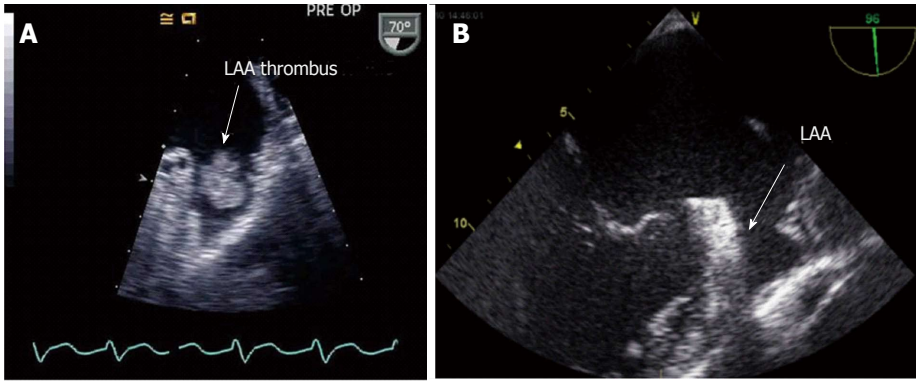

Figure 2 Left atrial appendage. A: With thrombus; B: Without thrombus. LAA: Left atrial appendage.

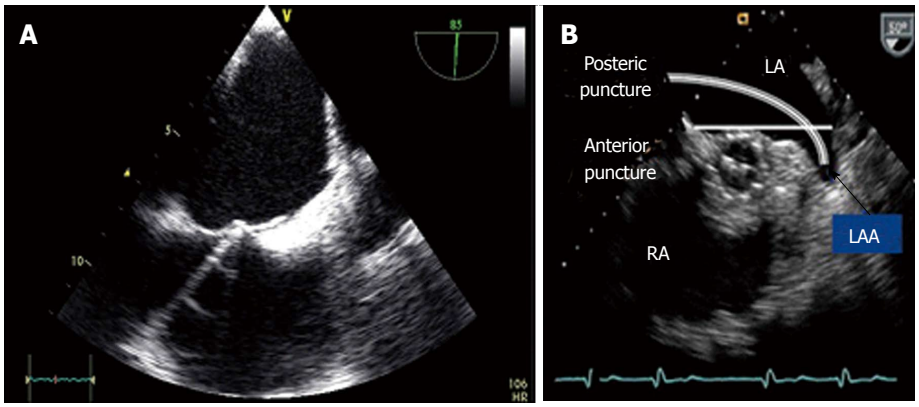

Figure 3 Echocardiography in transseptal puncture. A: Tenting of transseptal needle at the middle part of the atrial septum; B: Access sheath passing through the atrial septum within left atrium. LAA: Left atrial appendage.

Transseptal puncture

Transseptal puncture is described elsewhere[17]. Transseptal puncture should be ideally performed under pressure control and with TEE and fluoroscopic guidance. To enhance successful implantation of the LAA closure device the site of transseptal puncture should be at the posterior atrial septum. The more anterior or inferior the LAA is located, the higher transseptal puncture should be performed. The more cranial the axis of the LAA, the more inferior puncture site should be. This can be easily controlled by TEE in the bicaval view (90°) for cranio-caudal orientation and in the 45° view for anterior/posterior direction (Figure 3). Anterior puncture of the septum likewise going through an open PFO should be avoided due to the impossibility to turn the guiding catheter adequately to an anterior located axis of the LAA. An atrial septal defect may be used having enough space for turning the guide. After successful transseptal puncture heparin is given using a dosage of 100 U/kg body weight to achieve an ACT between 200 and 300 s. Heparin could be applied already before transseptal puncture, which, however might lead to an increased risk of bleeding during the

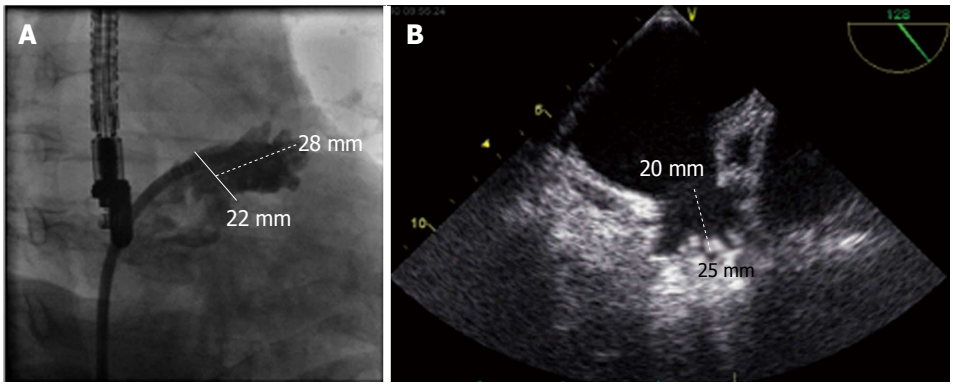

Figure 4 Measurement of the left atrial appendage during implantation procedure. A: After angiography (RAO 25°, caudal 20°)-measurement of ostium size (22 mm) and depth (28 mm); B: Echocardiographic measurement at around 135° of ostium size (20 mm) and depth (25 mm).

transseptal procedure.

Device implantation

Device implantation has been described previously[18] After exchange of the transseptal sheath over a stiff wire located best in the left upper pulmonary vein, the guiding catheter can be introduced. Wire positioning in the LAA with a loop may be also possible, however there is some risk of perforation. Two different curved sheaths (single and double curve) are available for implantation of the Watchman® device. For the Watchman device in more than 90% of patients the double curved sheath is used. To avoid undersizing of the LAA, the mean filling pressure of the left atrium should be in the high normal range (> 10 mmHg). For Watchman implantation a 4 or 5 French standard Pigtail catheter should be placed through the sheath. By turning the guiding catheter counter clockwise the pigtail can be advanced into the LAA under TEE and fluoroscopic guidance. The access sheath is then advanced slowly and carefully over the pigtail catheter into the LAA, thus at least reducing the risk of perforation. The marker bands on the access sheath of the Watchman® Device will help to determine the landing zone of the cover of the device at the site of the left atrium. The most suitable projections for angiographic visualization of the LAA seem to be RAO 30° with caudal 25° and cranial 20° for measurement of the orifice width and length of the LAA (Figure 4). Ideally the angiographic measurements will match the echocardiographic measurements in different angles from 0° to 135°, although they may vary by several mm depending on calibration methods.

Devices should be prepared according to the instructions for use. Careful flushing of the device and retrograde bleeding out of the access sheath is essential to avoid air embolism. Slight pressure on the patient's abdomen will increase venous pressure thus increasing backflow of blood through the sheath. A saline pressure infusion over the side branch of the guiding cath may be helpful.

Implantation of the watchman device

The Watchman device should be advanced in the sheath until the marker of the device catheter matches the most distal marker on the access sheath. The next step is to pull back the access sheath over the device until device catheter and access sheath are connected. At this point the device should remain in position and forward pushing of the device must be strictly avoided in terms of the risk of LAA injury or perforation with subsequent cardiac tamponade. The device is deployed by retracting the sheath and device catheter simultaneously while the device is held in place. Small amounts of contrast injections may help to visualize the relation between the tip of the device and the LAA wall during deployment (Figure 5).

Once the device is deployed within the LAA, correct positioning of the Watchman device at the LAA ostium must be demonstrated by echocardiography and angiography (Figure 6). To avoid embolism of the Watchman Device, there are four release criteria that should be evaluated before release: Position, Anchor, Size and Seal (PASS).

Position: To confirm that the device is properly positioned, ensure that the plane of maximum diameter of the device is at or just distal to the orifice of the LAA.

Anchor: To confirm the device is anchored in place, withdraw the access sheath/delivery catheter assembly 1-2 cm from the face of the device. After injecting a small puff of contrast gently retract and push the deployment knob to see the combined movement of the device and the LAA tissue.

Size: To confirm the correct device size, measure the plane of the maximum diameter of the device using TEE in the 4 standard views 0, 45, 90, and 135 degrees, ensuring the threaded insert is visible. The device size should be 80%-92% of the nominal diameter.

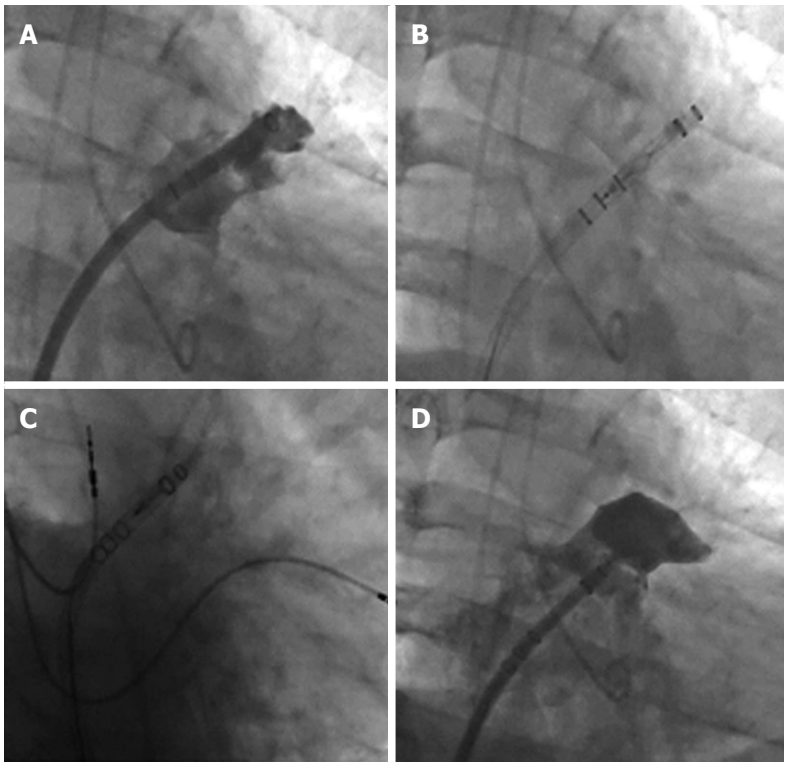

Figure 5 Deployment of a Watchman® device (fluoroscopic views). A: Deployment sheath in correct position; B: Watchman device loaded within the sheath before deployment; C: Watchman device deployment; D: Watchman device completely deployed within the LAA. LAA: Left atrial appendage.

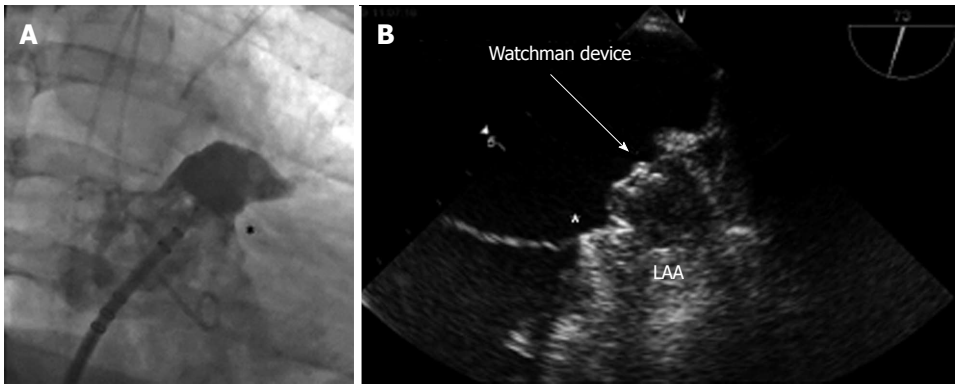

Figure 6 Optimal position of the Watchman device within the left atrial appendage. A: Angiographic view (RAO 25° caudal 20°); B: Echocardiographic view. Indicates inferior transition from left atrial appendage to LA (star).

Seal: Using colour Doppler, ensure that all of the lobes are distal of the device and are sealed. If there is a gap visible between the wall of LAA and the device with more than 3 mm, the device should be repositioned.

PREVENTION AND MANAGEMENT OF COMPLICATIONS

Pericardial effusion

Pericardial effusion as acute/subacute cardiac tamponade or as an asymptomatic effusion is one of the most serious complications in LAA-occlusion procedures[12,19]. The transseptal puncture itself[19], manipulation of stiff wires, guiding catheters and the device itself within the left atrium and the thin-walled LAA, as well as too aggressive movement of the device during stability testing may result in LAA wall injury leading to pericardial effusions.

In the Protect AF trial[12], the rate of pericardial effusion occurring within 7 d of Watchman implantation was 4.5%, 3.3% of patients required pericardiocentesis. These complications are mainly observed at the beginning of the learning curve and became less frequent with more experience[12]. In the CAP registry, where experienced operators implanted Watchman® devices after the randomized trial was completed, the rate of pericardial effusions decreased to 2.2%[20] and could be held in the same

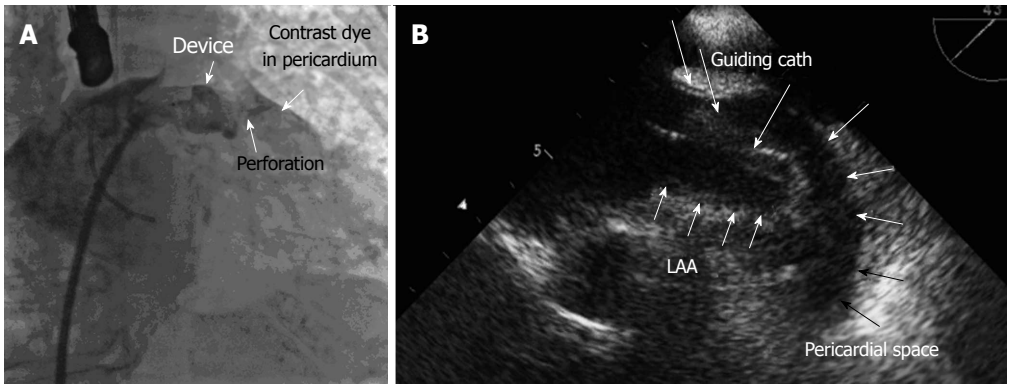

Figure 7 Pericardial effusion. A: Angiographic view; B: Echo view. LAA: Left atrial appendage.

range in the PREVAIL-trial with adequate training of new operators[21-23].

Several techniques to avoid pericardial effusions are: (1) TEE guidance and pressure monitoring to ensure a safe transseptal puncture at the correct site before advancing the sheath; (2) use of a 4 or 5 F. pigtail catheter inside the access sheath or a looped wire in the LAA to facilitate a safe movement of the guiding into the LAA; (3) slow and careful movements of the catheter and device manipulation within the left atrium; and (4) stability (tug-) testing after device implantation should be performed under TEE-control and fluoroscopy with injection of small amounts of contrast dye.

Cardiac tamponade with hypotension requires an aggressive approach with pericardiocentesis and reversal of anticoagulation (Figure 7). In case of recurrent tamponade, surgery is needed. Therefore early detection of an effusion by TEE is very important before hemodynamic deterioration arises from cardiac tamponade. Though most pericardial effusions occur early, subacute and late effusions are also possible. Therefore monitoring over a period of 48 h including 6 h close heart rate and pressure control and performance of trans-thoracic echocardiography at least before discharge to rule out pericardial effusion and to proof stable position of the device is recommended. Subacute effusions may arise from the anchors of the devices due to the thickness of only 0.5-0.8 mm of myocardial tissue of the LAA. If the effusion results in tamponade, pericardiocentesis and sometimes surgery is required. In case of late pericardial effusions also inflammatory processes may play a role, probably due to chronic injury of the hooks to the pericardium. In these cases anti-inflammatory therapy with non-steroidal antirheumatics like ASA, ibuprofen, diclofenac or even steroid therapy may be required at least for a certain period of time.

Air embolism

Air embolism is frequently a clinically silent event. However, acute coronary ischemia, stroke, hypotension, cardiac arrest and/or death are all possible outcomes. Holmes *et al*[12] report, that 5 of 449 patients with implantation of a Watchman® device suffered from a periprocedural stroke. The most common cause was air embolism, which is usually short-lived. Air emboli may enter the left atrium due to accidental injection of air, trapped air despite of flushing of the catheter or by air intrusion driven by a gradient between atmospheric and intracardiac pressure with a deep inspiration of the patient[24]. Therefore the left atrial pressure should be increased to normal or slightly high mean pressure using saline infusions. High pressure infusion through the side arm of the guiding catheter conversely can even lead to air embolism due to the Venturi-effect when opening the stop cock.

The management of air embolism is largely supportive. Hyperbaric oxygen has been shown to be of benefit in up to 80% of cases of cerebral air embolism, but controlled trials have not been performed yet[25]. However, as cerebral air embolism is mostly short-lived, there are usually no sequelae to be expected in longterm follow up.

Air embolism to the coronary circulation is most common in the right coronary artery because of the anterior position of the ostium (Figure 8). It often resolves within several minutes. However, marked ST-segment elevation, hypotension and ventricular arrhythmias may result, leading to cardiogenic shock. Aspiration of the air with an aspiration device (*e.g.*, EXPORT Catheter, Medtronic Inc. Minneapolis, MN, United States) or rigorous contrast dye injection into the coronary artery may be helpful in selected cases (Figure 8). In this case the arterial access may be helpful to advance a right coronary catheter quickly to the aortic rout or even having a right coronary diagnostic catheter already in place during transseptal puncture to mark the aortic rout Also Trendelenburg position is recommended; as air trapped anywhere within the heart (*e.g.*, the LAA) might be dislodged leading to another air embolism. If there is a large amount of air within the left or right atrium, the LAA or in the ascending aorta, this may also be aspirated through the sheath or *via* a coronary multipurpose or right coronary catheter.

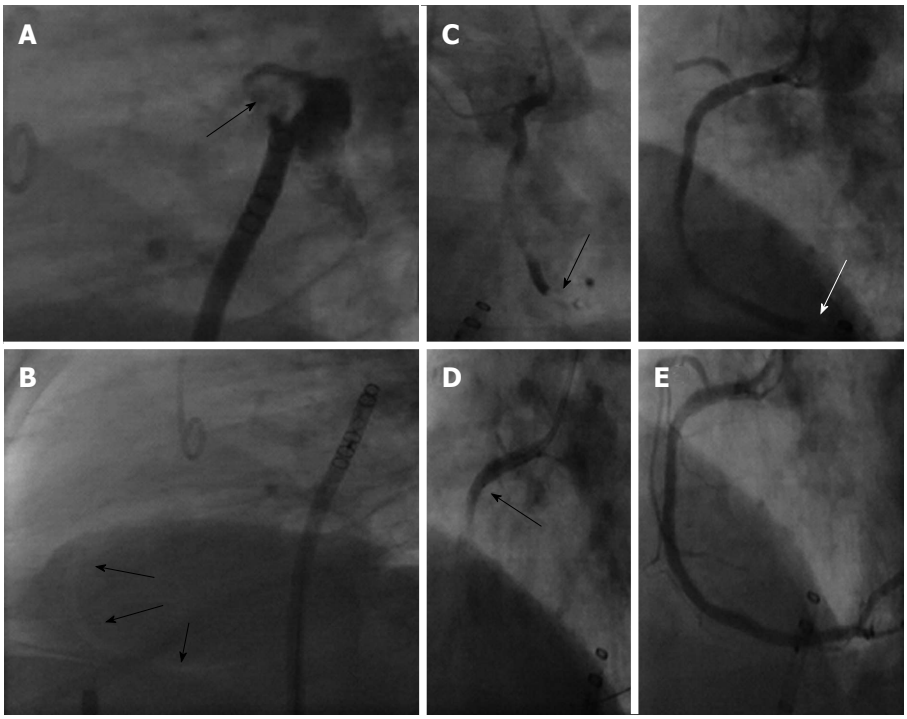

Figure 8 Air embolism. A: Air bubble within the left atrial appendage; B: Right coronary artery (RCA) filled with air (bright shadows); C: RCA with large air bubble; D: Placement of a aspiration catheter (EXPORT, Medtronic) within the RCA; E: RCA after successful aspiration of air bubbles without filling defect. LAA: Left atrial appendage

Aspiration of air should be performed before catecholamines are given for pressure management. This can protect from air movement into the brain circulation.

Thrombus formation during device implantation

During the procedure an activated clotting time of about 250 s should be aimed at using heparin and continious flushing of the guiding catheter is recommended to avoid thrombus formation within the catheter. In case of new thrombus formation in the LAA, the operator must decide if it is prudent to continue the procedure. If a good position of the delivery sheath has been achieved, implantation might be possible, especially if it is deemed to be more dangerous to retract the device and sheath. The membrane of the device, once deployed, is able to catch a thrombus in the LAA.

If the sheath has not yet been maneuvered into the LAA, aspiration of blood and possible thrombus through the side port should be attempted followed by removal of the sheath for an outside flushing.

If thrombus formation is observed outside the access sheath in the left atrium, the only option is to withdraw the sheath back into the right atrium applying pressure on both proximal carotid arteries to minimize the risk of cerebral thromboembolism followed by close neurological monitoring.

Early device embolization

Percutaneous closure of the LAA may be complicated by immediate or late device embolization. Device embolization occurs in about 0.2% of cases with the Watchman® device (Figure 9)[12] Selection of patients with favorable LAA morphology and appropriate device sizing are crucial to prevent embolization. Negative predictors are: large LAA ostial size, use of undersized devices, short LAA length for the Watchman and unusual LAA morphologies.

The Watchman-Device must be fully expanded and compressed by at least 10%-30% of its original size. If the device is too deep in the LAA and therefore not fully expanded, the device must be partially recaptured and repositioned. If the device is too proximal, a complete recapture and exchange of the device is necessary.

The above mentioned Device release criteria should be fulfilled before release.

Late device embolization

Routine post procedural TTE to exclude a new or expanding pericardial effusion and as a screen for device embolization is mandatory one to two days after implantation, even if the patient is asymptomatic[12,14]. The best way to visualize a LAA-occluder is from subxiphoidal. If the device cannot be seen, we recommend fluoroscopy to confirm correct position of the device.

Embolized devices that are found in the left atrium or left ventricle may be safely moved antegradely across the mitral and aortic valve by using coronary catheters. Snaring of devices within the heart itself is challenging and dangerous. However, retrieval from the descending aorta using a snare or bioptome

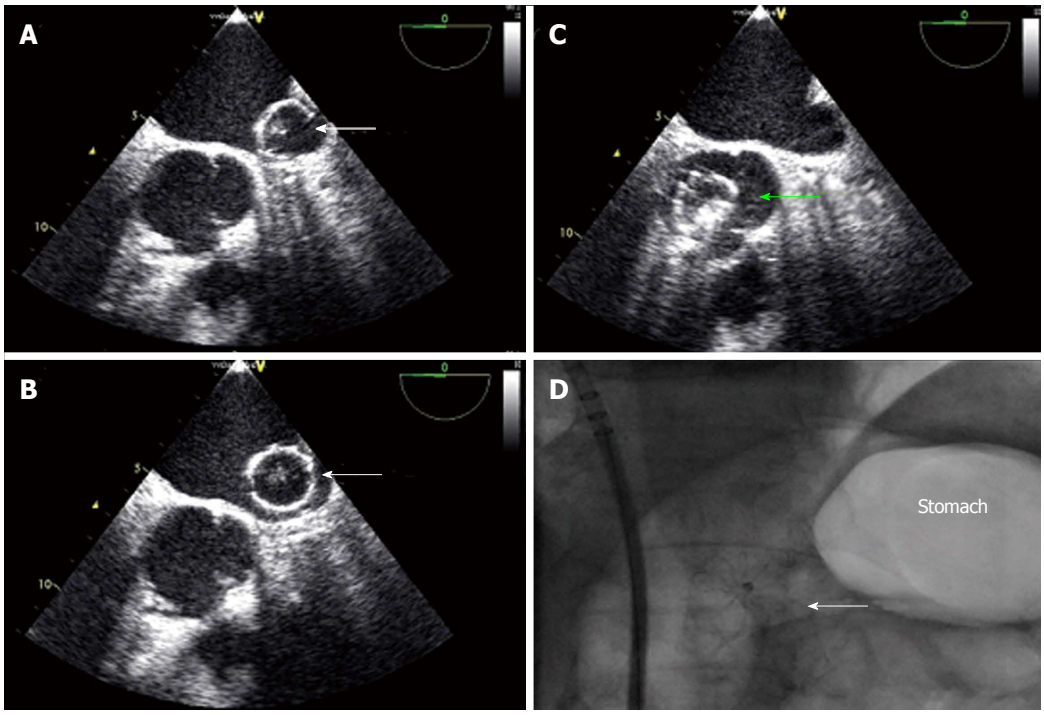

Figure 9 Device embolisation of watchman-device. A: Within the left atrial appendage immediately after release (arrow indicates device); B: Embolisation from the left atrial appendage; C: Passage of the aortic valve; D: Device within the lower thoracic aorta after embolization.

through an appropriately sized retrieval sheath (min. 14 Fr.) (Figure 10) is both safer and more feasible. In rare cases of embolisation of larger devices, surgical retrieval might be necessary even through the groin. Therefore, close collaboration with cardiac/vascular surgery ensures open access retrieval, if the percutaneous maneuver fails.

PROCEDURAL FOLLOW UP AND MEDICAL TREATMENT TO AVOID COMPLICATIONS

To avoid thrombus formation on the device for the Watchman® device, aspirin 100 mg/d and warfarin (INR goal 2-3x normal) should be administered until 45 d TEE control according to the PROTECT AF-trial[12]. If the device is still in position, the LAA occluded and no or minimal flow (residual jet < 5 mm) around the device, oral anticoagulation can be stopped and clopidogrel 75 mg/d should be added up to 6 mo after implantation. After another TEE-control aspirin alone should be administered lifelong at least according to the actual study results, though there are some centers that even stop aspirin therapy after three to six months. However, there are no data about long term results without any antiplatelet therapy. Within the Protect AF trial, patients having a residual gap < 5 mm were not likely to develop more stroke/TIA than patients without a gap, as a recent publication by Viles-Gonzalez was able to demonstrate[26]. If the 45 d TEE demonstrates a jet > 5 mm, warfarin must be continued with another TEE after 3 mo. If there is no change, the implant is deemed to have failed and the patient should remain on chronic oral anticoagulation therapy. If the gap has decreased to < 5 mm, therapy can be changed to ASA and clopidogrel.

In case of absolute or relative contraindications for warfarin therapy, another regimen recommends to give ASA and Clopidogrel immediately after implantation for 6 mo followed by ASA alone, which was similar effective in a small registry trial called ASAP[27]. If ASA could be even stopped at 6 mo or some time later is not clear yet. There are no data really supporting continuation or discontinuation of ASA lifelong except the experience of PROTECT AF.

In some cases, thrombus formation on the device may be detected by TEE (Figure 11). In these cases, low molecular weight heparin or oral anticoagulation should be restarted for another 4-8 wk. A repeat TEE will direct further treatment as described above.

CONCLUSION

Percutaneous closure of the left atrial appendage has been shown to be feasible with promising results in terms of reducing the rate of stroke and hemorrhagic complications, and has become an alternative therapy to standard anticoagulation therapy in patients with atrial fibrillation in Europe and other countries. The actual accepted indications for the use of LAA-occlusion therapy to be considered is published in an EHRA/EAPCI expert consensus statement[28].

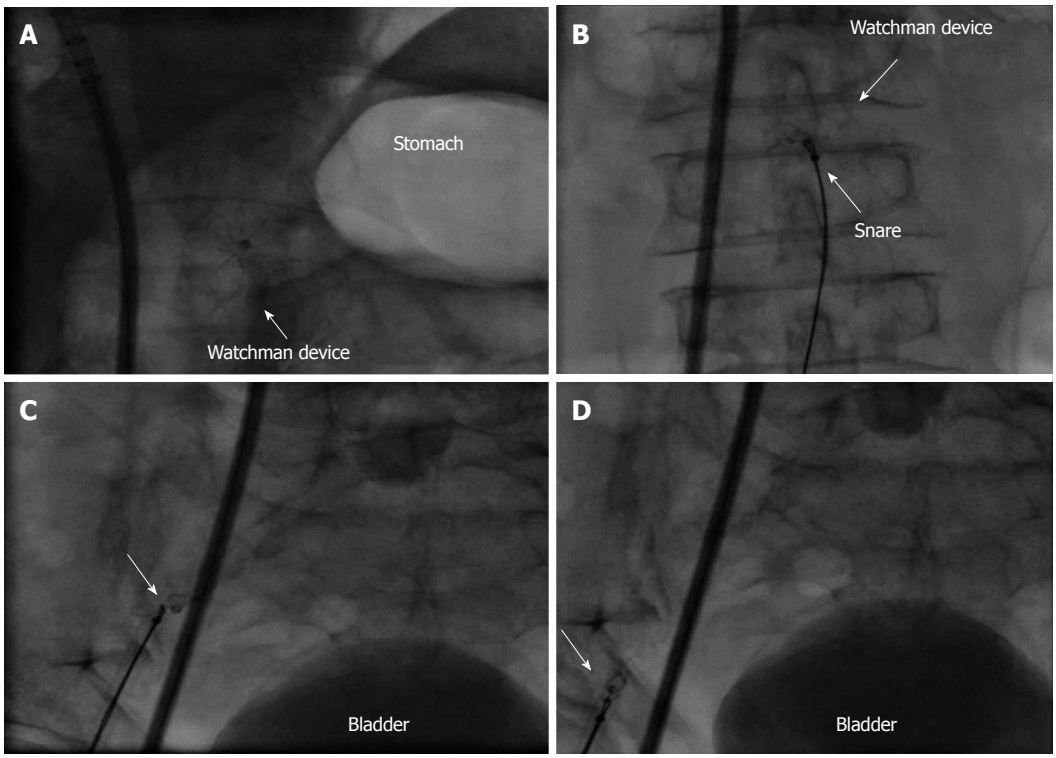

Figure 10 Device retrieval after embolization. A: Watchman device within the lower thoracic aorta after embolization; B: Snaring of the Watchman device with a goose neck snare 20 mm; C: Retraction of the device into the right arteria iliaca; D: Device retracted into a large (14 Fr.) sheath.

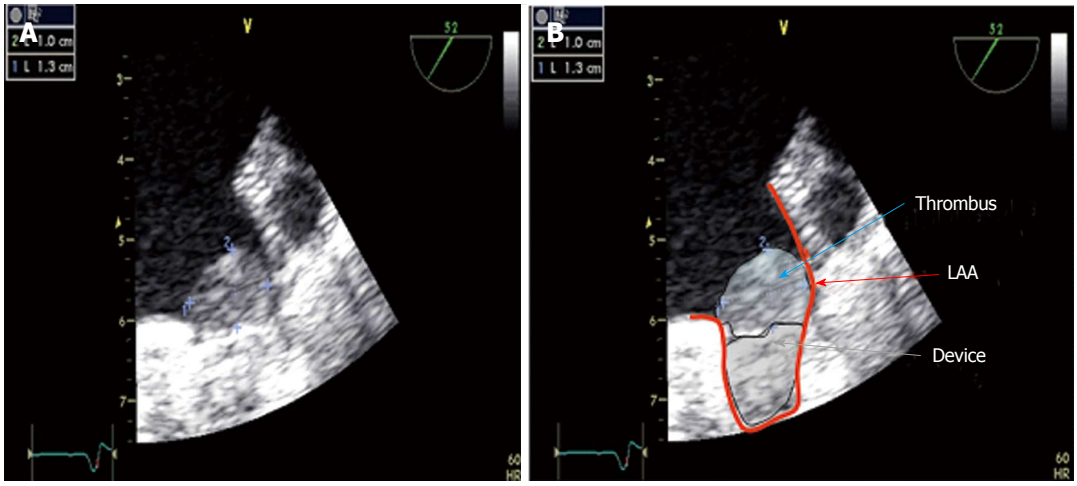

Figure 11 Thrombus formation (1.0 cm x 1.3 cm) 45 d after implantation on a Watchman device (A) and schematic view: Thrombus, device and left atrial appendage are highlighted (B).

Main indications are real contraindications for oral anticoagulation even for NOACs, patient refusal despite adequate information about different anticoagulation modalities, increased risk for bleeding due to a high HASBLED-score, need for prolonged triple therapy for example after coronary stenting, increased bleeding risk not reflected by the HASBLED-score and severe renal failure as a contraindication to NOACs. An individual risk benefit evaluation for each patient should be performed. FDA-approval may be expected in the beginning of the year 2015.

As a new invasive procedure, transcatheter LAA closure has several device- and procedure-specific complications, mainly pericardial effusions with or without tamponade, air embolism with subsequent stroke or device embolization. To minimize these complications, the procedure should be performed only by operators experienced in transseptal puncture and structural heart interventions. Additionally, TEE guidance by an experienced echocardiographer is important to ensure a complication-free and successful procedure. Profound knowledge of the

nature, management and prevention of complications is essential to optimize the outcome of transcatheter LAA closure.

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Flecainide: Current status and perspectives in arrhythmia management

George K Andrikopoulos, Sokratis Pastromas, Stylianos Tzeis

George K Andrikopoulos, Sokratis Pastromas, Stylianos Tzeis, Department of Cardiology, Henry Dunant Hospital, Athens, 11526 Attica, Greece
Author contributions: All authors contributed equally to this work.

Correspondence to: George K Andrikopoulos, MD, PhD, Director of Cardiology Department, Henry Dunant Hospital, Mesogeion ave 107, Athens, 11526 Attica, Greece. andrikop@hotmail.com
Telephone: +30-210-6972876
Fax: +30-210-6972200
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Abstract

Flecainide acetate is a class IC antiarrhythmic agent and its clinical efficacy has been confirmed by the results of several clinical trials. Nowadays, flecainide is recommended as one of the first line therapies for pharmacological conversion as well as maintenance of sinus rhythm in patients with atrial fibrillation and/or supraventricular tachycardias. Based on the Cardiac Arrhythmia Suppression Trial study results, flecainide is not recommended in patients with structural heart disease due to high proarrhythmic risk. Recent data support the role of flecainide in preventing ventricular tachyarrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia associated both with ryanodine receptor and calsequestrin mutations. We herein review the current clinical data related to flecainide use in clinical practice and some concerns about its role in the management of patients with coronary artery disease.

Key words: Flecainide; Class IC antiarrhythmic drugs; Atrial fibrillation; Ventricular tachycardia; Proarrhythmia

Core tip: Flecainide acetate is recommended as one of the first line antiarrhythmic drugs in patients with atrial fibrillation and/or supraventricular tachycardias for the restoration and maintance of sinus rhythm. Based on the Cardiac Arrhythmia Suppression Trial study results, flecainide is contraindicated for patients with structural heart disease due to high proarrhythmic risk. Recent data support the role of flecainide in preventing ventricular tachyarrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia associated both with ryanodine receptor and calsequestrin mutations.

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INTRODUCTION

Flecainide acetate is a class IC antiarrhythmic agent that was first synthesized in 1972. Its development began in 1966 in an attempt to generate new fluorinated anesthetic organic agents with the substitution of a trifluoroethoxy group on the aro-

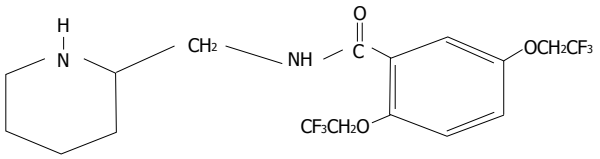

Figure 1 Chemical structure of flecainide acetate.

matic ring in the place of the amine group (Figure 1)[1]. The clinical efficacy of flecainide was confirmed by the results of several clinical trials, both in animals and humans[2-6]. Oral use of flecainide was approved in 1984 from the Food and Drug Administration for the suppression of sustained ventricular tachycardia since study results showed about 90% efficacy without significant adverse events[7]. However, safety data from 1330 patients between 1980 and 1985 demonstrated that flecainide use was associated with increased proarrhythmic events in patients with severe cardiac disease primarily in those who were starting therapy with high-dose[8].

The publication of the Cardiac Arrhythmia Suppression Trial (CAST) study in 1989, which was designed to investigate the efficacy of class Ⅰ antiarrhythmic agents moricizine, encainide or flecainide in patients after myocardial infarction with reduced ejection fraction and frequent ventricular ectopic beats, resulted in a major revision of the role of these antiarrhythmic drugs[9]. Thus, while flecainide suppressed ventricular ectopy in those patients, a threefold increase of arrhythmic death was recorded compared to placebo[9]. Based on CAST results, flecainide nowadays is not recommended for patients with structural heart disease and coronary artery disease. However, it is recommended as one of the first line therapies for pharmacological conversion as well as maintenance of sinus rhythm in patients with atrial fibrillation and/or supraventricular tachycardias without structural heart disease[10]. This review aims to present the existing data regarding the use, effectiveness and safety of flecainide in current clinical practice of arrhythmia management.

CLINICAL PHARMACOLOGY OF FLECAINIDE

Pharmacodynamics

Orally administrated flecainide is administrated twice daily and is absorbed rapidly without any significant interactions with food or antacid. Its bioavailability is around 90%, indicating no significant first pass effect through the liver. In normal subjects, plasma peak levels are reached after 2-3 h and steady state levels within 3-5 d. The half-life of flecainide ranges from 7 to 23 h and seems to be unaffected by dose[11,12]. Patients with ventricular ectopic beats have a longer half-life (mean 20 h) compared with normal subjects, mainly due to reduced renal function[13]. Flecainide levels are higher in cardiac tissues compared to plasma[14]. Two major metabolites, the active meta-O-dealkylated flecainide and its inactive lactam, are produced by hepatic oxidative metabolism *via* cytochrome CYP2D6 and CYP1A2. Both flecainide and metabolites are excreted mostly in urine, so patients with impaired renal function require close monitoring and dose reduction. Approximately 30% of flecainide is excreted unchanged into the urine[13,14]. It should be pointed that the antiarrhythmic efficacy of flecainide is closely correlated to the QRS duration. Therapeutic plasma levels range between 0.2 and 1.0 mg/mL and higher values are associated with toxic cardiac effects, such as bradycardia or conduction abnormalities[15]. The recommended starting dose in patients without renal insufficiency and paroxysmal supraventricular tachycardia or paroxysmal atrial fibrillation is 50 mg *bid* and may be increased in increments of 50 mg *bid* until efficacy is achieved (maximum recommended dose 300 mg/d). In patients with ventricular tachycardia and no contraindications for flecainide administration, the starting dose is 100 mg *bid* and the maximum recommended dose is 400 mg/d. Attention should be paid in patients concomitantly receiving amiodarone, although rare to encounter, who may require a dose reduction about 50%[11]. Additionally, caution is needed prior to but also following drug initiation, to exclude concomitant electrolyte disturbances, especially hypokalemia. In patients with severe hepatic insufficiency, flecainide administration should be carefully considered and monitoring of drug levels in plasma may be required[12].

Electrophysiological properties

As mentioned above flecainide belongs to the class IC antiarrhythmic agents which produce a potent and selective blockade of the cardiac fast inward sodium (Na^+) current resulting in conduction slowing[11]. A high affinity for open-state Na^+ channels and the slow unbinding kinetics from these channels during diastole has been described, explaining the slowing of the recovery time during the cardiac diastole and the prolongation of refractoriness[11,12]. Moreover, flecainide inhibits opening of potassium channels, especially the rapid component of the delayed rectifier K^+ current (I_{Kr}), prolonging the action potential duration (APD) in ventricular and atrial muscle fibers. In opposite, in Purkinje fibers, flecainide causes a shortening in the APD due to the Na^+ channel blockade[16-18]. Recent data suggest that flecainide blockades ryanodine receptor opening, thus reducing spontaneous sarcoplasmic reticulum Ca^{2+} release, which potentially results in afterdepolarization and triggered activity[19,20]. Therefore, flecainide has been used for the therapy of catecholaminergic polymorphic ventricular tachycardia (CPVT), which is an inherited arrhythmogenic disease with mutations of either the

cardiac ryanodine receptor or calsequestrin that can cause sudden cardiac death[21].

Regarding the impact of flecainide in the intra-cardiac intervals, flecainide increases the AH interval (15%-22%) and the HV interval (25%-50%), thus slightly slowing both intra-atrial and atrioventricular nodal conduction[22,23]. In patients with evidence of dual atrioventricular node pathway physiology, flecainide has shown to prolong mainly the retrograde refractoriness of the fast pathway[22]. Also, in patients with accessory atrioventricular pathways, flecainide can cause complete retrograde pathway block especially in patients with refractoriness more than 270 ms at baseline, although potentially decreases both anterograde and retrograde refractory periods[24]. Additionally, flecainide does not seem to affect normal sinus node function but in patients with sinus node dysfunction an increase of the corrected sinus node recovery time and the sinoatrial conduction time have been reported[25,26]. Finally, patients with implanted cardiac rhythm devices (pacemakers or internal cardiac defibrillators) and concomitant flecainide treatment may experience an increase in the pacing thresholds[25].

All the above mentioned electrophysiological properties of flecainide are deflected in the twelve lead surface electrocardiogram with an increase in PR, QRS and QT intervals duration. The QTc interval is not significantly increased since most of QT prolongation is due to the QRS widening[11,22]. During exercise, flecainide usually shortens QTc interval[27].

Proarrhythmic and inotropic effects of flecainide

It is well known that class IC antiarrhythmic drugs may potentially be associated with proarrhythmia, either as atrial flutter with 1:1 antrioventricular conduction or ventricular tachyarrhythmia. Flecainide can convert atrial fibrillation into atrial flutter, potentially resulting in a rapid tachycardia with more than 200 bpm in case of 1:1 atrioventricular conduction[28]. The reported rate of this proarrhythmic effect is 3.5% to 5.0% and has been associated with high adrenergic conditions[29]. Drugs with atrioventricular nodal blockade properties, such as β-blockers, verapamil and diltiazem, should be administered concomitantly in order to lower the risk.

Ventricular tachycardias due to proarrhythmic effect seem to be rare in patients without structural heart disease, electrolyte disturbances and coronary artery disease. Ventricular proarrhythmia manifests either as monomorphic or polymorphic tachycardia not only early but also late after the initiation of therapy according to the results of CAST study[9]. The incidence of ventricular proarrhythmia in patients receiving flecainide for acute cardioversion of atrial fibrillation was reported in a systematic review to be less than 3%[30].

Flecainide exerts a negative inotropic effect and therefore is contraindicated in patients with congestive heart failure, coronary artery disease and reduced ejection fraction. In this population flecainide significantly reduces stroke volume index and left ventricle ejection fraction and increases right atrial and pulmonary capillary wedge pressures[31,32]. Even in patients with normal ejection fraction, oral administration of flecainide can slightly reduce the ejection fraction. Intravenous administration of flecainide (2 mg/kg) in healthy subjects was associated with a reduction in cardiac output and stroke volume during the first 90 min after dosing[33]. These hemodynamic effects are related to the reduced Na^+ and Ca^{2+} entry into the myocardial cells. Moreover, as discussed above, flecainide has proved to blockade the ryanodine receptor opening and its interaction with the Ca^{2+} diastolic waves[34].

Mechanism of action of flecainide in maintenance of sinus rhythm and cardioversion in patients with atrial fibrillation

It is well known that atrial fibrillation causes both electrical and structural remodeling in atrial myocardium. Flecainide has proven its efficacy in the cardioversion of atrial fibrillation in sinus rhythm in both human and animal trials causing a shortening of the APD and prolongation of atrial refractoriness in a rate-dependent manner. Trial data have shown that the slow conduction properties of flecainide could result in a significant reduction of atrial wavelength so that atrial fibrillation cannot be maintained[34,35].

Atrial fibrillation has been associated with significant structural changes in the atria which subsequently cause remodeling of the myocardial fibers and mitochondrial dysfunction due to oxidative stress[34]. Several inflammatory adhesion molecules associated with oxidative stress and subsequent myocardial ischemia, such as nuclear factor kappa β ($NF_{\kappa}B$), reactive oxygen species and glycogen, impair the cellular physiology enhancing apoptotic process and cellular protein decomposition[36]. Rapid atrial activation encountered during atrial fibrillation results in intracellular Ca^{2+} accumulation, thus promoting ischemia and cellular dysfunction. This process is facilitated by the high transient intracellular Na^+ concentration during tachycardia, which accentuates the entry of Ca^{2+} *via* the Na^+/Ca^{2+} exchanger (Figure 2). Flecainide attenuates the intracellular Ca^{2+} accumulation by blocking Na^+ channels, thus reducing oxidative stress process and further atrial remodeling[37].

CLINICAL TRIALS

Acute conversion of atrial fibrillation of recent onset

The efficacy of flecainide, both oral and intravenous formulation, in terminating recent-onset atrial fibrillation has been evaluated in several studies (Table 1) and is affected from the study design

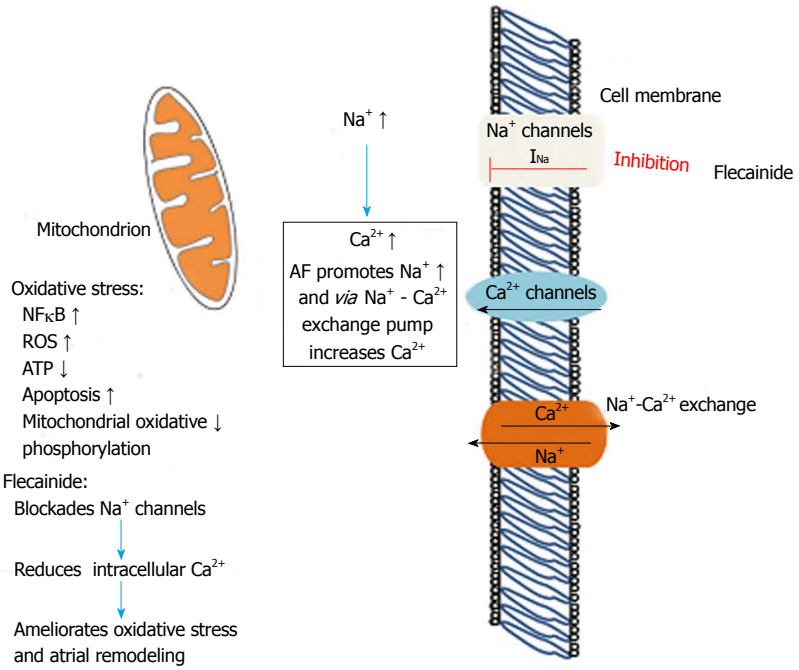

Figure 2 Mechanism of flecainide action during atrial fibrillation by inhibition of Na^+ channels which reduces intracellular Ca^{2+} accumulation and reduces oxidative stress and mitochondrial dysfunction. AF: Atrial fibrillation; INa: Fast inward Na^+ current; ROS: Reactive oxygen species; NFκB: Nuclear factor kappa β; ATP: Adenosine triphosphate.

Table 1 Reversion rate of recent-onset atrial fibrillation to sinus rhythm in clinical trials evaluating the conversion efficacy of oral or intravenous flecainide

Clinical trial	Patients in flecainide arm	AF duration	Formulation	Reversion rate
Capucci *et al*[38]	22 patients	≤ 7 d	Single oral dose (300 mg)	8 h → 91% 24 h → 95%
Donovan *et al*[39]	51 patients	≤ 3 d	*iv* (2 mg/kg-max 150 mg)	1 h → 57% 6 h → 67%
Donovan *et al*[40]	34 patients	≤ 3 d	*iv* (2 mg/kg-max 150 mg)	2 h → 59% 8 h → 68%
Boriani *et al*[41]	69 patients	< 8 d	Single oral dose (300 mg)	1 h → 13% 3 h → 57% 8 h → 75%
Martínez-Marcos *et al*[42]	50 patients	≤ 2 d	*iv* (2 mg/kg followed by 1 mg/kg at 8 h if not SR)	1 h → 58% 8 h → 82% 12 h → 90%
Romano *et al*[43]	138 patients	≤ 3 d	Intravenous	1 h → 73% 3 h → 80% 6 h → 86% 24 h → 90%

AF: Atrial fibrillation; SR: Sinus rhythm.

characteristics and the time intervals from drug initiation to assessment of reversion rate[38-42].

Several randomized controlled clinical trials have also compared the efficacy of flecainide to other antiarrhythmic agents in acute conversion of recent-onset atrial fibrillation. Capucci *et al*[38] found that a single oral loading dose of flecainide was significantly more efficient than intravenous amiodarone within 8 h but not at 24 h. In a randomized, double-blind trial, intravenous flecainide was also shown to result in earlier reversion of recent onset atrial fibrillation to sinus rhythm as compared to intravenous amiodarone[40]. In accordance, Martínez-Marcos *et al*[42] found that a significantly higher proportion of patients reverted to sinus rhythm when treated with intravenous flecainide as compared to intravenous amiodarone and propafenone, although the difference in reversion rate with intravenous propafenone reached statistical significance at 12 h but not at 8 h following treatment onset[42]. On the other hand, Romano *et al*[43] found a significantly higher efficacy of intravenous flecainide compared to intravenous propafenone in acute conversion of recent onset atrial fibrillation at 1, 3 and 6 h, although no difference was evident at 24 h. Finally, in a comparative study, Boriani *et al*[41] evaluated the conversion efficacy of different antiarrhythmic drug protocols and reported that oral flecainide had a similar conversion rate to oral propafenone.

Prevention of atrial fibrillation recurrences

Continuous treatment: The long-term safety and

efficacy of continuous treatment with flecainide for prevention of atrial fibrillation recurrences has been studied extensively in comparison to either placebo or other antiarrhythmic agents (Table 2). However, the majority of the trials published in the literature are hampered by methodological limitations, such as open-label design, small sample size, suboptimal follow-up and underestimation of AF burden mainly due to inability to document asymptomatic or short-lasting arrhythmia bouts. In the context of these limitations, oral flecainide has been demonstrated to be superior to placebo[44-48], and similar to quinidine[49,50], sotalol[48] and propafenone[51,52] in preventing AF recurrences. The clinical efficacy of flecainide in the maintenance of sinus rhythm has been proved in a meta-analysis of 60 trials showing that 65% of patients were responders to short term treatment and 49% in the long term treatment[53]. In terms of safety, flecainide is better tolerated than quinidine[46,50], and is associated with a lower rate of adverse events as compared to propafenone[51,52].

Special emphasis should be placed in two trials, the PITAGORA[54,55] and the FLEC-SL trial[56]. The PITAGORA trial was a multicenter, prospective, single-blind randomized trial which aimed to compare amiodarone with class IC antiarrhythmic agents (propafenone and flecainide) when administered as prophylactic treatment for sinus rhythm maintenance among pacemaker recipients with sinus node disease and history of atrial fibrillation[54]. The maintenance daily doses of tested antiarrhythmic agents were 200 mg amiodarone, 450-750 mg propafenone and 200 mg flecainide. The main strength of the study was the ability to quantify the frequency and burden of both symptomatic and asymptomatic atrial fibrillation episodes *via* pacemaker diagnostics. However, the primary endpoint of the study was not related to AF burden *per se* but was a composite endpoint defined as time to the first occurrence of one of the following adverse events: death, hospitalization for AF or heart failure, atrial cardioversion or AAD change owing to failure of AF prophylaxis or adverse events[54]. In terms of the incidence of this endpoint, flecainide and propafenone proved to be non-inferior to amiodarone[55]. Furthermore, when flecainide and propafenone, as single agents, were compared with amiodarone, only flecainide satisfied the criterion for noninferiority. Post-hoc analysis of arrhythmia burden data demonstrated that amiodarone and class Ic agents demonstrated similar efficacy in preventing arrhythmic episodes > 10 min, or > 1 d, with a trend in favor of amiofarone for prevention of episodes lasting more than a week[55].

The Flec-SL trial is the largest, prospective randomized clinical trial testing the efficacy of oral antiarrhythmic treatment for prevention of atrial fibrillation recurrence, with a meticulous follow-up and endpoint assessment[56]. The aim of the study was to evaluate whether short-term (4 wk) flecainide treatment is non-inferior to long-term (6 mo) treatment following cardioversion of persistent atrial fibrillation. The tested hypothesis was based on the concept that provision of antiarrhythmic protection n until completion of reverse atrial electrical remodeling might provide a long-term effect with an enhanced safety profile due to reduced drug exposure. In total, 635 patients were randomly assigned in three treatment arms (placebo *vs* short-term *vs* long-term oral flecainide treatment). Patients were followed up for 6 mo with daily telemetric electrocardiograph recordings and Holter ECGs when atrial fibrillation was noted in more than two consecutive telemetric recordings. The primary outcome measure of the study was time to first recurrence of persistent atrial fibrillation or death from any cause. Based on the study results, in the per-protocol population 46% of patients receiving short-term treatment presented a recurrence of persistent AF as compared to 39% in the long-term treatment group. Additionally, short-term treatment with flecainide was superior to placebo but failed to demonstrate non-inferiority as compared to long-term treatment. However, short-term treatment demonstrated about 80% of the 6-mo effect of long-term treatment, supporting that the former could be considered a viable treatment option in patients with infrequent AF recurrences or increased risk of proarrhythmia.

Pill-in-the-pocket strategy: The safety and feasibility of treatment with a single oral dose of either flecainide (200-300 mg) or propafenone (450-600 mg) on an outpatient basis, for termination of recent onset atrial fibrillation was validated in a pivotal trial including 268 patients without severe heart disease and a previously successful in-hospital treatment[57]. The tested "pill-in-the-pocket" strategy was successful in 94% of arrhythmic episodes (equally effective for both propafenone and flecainide), while in 84% of patients the treatment was effective during all the arrhythmic episodes. The mean conversion time to sinus rhythm was about 2 h, while only one case (0.6%) of atrial flutter with rapid ventricular rate was reported. Furthermore, the implemented treatment approach resulted in significant reduction of hospitalizations and visits to the emergency rooms.

The pill-in-the-pocket strategy can be used in symptomatic patients with infrequent recurrences of atrial fibrillation. Patients with sinus node dysfunction causing bradycardia and patients with bradycardia or syncope due to atrioventricular conduction defects may not be considered candidates for the pill-in-the-pocket strategy. Prerequisites for the safe implementation of this strategy are the initial in-hospital testing of its efficacy and safety as well as careful screening of candidate patients to rule

Table 2 Flecainide for prevention of atrial fibrillation recurrences - Randomized controlled clinical trials

Clinical trial	Patient population	Compared treatments	Endpoint of AF recurrence	Results	Comments
Steinbeck *et al*[44]	45 patients Paroxysmal AF	Quinidine + digoxin Flecainide + digoxin Digoxin	AF recurrence at 12 mo	Flecainide and digoxin superior to other regimens and safer than quinidine and digoxin	Quinidine is practically not used any more for sinus rhythm maintenance
Anderson *et al*[45]	64 patients Paroxysmal AF	Flecainide (median daily dose: 300 mg) Placebo	Patients without AF recurrences Time to first AF recurrence Time interval between AF recurrences	Flecainide superior to placebo Five-fold increase in time to first recurrence Four-fold increase in time interval between attacks Significantly increased percentage of patients free of AF recurrences Adverse cardiac events in 11% of patients during flecainide therapy	Transtelephonic monitoring Double-blind randomized crossover trial (8-wk observation period) Daily flecainide dose > 300 mg in 29% of patients
van Wijk *et al*[49]	26 patients Paroxysmal AF	Flecainide (200-300 mg daily) Quinidine (1.0-1.5 daily)	AF recurrence during 3-mo follow-up period	Flecainide superior to quinidine in the lower dosing regimen Flecainide similar efficacy to quinidine in higher dosing regimen	20% discontinuation rate with higher quinidine dosing regimen FU with 24-h Holter at the end of each month
van Gelder *et al*[46]	81 patients Persistent AF/ flutter	Flecainide Placebo	AF recurrence at 12 mo	Flecainide superior to placebo in preventing arrhythmia recurrences	Difficult to treat patients (mean AF duration: 12 mo)
Pietersen *et al*[47]	43 patients Paroxysmal AF/flutter	Flecainide (300 mg/d) Placebo	AF recurrence at 3 mo	Flecainide superior to placebo in preventing arrhythmia recurrences Adverse effects in 74% of patients treated with flecainide	Tolerable adverse events in flecainide group (only 2 withdrawals) One episode of sudden death
Carunchio *et al*[48]	66 patients Paroxysmal AF	Flecainide Sotalol Placebo	AF recurrence at 1, 3, 6 and 12 mo	Flecainide similar efficacy to sotalol and superior to placebo	
Aliot *et al*[51]	97 patients Paroxysmal AF/flutter	Flecainide (100-300 mg/d) Propafenone (600-1200 mg/d)	AF recurrence at 12 mo	Flecainide similar efficacy to propafenone Treatment discontinuation rate lower with flecainide (38% *vs* 53%, $P = 0.079$)	Multicenter, randomized, open-label study One episode of sudden death in the propafenone group
Chimienti *et al*[52]	200 patients Paroxysmal AF	Flecainide (200-300 mg/d) Propafenone (450-900 mg/d)	Palpitation recurrence on days 15, 30, 90, 180, 270, and 360	Flecainide similar efficacy to propafenone Similar rate of adverse cardiac and noncardiac events	Multicenter, open label, randomized, parallel study Suboptimal follow-up of AF recurrence
Naccarelli *et al*[50]	239 patients Paroxysmal AF	Flecainide (100-300 mg/d) Quinidine	AF recurrence at 12 mo	Flecainide similar efficacy to quinidine Flecainide better tolerated than quinidine	Multicenter, open label, randomized, parallel study Self-reporting of symptomatic AF recurrences (diary recording)
Gulizia *et al*[54]	176 pacemaker recipients with sinus node disease and paroxysmal AF	Ic AAD (flecainide or propafenone) Amiodarone	Primary endpoint: time to first occurrence of death, atrial cardioversion, cardiovascular hospitalization, or change of AAD	Class Ic AADs non-inferior to amiodarone in terms of the primary endpoint. Similar efficacy in freedom from AT recurrences based on post-hoc analyses	One patient experienced sudden cardiac death in flecainide group Capability of continuous rhythm monitoring by pacemaker AF recurrence and burden not included in primary endpoint
Kirchhof *et al*[56]	635 patients Persistent AF	Short-term flecainide Long-term flecainide Placebo	Time to first recurrence of persistent atrial fibrillation or death from any cause	Flecainide superior to placebo Short-term flecainide not non-inferior to long-term	Largest, prospective randomized clinical trial Meticulous follow-up

AF: Atrial fibrillation; AAD: Antiarrhythmic drug; AT: Atrial tachycardia.

out underlying structural heart disease. However, it should be noted that in-hospital testing should include only oral formulations of antiarrhythmic agents, since tolerance to intravenous administration of flecainide or propafenone has not been shown to predict adverse events during out-of-hospital self administration of these drugs[57].

RECOMMENDATIONS FOR FLECAINIDE USE IN ATRIAL FIBRILLATION - ESC GUIDELINES

Based on the guidelines of the European Society of Cardiology, *iv* flecainide (2 mg/kg over 10 min)

is recommended for cardioversion of recent onset AF (duration less than 48 h) when pharmacological cardioversion is preferred and there is no structural heart disease (Class ⅠA recommendation). In selected patients without significant structural heart disease, the pill-in-the-pocket strategy (single dose of 200-300 mg) should be considered if previously tested in a medically secure environment (Class Ⅱa recommendation). Flecainide is also recommended for long-term rhythm control in patients without significant underlying heart disease (Class ⅠA recommendation)[10].

Ventricular tachycardias

Ventricular tachycardias in patients with underlying heart disease: The role of flecainide in the treatment of ventricular tachycardias among patients with underlying heart disease has been formulated mainly by the results of the CAST trial[1]. This multicenter, randomized, placebo-controlled trial was conducted to evaluate whether suppression of asymptomatic or mildly symptomatic ventricular arrhythmias after myocardial infarction with antiarrhythmic drugs (flecainide, encainide or moricizine) would result in reduction of arrhythmic mortality. Eligible patients had prior myocardial infarction (6 d to 2 years), ventricular arrhythmias (≥ 6 ventricular extrasystoles per hour or ventricular tachycardia runs less than 15 beats) and impaired ventricular function (ejection fraction ≤ 0.55 if recruited within 90 d of the myocardial infarction, or ≤ 0.40 if 90 d or more after the myocardial infarction). Flecainide was not given to patients with an ejection fraction below 0.30. It should be highlighted that 789 of 1498 patients included in the study had ejection fraction ≥ 0.40[23]. The treatment arms of flecainide and encainide were prematurely discontinued after a mean follow-up of 10 mo due to a significantly increased risk of arrhythmia-related (2.64; 95%CI: 1.60-4.36) and all-cause mortality (2.38; 95%CI: 1.59-3.57)[1].

As regards the role of flecainide in patients with ventricular tachycardia without structural heart disease, type IC antiarrhythmic drugs seem to be useful in patients with right ventricular outflow ventricular tachycardia[58].

Catecholaminergic polymorphic ventricular tachycardia: Accumulating data have verified that flecainide inhibits the cardiac ryanodine receptor open state, thus directly targeting the molecular defect responsible for diastolic calcium release, delayed afterdepolarizations, and triggered arrhythmias in CPVT[33,59]. Case reports and series have reported that flecainide may prove useful in preventing ventricular tachyarrhythmias in patients with CPVT associated both with ryanodine receptor and calsequestrin mutations[21,60]. Van der Werf *et al*[61] reported the clinical experience from several international centers on the efficacy and safety of flecainide treatment in CPVT. The role of flecainide (median daily dose 150 mg in responders) was evaluated in 33 genotype-positive CPVT patients on optimal tolerated conventional treatment using as primary outcome measure the reduction of ventricular arrhythmias during exercise testing[61]. In total, 76% of patients had either a partial or complete suppression of exercise-induced ventricular arrhythmias by flecainide while no patient experienced worsening of exercise-induced ventricular arrhythmias[61]. Flecainide has also been shown to be effective in reducing ventricular arrhythmias during exercise testing and preventing arrhythmia events during long-term follow-up in patients with genotype-negative CPVT[62]. Marai *et al*[63] recently reported that the combination of flecainide and β-blockers can completely suppress exercise-induced ventricular arrhythmias and prevent recurrent ICD shocks in patients with calsequestrin-associated CPVT and high-risk features despite treatment with β-blockers.

PRACTICAL ASPECTS OF FLECAINIDE USE

The following practical considerations should be kept in mind in the management of patients under flecainide treatment: (1) performance of exercise stress test before treatment initiation to assess the presence of underlying coronary artery disease; (2) screening of candidates for sinus and atrioventricular node disease; (3) performance of regular ECG monitoring upon treatment initiation and upon dose titration. In case of QRS prolongation more than 25% as compared to baseline value, flecainide dosage should be halved and if QRS is not normalized thereafter it should be discontinued; (4) performance of exercise stress testing under flecainide treatment to assess increased risk of proarrhythmia, especially in the presence of slight QRS prolongation at rest. Flecainide exerts use-dependent properties and a potential minor or modest increase in QRS duration at rest may increase dramatically during exercise-related rapid heart rate; (5) control of pacing threshold in pacemaker recipients, especially if pacemaker-dependent. Flecainide may increase the pacing threshold occasionally to a significant extent; and (6) concomitant use of agents with negative dromotropic effect is recommended to avoid one-to-one atrio-ventricular conduction and very rapid ventricular rates, if atrial fibrillation is converted to atrial flutter.

IS THERE ANY ROOM FOR FLECAINIDE IN CORONARY ARTERY DISEASE PATIENTS?

The main precaution for flecainide administration is to rule out the presence of "structural" heart disease

and/or ischemic cardiomyopathy in order to avoid the associated increased risk of proarrhythmia. This caveat was mainly formulated on the basis of the CAST results which demonstrated that flecainide is associated with increased mortality when administered in patients with prior myocardial infarction. In this trial more than half of the enrolled patients had an ejection fraction > 40% and it is noteworthy that the hazard ratio of AAD therapy versus placebo for arrhythmic death was similar in patients with ejection fraction < 0.40 as compared to those with ejection fraction ⩾ 0.40. Furthermore, the presence of a non-Q wave myocardial infarction was the only variable which significantly interacted with encainide or flecainide for prediction of arrhythmic or all cause mortality (hazard ratio 7.9 in non-Q wave myocardial infarction patients as compared to 1.8 in those with Q-wave myocardial infarction)[57]. Therefore, it seems prudent to contraindicate flecainide treatment among patients with prior myocardial infarction (either Q or non-Q wave) even if left ventricular ejection fraction is preserved. Furthermore, flecainide administration should be precluded in the presence of myocardial ischemia since the latter increases the risk of proarrhythmia.

However, the group of coronary artery disease patients, with preserved ejection fraction, no prior myocardial infarction and no evidence of ischemia represents a grey zone where there is absolute paucity of data regarding safety of flecainide treatment. According to the 2012 update of the ESC guidelines for the management of atrial fibrillation[10], flecainide can be used in patients with "minimal structural heart disease", but it cannot be used in patients with coronary artery disease. Two main issues can be raised from these recommendations. Firstly, there is no commonly accepted and well established definition of "minimal structural heart disease". Secondly, there are no data supporting an increased proarrhythmic risk of flecainide among coronary artery disease patients in the absence of underlying scar and myocardial ischemia. Besides, even the definition of coronary artery disease may be obscured by the widespread use of advanced imaging techniques which may actually identify patients with coronary lesions but in low arrhythmic risk. Meanwhile, decision making in similar cases is not supported by solid evidence and all available treatment options, including catheter ablation in atrial fibrillation patients, should be taken into account.

Taking into consideration the limited therapeutic modalities that are currently available for the patients with coronary artery disease and atrial fibrillation, it is obvious that new studies need to be undertaken aiming to evaluate the safety of old antiarrhythmics, like flecainide, in the wide spectrum of patients with coronary artery disease, that we treat today. Nowadays, it is quite common to treat patients with stable coronary artery disease who have preserved left ventricular ejection fraction, lack of symptoms and absence of detectable myocardial ischemia. Flecainide and other antiarrhythmics may be useful in the management of these patients, who remain vulnerable to atrial fibrillation and have limited access to invasive management of atrial tachyarrhythmias.

Finally, there is one more unsolved issue in the area of antiarrhythmic therapy in patients with ischemic heart disease. There is lack of evidence for the potential benefit from flecainide treatment in patients with implantable cardioverter defibrillators (ICDs) who have "minimal heart disease" and present symptomatic arrhythmias, refractory to sotalol and/or amiodarone. Although there is no available evidence to support this hypothesis, given that ICDs ameliorate the proarrhythmic risk, flecainide may be an alternative treatment. Unfortunately, due to medical and economic restrains, the aforementioned hypotheses have not been properly addressed in the scheme of a specifically designed randomized clinical trial.

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ORIGINAL ARTICLE

Retrospective Study

Cardiac and non-cardiac causes of T-wave inversion in the precordial leads in adult subjects: A Dutch case series and review of the literature

Salah AM Said, Rene Bloo, Ramon de Nooijer, Andries Slootweg

Salah AM Said, Rene Bloo, Ramon de Nooijer, Andries Slootweg, Department of Cardiology, Hospital Group Twente, Almelo-Hengelo, 7555 DL Hengelo, The Netherlands

Author contributions: Said SAM, Bloo R, de Nooijer R and Slootweg A treated patients and collected material and clinical data from patients; Bloo R performed the assays; de Nooijer R analysed data; Said SAM wrote the paper; all authors approved the final version of the manuscript.

Ethics approval: The study is reviewed and approved by the Hospital Group Twente, Institutional Review Board. Verbal consent was taken from the patients and ethical clearance and permission to publish the cases is obtained from the Hospital Group Twente, Institutional Review Board.

Informed consent: All study participants provided verbal informed consent.

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Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at salah.said@gmail.com. Informed consent, neither verbal nor written, was obtained for data sharing but the presented data are anonymized and risk of identification is negligible.

Correspondence to: Salah AM Said, MD, PhD, FESC, Department of Cardiology, Hospital Group Twente, Almelo-Hengelo, Geerdinksweg 141, 7555 DL Hengelo, The Netherlands. salah.said@gmail.com
Telephone: +31-88-7085286
Fax: +31-88-7085289

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Abstract

AIM: To describe the electrocardiographic (ECG) phenomena characterized by T-wave inversion in the precordial leads in adults and to highlight its differential diagnosis.

METHODS: A retrospective chart review of 8 adult patients who were admitted with ECG T-wave inversion in the anterior chest leads with or without prolongation of corrected QT (QTc) interval. They had different clinical conditions. Each patient underwent appropriate clinical assessment including investigation for myocardial involvement. Single and multimodality non-invasive, semi-invasive and invasive diagnostic approach were used to ascertain the diagnosis. The diagnostic assessment included biochemical investigation, cardiac and abdominal ultrasound, cerebral and chest computed tomography, nuclear medicine and coronary angiography.

RESULTS: Eight adult subjects (5 females) with a mean age of 66 years (range 51 to 82) are analyzed. The etiology of T-wave inversion in the precordial leads were diverse. On admission, all patients had normal blood pressure and the ECG showed sinus rhythm. Five patients showed marked prolongation of the QTc interval. The longest QTc interval (639 ms) was found in the patient with pheochromocytoma. Giant T-wave inversion ($\geqslant$ 10 mm) was found in pheochromocytoma followed by electroconvulsive therapy and finally ischemic heart disease. The deepest T-wave was measured in lead V_3 (5 ×). In 3 patients presented with mild T-wave inversion (patients 1, 5 and 4 mm), the QTc interval was not prolonged (432, 409 and 424 msec), respectively.

CONCLUSION: T-wave inversion associated with or without QTc prolongation requires meticulous history taking, physical examination and tailored diagnostic

modalities to reach rapid and correct diagnosis to establish appropriate therapeutic intervention.

Key words: T-wave inversion; Coronary angiography; Pulmonary computed tomography angiography; Magnetic resonance imaging; Differential diagnosis

Core tip: Myriad of clinical conditions have been described in association with T-wave inversion in the anterior precordial leads. T-wave inversion associated with or without corrected QT prolongation may be encountered in a variety of clinical conditions. In the reversible (dynamic) types such as vascular coronary, cerebral and pulmonary disorders; metabolic disturbances and acute adrenergic stress cardiomyopathy; resolution of T-wave inversion may occur after days, weeks, months or years following the index event. Tailored diagnostic approach should be conducted avoiding overuse of diagnostic methods. Specific tailored therapeutic interventions were undertaken when high index of clinical suspicion was raised towards certain disease entity.

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INTRODUCTION

T-wave inversion is found in 1% of patients admitted to the coronary care unit[1] and in 14% of patients presented with unstable angina[2]. It has been stated that T-wave inversion in right precordial leads is relatively rare (0.5%) in the general population and not associated with adverse outcome[3]. The tendency to inversion of T-wave declines with increasing age. Normally in females, the T-wave in V_3 may be shallowly inverted. But in adult males, it is considered pathologic if the T-wave is inverted in V_{3-6}[4]. The T-wave in V_1 may be inverted normally at any age and in V_2 it is sometimes normally negative[5].

Generally, the T-waves are negative in leads aVR, V_1 and Ⅲ. Giant T-wave inversion in the precordial leads are seen in different pathologies, such as anterior myocardial wall ischemia in patients with acute coronary syndrome, apical hypertrophic cardiomyopathy, cerebral and pulmonary disorders and post-pacing or tachyarrhythmia states.

The definite diagnosis in the presence of inverted T-wave can usually be assessed by meticulous history taking including family history of sudden cardiac death or arrhythmias, physical examination as well as appropriate non-invasive, semi-invasive or invasive diagnostic investigations. This current review, will focus on T-wave inversion in the anterior chest wall leads and discuss its differential diagnosis with emphasis on the non-coronary non-cardiac disorders.

Diagnostic approach should be tailored according to the clinical presentation, medical and family history. We present a Dutch case series of eight patients with T-wave inversion in the precordial leads due to different etiologies and the literature is briefly reviewed.

MATERIALS AND METHODS

The study was reviewed and approved by the Hospital Group Twente, Institutional Review Board. All study participants provided verbal informed consent.

Eight representative adult patients were identified and evaluated. In all patients, physical examination, electrocardiography and transthoracic echocardiography (TTE) were routinely performed. When necessary for adequate clarification of the clinical presentation, tailored diagnostic methods were undertaken in the individual patient at the clinician' s discretion [TTE, n = 8; coronary angiography "coronary angiography (CAG)", n = 7; magnetic resonance imaging "magnetic resonance imaging (MRI)", n = 3; perfusion-ventilation scan, n = 2; computed tomography "computed tomography (CT)" abdomen, n = 1; CT brain, n = 1; CT thoracic aorta, n = 1; ^{123}Iodine-metaiodibenzylguanidine (MIBG) scan, n = 1 and DOPA- positron emission tomography (PET), n = 1].

Diagnostic criteria

The diagnostic criteria included presentation with T-wave inversion in the anterior chest leads on the admission ECG.

Definitions

Electrocardiography: The admission ECGs were analyzed using standard criteria for measurements of T-wave axis, T-wave amplitude and QT interval. ECGs were analyzed for the presence of Left ventricular hypertrophy (LVH) using the Sokolow criteria[6].

T-wave negativity: was defined as a voltage of giant negative T-wave ⩾ 10 mm in any of the leads[7-9], deep ⩾ 5 mm[8] and mild 1-3 mm[8].

Corrected QT prolongation: Corrected QT (QTc) interval for heart rate was performed in V_2 and was defined as QTc > 450 msec. according to Bazett[10] and Ahnve[11].

Measured serum biomarkers were creatine kinase and cardiac troponin T.

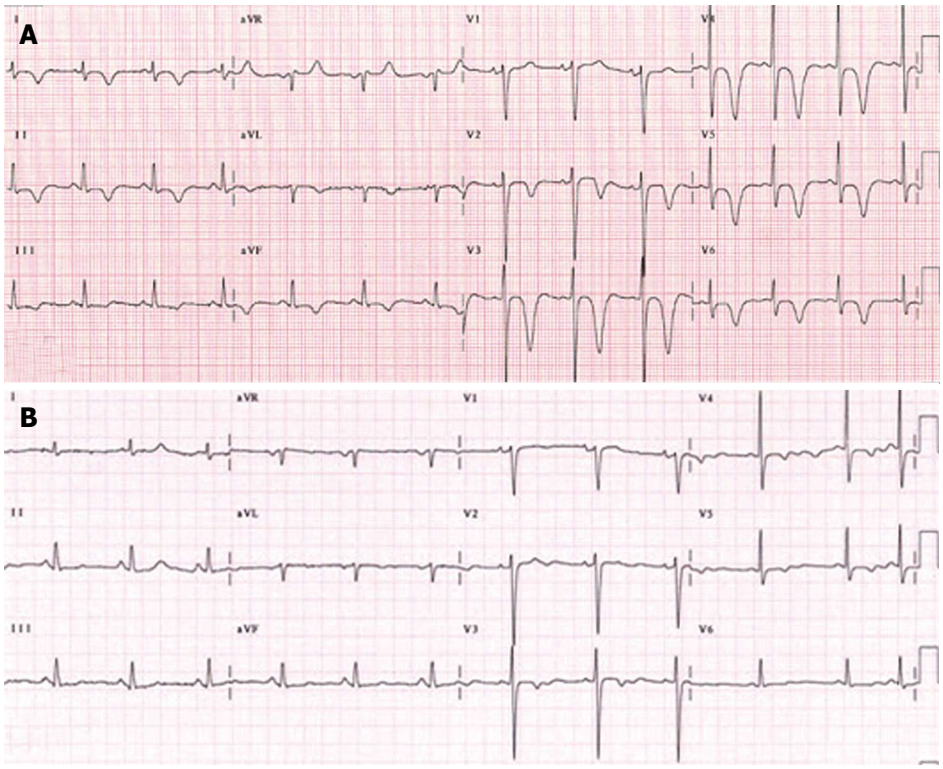

Figure 1 Patient 1. A: An electrocardiographic (ECG) tracing, illustrating the negative T-wave in the precordial leads $V_{2\text{-}6}$, of a 51-year-old-female patient presented with palpitation following electroconvulsive therapy for her depression. In 2001, she underwent catheter ablation for atrioventricular nodal reentry tachycardia. She complained of palpitation after a shock of electroconvulsive therapy for treatment of depression. Post-electroconvulsive therapy, the ECG showed giant T-wave inversion in the precordial leads. The cardiac enzymes were minimally raised. Takotsubo cardiomyopathy is suggested due to emotional stress and electrical shock. Transthoracic echocardiography demonstrated apical hypokinesia and basal normokinesia with trivial valvular regurgitation without massive pericardial effusion. The estimated right ventricular systolic pressure was 35 mmHg. Coronary angiography and cardiac magnetic resonance imaging demonstrated normal findings. Medical treatment with beta blocker was initiated and the symptoms disappeared and (B) the ECG returned to base line in 2 wk time. The antidepressant drug was not discontinued.

Transthoracic echocardiography: Left ventricular (LV) wall thickness as well as septal thickness were measured according to the established standards and guidelines of the American College of Cardiology/American Heart Association/European Society of Cardiology[12,13]. LVH was defined as a LV wall thickness > 13 mm.

Radionuclide studies: Radionuclide imaging and positron emission tomography: One patient (patient 7) underwent ^{123}I-MIBG and dihydroxyphenylalanine-Positron Emission tomography (DOPA-PET) scanning. In 2 patients, pulmonary perfusion/ventilation scintigraphy were performed.

Computed tomography: Abdominal, cerebral and thoracic aorta CT scanning were performed in one patient each.

Pulmonary computed tomography angiography (PCTA) was performed in one patient.

Coronary angiography contrast angiography was performed in standard views *via* the femoral approach.

Follow-up: Follow-up was obtained by direct contact with patients, their physicians or by chart review.

Statistical analysis

No statistical data are available.

RESULTS

A total of 8 adult patients presented with chest pain and negative T-wave in the anterior chest wall leads on the admission ECG were identified (Figures 1-8).

Clinical features

On presentation, the blood pressure was normal in all patients and all were in sinus rhythm (Table 1). Cerebral pathology was excluded by the absence of neurological signs. No neurological deficits were found.

All patients had on physical examination no neurological abnormalities. One patient known with a previous transient ischemic attack showed a complete recovery. Of the 8 patients, one presented with abnormal rest ECG, three with chest pain, one with palpitation, one with fatigue, one with left abdominal pain and psychomotor agitation and one with out-of-hospital cardiac arrest (OHCA).

None of the patients, except one (patient 8)

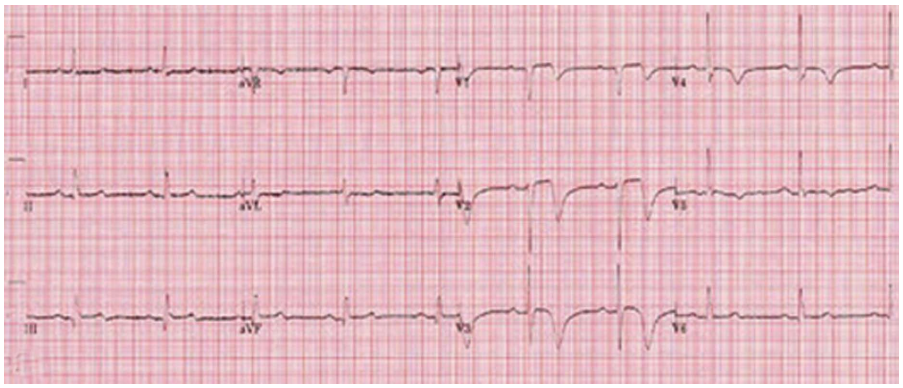

Figure 2 Patient 2. An electrocardiographic (ECG) tracing, illustrating the negative T-wave with minimal ST segment elevation in the precordial leads $V_{1\text{-}4}$, of a 82-year-old-female patient presented with acute coronary syndrome due to non ST elevation myocardial infarction. Her previous medical history included hormonal substitution for hypothyroidism, ablatio retinae, polymylagia rheumatica, arterial hypertension, mild concentric left ventricular hypertrophy, aortic valvular stenosis with a peak gradient of 18 mmHg and mild aortic regurgitation grade 2/4. In 2011, analysis with ambulatory ECG recording for a syncopal attack revealed no abnormalities. Transthoracic echocardiography demonstrated apicoinferior hypokinesia. The patient was treated medically and remained free of symptoms. Her maintenance drug therapy consisted of BB, aspirin (ASA), clopidogrel, Angiotensin-Ⅱ antagonist, prednisolon, diuretic and statin.

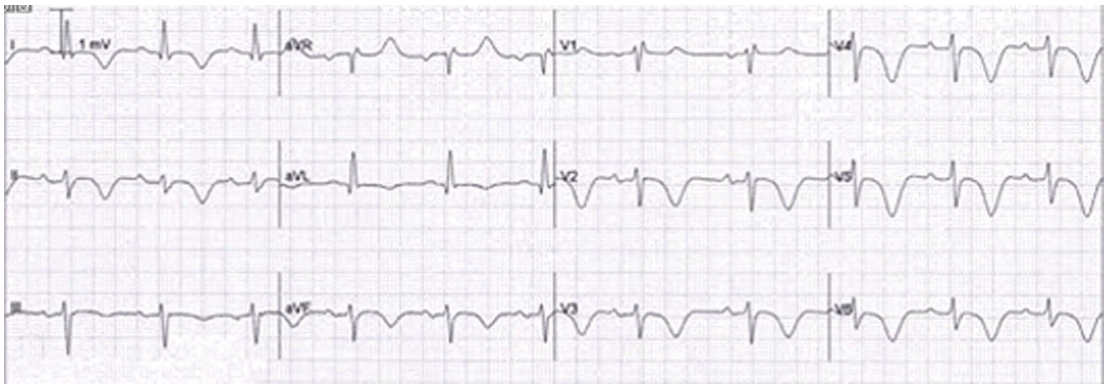

Figure 3 Patient 3. An electrocardiographic (ECG) tracing, illustrating the negative T-wave in the precordial leads $V_{2\text{-}6}$, of a 72-year-old-woman who underwent 6 mo earlier direct current electric cardioversion for persistent atrial fibrillation. Her concomitant medical history includes temporal arteritis, carotid endarterectomy and cluster headache. She presented with abnormal rest ECG during out-patient follow-up. Her spouse died 2 wk prior to presentation. The cardiac markers were minimally elevated. The diagnosis of Takotsubo cardiomyopathy was strongly suggested as the emotional stress may have been the trigger. Transthoracic echocardiography depicted biventricular normokinesia with hypertrophic LV. Pulmonary perfusion-ventilation scintigraphy, Coronary angiography and cardiac MRI were all normal. She did well on pharmacological treatment with oral vit K antagonist, class 3 antiarrhythmic drug, anti-depressive drug and diuretics. The ECG alterations returned to baseline over a 6 mo period.

performed endurance sports activities all had no family history of sudden cardiac death or arrhythmias.

Electrocardiography

The admission ECGs were analyzed using standard criteria for measurements of T-wave amplitude and QTc interval. Giant T-wave inversion (≥ 10 mm) was found in the patients with pheochromocytoma (20 mm) followed by electroconvulsive therapy (15 mm) then ischemic heart disease (10 mm). Deep T-wave inversion (≥ 5 mm) was detected in three and mild (1-3 mm) in two of the patients. The deepest T-wave was measured in lead V3 (5 ×), V4 (2 ×) and V2 (1 ×). Epsilon wave was present in one patient with arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) (patient 8) (Figure 8B). Electrocardiographic criteria for left ventricular hypertrophy was found in 4 patients (patients 2, 3, 6 and 7).

In 3 patients diagnosed with pulmonary embolism (PE) (patient 5), LVH (patient 6) and ARVC/D (patient 8) presented with mild T wave inversion (1, 5 and 4 mm), the QTc interval was not prolonged (432, 409 and 424 msec), respectively.

Corrected QT prolongation

Corrected QT prolongation defined as QTc > 450 msec. measured in lead V_2. The corrected QT interval exceeded 450 ms in 5 (452-639) with a mean of 530 ms and it was not prolonged in 3 (409, 424 and 432 ms) of the patients. The amplitude of the inverted T-wave varied significantly with the maximum negative T-wave amplitude ranging from one to 20 mm. A gradual complete resolution of the T-wave inversion and QT prolongation occurred in 5 of the patients.

Other ECG findings were as follow: two patients (patients 2 and 3) showed first degree AV block, one patient (patient 8) revealed microvoltage in the standard and limb leads, negative T wave in I and

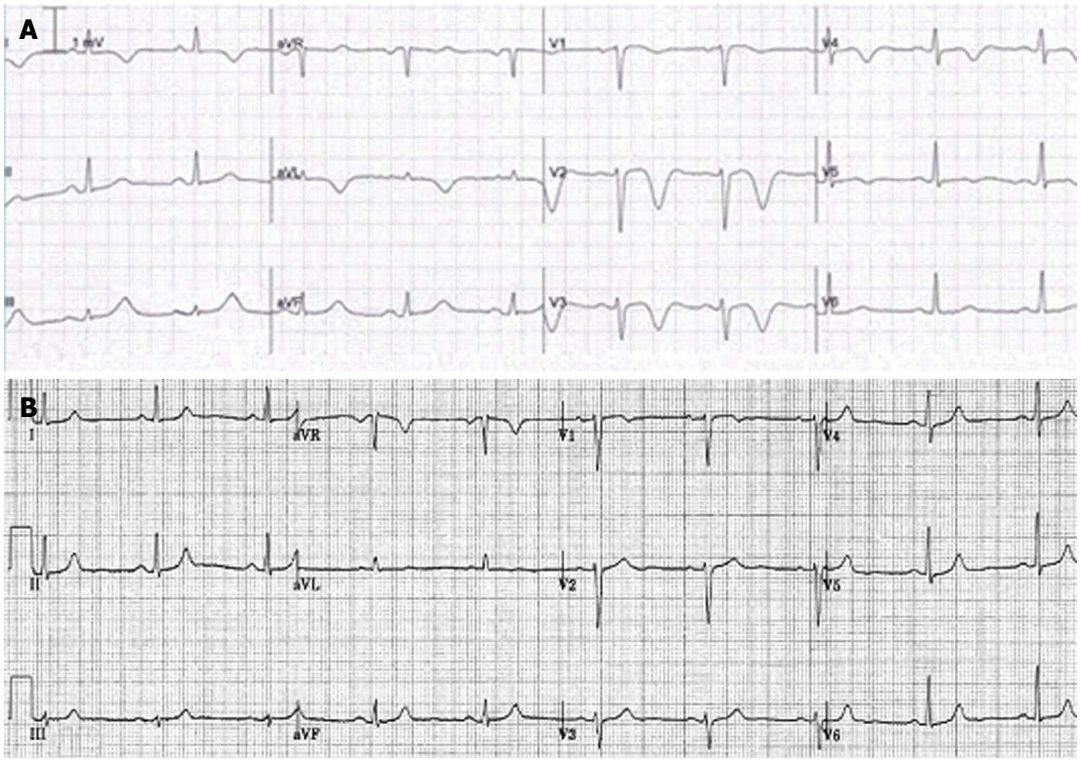

Figure 4 Patient 4. A: An electrocardiographic (ECG) tracing, demonstrating negative T wave in the precordial leads $V_{2\text{-}5}$, of a 69-year female patient with past medical history of transient ischemic attack two years previously, presented with interscapular pain. She had no emotional or physical stress. Normal results were found on transthoracic echocardiography, perfusion-ventilation scintigraphy, Coronary angiography and cardiac MRI. Brain CT scan revealed mild cerebral atrophy and minimal ischemic changes; B: The ECG showed spontaneous regression in 2 mo time. The etiology of the negative T wave inversion remains undetermined. Her medical regimen included aspirin, beta blocker, statin and diuretic.

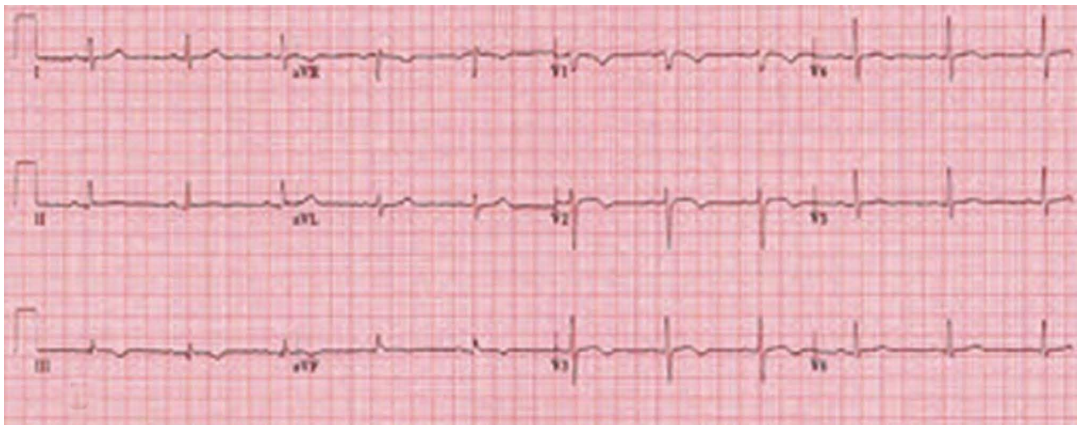

Figure 5 Patient 5. An electrocardiographic (ECG) tracing, showing negative T wave in the precordial leads $V_{1\text{-}3}$ and S_1 Q_3 T_3, of a 55-year obese male patient without antecedent medical history presented with chest pain and minimally elevated serum cardiac biomarkers. Transthoracic echocardiography revealed dilated and hypokinetic RV with pulmonary hypertension and distended inferior caval vein. Coronary angiography was normal and pulmonary CT angiography confirmed the clinical diagnosis showing massive bilateral pulmonary embolism with central and peripheral localization. A continuous positive airway pressure was implemented for newly detected severe obstructive sleep apnea syndrome. He was successfully treated with medical regimen and on follow-up he became asymptomatic and the abnormal ECG findings gradually disappeared.

aVL in 2 patients (patients 4 and 7), negative T wave in leads Ⅰ, Ⅱ, Ⅲ, aVL and aVF in (patients 1 and 3), negative T wave in II, III and aVF without (patient 5) or with (patient 6) ST segment depression.

Serum biomarkers

Cardiac troponin T were assessed. Myocardial infarction was ruled out in 7 patients. One (patient 2) with non ST elevation myocardial infarction (NSTEMI) showed typical biomarker rise and fall course with markedly elevation of troponin level. Mild elevation of troponin value was found in 4 patients (patients 3, 4, 5 and 7). No elevation was detected in 3 patients (patients 1, 6 and 8).

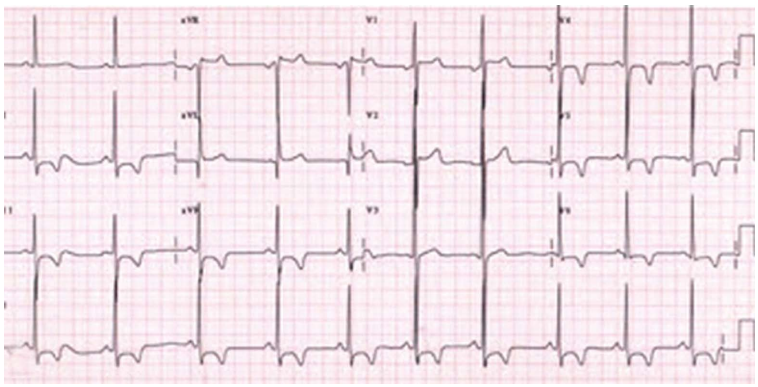

Figure 6 Patient 6. An electrocardiographic (ECG)tracing, showing voltage criteria for left ventricular hypertrophy with ST depression and negative T wave in the precordial and inferior leads $V_{3\text{-}6}$, of a 52-year female patient with non-obstructive hypertrophic cardiomyopathy (thickness of septum 20 mm and posterior wall of 24 mm without septal anterior movement or obstruction of outflow tract). Normal coronary arteries were found on coronary angiography. She refused genetic counseling and invasive intervention. She was treated medically with beta blocker.

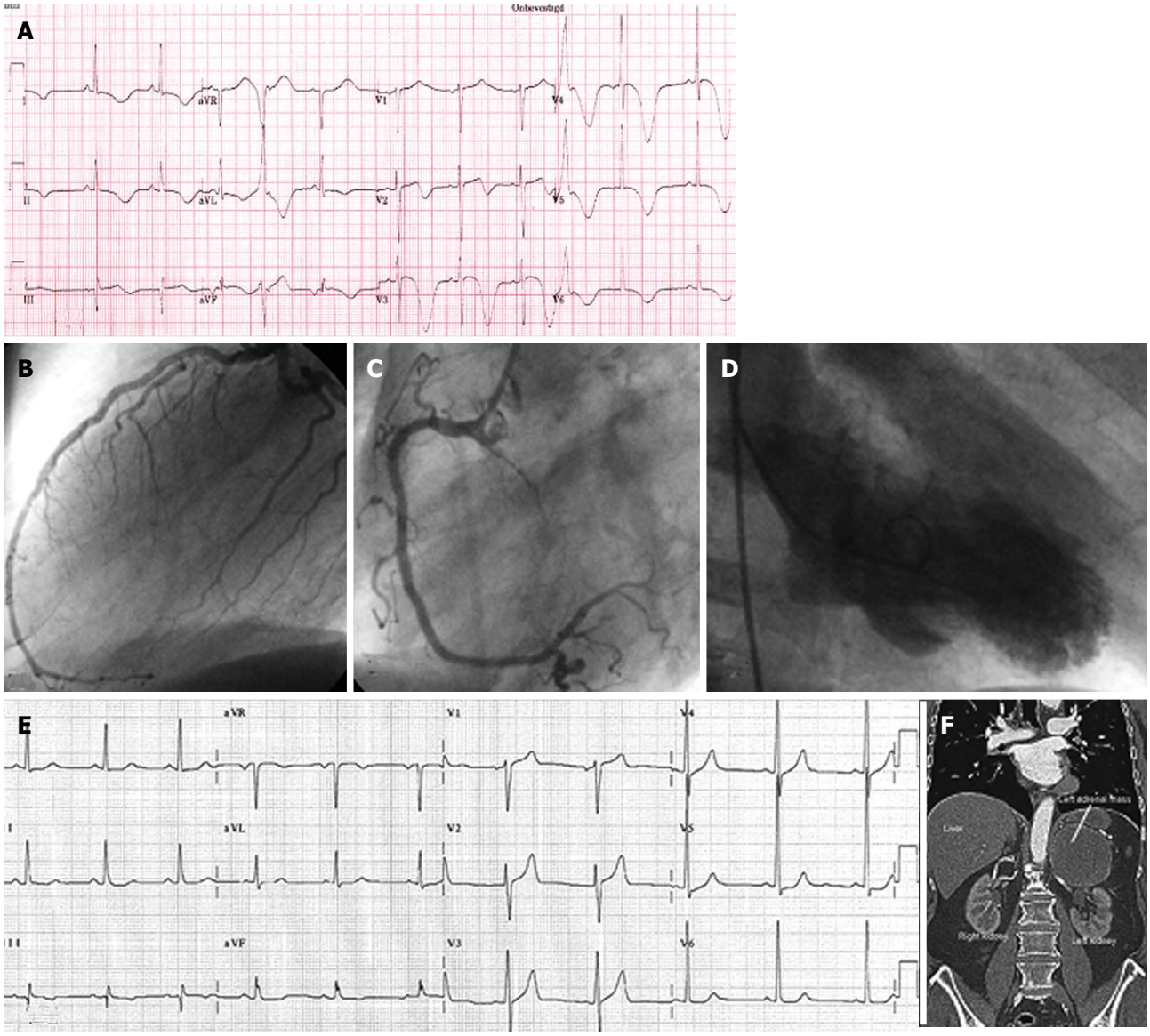

Figure 7 Patient 7. A: An electrocardiographic (ECG) tracing, showing giant T wave inversion in the precordial leads $V_{2\text{-}6}$, of a 76-year Caucasian male with a past medical history of an old inferior myocardial infarction, percutaneous coronary intervention of the right coronary artery (RCA) and left anterior descending coronary artery, acutely presented with left abdominal pain, psychomotor unrest, diaphoresis and blood pressure difference between the right and left arm. Acute aortic dissection was excluded as well as recurrent MI. Coronary angiography frame of (B) the left coronary artery and (C) the RCA depicting no significant stenosis of the arterial tree. Serum cardiac markers were slightly elevated. Echocardiographic (hypokinesia of the mid and apical regions and hyperkinesia of the basal segments) findings and (D) ventriculography (apical ballooning) were all compatible with Takotsubo cardiomyopathy; E: Base line ECG. The abdominal ultrasound and (F) CT demonstrated a pheochromocytoma in the left adrenal region which was confirmed with 123I-MIBG scan and dihydroxyphenylalanine-Positron Emission tomography and proved by pathological results. Plasma and urine metanephrin and normetanephrin were highly elevated. After removal of the hormonally active tumor, the patient became symptom free and the ECG normalized. The medical treatment continued including calcium reentry blocker, beta blocker, aspirin, angiotensin converting enzyme inhibitor, statin and an α-blocker.

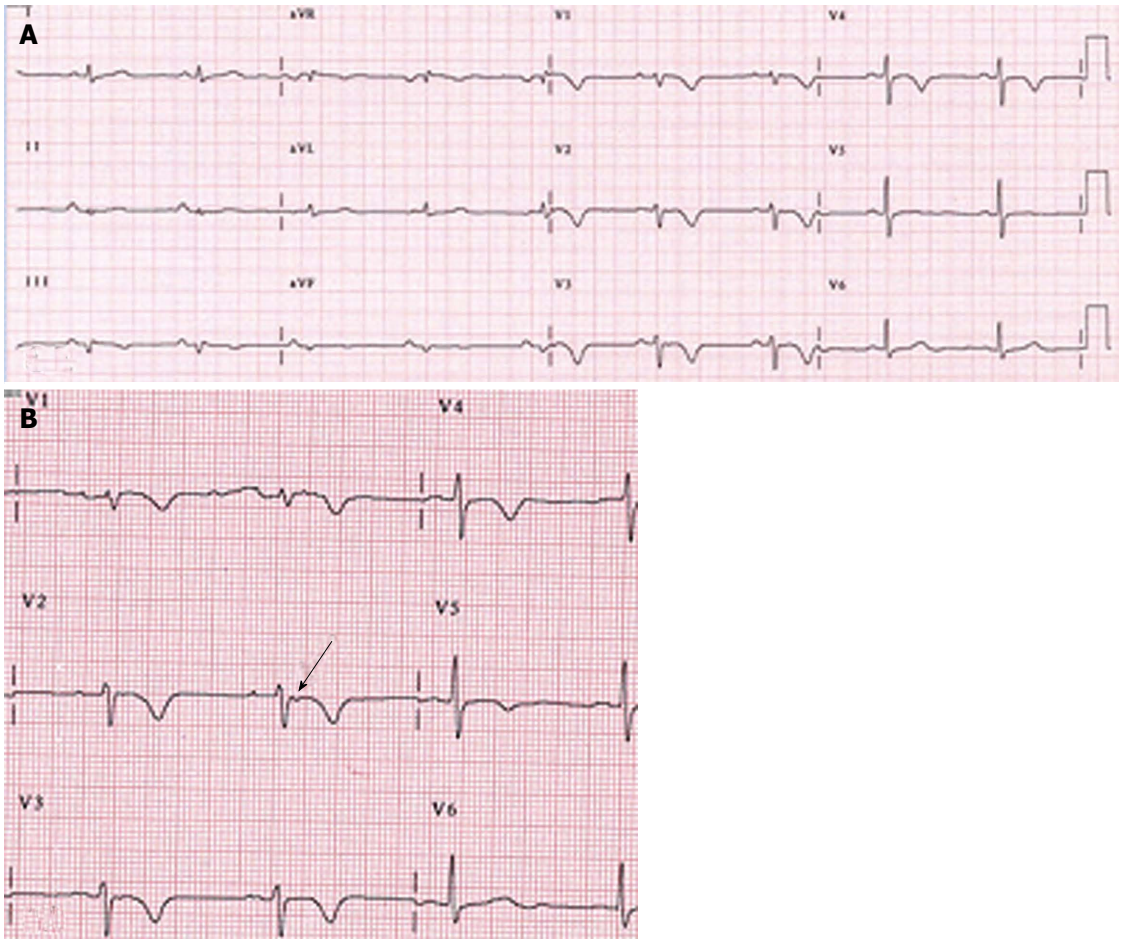

Figure 8 Patient 8. Electrocardiographic (ECG) tracing, demonstrating negative T-wave in the precordial leads V_{1-5}, microvoltage in the standard leads and an Epsilon wave (arrow) which can be appreciated on magnification of ECG lead V_2, of a 73-year-old man, amateur marathon runner after successful resuscitation for OHCA due to ventricular fibrillation. His coronary angiography and transthoracic echocardiography were normal. Genetic counseling revealed mutation 1238C > A (Tyr616X) in the plakophilline2 gen, compatible with AC. He refused implantable cardioverter defibrillator implantation and remained on medical treatment including class III antiarrhythmic drug. He was advised to refrain from marathon running and other strenuous exercise.

Transthoracic echocardiography

Transthoracic echocardiography was performed in all patients. Mild and moderate tricuspid regurgitation was detected in 6 (patients 1, 3, 4, 5, 6 and 7) and one (patient 2) of the patients, respectively. In 3 patients (patients 2, 3 and 7) trivial aortic regurgitation was present. Mild and moderate mitral regurgitation was demonstrated in 4 (patients 3, 4, 5 and 7) and 3 (patients 1, 2 and 6) patients, respectively. Dilatation of the right ventricle (RV) was demonstrated in one patient (patient 8). In five patients (patients 1, 2, 3, 4 and 5) the estimated RV systolic pressure was 35, 28, 35, 32 and 59 mmHg, respectively. Echocardiographic criteria for LVH was found in 4 patients (patients 2, 3, 6 and 7). Apical hypokinesia was detected in two patients (patients 1 and 7).

Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging was performed in three patients (patients 1, 3 and 4). All had normal findings.

Pulmonary computed tomography angiography

Pulmonary computed tomography angiography was performed in one patient (patient 5). Tailored individual diagnostic investigation was performed. In 2 patients (patients 3 and 4), pulmonary perfusion/ventilation scintigraphy were performed and pulmonary embolism was ruled out.

Computed tomography

Abdominal CT scan was performed in one which revealed an abdominal mass in the left adrenal region, a CT scan of the thoracic aorta excluding acute aortic dissection (patient 7) and brain CT scan in another (patient 4) showing mild atrophy and minimal ischemic changes.

Radionuclide imaging and positron emission tomography

One patient (patient 7) underwent ^{123}I-MIBG scan and DOPA-PET scanning. This revealed MIBG uptake in the left adrenal region and a solitary lesion was detected at the left adrenal area with central necrosis

Table 1 Demographic features, clinical presentations, diagnostic modalities and management

Case/ gender/ age	Clinical presentation	ECG (SR) T-wave inversion	QTc (msec)	Associated disorders	TTE	Diagnostic modalities	Management	Condition
1- F51	Palpitation	15 mm in V_{2-6}	452	AVNRT Depression	Apical hypokinesia	TTE CAG MRI	MM	Post-ECT. TTC (electrical stress)
2-F82	Chest pain	10 mm in V_{1-4}	484	Mild AS PG 18 mmHg	Apicoinferior hypokinesia LVH	TTE	MM	NSTEMI
3-F72	Abnormal rest ECG	5 mm in V_{2-6}	553	PAF 2011 Temporal arteritis Carotid endarterectomy Cluster headache	LVH	TTE CAG PV scan MRI	MM	TTC (emotional stress, spouse died 2 wk earlier)
4-F69	Inter-scapular pain	9 mm in V_{2-5}	520	TIA 2010	Normal	TTE CAG PV scan MRI CT brain	MM	Undetermined
5-M55	Chest pain	1 mm in V_{1-3} S_1 Q_3 T_3	432	-	Dilated hypokinetic RV ePAP 75-80 mmHg	TTE CAG PCTA	MM	Pulmonary embolism
6-F52	Fatigue	5 mm in V_{3-6}	409	-	LVH	TTE CAG	MM	HCM
7-M76	Left abdominal pain and psychomotor agitation	20 mm in V_{2-6}	639	IMI 1990 PCI RCA 1990 and 2004 PCI LAD 1991	Apical Hypokinesia LVH	TTE CAG CT thoracic aorta Ultrasound abdomen CT abdomen 123I-MIBG DOPA-PET Pathology	Surgical left adrennalectomy MM	TTC. Pheochromocytoma (hormonal stress)
8-M73	VF, OHCA	Epsilon V_2 4 mm in V_{1-5}	424	Negative family history	RV dilatation	TTE CAG Genetic counseling	MM, AAD Refused ICD implantation Advise to refrain from strenuous exercise	ARVC/D

AAD: Antiarrhythmic drug; ARVC/D: Arrhythmogenic right ventricular cardiomyopathy/dysplasia; AS: Aortic valvular stenosis; AVNRT: Atrioventricular nodal reentry tachycardia; BB: Beta blocker; CAD: Coronary artery disease; CAG: Coronary angiography; ECT: Electroconvulsive therapy; MM: Medical management; CRB: Calcium reentry blocker; CT: Computed tomography; ePAP: Estimated pulmonary artery pressure; F: Female; HCM: Hypertrophic cardiomyopathy; IMI: Inferior myocardial infarction; LAD: Left anterior descending coronary artery; LVH: Left ventricular hypertrophy; M: Male; MRI: Magnetic resonance imaging; NSTEMI: Non ST elevation myocardial infarction; OHCA: Out of hospital cardiac arrest; PAF: Persistent atrial fibrillation; PCI: Percutaneous coronary intervention; PCTA: Pulmonary computed tomography angiography; PET-CT: Positron emission tomography-computed tomography; PG: Peak gradient; PV scan: Perfusion-ventilation scan; RCA: Right coronary artery; RV: Right ventricle; SR: Sinus rhythm; TIA: Transient ischemic attack; TTC: Takotsubo cardiomyopathy; TTE: Transthoracic echocardiography; VF: Ventricular fibrillation; ECG: Electrocardiographic.

with DOPA-PET scanning.

Coronary angiography

Seven patients underwent selective contrast angiography which revealed non-obstructive coronary artery disease in one patient and normal coronary arterial tree in 6 patients.

Follow-up: Follow-up was obtained by direct contact with patients, general practitioner, their physicians or by chart review.

DISCUSSION

In middle-aged subjects, T-wave inversion in the precordial leads is relatively rare in the general population occurring in 0.5% (54/10899[3] of the subjects. T-wave inversion in the anterior chest wall leads is relatively common in children and adolescents[9] but infrequently found in healthy adults and is considered as "normal variants"[4]. This pattern is more common in young females and young adults (1%-3%)[14,15]. The prevalence was associated with gender difference, which was higher (0.9%) in women than in men (0.1%)[3].

Primary and secondary T-wave abnormalities

Primary T-wave abnormalities (ischemia or injury) are due to alterations in myocardial cellular electrophysiology and secondary T-wave abnormalities

(bundle branch block or ventricular Hypertrophy) are subsequent to alterations of sequence of ventricular activation.

Differential diagnosis of T-wave inversion

In the 1960s of last century, Jacobson and Schrire described the differential diagnosis of T-wave inversion that included heart block, ischemic heart disease, bradycardia, right ventricular hypertrophy, right bundle branch block, metabolic disturbances, changes during diagnostic coronary angiography and cerebral disturbances[16]. Nowadays, the current differential diagnosis of T-waves inversion has expanded including, besides the abovementioned citations of Jacobson and Schrire, LV anterior wall ischemia, acute central nervous system disorders, acute adrenergic stress (Takotsubo cardiomyopathy "TTC")[17,18], pulmonary edema[19,20], antiarrhythmic drug effects[21], pulmonary embolism[22], cardiac memory secondary to transient tachycardia[8], post-ventricular pacing states[23], idiopathic[24] or in relation to cocaine use[25,26]. In a recent review, reversible or permanent inverted T-waves were found in 38% of patients with congenital coronary artery-ventricular multiple micro-fistulas (MMFs)[27]. Hence, congenital MMFs may be included in the differential diagnosis of anterior chest wall T-wave inversion.

Transient and permanent T-wave inversion

Transient T-wave inversion may occur in the following conditions: Acute coronary syndrome[1], cardiac memory T-wave[8,23], cardiogenic non-ischemic pulmonary edema[19], gastroenteritis[28], post max-illofacial surgery[29], subarachnoid hemorrhage[30], electroconvulsive therapy[31-33], Takotsubo cardio-myopathy[18,34], pheochromocytoma[35] and indeter-minate origin[24]. On the other hand, permanent T-wave inversion may accompany a variety of disorders associated with LV or RV cardiomyopathy such as apical hypertrophic cardiomyopathy (AHCM)[4,7,36,37] and arrhythmogenic right ventricular cardiomyopathy/dysplasia[38-41].

Non-coronary cardiac and non-cardiac disorders

Several non-coronary cardiac and non-cardiac diso-rders have been associated with the development of T-wave inversion. Among the Non-coronary cardiac disorders: are pericarditis, myocarditis, cardiac metastasis, athletic heart syndrome, AHCM[4], hyper-trophic cardiomyopathy[37], post-tachycardia and right ventricular pacing (cardiac memory)[8,23].

Prognosis

In the middle-aged population, inverted T-wave in the right precordial leads V_{1-3} was associated with good prognosis in contrast to inverted T-wave in leads other than V_{1-3} which predicted adverse outcomes such as increased risk of hospitalization due to congestive heart failure and coronary artery disease[3]. Fisch *et al*[42], stated that inverted shallow asymmetric T-wave with the descending limb longer than the abruptly ascending limb seen in middle-aged women are not associated with cardiac disease.

Non-coronary non-cardiac disorders

Non-coronary non-cardiac disorders (T-wave wide asymmetric and associated with prolonged QT interval) include severe brain injury (subarachnoid hemorrhage "SAH", intracranial hemorrhage)[30,43], traumatic head injury, maxillofacial surgery[29], bilateral carotid endarterectomy, after vagotomy, cocaine abuse[44], flecainide use[21], pheochromocytoma[45] and gastrointestinal emergencies (perforated ulcer, acute pancreatitis and acute cholecystitis)[28,46-49].

Transient T-wave inversion

Acute coronary syndrome[1]: T-wave inversion in the precordial leads have been reported since 1982 as narrow, sharp, and symmetrical waves; the so called "coronary type" reflecting high-grade stenosis of the proximal left anterior descending coronary artery due to regional delay in ventricular repolarization as generally found in ischemic heart disease[50,51]. In this condition, T-wave inversion may persist for days or weeks.

Cardiac memory pattern: Memory T wave was first presented by Chatterjee *et al*[52], in 1969 in 94% of patients with intermittent right ventricular pacing. Transient T-wave inversion occurring after conversion to sinus rhythm from tachycardia or artificial pacing which is caused by abnormal ventricular activation. This pattern of transient T-wave inversion in the precordial leads is associated with tall T-wave in leads I and aVL and are common in patients with permanent pacemakers or ensue after recovery from ventricular or supraventricular tachycardia[23]. It is a diagnosis of exclusion. This pattern of T-wave inversion in RV pacing is caused by dyssynchronous LV activation and involvement of potassium ion channels are postulated to be the key issues in its pathogenesis[53].

Electroconvulsive therapy: ECT may induce ECG changes with simultaneous echocardiographic regional wall motion abnormalities especially when arterial blood pressure and heart rate are markedly elevated[54]. It has been reported that transient T wave inversion Occurs in 4% of the patients undergoing ECT[55]. Transient T-wave inversion, not associated with cardiac abnormalities, has been reported due to increased sympathetic stimulation associated with ECT[31,32]. Conversely, Tuininga reported two cases of T-wave inversion following ECT with significant obstructive coronary artery disease requiring anti-anginal therapy and percutaneous coronary intervention[33]. It was recommended that further investigation to rule out significant coronary

artery disease should be performed especially in patients with cardiovascular risk factors. Moreover, transient T-wave inversion has been reported after electroconvulsive therapy but not following transthoracic direct current electrical cardioversion for treatment of atrial fibrillation[56]. In representative case (patient 1), the findings of CAG and cardiac MRI were normal.

Cardiac sarcoidosis: Cardiac sarcoidosis (CS) is a systemic inflammatory disease with unknown etiology characterized with non-caseating granulomas in multiple organ systems and may be associated with negative T-wave in the anterior precordial leads.

Subarachnoid hemorrhage: In the 1960s, electrocardiographic changes mimicking myocardial ischemia and infarction associated with spontaneous intracranial hemorrhage have been reported[57]. Reversible T-wave abnormalities accompanied with prolongation of QT interval were found in 32% of patients with SAH[30]. ECG changes and arrhythmias occurred within the first 48 h after SAH[43,58]. The postulated pathogenesis is cardiomyocytolysis due to excessive sympathetic stimulation. Possible mechanisms[30] are autonomic neural stimulation from the hypothalamus and elevated levels of circulating catecholamine. The care of patients with subarachnoid hemorrhage has improved dramatically over the last few decades. These gains are the result of improved microsurgical, endovascular, and medical management techniques. This intensive management subjects patients to multiple radiographic studies and thus increased radiation exposure. Thus, tailored diagnostic modalities are required for early and correct establishment of the diagnosis. This to avoid over exposure to ionizing radiation and other invasive procedures. T-wave abnormalities in patients suffering SAH are subsequent to TTC secondary to elevated levels of circulating catecholamine and excessive sympathetic stimulation[29].

Pulmonary embolism: Acute pulmonary embolism may occasionally result in reversible deep T-wave inversion with QT interval prolongation[59]. T-wave inversion associated with PE was first described in 1938 by Love *et al*[60]. T-wave inversion in the precordial leads have been noticed in a moderate-size PE[61], partially occlusive[22] and non-occlusive[23] PE. Precordial T-wave inversion was the most common abnormal ECG finding (68%), this anterior wall ischemic pattern was found in 85% of massive PE and in 19% of mild-moderate PE followed by S1 Q3 T3 pattern detected in 54% of the patients[62]. The proposed mechanisms include RV strain and decreased perfusion of LV anterior wall caused by hypotension consequent to pulmonary embolism[63]. The above mentioned findings were present in the patient with PE (patient 5).

Diagnostic workup for T-wave inversion should always focus on the most likely causes and patient individually tailored diagnostic program should be followed. This to avoid and to limit unnecessary radiation exposure including diagnostic invasive cardiac catheterization.

Pulmonary edema: T wave inversion in the precordial leads has rarely been reported: Possible postulated mechanisms are: an acute rise in the cardiac sympathetic tone either *via* an increased sympathetic discharge from the central nervous system or through subendocardial ischemia due to elevated wall stress, high end-diastolic pressure and decreased coronary arterial blood flow and the electrical heterogeneity in the ventricular wall[19].

Pheochromocytomas: Pheochromocytomas are catecholamine secreting tumours that arise from the chromaffin cells of the adrenal gland. Biochemical diagnosis is established by measuring plasma free metanephrins or nor-metanephrin levels. Localization of the tumour is reached by performing computed tomography or magnetic resonance imaging scans and specifically using metaiodibenzylguanidine scan. The latter is considered the gold standard. Finally, it is confirmed by histopathologic examination[64]. Occasionally, pheochromocytoma may resemble acute coronary syndrome[35,65]. Pheochromocytoma-related cardiomyopathy has incidentally been reported with inverted takotsubo contractile pattern[45]. In patient No. 7, diagnosed with pheochromocytoma (Figure 7F) presented with chest pain and psychomotor agitation, showed periodic fluctuations of blood pressure and ECG abnormalities mimicking acute coronary syndrome (Figure 7A) without significant obstructive coronary artery disease (CAD) on his CAG (Figure 7B and C).

Pheochromocytomas are rare neuroendocrine tumours with a highly variable clinical presentation but most commonly presenting with bouts of headaches, sweating, palpitation and hypertension. Imaging techniques such as CT or MRI and functional assessments using ^{123}I-MIBG are applied to localize biochemically active tumors. In our patient (patient 7), full recovery occurred after left adrenalectomy was successfully performed in an academic hospital. The deepest T-wave inversion (Figure 7A) was found in this patient.

Takotsubo cardiomyopathy: TTC in relation to neurohormonal active adrenal tumor pheochromocytoma or after pulmonary resection for bilateral non-small cell lung neoplasms has rarely been reported[45,66]. TTC accounts for 2% of total hospital admissions for suspected acute coronary syndrome[67]. It Accounts for approximately 1% of admissions for suspected acute myocardial infarction in Japan[68]. Satoh *et al*[69] and Dote and

associates first described this syndrome in Japanese patients[70]. In 2001, Tsuchihashi *et al*[17] reported on cardiomyopathy with apical ballooning mimicking acute myocardial infarction (MI) without obstructive epicardial CAD, 97% of patients demonstrated T-wave inversion in the precordial leads with female predominance (86%). They observed that in 70% of the subjects there was a preceding heavy psychological or physical stress[17]. While the pathogenesis of TTC is not fully understood and remains to be elucidated, several hypotheses, including multivessel epicardial coronary artery spasm, storm of catecholamine excess and coronary microvascular disorder have been proposed[17,71,72]. In a comparison between patients with acute MI and heart failure and patients admitted with a LV systolic dysfunction after sudden emotional stress (95% female subject), Wittstein *et al*[18], found CAD in only 5% in the latter group with significantly higher plasma catecholamine levels suggesting a relation between an exaggerated sympathetic stimulation and transient LV dysfunction[18]. The following criteria are required for establishing the diagnosis TTC; transient LV systolic dysfunction frequently emerging following a stressful trigger, not associated with significant obstructive CAD, novel ECG changes with ST-segment elevation or T-wave inversion usually accompanied with slightly elevation of cardiac markers and no signs of myocarditis and pheochromocytoma[73-75]. Occasionally, in the hyperacute phase of TTC, transient J wave may precede T-wave inversion[72]. Diagnostic work-up may include history, ECG, echocardiography, CAG, ventriculography and less frequent Cardiac magnetic resonance imaging[76]. Cardiac MRI has been useful to differentiate stress TTC from anterior ST-Elevation MI with segmental wall necrosis, by absence of late enhancement in the former condition on delayed image sequence. Other pivotal MRI findings for the diagnosis of TTC are diffuse edema of the left ventricular apical or mid wall associated with akinesia or hypokinesia and absence of perfusion defects[34]. Furthermore, T2-weighted MRI delineated the ECG characterizations (dynamic negative T waves and QTc prolongation) in TCC, resembling the ischemic-like Wellens' ECG pattern, correlating with the apicobasal gradient of myocardial edema, reflecting the edema-induced transient apicobasal inhomogeneity[77]. Recently, positron emission tomography computed tomography has been used to differentiate takotsubo cardiomyopathy TTC from acute coronary syndrome[78]. TTC may occur subsequent to aneurysmal subarachnoid hemorrhage[79]. The relation between TTC and SAH is well known. Many reports have shown the reversible pattern of T-wave inversion associated with SAH[29,78].

Athletic heart: In 1899, Henschen[80] described cardiac enlargement in cross-country skiers. Several ECG changes have been observed in athletes engaged in high intensity dynamic endurance sport activities which may mimic pathological and structural heart diseases. Among others, T-wave inversion in the precordial leads and relative bradycardia were reported[81]. Echocardiographic features of athletic left ventricular hypertrophy may include mild concentric LVH, mild LV dilatation, normal diastolic filling and normal systolic function[82]. It is important to distinguish between physiological adaptive ECG changes and pathological ECG abnormalities to prevent unnecessary distress [83,84]. Regression of LVH occurs when athletes decide to decondition. Some authors consider this adaptation of endurance sports athletic heart as pathologic since LVH regresses on cessation of endurance training in a similar response to a successful treatment of aortic stenosis or arterial hypertension[85].

Permanent T-wave inversion

Arrhythmogenic right ventricular hypertrophy was first described by Dalla volta *et al*[86] in 1961 in Italy and it was brought comprehensively under attention by Frank *et al*[87] in 1978. They reported individuals with extending fibro-fatty non-ischemic changes of the right ventricle. Sudden death may be the first sign of disease[88]. Prevalence is 1/5000 individuals[89]. They have an autosomal dominant or recessive mode of inheritance with incomplete penetrance. ARVC/D affects mainly the right ventricular myocardium characterized with progressive fibro-fatty replacement and infiltration of the RV myocardium and is considered a major cause of sudden arrhythmic death. Recently, involvement of the left ventricle at a later stage may be associated with severe manifestation and carry a worse prognosis[90]. It has been suggested by Gallo *et al*[91] and others for the implementation of a broader term as arrhythmogenic cardiomyopathy (AC)[91,92]. In AC, two major criteria or one major and 2 minor criteria are required to establish the diagnosis[38]. T wave inversion may be the first presentation[3]. Recently, in 2010, the revised task force criteria and guidelines for the clinical diagnosis of AC have been updated and T-wave inversion in the right precordial leads V_{1-3} or beyond was upgraded to a major criterion, in subjects > 14 years of age in the absence of complete right bundle branch block[93], as was found in patient No. 8. The 12-lead ECG demonstrated abnormal changes in 90% of the cases with T-wave inversion in V_{1-3} and sometimes across V_6 as the most common finding[38]. Right precordial T-wave inversions were present in 48%-85% of suspected subjects and Epsilon wave (terminal notch in the QRS complex due to slowed intraventricular conduction) was found in 8%-33% of patients with AC[38-40] as was the case in patient No. 8. In subjects with AC, global and or regional dysfunction and structural alterations may be detected by echocardiography,

angiography, radionuclide scintigraphy or MRI. Cardiac MRI has a high negative predictive value with sensitivity of 100% and specificity of 87%[94]. Mutations in the genes responsible for coding of connecting proteins, called desmosomes are the culprit. In the Dutch population, a founder mutation of p.Arg79X in plakophillin-2 (PKP2) gen with the same desmosome gene mutation in AC has been described[95]. Analysis of DNA in patient No. 8, showed a mutation of plakophillin-2 gen (1248C>A Tyr 616X). Rarely, cardiac sarcoidosis may mimic AC[96].

Apical hypertrophic cardiomyopathy[4,7,36,37]**:** It Is also called (Yamaguchi syndrome) and is considered a rare variant of hypertrophic cardiomyopathy. In the majority of cases (93%) of AHCM, negative T-wave in the precordial leads is the most frequent finding[7]. Its prevalence (15%) in Japan is high in comparison to the United States (3%)[97] and in Europe (< 5%)[98]. Giant negative T-wave exceeding 10 mm is found in 47% of patients with AHCM[7]. Typical findings were first described by Sakamoto in 1976[99] and Yamaguchi *et al*[100] in 1979. In the retrospective study of Eriksson *et al*[7], T-wave inversion was found in (98/105) 93% of the patients. Apical wall thickness of 15 mm was based on TTE or MRI measurements. The T-wave inversion is permanent without tendency for recovery. The permanent ECG features of AHCM are among others giant negative T waves in the precordial leads, ST depression and negative U waves in Ⅱ, Ⅲ, aVF, V4-V6, a prolonged QTc and tallest R wave in V4; however, these permanent features may vary over time[101].

Awareness of the differential diagnosis of T-wave inversion in the precordial leads will help trainees and physicians to discern different entities and will prevent some patients from undergoing unnecessary invasive investigations and procedures. Tailored individual diagnostic investigation was performed and specific diagnostic tools were undertaken when high index of clinical suspicion was raised towards a certain disease entity.

COMMENTS

Background

Myriad of clinical conditions have been described in association with T-wave inversion in the precordial leads. T-wave inversion associated with or without corrected QT prolongation may be encountered in a variety of clinical conditions.

Research frontiers

In patients with T-wave inversion in the precordial leads, tailored diagnostic approach should be conducted avoiding overuse of diagnostic methods. Specific tailored diagnostic modalities and directed therapeutic interventions may be undertaken when high index of clinical suspicion is raised towards certain disease entity.

Innovations and breakthroughs

This study is a retrospective analysis of patients presented with T-wave inversion in the anterior chest leads. The T-wave inversion may be accompanied with or without QTc prolongation. Classification has been made into reversible and irreversible types to facilitate its differential diagnostic approach.

Applications

Awareness of the differential diagnosis of T-wave inversion in the precordial leads will help trainees and physicians to discern different entities and will prevent some patients from undergoing unnecessary invasive investigations and procedures.

Peer-review

In this paper, authors report the various clinical conditions of patients with T wave inversion in the anterior chest wall leads. This review article is interesting and very educational.

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CASE REPORT

Pulmonary arterial dissection in a post-partum patient with patent ductus arteriosus: Case report and review of the literature

Mehmet Yaman, Uğur Arslan, Ahmet Hakan Ateş, Aytekin Aksakal

Mehmet Yaman, Uğur Arslan, Ahmet Hakan Ateş, Aytekin Aksakal, Department of Cardiology, Samsun Education and Research Hospital, 55400 Samsun, Turkey
Author contributions: All authors contributed to this work.

Correspondence to: Uğur Arslan, MD, Associate Professor of Cardiology, Department of Cardiology, Samsun Education and Research Hospital, Barış Avenue, No:199 Ilkadim, 55400 Samsun, Turkey. ugurarslan5@yahoo.com
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Abstract

Pulmonary arterial dissection is an uncommon but usually a deadly complication of chronic pulmonary hypertension. A 26-year-old female patient was admitted to our clinics with sudden dyspnea and chest discomfort one hour after giving birth to twins by vaginal delivery. An echocardiography was performed with a pre-diagnosis of pulmonary embolism. However, echocardiographic examination revealed a dilated main pulmonary artery and a dissection flap extending from main pulmonary artery to left pulmonary artery. In summary, in this report, we described a very rare case of pulmonary artery dissection in a pregnant patient with a previously un-diagnosed patent ductus arteriosus without an obvious rise in pulmonary artery pressure and reviewed the relevant literature.

Key words: Pulmonary dissection; Patent ductus arteriosus; Pregnancy; Echocardiography; Computed tomography

Core tip: A 26-year-old female patient was admitted to our clinics with sudden dyspnea and chest discomfort one hour after giving birth to twins by vaginal delivery. Dilated main pulmonary artery and dissection flap extending from main pulmonary artery to left pulmonary artery were found in echocardiographic examination, there after, pulmonary dissection was confirmed by a computed tomography and the patient underwent surgery. In summary, in this report, we described a very rare case of pulmonary artery dissection in a pregnant patient with a previously un-diagnosed Patent ductus arteriosus without an obvious rise in pulmonary artery pressure and reviewed the relevant literature.

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INTRODUCTION

Pulmonary arterial dissection is an uncommon but usually a deadly complication of chronic pulmonary hypertension. However, with the development of diagnostic and therapeutic interventions in recent

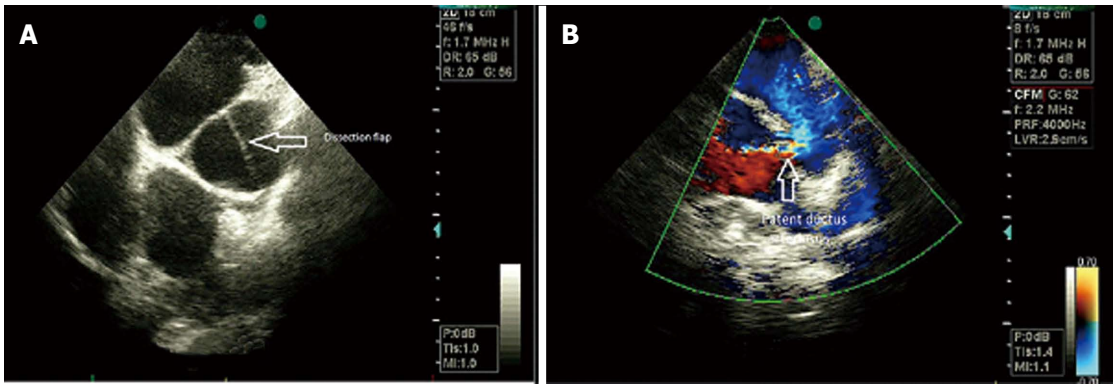

Figure 1 Echocardiographic views of pulmonary dissection (A) and patent ductus arteriosus (B).

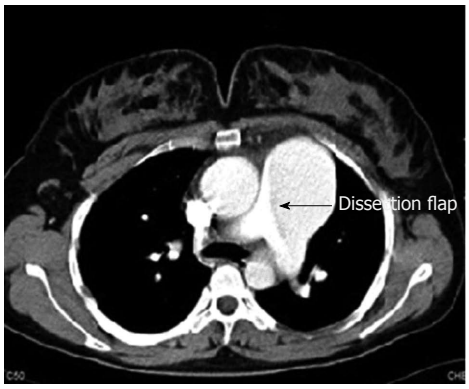

Figure 2 Computed tomography views of pulmonary dissection.

years, a few reports in surviving patients with pulmonary artery dissection have been reported[1].

Patent ductus arteriosus (PDA) is a congenital anomaly caused by a failure to close of a fetal vessel which connects the pulmonary artery directly to the ascending aorta after birth. A rare but lethal complication of PDA is pulmonary artery dissection and dissection usually occurs as a result of chronic pulmonary arterial hypertension associated with PDA and is typically seen in patients with Eisenmenger's syndrome[2-5]. In this report, we described pulmonary artery dissection in a pregnant patient with a previously un-diagnosed PDA without an obvious rise in pulmonary artery pressure.

CASE REPORT

A 26-year-old female patient was admitted to our clinics with sudden dyspnea and chest discomfort one hour after giving birth to twins by vaginal delivery. She had no previous medical history. On physical examination, her blood pressure was 100/60 mmHg and pulse rate was 125/min. The ECG was consistent with sinus tachycardia. An echocardiography was performed with a pre-diagnosis of pulmonary embolism. However, echocardiographic examination revealed a dilated main pulmonary artery and a dissection flap extending from main pulmonary artery to left pulmonary artery (Figure 1A). Besides, in suprasternal and parasternal views, a PDA was observed (Figure 1B). Moderate tricuspid regurgitation was present with a peak continuous wave velocity 2.7 m/s. A thorax computed tomography examination was then performed which showed the dissection flap and the false lumen in the main pulmonary artery (Figure 2). The patient underwent emergent surgery after clarifying the diagnosis. A Dacron graft was positioned in the main pulmonary artery and the PDA was closed. She was discharged from the hospital at the 12th post-operative day. She was in a good clinical condition and asymptomatic at her regular policlinic controls.

DISCUSSION

PDA is a congenital heart disease resulting from the postnatal closure defect of the ductus arteriosus. It represents 5%-10% of all congenital heart diseases, with a female-to-male ratio of 2:1. Delayed treatment may lead to certain complications including pulmonary hypertension, Eisenmenger's syndrome, congestive heart failure, and infective endarteritis. Though rare, pulmonary artery dissection may also be included in the above list of complications.

Posing a high mortality risk, pulmonary artery dissection results from congenital heart diseases, pulmonary hypertension and cardiac interventions[1]. Right heart endocarditis, amyloidosis, trauma and severe atherosclerosis may also result in pulmonary artery dissection[6]. The most common cause is pulmonary hypertension associated with congenital heart disease. Interestingly, in our case, the patient did not have pulmonary hypertension.

The main pulmonary artery is involved in 80% of the pulmonary artery dissection cases. However, isolated cases with right or left pulmonary artery involvement can also be seen. Localized small dissections are rare. Because rupture causes cardiogenic shock and sudden death, diagnosis is rare in the living subject and pulmonary artery dissection is frequently detected in autopsy[5]. Khattar *et al*[1] stated that only

8 (12.6%) out of 63 cases were diagnosed to have pulmonary artery dissection while living, and that 34 (53.9%) of the cases had congenital heart disease[1]. In our case report, pulmonary dissection was reliably diagnosed by transthoracic echocardiography, and thereafter the diagnosis was confirmed by computed tomography examination.

Pulmonary artery dissections usually occur in patients with medial degeneration and pulmonary arterial dilatation due to chronic increases in pulmonary arterial pressures[7-10]. Medial degeneration is a common cause of weakened pulmonary arterial wall and dilatation of the vessel and if intravascular pressure and shear stresses increase due to pulmonary hypertension, intimal tear may develop and cause dissection in the arterial wall. In our case, we thought that pregnancy predisposed the patient to dissection due to weakening of the connective tissue and pulmonary dissection occurred during difficult twin labour which increases venous return and blood pressure in the alreadily dilated pulmonary bed due to PDA.

In aortic dissection, the false lumen usually extends distally and develops a re-entry site. However, in pulmonary dissection, the false lumen usually ruptures causing sudden death of the patient[7-10]. Cardiac tamponade due to rupture seems to be the most common mechanism of death in case of pulmonary dissection[10]. We were lucky because our patient survived this serious clinical situation.

For the first time in literature, we reported a very rare case of pulmonary dissection after giving birth to twins. Presence of PDA without an obvious increase in pulmonary arterial pressures made us think that a transient increase in venous return and pressure during labour caused the dissection to occur. With the development of new imaging techniques providing high quality images in acceptably short time intervals, this mortal disease has become to be diagnosed before death. Moreover, successful repair of pulmonary dissection with surgery has been reported in recent reports including ours. In conclusion, the success of early diagnosis and surgical intervention in pulmonary dissection necessitates an increased awareness of this highly mortal condition.

COMMENTS

Case characteristics

A post-partum patient with sudden dyspnea and chest discomfort after giving birth was admitted.

Clinical diagnosis

Tachycardia and a systolic murmur were found on physical examination.

Differential diagnosis

Pulmonary embolism, aortic dissection.

Imaging diagnosis

Echocardiography and computed tomography were used for the diagnosis of patent ductus arteriosus and pulmonary dissection.

Treatment

Surgical treatment for pulmonary dissection and patent ductus arteriosus closure was performed.

Related reports

Few reports are present about pulmonary dissection in the literature and this is the first report regarding the presence of pulmonary dissection in a post-partum patient without an obvious rise in pulmonary arterial pressures.

Experiences and lessons

An increased awareness of pulmonary dissection with a view to early diagnosis and corrective intervention is necessary in selected cases.

Peer-review

It is an excellent work.

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CASE REPORT

Coronary stenting with cardiogenic shock due to acute ascending aortic dissection

Yuichi Hanaki, Kazuhiko Yumoto, Seigen I, Hajime Aoki, Tomoyuki Fukuzawa, Takahiro Watanabe, Kenichi Kato

Yuichi Hanaki, Kazuhiko Yumoto, Seigen I, Hajime Aoki, Tomoyuki Fukuzawa, Takahiro Watanabe, Kenichi Kato, Department of Cardiology, Yokohama Rosai Hospital, Yokohama 222-0036, Japan

Author contributions: Hanaki Y, Yumoto K and Kato K designed the report; Hanaki Y, Yumoto K and Fukuzawa T performed percutaneous coronary intervention; Aoki H analyzed IVUS findings; I S analyzed CT findings; Watanabe T collected the patient's clinical data; Hanaki Y and Yumoto K wrote the paper.

Correspondence to: Kazuhiko Yumoto, MD, PhD, Department of Cardiology, Yokohama Rosai Hospital, 3211 Kozukue-cho, Kohoku-ku, Yokohama 222-0036, Japan. kyumoto@db3.so-net.ne.jp
Telephone: +81-45-4748111
Fax: +81-45-4748322

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Abstract

A 65-year-old man developed chest pain under cardiogenic shock. Coronary angiography revealed severe stenosis from the ostium of the left main coronary artery (LMCA) to the left anterior descending artery (LAD). Intravascular ultrasound (IVUS) identified a large hematoma that originated from the aorta and extended into the LAD, thereby compressing the true lumen. Type A aortic dissection (TAAD) that involved the LMCA was diagnosed by IVUS. Coronary stenting was performed *via* the LMCA to the proximal LAD, which resulted in coronary blood flow restoration and no further propagation of dissection. Elective surgical aortic repair was performed 2 wk after the stenting. LMCA stenting under IVUS guidance is effective for prompt diagnosis and precise stent deployment in patients with cardiogenic shock due to TAAD with LMCA dissection.

Key words: Aortic dissection; Left main coronary artery; Myocardial infarction; Intravascular ultrasound; Coronary artery stenting

Core tip: Type A aortic dissection (TAAD) involving the left main coronary artery (LMCA) is a rare but potentially lethal condition. However, the precise diagnosis of TAAD prior to the treatment of acute myocardial infarction is difficult, and percutaneous intervention for LMCA obstruction secondary to TAAD is often complicated. This case report represents successful LMCA stenting under intravascular ultrasound (IVUS) guidance in a patient with cardiogenic shock due to TAAD with LMCA dissection. This procedure, particularly in terms of the use of IVUS, may be effective for rapid hemodynamic stabilization in patients in critical condition.

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INTRODUCTION

Acute myocardial infarction (AMI) concomitant

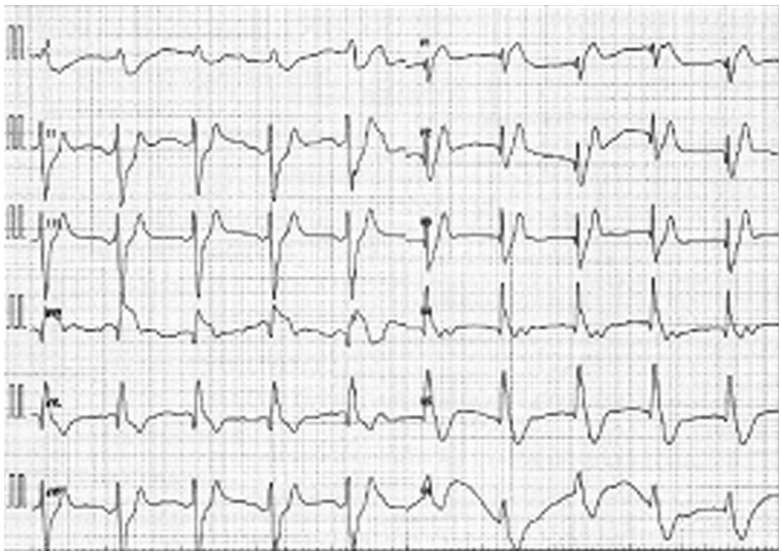

Figure 1 Electrocardiogram on admission. Electrocardiogram shows an idioventricular rhythm with wide QRS complexes. A clear ST elevation is shown in lead aVR.

with acute type A aortic dissection (TAAD) is associated with a high hospital mortality rate despite improvements in TAAD surgical outcomes[1-3]. In particular, TAAD involving the left main coronary artery (LMCA) is a rare but lethal condition associated with low output syndrome, which results from extensive myocardial necrosis regardless of whether an aortic repair surgery is successful. Early coronary revascularization should be performed to minimize cardiac dysfunction[3]. The treatment of dissected coronary arteries with stent implantation achieves prompt and adequate myocardial blood flow and helps prevent extensive myocardial damage. However, an accurate diagnosis of TAAD prior to treatment for AMI is difficult, particularly in patients with hemodynamic instability[4,5]. Furthermore, percutaneous coronary intervention for LMCA obstruction due to TAAD is a complicated procedure unless the mechanism of the LMCA obstruction has been clarified[6]. Here, we describe a case of successful coronary intervention under intravascular ultrasound (IVUS) guidance in a patient with shock due to an unusually localized TAAD with LMCA obstruction.

CASE REPORT

A 65-year-old man was admitted due to sudden-onset chest pain accompanied with cold sweats. The patient had previously undergone a drug-eluting stent implantation in the left anterior descending artery (LAD) 5 years earlier to treat stable angina. His hypertension and hyperlipidemia were well controlled with medication, and he had continued dual antiplatelet therapy (DAPT) since the stent implantation. The patient was transported by ambulance to our hospital within an hour of onset, and his status on arrival included an unmeasurable blood pressure below 60 mmHg and a heart rate of 50 beats/min. The initial electrocardiogram demonstrated bradycardia with an idioventricular rhythm, wide QRS complexes, and ST elevation in the lead aVR (Figure 1). Transthoracic echocardiography (TTE) revealed marked left ventricular dysfunction and a left ventricular ejection fraction of < 30% based on visual estimation. No aortic intimal flap, severe aortic regurgitation, or pericardial effusion was observed. The patient was immediately transferred to the cardiac catheterization laboratory because of ongoing myocardial ischemia and hemodynamic instability. An intra-aortic balloon pump was immediately inserted into the left femoral artery, and coronary angiography was performed from the right femoral artery without difficulty. The right coronary artery did not exhibit stenosis or collateral vessels. The left coronary artery exhibited severe stenosis from the ostium of the LMCA to the proximal LAD, which involved the ostium of the left circumflex artery (LCX) with TIMI grade 1 flow (Figure 2). Coronary intervention was subsequently performed. Run-through NS guidewire (Terumo, Tokyo, Japan) and SION guidewire (Asahi Intecc, Aichi, Japan) were inserted into the LAD and LCX, respectively. To confirm the wire position and evaluate the LMCA obstruction, IVUS (ViewIT, Terumo, Tokyo, Japan) was performed with quick pull-back. The IVUS revealed a large hematoma that originated from the aorta and extended into the LMCA and LAD (Figure 3A-C). The true lumen was compressed by the false lumen throughout the LAD immediately prior to the bifurcation of the diagonal branch (Figure 2D). A large intramural hematoma that involved the ostium of the LCX (Figure 3B) continued into the aortic wall in accordance with the double contour image in the coronary angiogram (Figure 3). We diagnosed an LMCA obstruction due to TAAD; thus, we decided to place a stent for immediate restoration of the coronary blood flow followed by aortic surgery. A 4.0- × 22.0-mm bare metal stent (Integrity; Medtronic, Minnesota, United States) was deployed *via* the

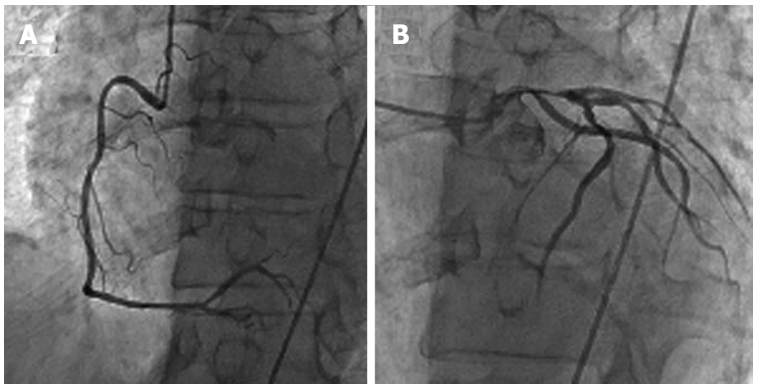

Figure 2 Urgent coronary angiography. A coronary angiography of RCA reveals no stenosis (A), but there is severe stenosis from the ostium of the left main coronary artery to the LAD with TIMI grade 1 flow (B). RCA: Right coronary artery; LMCA: Left main coronary artery; LAD: Left anterior descending artery; TIMI: Thrombolysis in myocardial infarction.

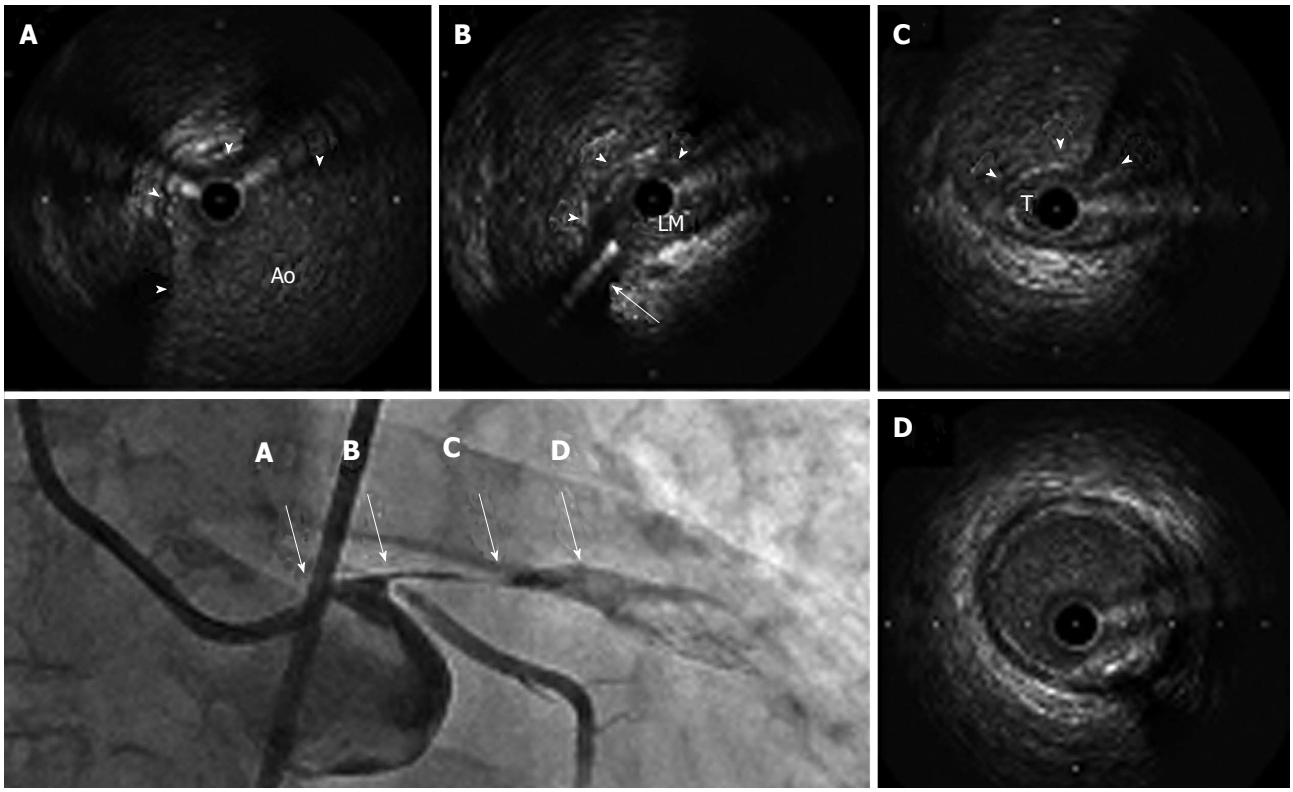

Figure 3 Coronary angiography and intravascular ultrasound findings prior to stent implantation. IVUS shows compression of the true lumen from the LMCA ostium to the LAD by a large false lumen. A: The large hematoma (arrowhead) eviscerates into the aorta (Ao); B: The false lumen (arrowhead) compresses the LMCA (LM), including both the LAD and LCX ostium. The arrow indicates the guidewire in the LCX; C: The large false lumen (arrowhead) compresses the true lumen (T) in the proximal LAD; D: The false lumen disappears before the bifurcation of the diagonal branch. Scale measures in 1 mm. IVUS: Intravascular ultrasound; LCX: Left circumflex artery; LAD: Left anterior descending artery.

LMCA through the LAD across the LCX ostium with satisfactory restoration of coronary blood flow. IVUS confirmed that the implanted stent appeared well-expanded and completely sealed the false lumen. No propagation of the false lumen into the distal LAD or the ostium of the LCX was observed (Figure 4). The patient's hemodynamic failure and symptoms improved immediately after stenting. We removed the intra-aortic balloon pump immediately after hemodynamic stabilization because of the potential exacerbation of TAAD. Contrast-enhanced computed tomography (CT) following the coronary intervention revealed a localized retrograde dissection of the ascending aorta that extended to the ostium of the LMCA (Figure 5). The implanted stent protected the LMCA ostium from TAAD. Marked lung congestion was present. The following day, the patient's maximum creatine kinase and creatine kinase-myocardial band levels were 16190 and 829 IU/L, respectively. The DAPT comprised aspirin (100 mg/d) and clopidogrel (75 mg/d). An elective ascending aortic repair surgery was performed pending improvement of congestive heart failure under continued DAPT 2 wk after coronary stenting. Ascending aortic replacement with an interposition vascular prosthesis graft was performed; coronary artery bypass grafting (CABG), which included the left internal thoracic artery (LITA) to the distal portion of the LAD and a

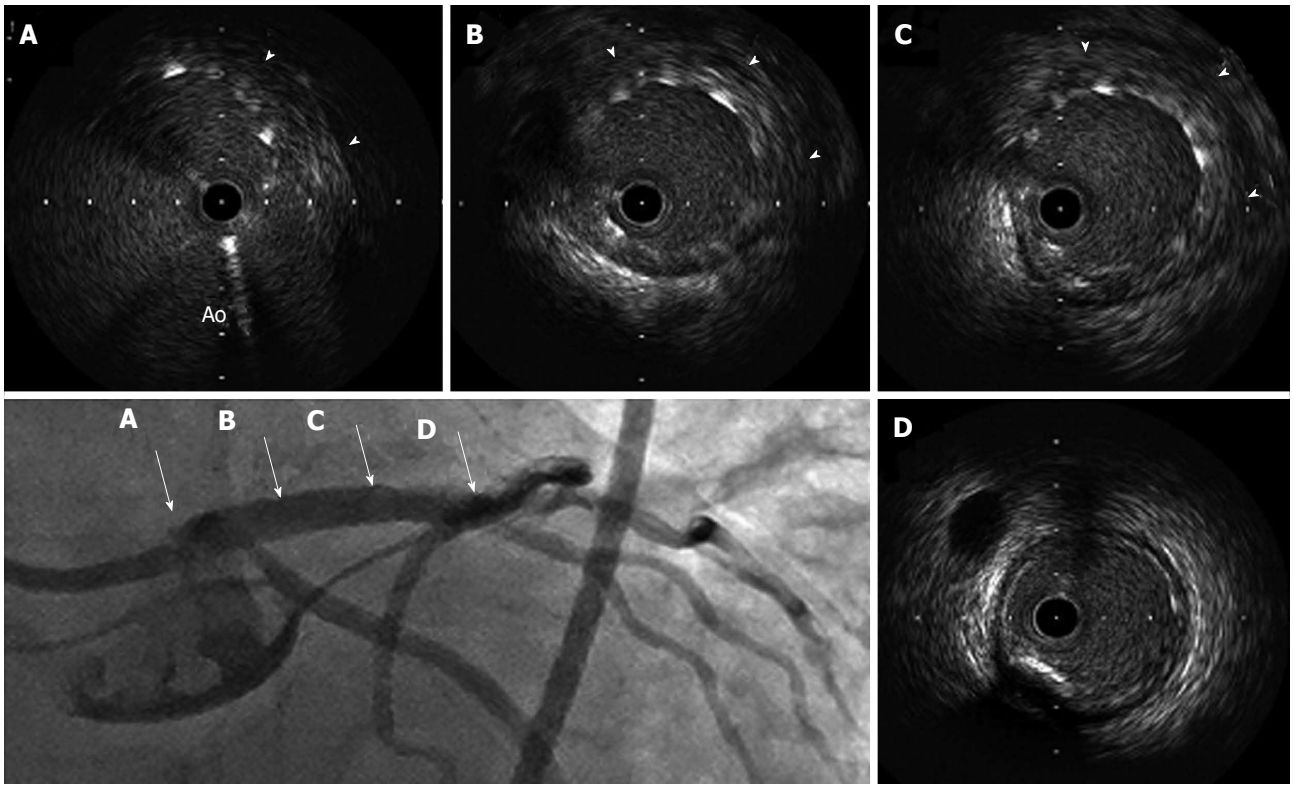

Figure 4 Coronary angiography and intravascular ultrasound findings after stent implantation. The implanted stent appears to be well expanded and completely seals the false lumen. There was no evidence of distal propagation of the false lumen or a flow limitation of the LCX. A: The hematoma (arrowhead) in the aorta is well covered by the stent; B, C: Implanted stent completely sealed the hematoma (arrowhead); D: No extension of the hematoma beyond the stent. Scale bars represent 1 mm.

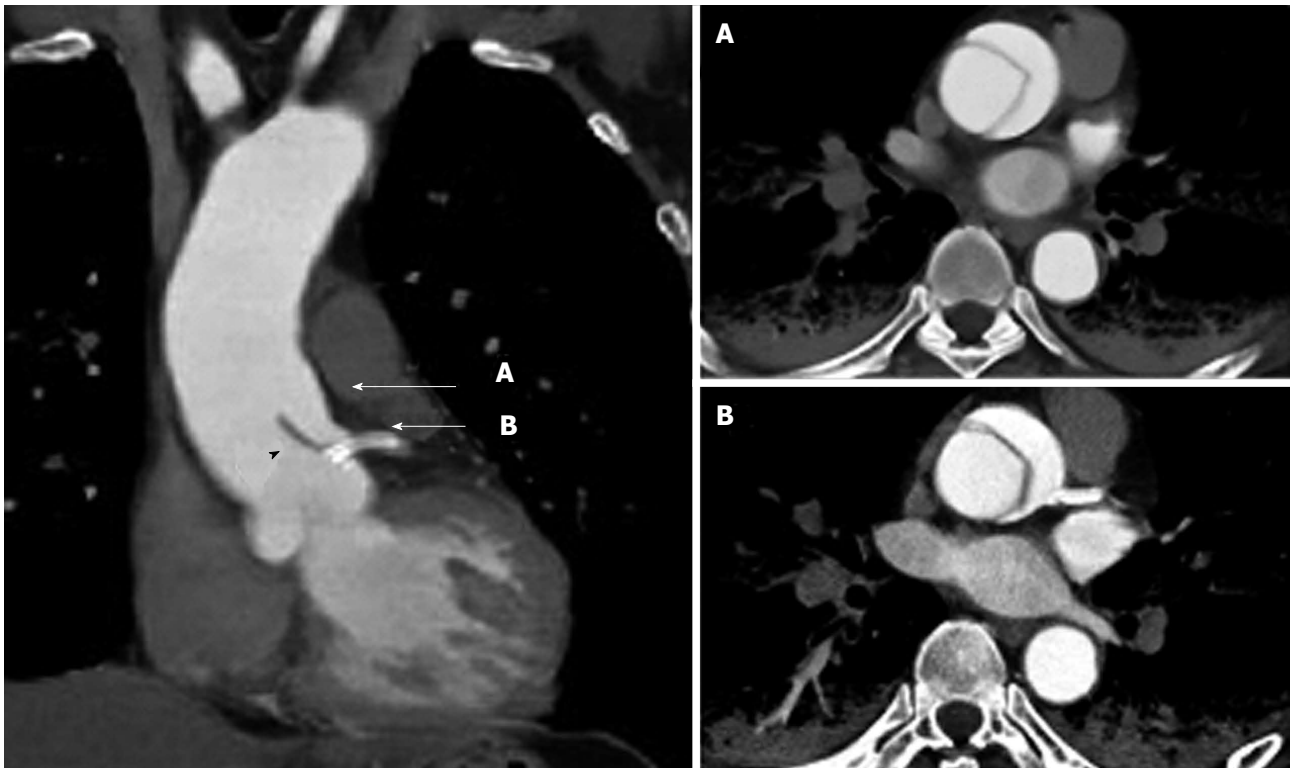

Figure 5 Contrast-enhanced computed tomography after coronary stenting. The sagittal view (left image) reveals the localized dissection of the ascending aorta that extends to the left main coronary artery ostium. The implanted coronary stent (arrow) protects against the intrusion of the dissection flap (black arrowhead) into the left coronary artery. Ascending aortic dissection is clearly visible in the horizontal view (right images) in accordance with A and B in the sagittal view.

Table 1 Previously reported cases of coronary stenting for left main coronary artery due to acute ascending aortic dissection

Ref.	Patient (age/sex)	Hemodynamic status	Timing of diagnosis	Diagnostic modality	Timing of operation	CABG	Outcome
Saxena *et al*[7]	56 Male	Unstable	During PCI	Aortogram	< 24 h	-	Alive
Ohara *et al*[8]	67 Male	Unstable	After PCI	CT	Not performed	-	Dead
Barabas *et al*[9]	74 Male	Stable	During PCI	Aortogram	< 24 h	+	Alive
Ravandi *et al*[17]	86 Male	Unstable	During PCI	Aortogram	Not performed	-	Uncertain
Imoto *et al*[18]	71 Male	Unstable	Before PCI	CT	3 d later	-	Alive
Cardozo *et al*[19]	68 Male	Stable	During PCI	Aortogram	Not performed	-	Dead
Camero *et al*[20]	52 Female	Unstable	After PCI	TEE	< 24 h	-	Alive
Present case	65 Male	Unstable	During PCI	IVUS	14 d later	+	Alive

PCI: Percutaneous coronary intervention; CABG: Coronary artery bypass graft; CT: Computed tomography; TEE: Transesophageal echocardiogram.

saphenous vein graft (SVG) to the middle portion of the LCX, was performed at the surgeon's discretion. Intraoperatively, an intimal tear in the ascending aorta with the false lumen that extended to the ostium of the LMCA was identified without an intimal rupture or aortic valvular destruction. Satisfactory restoration of the intracoronary stent for the left coronary artery and wide patency of the ostia of the left and right coronary arteries were visibly confirmed at the time of surgery. The intraoperative bleeding volume was 1230 mL, which necessitated a blood transfusion of 650 mL. The patient was discharged 51 d after admission. The physical status at the one-month follow-up visit was characterized as New York Heart Association class 1 despite a high concentration of brain natriuretic peptide (400 pg/mL: normal range < 20 pg/mL). The TEE demonstrated reduced antero-septal wall motion; however, the overall ejection fraction recovered to 50%. A follow-up coronary angiography 6 mo after discharge revealed that the left coronary artery maintained excellent blood flow without in-stent restenosis. The SVG was patent; however, the LITA revealed shrinkage as a non-functional bypass.

DISCUSSION

AMI with TAAD is associated with a high risk of extensive and irreversible myocardial damage and hemodynamic instability, which leads to high mortality regardless whether the surgical repair is successful[1,2]. TAAD that involves the LMCA is associated with a particularly high incidence of preoperative cardiopulmonary arrest and high operative mortality[3]. Postoperative low output syndrome due to extensive myocardial damage from AMI involving the LMCA is a major concern in surgical management. Although stent implantation in the treatment of dissected coronary arteries achieves immediate restoration of coronary blood flow and prevents extensive myocardial damage, the confirmation of the correct diagnosis and the performance of the optimal treatment procedure remain challenging[7-9] (Table 1).

AMI with shock might be overlooked as an underlying factor of TAAD[4]. The diagnosis of TAAD with concomitant AMI is difficult because TAAD may be hidden in patients in critical condition. The present patient did not have back pain or a widened mediastinum on the chest X-ray obtained for suspected TAAD. Furthermore, the TTE revealed no evidence of localized TAAD. Although early revascularization should be strongly considered for patients with AMI secondary to cardiogenic shock[10], subsequent thrombolytic therapy and/or coronary intervention can be complicated in patients with underlying TAAD[5]. CT is recommended as the first line of investigation for patients with suspected TAAD. However, CT imaging is more time consuming for critical shock patients with an ST segment elevation myocardial infarction[11]. TTE is also useful; however, it is a limited screening technique for the quick diagnosis of TAAD because of the unavoidable operator dependency, reduced image resolution, and limited field of view[12]. The IVUS findings in the present case enabled the determination of the precise diagnosis of TAAD with typical findings of coronary artery compression[13,14].

Although coronary stenting is helpful in patients with TAAD, it remains challenging because of technical difficulties. The technical issues regarding coronary stenting include the navigation of the guidewire through the true lumen. IVUS imaging can be used to detect the orifice of the dissection, confirm the correct wire placement in the true lumen, and assist in the determination of the precise stent position, size, and length in a short time. Inappropriate ballooning or stenting that fails to completely seal the dissection might propagate the false lumen to the distal or proximal region. Repeated contrast injections should also be avoided because the extension of the dissection may result in the deterioration of the patient's condition[15].

Imoto *et al*[3] reported that preoperative cardiopulmonary arrest and myocardial ischemia, particularly of the left coronary artery territory, negatively affected the survival outcomes in patients undergoing surgery for TAAD with coronary artery dissection. Early coronary intervention *via* stent implantation effectively prevents postoperative low cardiac output

syndrome. Several bridge approaches to surgery have consequently been developed to facilitate early coronary intervention and reduce the extent of myocardial cell necrosis.

The timing of surgical repair for TAAD after coronary stenting is important. A delay in surgical repair may lead to the propagation and rupture of the aortic dissection. Prompt intervention can serve as a bridge approach to gain time for critically unstable patients prior to definitive surgery. In contrast, perioperative stent thrombosis is a serious complication that is associated with a significant increase in mortality, particularly in LMCA stenting. This complication is caused by antiplatelet therapy discontinuation and a surgery-induced prothrombotic situation. DAPT is necessary after coronary artery stenting, particularly in the acute phase. Hansson *et al*[16] studied the association of antiplatelet therapy with bleeding complications and mortality in patients undergoing operations for TAAD. The patients with ongoing platelet inhibition had significantly larger intraoperative and postoperative bleeding volumes; furthermore, the patients on DAPT had high 30-d mortality rates. In the present case, surgical aortic repair was performed after 2 wk while DAPT was continued. The duration of DAPT may be shortened, thus reducing bleeding during aortic surgery, by using a large bare metal stent with IVUS guidance to confirm the proper stent apposition. However, premature cessation of DAPT is likely to induce critical stent thrombosis. The addition of CABG is encouraging even after successful recanalization with stenting during the preparation for stent thrombosis after aortic surgery[3]. The optimal timing of surgical repair, the duration of DAPT and the efficacy of CABG addition after coronary artery stenting have not been established (Table 1).

LMCA stenting prior to the surgical repair of TAAD with LMCA dissection could be effective for an immediate improvement in hemodynamic instability. We emphasize the use of IVUS during the treatment of AMI because LMCA obstruction is necessary to exclude the presence of TAAD.

COMMENTS

Case characteristics

A 65-year-old male with a history of sudden-onset chest pain with hemodynamic instability.

Clinical diagnosis

The shock status, peripheral coldness and electrocardiogram indicated a severe myocardial infarction with hemodynamic instability.

Differential diagnosis

Coronary artery disease with or without coronary atherosclerosis, for example coronary spasm, thrombosis, or Takotsubo cardiomyopathy.

Laboratory diagnosis

The cardiac enzyme levels were extremely elevated after the catheter procedure, with the following results: WBC 21.6 k/uL; AST 1706 U/L; LDH 2945 U/L; CK 16190 U/L; CK-MB 829 U/L; and D-dimer 10.18 μg/mL.

Imaging diagnosis

The intravascular ultrasound (IVUS) findings during coronary intervention revealed a large hematoma that originated from the aorta and extended into the left main coronary artery (LMCA) and left anterior descending artery. An enhanced computed tomography scan revealed a localized retrograde dissection of the ascending aorta that extended to the ostium of the LMCA.

Pathological diagnosis

No specimen materials were collected.

Treatment

The patient was treated with a percutaneous coronary intervention and surgical procedure.

Related reports

Type A aortic dissection (TAAD) involving the LMCA is rare; however, there are a few case reports of the efficacy of LMCA stenting prior to surgical repair for immediate improvement in hemodynamic instability.

Experiences and lessons

The use of IVUS during acute myocardial infarction secondary to LMCA obstruction is effective for not only achieving appropriate stenting but also excluding the presence of TAAD.

Peer-review

A well-written and interesting case report nicely outlining management strategy of acute myocardial infarction secondary to involvement of left main coronary artery by type A acute aortic dissection.

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EDITORIAL

Catheter ablation of persistent atrial fibrillation: The importance of substrate modification

Konstantinos P Letsas, Michael Efremidis, Nikolaos P Sgouros, Konstantinos Vlachos, Dimitrios Asvestas, Antonios Sideris

Konstantinos P Letsas, Michael Efremidis, Nikolaos P Sgouros, Konstantinos Vlachos, Dimitrios Asvestas, Antonios Sideris, Second Department of Cardiology, Laboratory of Cardiac Electrophysiology, Evangelismos General Hospital of Athens, 10676 Athens, Greece

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Correspondence to: Konstantinos P Letsas, MD, FESC, Second Department of Cardiology, Laboratory of Cardiac Electrophysiology, Evangelismos General Hospital of Athens, Ipsilantou 45-47, 10676 Athens, Greece. k.letsas@mail.gr
Telephone: +30-213-2041467
Fax: +30-213-2041344

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Abstract

Accumulating data have shown that elimination of atrial fibrillation (AF) sources should be the goal in persistent AF ablation. Pulmonary vein isolation, linear lesions and complex fractionated atrial electrograms (CFAEs) ablation have shown limited efficacy in patients with persistent AF. A combined approach using voltage, CFAEs and dominant frequency (DF) mapping may be helpful for the identification of AF sources and subsequent focal substrate modification. The fibrillatory activity is maintained by intramural reentry centered on fibrotic patches. Voltage mapping may assist in the identification of fibrotic areas. Stable rotors display the higher DF and possibly drive AF. Furthermore, the single rotor is usually consistent with organized AF electrograms without fractionation. It is therefore quite possible that rotors are located at relatively "healthy islands" within the patchy fibrosis. This is supported by the fact that high DF sites have been negatively correlated to the amount of fibrosis. CFAEs are located in areas adjacent to high DF. In conclusion, patchy fibrotic areas displaying the maximum DF along with high organization index and the lower fractionation index are potential targets of ablation. Prospective studies are required to validate the efficacy of substrate modification in left atrial ablation outcomes.

Key words: Ablation; Atrial fibrillation; Persistent; Substrate; Dominant frequency

Core tip: A combined approach using voltage, complex fractionated atrial electrograms and dominant frequency mapping may be helpful for the identification of atrial fibrillation sources, and therefore for sufficient substrate modification in patients with persistent atrial fibrillation undergoing left atrial ablation.

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INTRODUCTION

Catheter ablation of atrial fibrillation (AF) is indicated in patients with symptomatic AF, refractory or intolerant to at least one class Ⅰ or Ⅲ antiarrhythmic medication[1-3]. Indications for catheter ablation of AF have expanded to include increasingly complex cases including patients with long-standing persistent AF and structural heart disease. Circumferential pulmonary vein antral isolation (PVAI) has become a standard therapy for paroxysmal AF[4]. On the contrary, PVAI displays a significantly lower success rate in patients with persistent or long-lasting persistent AF[1,2,5,6]. This difference suggests that the mechanisms underlying the maintenance of persistent AF are different in relation to paroxysmal AF. Recently, catheter ablation of stable rotors or focal sources in individuals with paroxysmal and persistent AF has given promising results[7-9]. Additional substrate modification is therefore required in the setting of persistent AF. However, the optimal ablation approach in these complex cases remains uncertain.

PATHOPHYSIOLOGY OF AF

AF represents the final common phenotype for multiple disease pathways and mechanisms that are incompletely understood. The multiple re-entrant wavelet hypothesis as a mechanism of AF was described by Moe and colleagues in 1959[10] with supportive experimental work by Alessie[11]. The "multiple re-entrant wavelet hypothesis" supports that fractionation of wavefronts propagating through the atria results in self-perpetuating "daughter wavelets". Multiple re-entrant wavelets are separated by lines of functional conduction block. The lines of the conduction block can occur around the anatomical structures within the atria with different inherent electrophysiological properties, such as scars, patchy fibrosis and myocardium, at different stages of recovery and excitability. However, this hypothesis can not easily explain why AF exhibits consistent spatial non-uniformities in rate and activation vector[12-14], how ablation may terminate AF relatively early in some cases before compartmentalization of meandering wavelets[1,6], or why extensive ablation often has little acute impact[1,15]. Alternatively, the "localised source hypothesis" is supported by elegant experiments in which localised spiral waves (rotors)[16,17] or focal sources[14] disorganise into AF. Stable microreentrant sources appears to be the most likely underlying mechanism of AF in experimental models[18,19]. Recent developments of patient-tailored and physiology-based computational mapping systems have identified localized electrical spiral waves, or rotors, and focal sources as mechanisms that may represent novel targets for therapy[7-9].

Studies have emphasised the importance of ion channel remodelling, changes in signalling pathways, oxidative stress, altered calcium handling, changes in atrial architecture, and altered connexin expression in the pathogenesis and maintenance of AF[20,21]. AF in turn causes AF-promoting abnormalities in each of these areas and enhances the vulnerability of the heart to AF induction and maintenance (AF begets AF)[21]. In particular, structural remodeling is characterized by atrial enlargement and tissue fibrosis. The presence of interstitial fibrosis leading to changes in cellular coupling results in spatial "non-uniform anisotropic" impulse propagation and is a potential cause of atrial activation abnormalities that may underlie the initiation and perpetuation of re-entrant arrhythmias including AF[22,23]. As AF progresses from paroxysmal to persistent, the atrial substrate becomes increasingly abnormal and displays a more prominent role in maintaining the arrhythmia[1,20,21]. In patients with persistent AF, a better understanding of arrhythmia mechanisms is therefore needed so that ablation approaches can be targeted to a clearly shown mechanism.

LEFT ATRIAL ABLATION

Pulmonary vein antral isolation

Despite the evolution of left atrial ablation strategies, PVAI remains the cornerstone of in both paroxysmal and persistent AF ablation procedures[1,2]. Isolation of wide circumferential areas around both ipsilateral pulmonary veins (PVs) with verification of conduction block is more effective than isolation of each individual PV using a segmental approach[4]. A lower success rate of PVAI as a stand-alone strategy has been reported in patients with persistent or long-lasting[1,2,5,6]. However, relatively new data on this topic have given contradictory results. The RASTA study have demonstrated that additional substrate modification beyond PVAI including ablation of non-PV triggers and ablation of complex fractionated electrogram sites does not improve single-procedure efficacy in patients with persistent AF[24]. The recently published STAR AF Ⅱ trial has clearly showed that additional substrate modification (fractionated atrial electrograms or linear lesions) following PVAI has no benefit in AF reduction[25]. A high incidence of PV reconnection is similarly observed in patients with and without recurrence of AF[26], suggesting that sustained PV isolation is not required for freedom from clinical recurrence of AF. This finding may be explained by the important substrate modification performed after the circumferential lines. In CONFIRM trial, AF sources were ablated coincidentally in 45% of cases after wide area circumferential ablation and left atrial roof line in persistent AF cases[8]. These data provide an alternative potential explanation for why PVAI treats AF in some patients and not others. Elimination of AF sources may explain why wide-area ablation is more

effective than ostial PV isolation, why AF may not recur in patients whose PVs have reconnected, why non-PV encircling lines or fractionated electrogram ablation may be effective and, potentially, why ablation success correlates with the extent of ablated tissue in persistent AF[8].

Linear lesions

Based on surgical MAZE procedures, linear lesions including roof and mitral isthmus lines have been also adapted in percutaneous left atrial ablation procedures providing additional substrate modification[27]. The additional benefit of linear lesions on top of ostial PV isolation has been prospectively demonstrated[28,29]. However, in these studies an ostial and not a wide circumferential PV isolation was performed. The intrapulmonary region has been also implicated as an important source of PV triggers[30]. In a randomized study, we investigated the efficacy of additional radiofrequency energy delivery in the interpulmonary isthmus following PVAI[31]. A continuous line in the interpulmonary isthmus connecting the anterior and the posterior part of the ipsilateral circumferential line creating a "theta" model (the Greek letter θ) was performed. Although patients with additional energy delivery in the interpulmonary isthmus displayed a better long-term outcome (free from arrhythmia recurrence), this was not statistically significant. Nevertheless, left atrial linear lesions remain technically challenging. Incomplete linear lesions may have a proarrhythmic effect[5,6]. Most atrial tachycardias result from gaps in the ablation lines.

DIRECT SUBSTRATE MODIFICATION STRATEGIES

Voltage mapping: Identification and modification of heterogeneous substrate and local barriers

As previously stated, interstitial fibrosis play a key-role in the pathophysiology of AF[22,23]. Delayed enhancement-cardiac magnetic resonance imaging (DE-CMRI) has demonstrated fibrosis and scarring in the left atrium of patients undergoing AF ablation[32,33]. Among patients with AF undergoing catheter ablation, atrial tissue fibrosis estimated by DE-CMRI has been independently associated with likelihood of recurrent arrhythmia[34,35]. Electroanatomic bipolar voltage mapping has been described to define the relationship between anatomic and electrophysiological abnormalities. Although, specific bipolar and unipolar voltage cut-off values have been reproducibly shown to accurately identify scar and/or fibrosis in the ventricles, data regarding voltage cut-off values in the atria are limited. In preliminary studies, the bipolar voltage cut-off value were set at < 0.05 mV for the identification of atrial scar, partly influenced by the background noise from early electroanatomic mapping systems, and < 0.5 mV for low-voltage regions[36,37]. In a recent study, the global mean left atrial bipolar and unipolar voltage amplitude in SR was 2.83 ± 2.25 and 4.12 ± 2.14 mV, respectively; 95% of all bipolar and unipolar electrograms recorded from the LA were > 0.50 and > 1.57 mV, respectively[38]. There was no difference in the segmental distribution of low-voltage areas between patients with AF and healthy controls. Jadidi *et al*[39] have demonstrated bipolar voltages of 0.63 ± 0.8 in dense DE-CMRI areas, compared with 0.86 ± 0.89 in non DE-MRI areas. These measurements were performed during AF. By using both electroanatomic mapping system and DE-MRI, Spragg *et al*[32] have demonstrated that the mean atrial voltage in areas identified as scar by DE-MRI was 0.39 ± 0.61 mV, while in areas identified as normal by DE-CMRI was 1.38 ± 1.23 mV. In a similar study, a bipolar voltage of < 0.38 ± 0.28 mV was associated with fully scarred atrial myocardium[40]. Kapa *et al*[41] using electroanatomic mapping along with DE-CMRI have shown that a bipolar voltage cut-off of 0.27 mV performed best for delineating scar (sensitivity: 90%, specificity: 83%).

Substrate mapping in patients with postinfarction cardiomyopathy and ventricular tachycardia may involve lowering the voltage cut-off that defines the scar in order to identify "channels" of relative higher voltage within the scar[42,43]. Conducting channels within the unexcitable scar areas (particularly those displaying late potentials) are considered as an appropriate ablation target[43]. In a similar way, scar homogenization may be also performed in left atrium. Rolf *et al*[44] have recently shown that catheter ablation at low voltage areas aiming to homogenize the diseased left atrium in addition to PVAI resulted in better long-term outcomes compared to PVAI alone. Catheter ablation of stable rotors in patients with paroxysmal and persistent AF has given promising results[7-9]. It is quite possible that these stable sources correlate with areas of atrial fibrosis where the site-specific micro-architecture of connective tissue fibres and the remaining myocardial fibres allows reentrant/rotor activation to occur and to sustain. The combination of localizing atrial fibrosis plus mapping of specific functional areas allowing re-entrant/rotor activation may hold promise for catheter based AF substrate modification in the future.

Mapping and ablation of complex fractionated atrial electrograms

Complex fractionated atrial electrograms (CFAEs) are seen in all forms of AF (paroxysmal and persistent). The underlying mechanisms of CFAEs remain controversial. Two leading hypothesis have been proposed for CFAEs formation. First, the "rotor hypothesis" where the rotor encounters heterogeneous substrate (*e.g.,* dispersion of refractoriness), and the CFAEs are the by-product of the reentrant rotor that breaks down at its boundary; the posterior left atrium is a histologically and

electrically complex region, in which firing from the PVs meets regions of functional block due to anisotropic conduction. The atrial signals in the area of this line of block are frequently fractionated[45]. As paroxysmal AF progresses to persistent AF, the progressive fibrotic and microarchitectural changes determine the propagation, collision, and fragmentation of the wave front as it emanates from focal triggers[46]. Second, the "autonomic hypothesis" where CFAEs indicate sites of ganglionated plexi[47,48]. Lin *et al*[48] showed in a canine model that CFAEs can be produced locally at the site where acetylcholine was topically applied. Moreover, CFAEs can be eliminated by ablating the GP at a distance, indicating that activating the "network" of the intrinsic cardiac autonomic nervous system may be a critical element in the formation of CFAEs.

The use of CFAEs has become an important tool in the clinical electrophysiology laboratory to guide catheter ablation of AF sources. However, their clinical significance is questionable. In Nademanee's original report, CFAEs were defined as (1) fractionated electrograms composed of >2 deflections and/or perturbation of the baseline with continuous deflection of a prolonged activation complex; and (2) atrial electrograms with very short cycle length (< 120 milliseconds)[49]. Nademanee *et al*[49] have demonstrated 92% freedom from AF one year after CFAEs ablation without PV isolation after two procedures. This level of success has not been reproduced by other groups. Ablation of CFAE as a stand-alone ablation strategy seems insufficient for the treatment of patients with persistent AF[50,51]. In addition, there are clear data that CFAEs ablation as an adjunct therapy to PVAI does not improve the success rate of left atrial ablation[52]. As previously reported, the recently published STAR AF II trial has clearly showed that additional substrate modification (fractionated atrial electrograms or linear lesions) following PVAI has no benefit in AF reduction[25].

Whether certain subtypes of CFAE, such as those exhibiting continuous fractionation or particular types of activation gradients, are more important than others is not known[53]. Using multipolar catheters and monophasic action potentials (MAPs) to define local activation and repolarization, Narayan *et al*[54] identified four types of CFAEs in human AF: (1) CFAEs with discrete rapid MAPs and pansystolic local activation (8%); (2) CFAEs with discrete MAPs after AF acceleration (8%); (3) CFAEs pattern with distinct MAPs and dissociated superimposed signals consistent with far-field electrograms (67%); and (4) CFAEs pattern without discrete MAPs (17%), consistent with spatial disorganization. CFAEs with discrete MAPs and pansystolic activation had shorter cycle length and lower voltage and trended to have higher dominant frequency than other CFAEs sites. The majority of CFAEs were the result of superimposed far-field atrial activations from overlying atrial structures. In contrast, only a small proportion of CFAEs exhibited rapid, discrete, organized MAP recording activity consistent with an AF driver. Jadidi *et al*[39] have elegantly shown that the distribution of fractionated electrograms is highly variable, depending on direction and rate of activation. Fractionation in sinus rhythm and pacing rhythms mostly resulted from wave collision. All sites with continuous fractionation in AF displayed normal voltage in sinus rhythm, suggesting absence of structural scar[39]. Thus, many fractionated electrograms are functional in nature, and their sites dynamic. The same group of investigators has demonstrated an inverse relationship between fractionated electrograms and atrial fibrosis in persistent atrial fibrillation[55]. Ninety percent of continuous CFAE sites occur at non-delayed-enhancement and patchy delayed-enhancement LA sites. Finally, there are many limitations arising from the definition of CFAEs. Lee *et al*[56] aimed to determine the prevalence and spatial correlation of CFAEs using two definitions: (1) multicomponent/continuous electrograms; and (2) AF cycle length < 120 ms. Multicomponent/continuous electrograms and sites of short CL activity (< 120 ms) identified different atrial regions.

Dominant frequency mapping

Dominant frequency (DF) mapping is aimed at identifying localized sites of high DF during AF[57]. Initial reports using optical mapping systems have identified the presence of localized regions of high-frequency activity demonstrating spatiotemporal periodicity which may act as drivers of AF[19]. Most importantly, the highest DF using spectral analysis of the biatrial electrogram correlates well with the rotor frequency observed during optical mapping[58], suggesting that this technique of signal decomposition and DF analysis may allow identification of reentrant circuits sustaining AF. Sites of high DF typically show rapid periodicity but lack significant fractionation[47]. In paroxysmal AF, the high DF sites are more prevalent within the PVs, whereas in persistent AF, the high DF sites commonly exist within the left atrium[57]. Retrospective analyses have shown that ablation at such high DF sites results in slowing and termination in a significant proportion of paroxysmal AF patients, indicating their role in AF maintenance[57,59,60]. The higher AF recurrence rate in patients with non-ablated high DF sites at the end of the procedure supports the important role of extrapulmonary sites in persistent AF maintenance[60].

Sites showing DFs that were at least 20% higher than their surrounding points were identified as primary and secondary high DF sites[57,59,60]. Two indices from fast Fourier transform (FFT) power spectrum analysis describe the degree of organization within AF: (1) the regularity index, defined as the ratio of the area under the dominant peak in the power spectrum to the total area[47]; and (2) the organization index, a ratio consisting of the sum of the areas under the dominant peak and its harmonics divided by the

total area[61].

High DF sites typically show rapid periodicity but lack significant fractionation[47]. Kumagai *et al*[62] have shown that high DF sites and continuous CFAEs sites overlap in only 14% of mapped areas after PVAI. Lee *et al*[56] have demonstrated a poor direct spatial correlation between sites of multicomponent/continuous electrograms and sites of high DF, with only 23.1% of multicomponent/continuous electrograms sites occurring at the same location as a site of high DF. Spatial analysis confirmed that the vast majority (84%) of the multicomponent/continuous electrograms sites occurred directly adjacent (< 2.5 mm) to a site of high DF[57]. If high DF identifies a focal source, then visual fractionation may represent wave front breakup at the periphery. Stiles *et al*[63] reported similar findings. Correlation between CFAEs and DF was poor. Exploration of their spatial relationship demonstrates CFAEs in areas adjacent to high DF (within 10 mm in 80% and 10-20 mm in 10%). Lin *et al*[64] demonstrated that the most consistent CFAEs activity is observed near maximum DF sites and that the core of the widely distributed continuous CFAEs is correlated with the sites of maximum DF. Maximal fractionated sites are observed in the center or the boundary region of maximum DF sites.

High DF sites have been negatively correlated to the amount of fibrosis, whereas fractionation index was positively correlated with fibrosis in the posterior left atrium[65]. Atrial fibrosis as defined by DE-CMRI have been associated with slower and more organized electrical activity but with lower voltage than healthy atrial areas[55]. As suggested by Koduri *et al*[65], the increased regularity of electrograms (indicated by increased organization index) in the presence of slower activation rates (indicated by lower DFs and higher fractionation index sites) in experimental heart failure models may indicate the presence of regions of underlying fibrosis.

Currently, there are several limitations of DF mapping as an ablation strategy. These factors include lack of high-resolution mapping to precisely locate DF sites, real-time analysis of DF, and spatiotemporal stability of DF sites[66].

HOW TO PERFORM SUBSTRATE MODIFICATION IN PERSISTENT AF ON TOP OF PVAI?

Summarizing the above data, it's clear that the additional substrate modification involving linear lesions and CFAEs sites in patients with persistent AF is debatable. Based on the recent findings by Narayan's group[7-9], elimination of AF sources (principally rotors) should be the goal in persistent AF ablation. How can we localize these AF sources with current diagnostic modalities? A combined approach using voltage, CFAEs and DF mapping may be helpful for this purpose. Voltage mapping may assist in the identification of fibrotic areas. Tanaka *et al*[67] have demonstrated that the largest fibrotic patches and the PV ostia are potential anchoring sites for "micro-anatomical" reentry. In their experiments, the fibrillatory activity is maintained by intramural reentry centered on fibrotic patches and that it appeared at the posterior left atrial wall as breakthroughs. The average area of fibrosis in the periphery is significantly larger than in the center. Differences in voltage amplitude may be important to identify relatively healthy areas within the patchy fibrotic tissue. For this purpose, upper and the lower voltage thresholds have to be decreased in decrements.

Stable rotors display the higher DF and possibly drive AF[18,19,57,58]. Furthermore, the single rotor is usually consistent with organized AF electrograms without fractionation[68]. It is therefore quite possible that rotors are located at relatively "healthy islands" within the patchy fibrosis. This is supported by the fact that high DF sites have been negatively correlated to the amount of fibrosis[65]. This assumption also explains why CFAEs are the by-product of the reentrant rotor that breaks down at its fibrotic boundaries[67]. As previously reported, correlation between CFAEs and high DF sites is poor. CFAEs are located in areas adjacent to high DF (within 10 mm)[62]. Regularity index showed that fractionation is low within the area with the maximum DF and high within a band of approximately 3 mm at boundaries with lower-frequency domains[47]. CFAEs mapping has to be therefore performed with great caution. Only CFAEs with a discrete MAP should be targeted for ablation[53]. Of note, these are low voltage and high DF sites[53]. In conclusion, areas with relatively higher voltage compared to the surrounding tissue displaying the maximum DF along with high organization index are potential targets of ablation. Prospective studies are required to validate the efficacy of substrate modification in left atrial ablation outcomes.

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EDITORIAL

Coronary artery disease in type 2 diabetes mellitus: Recent treatment strategies and future perspectives

Ryo Naito, Takatoshi Kasai

Ryo Naito, Department of Cardiology, Juntendo University Graduate School of Medicine, Tokyo 113-8421, Japan
Takatoshi Kasai, Cardio-Respiratory Sleep Medicine, Department of Cardiology, Juntendo University Graduate School of Medicine, Tokyo 113-8421, Japan
Author contributions: All authors contributed equally to this editorial.
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Correspondence to: Takatoshi Kasai, MD, Cardio-Respiratory Sleep Medicine, Department of Cardiology, Juntendo University Graduate School of Medicine, 2-1-1 Hongo Bunkyo-ku, Tokyo 113-8421, Japan. kasai-t@mx6.nisiq.net
Telephone: +81-3-38133111
Fax: +81-3-56890627
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Abstract

Patients with type 2 diabetes mellitus (T2DM) are at a higher risk of developing coronary artery disease (CAD) than are non-T2DM patients. Moreover, the clinical outcomes in CAD with T2DM are poor despite improvements in medications and other interventions. Coronary artery bypass grafting is superior to percutaneous coronary intervention in treating multivessel coronary artery disease in diabetic patients. However, selecting a revascularization strategy depends not only on the lesion complexity but also on the patient's medical history and comorbidities. Additionally, comprehensive risk management with medical and non-pharmacological therapies is important, as is confirmation regarding whether the risk-management strategies are being appropriately achieved. Furthermore, non-pharmacological interventions using exercise and diet during the earlier stages of glucose metabolism abnormalities, such as impaired glucose tolerance, might be beneficial in preventing the development or progression of T2DM and in reducing the occurrence of cardiovascular events.

Key words: Diabetes; Comprehensive risk management; Multivessel disease; Drug-eluting stents; Percutaneous coronary intervention

Core tip: Clinical outcomes in coronary artery disease with type 2 diabetes mellitus (T2DM) are poor despite improvements in medications and other interventions. Although coronary artery bypass grafting is superior to percutaneous coronary intervention in multivessel coronary artery disease with T2DM, selecting the revascularization strategy depends not only on the lesion complexity but also on the patient's medical history and comorbidities. In these patients, comprehensive risk management with medical and non-pharmacological therapies is indispensable, and confirming whether such risk management is being appropriately achieved is also important. Furthermore, interventions with exercise and diet therapy during the early stages of glucose abnormalities might be effective in preventing the development or progression of T2DM and in reducing the occurrence of cardiovascular events.

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INTRODUCTION

Patients with type 2 diabetes mellitus (T2DM) have a higher risk of developing coronary artery disease (CAD) than do patients without T2DM[1]. Additionally, 75% of T2DM patients die as a consequence of cardiovascular diseases, including CAD[2]. In patients with T2DM, CAD tends to be a more complex disease characterized by small, diffuse, calcified, multivessel involvement [multivessel disease (MVD)][3,4] and often requires coronary revascularization in addition to optimal medical therapy to control angina[5]. Regarding coronary revascularization, recent advances in the techniques and devices used during percutaneous coronary intervention (PCI) have expanded the indication of PCI to more complex lesions[6-8]. In particular, drug-eluting stents (DES) have reduced the restenosis and repeat revascularization rates[9,10]. However, the morbidity and mortality of CAD in patients with T2DM continues to be high, even in the current DES era[11]. Although most clinical trials comparing outcomes among T2DM patients with MVD have shown that coronary artery bypass grafting (CABG) was superior to PCI in terms of repeat revascularization and the incidence of myocardial infarction and mortality[12-17] (Table 1), it is not feasible to perform CABG in all diabetic patients with MVD. Because CABG is highly invasive in contrast to PCI, selecting a revascularization therapy depends not only on the lesion complexity but also on a patient's medical history and comorbidities. SYNTAX score is a reliable score to assess coronary anatomical features and lesion complexity[16]. EuroSCORE is also a useful scoring system that is based on the clinical background information of an individual patient, which might predict the operative mortality for patients undergoing cardiac surgery[18]. Recently, revised versions of these two scoring systems were proposed. Because combining the SYNTAX score and other clinical variables have been demonstrated to be more accurate in identifying the risk of patients with complex CAD compared with the SYNTAX score alone, the SYNTAX score Ⅱ was constructed, which included the original SYNTAX score and the following variables: the presence of unprotected left main CAD, female gender, chronic obstructive pulmonary disease, age and left ventricular ejection fraction[19]. Similarly, EuroSCORE Ⅱ is an updated version of the original EuroSCORE, reconstructed from a large database of 22381 consecutive patients undergoing cardiac surgery in 43 countries in 2010 using a logistic regression model[20]. These scoring systems may provide additional and reliable information to better decide revascularization strategies. In clinical trials, higher-risk surgical patients, such as the elderly and those with more comorbid diseases, have been excluded. Therefore, selecting a revascularization therapy for CAD with T2DM requires a thorough discussion of the patient's coronary anatomical features and lesion characteristics, age, and comorbid conditions.

Considering this issue, several important and as yet unresolved questions are raised including the following: (1) whether the newer DES are superior or similar in terms of repeat revascularization, incidence of myocardial infarction and mortality; (2) what can be done in conjunction with optimal medical and revascularization therapy to improve patient outcomes; and (3) whether early detection and intervention for CAD patients with undiagnosed T2DM or impaired glucose tolerance may improve mortality. In this editorial, we aim to provide novel insights into each of these specific questions and to consider the directions for future research.

REVASCULARIZATION THERAPY-THE POTENTIAL OF NEWER DRUG-ELUTING STENTS AND BIORESORBABLE VASCULAR SCAFFOLDS

First, it is essential to understand what types of outcome measures were used in clinical trials to evaluate the effectiveness of a given revascularization strategy or to determine the superiority of one revascularization therapy over another. Clinical trials for cardiovascular diseases often use a composite assessment of major adverse cardiovascular events as outcome measures including all-cause mortality, myocardial infarction, stroke and repeat revascularization. Because death and myocardial infarction are considered to be hard and preferably primary endpoints, whereas repeat revascularization is a less hard and secondary endpoint according to the severity of each case, the primary and secondary endpoints should be treated as two distinct endpoints.

Advances in PCI have prompted the selection of this procedure in more complex lesions that previously had been indicated for CABG. However, MVD in T2DM patients is associated with a high incidence of repeat revascularization after PCI with DES; therefore, CABG remains superior to PCI in such lesions. A meta-analysis has demonstrated that the superiority of CABG to PCI with balloon angioplasty or bare metal stents in terms of all-cause mortality was greater in patients with than without T2DM[21].

To date, several clinical trials have been conducted at 85 centers in the United States and Europe to compare CABG and PCI with DES. The SYNergy between percutaneous coronary intervention with TAXus and cardiac surgery (SYNTAX) was a prospective randomized trial that compared the efficacy of CABG and PCI with paclitaxel-eluting stents (PES) for patients with de-novo left main

Table 1 Clinical trials of percutaneous coronary intervention with coronary artery bypass grafting in diabetic patients

Trial	Type of trial years of recruitment	Number of study population	Type of PCI	Endpoint	Main results (PCI *vs* CABG)
ARTS I [12]	Randomized 1997-1998	208	BMS	1 yr freedom from death, stroke, MI or revascularization)	63.4% *vs* 84.4% ($P < 0.001$)
MASS II [13]	Randomized 1995-2000	115	N/A	1 yr death	5.3% *vs* 6.8% ($P = 0.5$)
BARI-2D[14]	Randomized	1605	1st DES: 34.7%	5 yr freedom from death, MI, repeat revascularization	PCI *vs* medical (77.0 *vs* 78.9; $P = 0.15$)
	Comparison between revascularization and medical 2001-2005		BMS: 56.0%		CABG *vs* medical (77.6% *vs* 69.5%; $P = 0.01$)
			Others: 9.3%		P for interaction 0.002
CARDIa[15]	Randomized 2002-2007	510	1st DES: 61% BMS: 31%	1 yr death, stroke, or MI	13.0% *vs* 10.5% ($P = 0.39$)
SYNTAX[16]	Randomized 2005-2007	452	1st DES	5 yr death, stroke, MI, or revascularization	46.5% *vs* 29.0% ($P < 0.001$)
FREEDOM[17]	Randomized 2005-2010	1900	1st DES	5 yr death	16.3% *vs* 10.9% ($P = 0.049$)
				5 yr death, nonfatal MI, or nonfatal stroke	26.6% *vs* 18.7% ($P = 0.005$)

PCI: Percutaneous coronary intervention; CABG: Coronary artery bypass grafting; DES: Drug-eluting stent; ARTS: Arterial revasucularization Therapies Study; BMS: Bare metal stent; MACE: Major adverse cardiovascular event; MI: Myocardial infarction; MASS: Medicine, Angioplasty, or Surgery Study; 1st DES: First generation DES.

coronary disease, three-vessel disease or both, which were considered equally suitable for CABG or PCI by both a cardiac surgeon and an interventional cardiologist at each center[22]. In the trial, 452 (25.1%) of the study population patients were diabetic, and these patients were included in a pre-specified sub-analysis. For 3-year major adverse cardiac and cerebrovascular events in the diabetic cohort, the incidence was 37.0% in the PCI group and 22.9% in the CABG group ($P = 0.002$). The rate of revascularization was also higher in the PCI group (PCI, 28.0% and CABG, 12.9%, $P < 0.001$)[23]. In 2012, a large-scale randomized trial known as the future revascularization evaluation in patients with diabetes mellitus (FREEDOM) trial was conducted. A total of 1900 diabetic patients with MVD were randomly assigned to CABG or to PCI with mainly sirolimus-eluting stents (SES) and PES[17]. The incidence of all-cause mortality and myocardial infarction was significantly lower in the CABG group during the mean follow-up period of 5 years compared with the DES group (CABG, 18.7% *vs* DES, 26.6%). Based on these results, the latest guidelines from the European Cardiology Society for the management of T2DM patients stated that PCI for MVD was a Class IIb indication for relieving symptoms as an alternative to CABG in patients with low SYNTAX scores[24]. However, in the FREEDOM trial, almost all patients in the PCI group were treated with first-generation DES that were replaced by newer-generation DES used in current clinical practice. The newer generation DES have overcome the critical issue of stent thrombosis; in particular, the everolimus-eluting stent (EES) reduced myocardial infarction and stent thrombosis compared with other DES in a meta-analysis[25]. Recently, Bangalore and colleagues reported a meta-analysis of 68 randomized clinical trials to compare clinical outcomes in CAD patients with T2DM between those who received CABG and DES, including SES, PES and EES[26]. All-cause mortality was higher in the patients who received SES and PES compared with CABG, whereas the mortality rates in the EES group were similar to those of the CABG group (reference rate ratio to CABG, 1.31, 95%CI: 0.74-2.29). These results should be carefully interpreted because they were generated from an indirect comparison of individual clinical trials. Ongoing randomized trials in evaluation of the Xience Prime or Xience V stents *vs* coronary artery bypass surgery for the effectiveness of left main revascularization (EXCEL) and bypass surgery *vs* everolimus-eluting stent implantation for approaching multivessel disease (BEST) aim to determine the effectiveness of EES. EXCEL is a randomized trial comparing EES and CABG in patients with left main trunk lesions and SYNTAX scores of 32 or less. The BEST trial aims to compare EES and CABG in MVD. In both trials, a sub-analysis for diabetic patients is intended.

Regarding other novel devices, bioresorbable vascular scaffolds (BVS) may be a candidate treatment of CAD in diabetic patients. BVS are novel intra-coronary devices that have potential advantages over metallic DES in terms of adverse coronary events such as stent thrombosis because unlike metallic DES, no uncovered struts or polymers exist after the scaffolds are resorbed[27]. To date, only a single clinical study has reported on the efficacy of BVS in diabetic patients. Muramatsu *et al*[27] compared BVS and EES in diabetic patients using different clinical trials of each device and reported that the incidence of the clinical outcome, which was a composite of cardiac death, target vessel MI, or ischemia-driven target lesion revascularization, was similar between BVS and EES in diabetic patients (3.9% for the BVS *vs* 6.4% for EES, $P = 0.38$)[28].

As described by the authors, the data analysis was performed using different pooled data and the study population number was quite small (n = 102 in the BVS group and 172 in the EES group). Further studies in a larger cohort of diabetic patients are required to demonstrate the safety and efficacy of BVS.

COMPREHENSIVE RISK MANAGEMENT AND INTERVENTIONS

Because clinical outcomes in T2DM patients with CAD are poor, aggressive medical and non-pharmacological therapies are indispensable, regardless of the revascularization strategy pursued. The bypass angioplasty revascularization investigation in type 2 diabetes (BARI-2D) trial examined and compared long-term clinical outcomes between medical therapy alone and revascularization by PCI or CABG in T2DM patients[14]. No significant difference was observed between the PCI and CABG groups in all-cause mortality or in the event-free survival rates for cardiovascular events during the 5-year follow-up period. These data indicated the importance of comprehensive risk management with glycemic control and the administration of statins, angiotensin receptor blockers, angiotensin converting enzyme inhibitors and antiplatelet therapy in T2DM patients with CAD[21]. Guidelines for the management of diabetes mellitus from the American Diabetes Association, the American College of Cardiology and the American Heart Association recommend the following prevention strategies for CAD: blood pressure 130/80 mmHg or less, low-density lipoprotein cholesterol (LDL-C) below 100 mg/dL (below 70 mg/dL for CAD patients) and prompt smoking cessation[29-31]. However, a previous study examining the achievement of risk management in the large-scale clinical trials of clinical outcomes utilizing revascularization and aggressive drug evaluation (COURAGE), BARI-2D and FREEDOM, showed unexpectedly low achievement rates[32]. One-year risk management achievement rates (LDL-C < 100 mg/dL (70 mg/dL in the FREEDOM trial), systolic blood pressure < 130 mmHg, glycated hemoglobin < 7.0% and smoking cessation) were 18%, 23% and 8% in the COURAGE, BARI-2D and FREEDOM trials, respectively. Although the achievement rate was not originally included in the clinical trial endpoints, these results prompted us to review our clinical practices regarding not only adherence to evidence-based medical therapy but also whether risk management is being properly achieved. Furthermore, non-pharmacotherapeutic strategies including exercise, diet and smoking cessation should be pursued.

INTERVENTIONS FOR IMPAIRED GLUCOSE TOLERANCE

Considering that patients with T2DM tend to have macro- and microvascular complications and that the clinical outcomes of CAD patients are poor, interventions are desirable during the earlier stages of T2DM, such as impaired glucose tolerance (IGT). We understand that IGT is not simply an early stage of T2DM but rather an important state predisposing to T2DM. In fact, progression to diabetes was observed in 10% of IGT patients[33]. Additionally, it was suggested that IGT itself might have an impact on CAD morbidity and mortality[34]. However, it is not fully elucidated whether IGT in CAD patients might be a treatment target for secondary prevention the effects of anti-diabetic agents on reducing progression to diabetes or the incidence of cardiovascular events in such patients. Nevertheless, non-pharmacological therapies such as nutrition and exercise are important even in IGT patients. Previous studies reported that about one-third of CAD patients who had not been diagnosed with diabetes were actually diabetic[35,36]. Thus, aggressive evaluation for diabetes and IGT are required in CAD patients. In current clinical practice, although fasting blood glucose and glycated hemoglobin diabetes testing is routinely performed, the glucose tolerance test is not frequently performed in CAD patients unless the fasting blood glucose or glycated hemoglobin levels are above the upper limits of normal. To detect diabetes at an earlier stage, blood glucose, glycated hemoglobin and glucose tolerance tests for diabetes are considerably important.

CONCLUSION

When selecting revascularization strategies in diabetic patients, physicians must thoroughly consider not only a patient's coronary artery lesions but also his/her medical history. Additionally, comprehensive risk management with medical and non-pharmacological therapies should be performed and the proper achievement of risk management should be confirmed. Furthermore, non-pharmacological interventions through exercise and diet therapy during the earlier stages of glucose metabolism abnormalities such as IGT may also be beneficial in preventing the development or progression of T2DM and in reducing the occurrence of cardiovascular events by either primary or secondary prevention of CAD.

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EDITORIAL

Making cardiomyocytes with your chemistry set: Small molecule-induced cardiogenesis in somatic cells

Woong-Hee Kim, Da-Woon Jung, Darren Reece Williams

Woong-Hee Kim, Da-Woon Jung, Darren Reece Williams, New Drug Targets Laboratory, School of Life Sciences, Gwangju Institute of Science and Technology, Gwangju 500-712, South Korea

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Correspondence to: Darren Reece Williams, PhD, New Drug Targets Laboratory, School of Life Sciences, Gwangju Institute of Science and Technology, Gwangju 500-712, South Korea. darren@gist.ac.kr
Telephone: +82-62-7152509
Fax: +82-62-7152484

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Abstract

Cell transplantation is an attractive potential therapy for heart diseases. For example, myocardial infarction (MI) is a leading cause of mortality in many countries. Numerous medical interventions have been developed to stabilize patients with MI and, although this has increased survival rates, there is currently no clinically approved method to reverse the loss of cardiac muscle cells (cardiomyocytes) that accompanies this disease. Cell transplantation has been proposed as a method to replace cardiomyocytes, but a safe and reliable source of cardiogenic cells is required. An ideal source would be the patients' own somatic tissue cells, which could be converted into cardiogenic cells and transplanted into the site of MI. However, these are difficult to produce in large quantities and standardized protocols to produce cardiac cells would be advantageous for the research community. To achieve these research goals, small molecules represent attractive tools to control cell behavior. In this editorial, we introduce the use of small molecules in stem cell research and summarize their application to the induction of cardiogenesis in non-cardiac cells. Exciting new developments in this field are discussed, which we hope will encourage cardiac stem cell biologists to further consider employing small molecules in their culture protocols.

Key words: Cardiogenesis; Cell reprogramming; Somatic cells; Small molecules; Cardiovascular disease

Core tip: There are a plethora of methods to manipulate the phenotype of somatic cells and convert them into different cell types, such as cardiac cells. The use of small molecules provides numerous advantages, such as ease of use, tight temporal control and reversible effects on target proteins. Significantly, the production of small molecules is cheap and synthesis can be readily standardized. This would allow non-specialist stem cell laboratories to readily adopt small molecule-based methods to produce functional cardiac cells from multiple

cell sources, including therapeutic applications requiring the somatic cells of patients with cardiovascular disease.

Kim WH, Jung DW, Williams DR. Making cardiomyocytes with your chemistry set: Small molecule-induced cardiogenesis in somatic cells. *World J Cardiol* 2015; 7(3): 125-133 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i3/125.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i3.125

WHY SHOULD CARDIOMYOCYTES BE PRODUCED USING NON-CARDIAC CELLS?

Heart contraction is produced by cardiomyocytes, which comprise the cardiac muscle cell population. It was thought that the heart is a refractory organ that is incapable of replacing cardiomyocytes that are lost by normal tissue damage or cardiac disease. However, over the past decade this view has been challenged by numerous studies indicating that the heart can regenerate cardiomyocytes; at least with a capacity to replace those cells lost by regular tissue turnover[1]. Unfortunately, this regenerative capacity is significantly lower compared to skeletal muscle. Thus, major disease insults, such as myocardial infarction (MI), result in an irreversible, catastrophic loss of cardiomyocytes. Typically, MI results in the death of around 20% of the total cardiomyocytes population in the heart. The ventricle is a major site of cardiomyocyte death, with billions of dead cells being eventually replaced by fibrous scar tissue[2-4]. Acute MI produces significant mortality (for example, 36% fatality in the United Kingdom, over the years 2002-2010[5]). For those patients that survive, the poor capacity of heart regeneration means that many are predisposed to eventually develop clinical heart failure[2,6,7].

It can be envisaged that one potential therapy for preventing the progression of MI to heart failure would be the transplantation of functional cardiomyocytes to the infarction site. This cell therapy approach has been demonstrated as an achievable cure for degenerative diseases, such as the transplantation of hematopoietic multipotent stem cells to treat certain types of leukemia[8]. Cardiac cell therapy could prevent progression to heart failure by allowing functional recovery of the heart. Most cell therapy approaches involving cardiomyocyte transplantation aim to treat the consequences of MI, because of the significant impact of this disease on human health.

Cell therapy approaches have been developed to treat cardiac dysfunction, such as MI (reviewed in[9]). Multiple strategies exist for cell delivery and cell source. For example, different types of stem cells have been used for transplantation, including mesenchymal stem cells or cardiac stem cells. More differentiated cells have also been utilized, such as skeletal muscle cells and cardiomyocytes. Unfortunately, the results of clinical trials have only shown a modest improvement after MI. One approach to improve the outcome of cell therapy for MI would be the development of an ideal, optimized cell type for transplantation. This would also require the development of a rigorous, defined experimental methodology to ensure quality control for the cells prior to grafting. However, standardized protocols for culturing transplantable cells are lacking and laboratories tend to develop thief own "in house" techniques and culture media recipes.

The research field of chemical biology is ideally suited to provide reagents that can enhance cell culture and scale-up for transplantation. Chemical biology is a multidisciplinary field that uses chemical "tools" or probes provided by synthetic chemistry to understand and manipulate biological systems[10,11]. These chemical tools are usually small molecules, which are defined as organic compounds with a molecular weight below 800 Daltons. This allows diffusion across the cell membrane and is an upper limit for oral bioavailability[12,13]. A significant example of the contribution of chemical biology to cell research is the generation of induced pluripotent stem cells (iPSCs) from differentiated adult cells[14]. This was originally achieved by overexpressing four "Yamanaka" transcription factors: Oct4, Sox2, c-Myc, and Klf4. Within just a few years, the protocol to produce iPSCs was optimized and simplified by chemical biologists. It was shown that iPSCs could be generated by expressing only the Oct4 transcription factor and a two-step combination of small molecule inhibitors of cell signaling pathways and gene regulatory mechanisms[13]. This is an important example of the ability of small molecules to substitute for transcription factors that have a global influence on genes regulating cell differentiation. Small molecules also possess significant advantages compared to other technologies for controlling cell phenotype, such as genetic methods. In the following section, we briefly discuss these advantages and provide examples of the application of small molecules to the derivation of cardiogenic cells for cell therapy.

Why use small molecules to control cardiogenesis?

Small molecules allow flexibility over the manipulation of the target protein[13,15]. This is not always possible with alternative approaches, such as genetic manipulation. In addition, the effects of small molecule treatment are usually reversible. This means that the target protein can be manipulated with relatively precise timing. An additional level of control can be achieved by fine-tuning the treatment concentration. Small molecules do not always modulate a single protein target in cells; they can produce multiple effects by binding to different protein classes. An example is the molecule BIX-01294, which inhibits different histone-modifying enzymes[16]. Thus, different

small molecules can act synergistically to produce multiple effects, with the potential to produce dramatic changes in cell phenotype. The structural diversity of small molecule libraries developed exponentially in the 1990s, due to advances in chemical synthesis, such as combinatorial chemistry and diversity-orientated synthesis[17,18]. This greater diversity increases the potential to control molecular interactions with target proteins. Small molecules also provide logistical advantages for researchers. Compared to protein or nucleic acid reagents, they are cheap to produce, simple to store in the laboratory and more amenable to quality control. Small molecule-based methods do have some disadvantages, such as the potential for off-target effects on other proteins possessing similar structural elements. Notwithstanding, small molecules have become prominently used in stem cell biology and regenerative medicine, including the production of cardiomyocytes or cardiogenic stem cells[3,15,19]. Next, we discuss some prominent examples of small molecule-based strategies for cardiac cell therapy.

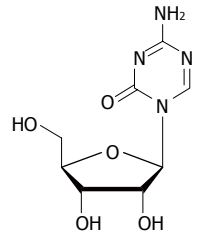
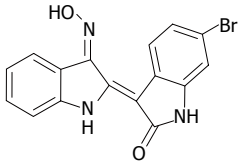
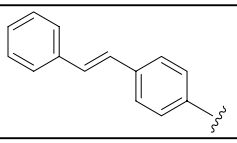
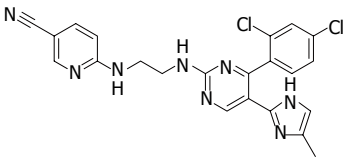
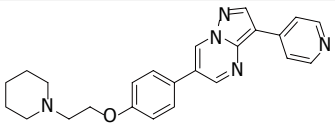
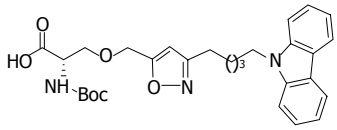
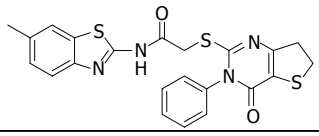
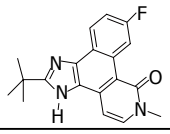
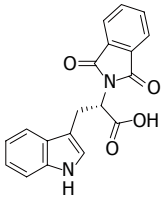
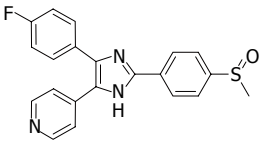
Due to the major health impact of cardiac disease, many small molecules have been developed to simplify the generation of cardiomyocytes or enhance the production of cardiogenic stem cells for potential cell therapy. A selection of these small molecules is shown in Figure 1. The use of small molecules to generate cardiomyocytes can be traced back to 1982, with the discovery that the small organic molecule, DMSO, could induce cardiomyocytes differentiation in murine teratocarcinoma-derived embryonic stem cells[20]. However, it was the development of cell therapy applications for cardiac diseases, such as MI in the 1990s[21] that spurred the discovery of bioactive compounds for enhancing cardiogenesis.

A significant advance came with the report that a small molecule inhibitor of DNA methylation, 5-azacytidine, could induce cardiomyocyte differentiation in murine bone marrow stromal cells[22]. DNA methylation is an epigenetic modification that regulates global gene expression patterns. Thus, it was demonstrated that a small molecule could modulate the epigenetic status of the cell genome to make it amenable to differentiation when exposed to a cardiogenic environment, such as transplantation into to heart or exposure to cardiomyocyte differentiation factors. A follow-up study showed that 5-azacytidine could also induce cardiomyocyte differentiation in embryonic stem cells (ESCs)[23]. Consequently, numerous studies focused on the application of small molecules to derive cardiomyocytes from ESCs. Prominent examples included the discovery of cardiogenol, which activates the Wnt cell signaling pathway and chromatin remodeling enzymes[24]; dorsomorphin, which inhibits the bone morphogenetic (BMP) signaling pathway[25]; and a series of isoxazoyl serines, which act as peroxisome activated proliferation receptor (PPAR) agonists[26] (Figure 1).

These small molecules are not only useful as tools to induce cardiomyocyte differentiation. Characterizing their biological activity also gives insights into the cellular mechanisms that regulate the differentiation process. For example, the pivotal role of the Wnt signaling pathway was confirmed in a screening study for small molecule inducers of cardiogenesis[27]. Cardiogenesis is initiated in ESCs by the formation of embryonic bodies that induce formation of the mesodermal lineage, from which cardiomyocytes are eventually derived[28]. Small molecule screening at 6 d after embryoid formation revealed that Wnt signaling inhibitors significantly enhance cardiomyocyte differentiation, confirming the important role of this signaling pathway in cardiogenesis. This finding also contrasts with the known importance of Wnt signaling for mesodermal induction at the earlier stage of cardiogenesis[28]. This was confirmed in a study which used the Wnt activating molecule, BIO (Figure 1), at the embryoid body stage to increase the number of beating cells after differentiation[29]. Another interesting finding was that inactivation of the mitogen-activated protein kinase (MAPK) signaling pathway by small molecule SB203580 (Figure 1) enhanced cardiomyocyte differentiation from ESCs[30]. SB203580 was treated to embryoid bodies at 24 h after formation, indicating the important role of the MAPK pathway in maintaining the undifferentiated cell state and inhibiting cardiogenesis.

The development of iPSCs (described above) also provided extra impetus to develop small molecule-based methods to induce cardiogenesis, because iPSCs circumvent the ethical and technical problems associated with using ESCs[31]. In addition, iPSCs can be derived from somatic cells, which offers an opportunity to derive cardiac cells from differentiated cells residing in non-cardiac tissues. An interesting example of a small molecule based approach to induce cardiac differentiation in iPSCs utilizes JAK inhibitor-1 (Figure 1), which blocks signaling by janus protein tyrosine kinase and repression of the JAK-STAT pathway (the major alternative second messenger system in cells)[32]. The usefulness of this approach is that it bypasses the need to induce fully reprogrammed iPSCs from somatic cells. Application of JAK-1 during the iPSC generation step blocked the acquisition of full "stemness" and produced a cell population that could be efficiently induced to form functional cardiomyocytes by culture in chemically defined cardiogenic media. Impressively, 100% of the treated cells underwent spontaneous contractions and the protocol was significantly faster than alternative methods, such as the forced expression of master cardiogenic transcription factors[33].

Pluripotent stem cells, such as ESCs and iPSCs, are not the only cell source that has been used for small molecule-induced cardiogenesis. Cells from a diverse range of tissues have been demonstrated to be amenable to cardiomyocyte differentiation (Figure 2). These tissue-specific stem and precursor

Name	Structure	Mechanism	Ref.
5-azacytidine		DNA methyltransferase inhibitor	[22,23]
BIO		Wnt signaling pathway activator	[29]
Cardiogenol		Wnt signaling pathway activator	[24,54]
CHIR99021		Wnt signaling pathway activator	[48]
DMSO		Unknown (possible scavenger of hydroxyl radicals or modulates protein conformation)	[20]
Dorsomorphin		BMP signaling inhibitor	[25]
Isoxazolyl serine		Peroxisome proliferator activated receptor activator	[26]
IWP2		Wnt signaling pathway inhibitor	[48]
JAK inhibitor-1		JAK-STAT pathway inhibitor	[32]
RG108		DNA methyltransferase inhibitor	[35]
SB203580		MAPK signaling pathway activator	[30]

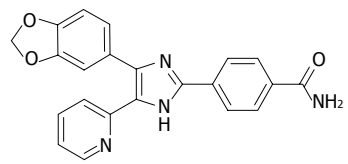
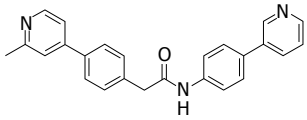
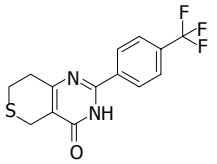
SB431542		TGF-β signaling pathway activator	[38]
Wnt C-59		Wnt signaling pathway inhibitor	[41]
XAV939		Wnt signaling pathway inhibitor	[48]

Figure 1 Selected small molecules that are used to regulate cardiogenesis (in alphabetical order). BMP: Bone morphogenetic; MAPK: Mitogen-activated protein kinase; TGF-β: Transforming growth factor-β.

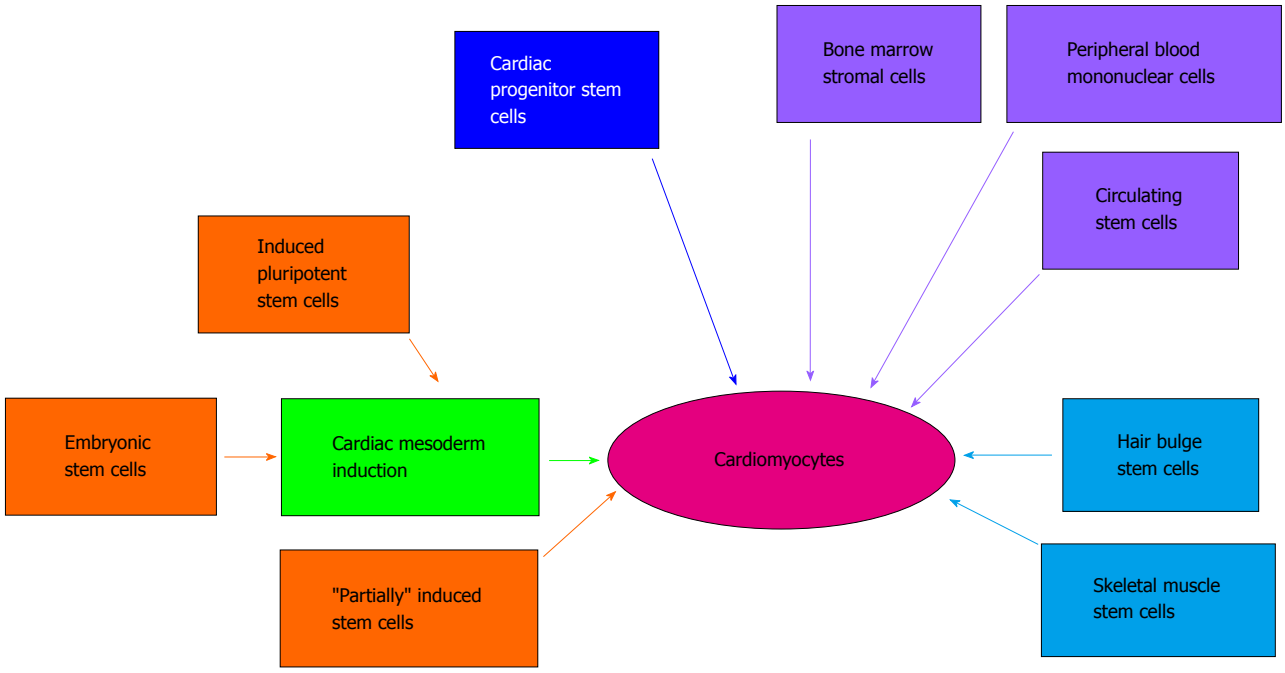


Figure 2 Pathways of small molecule-mediated cardiomyocyte production. It is now established that cardiomyocyte differentiation can be induced in multiple cells types using small molecule-based approaches. Embryonic stem cells and induced pluripotent stem cells are typically induced to undergo cardiac mesoderm differentiation before culture conditions are switched to cardiomyocyte differentiation media. "Partially" induced stem cells are somatic cells that were transfected with induced pluripotent stem cells reprogramming factors and then treated with the small molecule, JAK inhibitor-1 (as described in the text).

cells possess different levels of potency, such as skeletal muscle stems (unipotent) or bone marrow stromal cells (multipotent)[34]. These approaches may be useful for patient-specific cell therapy, because tissues such as skeletal muscle and blood are readily assessable. As an example of this small molecule-based approach, the DNA methyltransferase inhibitor RG108 was successfully used to convert skeletal muscle stem cells into a pluripotent state. These cells could then be induced to form embryoid bodies and undergo cardiogenic differentiation[35]. Significantly, transplantation of these cells could improve cardiac performance and reduce scarring in animal models of MI. Interestingly, a stem cell population has also been found to reside at the base of hair follicles (bulge stem cells)[36] and treatment with the small molecule Wnt pathway activator, cardiogenol C, induced expression of cardiomyocyte markers in these cells[37]. Thus, there are multiple "paths" to generate cardiac cells from diverse tissue types, which are facilitated by small molecule treatments (Figure 2). Moreover, small molecule methods can significantly enhance genetic-based approaches for cardiogenesis in somatic cells. For example, compound SB431542 (Figure

1), which inhibits the transforming growth factor-β (TGF-β) can produce a five-fold increase in the direct reprogramming of fibroblasts into cardiomyocytes using master cardiomyocyte transcription factors[38].

It can also be observed that, over time, these small molecule-based approaches are being optimized and simplified by the research community to allow easier derivation of cardiogenic cells. An important development in this regard is the development of chemically defined culture media for cardiogenic differentiation. This is important because serum should be eliminated from cell therapy applications. Serum supplies can suffer from batch variability and contain unspecified growth factors that may interfere with the differentiation process. Serum may also contain xenoantigens or infectious agents, which may induce an immune response in the host after transplantation[39]. To address this problem, a recently published study describes the development of a small molecule-based protocol to induce cardiomyocytes from bone marrow stem cells[40]. The development of these chemically-defined cardiogenic media cocktails are discussed in more detail below.

Latest progress in small molecule mediated cardiogenesis: Development of chemically defined induction media

A recently published study indicates the rapid progress that has been made for developing small molecule-based methods for cardiomyocyte differentiation. The study by Burridge *et al*[41] represents an impressively detailed investigation to define an optimized, chemically defined protocol for cardiomyocyte differentiation from human iPSCs. Their previous protocol for cardiomyocyte induction required the supplement B27, which is a complex mixture of 21 components. Some of these components are derived from animals, which necessitated the need to develop a chemically defined cardiogenic media. Interestingly, the iPSCs used in this study were also generated using a chemically defined methodology: human primary fibroblasts were transduced/transfected with the Yamanaka factors and cultured sequentially in the defined E8 and E7 culture media containing the small molecule histone deacetylase inhibitor, sodium butyrate. iPSC colonies could be selected after three weeks. The induction of cardiomyocyte differentiation was rigorously investigated using different combinations of small molecules and matrix scaffolds for cell attachment. Before the onset of cardiogenesis, cells were incubated with the small molecule thiazovivin, which is a selective inhibitor of Rho-associated coiled-coil containing protein kinase (ROCK) that has been shown to facilitate iPSC generation[13,42]. As mentioned above, modulation of the Wnt cell signaling pathway is a crucial aspect of the differentiation process, with positive signaling required for mesoderm specification and inhibition required for subsequent cardiomyocyte differentiation. A wide range of small molecule Wnt pathway modulators was tested to find the optimal combination for cardiac induction. Cardiomyocyte differentiation was monitored by measuring expression of the early cardiomyocyte marker gene, troponin T (TNNT)[43]. Interestingly, different small molecule Wnt pathway activators showed markedly diverse effects on cardiogenesis. Of six activators tested, only two [CHIR99021 and BIO (Figure 1)] facilitated the production of TNNT expressing cells without causing cell death. This finding emphasizes the need to compare small molecules that target identical pathways to eliminate the potential for significant off-target effects.

Further optimization of this cardiomyocyte differentiation protocol involved comparison of small molecule inhibitors of various signaling pathways (such as BMP or TGF-β) during the mesoderm induction step and Wnt signaling inhibition during the later cardiomyocyte differentiation step. Remarkably, these optimizations led to the development of a simplified induction protocol that could produce 95% TNNT positive contractile cardiomyocytes, with around 100 cells being derived from a single human iPSC. This protocol is based on the CDM3 media, which contains the chemically defined RPMI-1640 base media and is supplemented with just two reagents: l-ascorbic acid 2-phosphate and human recombinant albumin. iPSCs are cultured with CDM3 plus the Wnt pathway activator, CHIR99021 for two days, followed by two days incubation with CDM3 plus the small molecule Wnt pathway inhibitor, Wnt C-59 (Figure 1). Impressively, cardiomyocyte differentiation was observed just 96 h after this step, using additional incubation with CDM3 media alone. The cardiomyocyte phenotype was assessed by nanopillar-based microscopic observation and it was observed that the majority of cardiomyocytes (> 60%) were ventricular-like compared to atrial-like, with no nodal cardiomyocytes being observed. This observation is important, because new strategies to induce cardiomyocyte differentiation aim to produce specific, adult cardiomyocyte subtypes. The role of small molecules in these differentiation strategies are discussed in the next section.

New small molecule-based methods to induce epicardial cells for facilitating regeneration

As mentioned in the introduction for this editorial, cell therapy approaches for degenerative diseases require a high quality source of purified, functional cells for transplantation. This is also relevant for other applications, such as disease modeling, developmental studies and cell-based drug screening[44]. Early small molecule-based methods for inducing cardiogenesis, such as treatment with 5-azacytidine, were found to produce mixed populations of cells containing approximately 30% cardiomyocytes[22]. As described above, recent developments in producing chemically defined conditions for cardiogenesis from stem cells

allows the derivation of cell populations containing up to 95% cardiomyocytes[41]. However, the heart comprises multiple cell types and cardiomyocytes account for only around 30% of total cells[45]. Therefore, for applications such as modeling cardiac development or cell therapy approaches for MI, it would be useful to generate these non-cardiomyocyte cell types. This is especially relevant for the epicardial cell type, because it is known that epicardial tissue plays a pivotal role in both cardiac development and cardiomyocyte regeneration after disease[46,47]. A recently published study demonstrates that it is indeed possible to derive epicardial cells from iPSCs, with small molecules being used as "control switches" to regulate cell differentiation potential[48].

Epicardial cells can be defined by their cobblestone morphology, specific gene marker expression [Wilms tumor protein (WT1) and T-box 18 (TBX18)] and the ability to synthesize retinoic acid[49-51]. In this study, human ESCs were induced to form embryoid bodies and undergo mesoderm formation by treatment with BMP4 alone for one day, followed by treatment with combined BMP, fibroblast growth factors (FGF) and activin A (a TGF pathway stimulator). Crucially, after 96 h the TGF pathway was inhibited with the small molecule SB431542 (Figure 2). This small molecule was removed after 48 h to facilitate the derivation of cardiomyocytes. In an attempt to block this cardiomyocyte differentiation and allow epicardial cells to be generated, the authors of this study focused on manipulating the Wnt signaling pathway after the addition of BMP. Such precise manipulation of Wnt signaling within this time window of differentiation could be achieved using small molecules. The effects of small molecule Wnt inhibitors (XAV939 and IWP2) were compared with a small molecule activator (CHIR99021). The effect of BMP-mediated signaling on differentiation was assessed using the small molecule inhibitor, dorsomorphin. The use of these small molecule combinations showed that BMP signaling has no effect on the canonical Wnt signaling pathway. Most significantly, it was observed that maintaining Wnt signaling using CHIR99021 allowed the derivation of epicardial lineage cells, as assessed by gene marker expression, morphological characteristics, differentiation into vascular or smooth muscle cells and the ability to synthesize retinoic acid[48]. The population of cells expressing the epicardial marker WT1 could be expanded to account for over 95% of the differentiated cell population, with one mesodermal lineage cell at day 4 of differentiation producing 4-5 epicardial cells. This study provides a high profile example of the advantages that small molecule methodologies offer to cell biologists. In this study, small molecules were used with precise timing to derive a valuable cardiac cell population which has been of great interest to investigators of heart development and regeneration after injury.

CONCLUSION

In this editorial, we have described some prominent examples of small molecule-based methods to facilitate the production of cardiac cells from diverse cell sources. We have shown that small molecules have numerous advantages as tools to control cell differentiation. They can be viewed as cheap, simple and reliable "switches" that provide rapid and reversible control of key cell signaling pathways. Our editorial has focused on the generation of cardiomyocytes from non-cardiac cells, but small molecules are also used in many other areas of cardiac regeneration research. Examples include improving the survival of cardiac tissue grafts, inducing cardiomyocyte dedifferentiation/proliferation and activating endogenous cardiac progenitor cells[3,52]. In addition, the impact of small molecule approaches is shown in the recent demonstration that iPSCs can be derived from somatic cells using just these chemicals alone, *i.e.,* without the need for expressing reprogramming transcription factors[53]. This was achieved using a stepwise protocol requiring only seven small molecules: RepSox (a TGF signaling pathway inhibitor), PD0325901 (a MAPK pathway inhibitor), CHIR99021 (a Wnt pathway activator), TTNPB (a retinoic acid analog), 3 deazaneplanocin-A (which reduces histone methylation), valproic acid (which increases histone acetylation) and forskolin (which increases protein kinase A signaling). Remarkably, the efficiency of iPSC generation using this small molecule method was similar to that achieved using the reprogramming factors. It can be envisaged that this iPSC method could be joined with the chemically defined, small molecule method for cardiomyocyte differentiation, described above. Theoretically, this would allow the derivation of cardiac cells from almost any somatic cell source in the body.

Overall, we hope that this editorial has provided convincing evidence of the many advantages of using small molecules in biological research and cardiac regenerative medicine in particular. Chemical biologists continue to develop new bioactive small molecules or optimize the structures of existing compounds, to improve specificity and/or lower effective concentration. In concert with this research effort, cell biologists are also discovering new applications for known bioactive molecule or developing novel small molecule cocktails to manipulate cell behavior. Therefore, it seems likely that even more diverse and exciting progress is the field of cardiac regeneration can be achieved using small molecules.

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REVIEW

Role of *Helicobacter pylori* infection in pathogenesis of atherosclerosis

Rajesh Vijayvergiya, Ramalingam Vadivelu

Rajesh Vijayvergiya, Ramalingam Vadivelu, Department of Cardiology, Advanced Cardiac Centre, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India
Author contributions: Both the authors have contributed equally to this review article; Vijayvergiya R has done literature search, critical review and analysis of the literature and a revision of the manuscript; and Vadivelu R has performed literature search and review, articles extraction, writing of first draft of the manuscript.

Correspondence to: Rajesh Vijayvergiya, MD, DM, FSCAI, FISES, FACC, Additional Professor, Department of Cardiology, Advanced Cardiac Centre, Post Graduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India. rajeshvijay999@hotmail.com
Telephone: +91-172-2756518
Fax: +91-172-2744401
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Abstract

Though a century old hypothesis, infection as a cause for atherosclerosis is still a debatable issue. Epidemiological and clinical studies had shown a possible association but inhomogeneity in the study population and study methods along with potential confounders have yielded conflicting results. Infection triggers a chronic inflammatory state which along with other mechanisms such as dyslipidemia, hyper-homocysteinemia, hypercoagulability, impaired glucose metabolism and endothelial dysfunction, contribute in pathogenesis of atherosclerosis. Studies have shown a positive relations between Cytotoxic associated gene-A positive strains of *Helicobacter pylori* and vascular diseases such as coronary artery disease and stroke. Infection mediated genetic modulation is a new emerging theory in this regard. Further large scale studies on infection and atherosclerosis focusing on multiple pathogenetic mechanisms may help in refining our knowledge in this aspect.

Key words: Atherosclerosis; Coronary artery disease; *Helicobacter pylori* ; Infection; Stroke

Core tip: Though a century old hypothesis, infection as a cause of atherosclerosis is still a debatable issue. Clinical and epidemiological studies had shown a possible association, however in-homogeneity in the study population and methodology has yielded conflicting results. We performed a literature search on MEDLINE electronic database using keywords such as *Helicobacter pylori* (*H. pylori*), infection, atherosclerosis, coronary artery disease, myocardial infarction, stroke, cerebro-vascular disease and peripheral arterial disease using MeSH terms, to review this subject. The association between *H. pylori* and atherosclerosis is not strong and a causal role is not yet established. Large scale studies on infection and atherosclerosis focusing on multiple pathogenetic mechanisms may help in refining our knowledge in this aspect.

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INTRODUCTION

Though a century old hypothesis, infection is still debated as a cause of atherosclerosis[1]. Infection triggers a chronic inflammatory state which along with other mechanisms such as dyslipidemia, hyperhomocysteinaemia, hypercoagulability, impaired glucose metabolism and endothelial dysfunction contribute in pathogenesis of atherosclerosis. Studies have shown a positive relations between Cytotoxic associated gene-A (Cag-A) positive *Helicobacter pylori* (*H. pylori*) strains with vascular diseases such as coronary artery disease (CAD) and stroke. Infection mediated genetic modulation is a new emerging theory in this regard. Minick and Fabricant's work on infection and atherosclerosis in animal model had made the ground for revolutionary research in this field[2,3]. Chronic infection triggers T1 Helper cell (Th1) mediated inflammatory reaction, which plays a crucial role in atherosclerosis. Markers of infection and inflammation were also studied as the risk factors for atherosclerosis[4-6]. An association between infection and atherosclerosis was established following detection of infectious agents from arterial vessels, positive immunehistochemistry studies, detection of microbial DNA sequences in atherosclerotic plaques by PCR method, positive serological response with higher titres in infected patients, and a positive correlation of infection with atherosclerotic burden and dyslipidaemia[7-23]. The microbial agents that have been implicated in the etiopathogenesis of atherosclerosis are presented in Table 1, Figure 1.

This review has been divided into two parts. Part Ⅰ elucidates different mechanisms of *H. pylori* related atherosclerosis and relevant studies. Part Ⅱ reviews the literature about *H. pylori* association with atherosclerotic diseases such as CAD, stroke and peripheral arterial disease (PAD).

MECHANISMS OF *H. PYLORI* RELATED ATHEROSCLEROSIS

Development of CAD in patients without conventional risk factors suggests a possible role of an additional unexplored mechanism. The evolution of atherosclerosis in the background of chronic inflammatory milieu involves multiple pathways (Table 2, Figure 1). Some of these pathways will be discussed in following section.

H. pylori and endothelial dysfunction

Infection related chronic vascular inflammation can result in endothelial dysfunction. Tousoulis *et al*[24] first proposed an inflammatory mechanism for endothelial dysfunction. C-reactive protein (CRP) and inflammatory adhesion molecule such as intracellular adhesion molecule-1 (ICAM-1) are elevated in patients with *H. pylori* infection, suggesting a possible link between infection and endothelial dysfunction[25].

Table 1 Microbial agents associated with atherosclerosis

Bacteria	Viruses
Chlamydia pneumonia	H simplex virus type 1 and 2
Helicobacter pylori	Cytomegalovirus
Helicobacter cinaedi	Epstein- Barr virus
Hemophilus influenza	
Mycoplasma pneumonia	

Chronic infection triggers release of inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor-α (TNF-α), which affects microvascular vasomotor functions, resulting into vasoconstriction and endothelial dysfunction. Coskun *et al*[26] studied a possible relation between *H. pylori* infection in children and endothelial dysfunction as a precursor for future atherosclerosis. There was no significant association between *H. pylori* seropositivity and CRP levels with flow mediated vasodilation. Another evidence is about increase prevalence of slow flow in the major epicardial coronary arteries in patients with *H. pylori* infection[27]. The possible mechanism of slow flow was endothelial dysfunction secondary to raised homocysteine levels. *H. pylori* infection causes malabsorption of vitamin B12 and folic acid and thus increases serum homocysteine levels. Evrengul *et al*[27] reported a mean TIMI frame count of coronary flow as 46.3 ± 8.7 and 24.3 ± 2.9 in patients with and without *H. pylori* infection, respectively. An association between *H. pylori* infection and functional vascular disorders such as cardiac syndrome-X, migraine and primary Reynaud phenomenon provides evidence about its role in endothelial dysfunction and atherosclerosis[28-32].

Chronic inflammation

Presence of chronic, persistent inflammation provides a vital clue for infectious theory of CAD. Chronic *H. pylori* infection induces a pro-inflammatory state, resulting into an increase in cytokines levels such as TNF-α, Interleukins (IL-1, IL-6, IL-8), gamma interferon, coagulant factors - fibrinogen, thrombin and soluble adhesion molecules such as intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1)[33-35]. Eradication of *H. pylori* infection by use of antibiotics leads to reduction in cytokines levels[34,36]. These evidences suggest that *H. pylori* induced inflammatory cascade plays an active role in atherosclerosis. Activated T lymphocytes and macrophages following cytokines release induce proliferation of smooth muscle cells and extracellular matrix, which plays a crucial role in pathogenesis of atherosclerosis. It also stimulates metalloproteinases production, which causes rupture of atheroma cap and leads to acute coronary syndromes. However, a large population based study failed to support the association between *H. pylori* and increased inflammatory cytokines[37].

Recent research has unveiled novel molecular

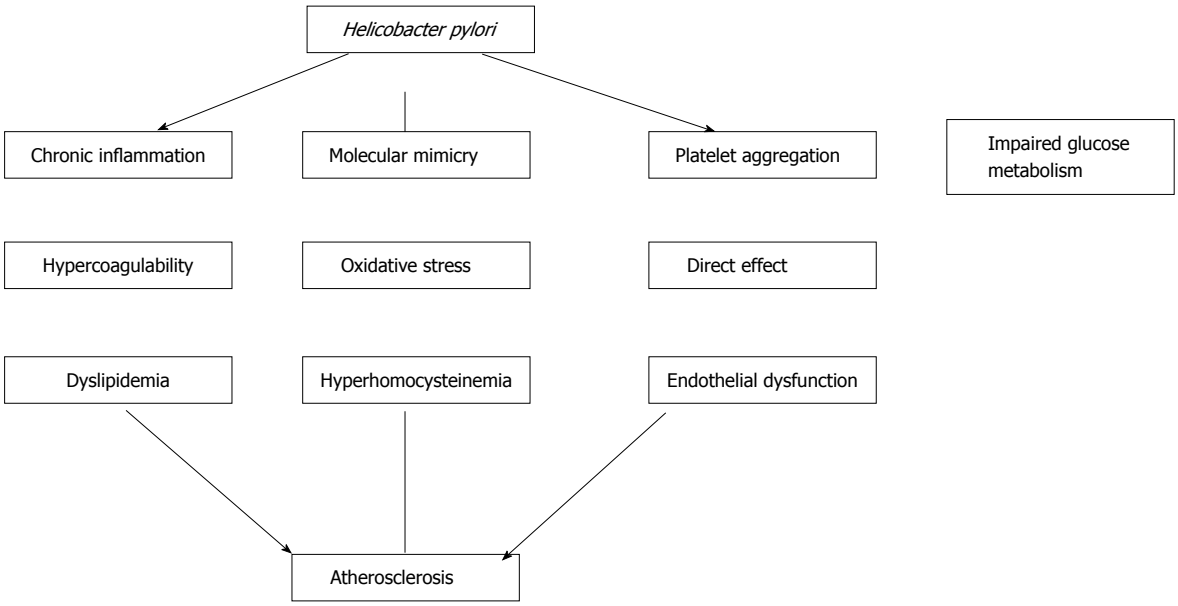

Figure 1 Theories of infection related atherosclerosis.

Table 2 Mechanisms of *Helicobacter pylori* related atherosclerosis

Induction of inflammatory response secondary to chronic infectious state
Endothelial damage
Chronic low grade activation of coagulation cascade
Dysregulation of lipid metabolism resulting in increased total cholesterol and triglyceride levels and reduced high density lipoprotein levels
Hyperhomocysteinaemia

While the proponents support the possible association[52,70,104], the opponents refute this hypothesis[46,71,105].

mechanisms of *H. pylori* mediated inflammation[38-41]. *H. pylori* infection exerts an immune-inflammatory reaction by activating cyclooxygenase enzyme-2 (COX-2), which causes increase production of prostaglandin (PGE_2) and nitric oxide (NO). *H. pylori* cell wall lipopolysaccharide (LPS) triggers toll-like receptor-4, which activates various secondary mediators such as mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase, c-Jun N-terminal kinase (JNK) and p38 kinase resulting in enhanced stimulation of *NOS* and *COX-2* gene expression[38,39]. LPS-induced activation of MAPK cascade is also associated with epidermal growth factor receptor (EGFR) transactivation which is a key protein regulating cellular proliferation, differentiation, migration and modulation of apoptosis[41]. Gherlin, a peptide hormone activates NO synthase, thereby inhibiting *H. pylori* LPS induced activation of COX-2 and other inflammatory pathways[40].

H. pylori and hyper-homocysteinaemia

H. pylori causes atrophic gastritis, which is associated with malabsorption of vitamin B12 and folic acid. Deficiency of these vitamins causes hyper-homocysteinaemia due to interruption of re-methylation pathway[42-45]. Hence, it may have a role in the pathogenesis of premature atherosclerosis[45]. In a study by Kutluana *et al*[45], carotid intima media thickness was found to be higher in patients with *H. pylori* related atrophic gastritis. In this study, *H. pylori* positive patients had significantly higher homocysteine levels compared to controls (14.17 ± 9.24 μmol/L *vs* 9.81 ± 3.42 μmol/L, $P = 0.01$). Senmaru *et al*[46] reported a higher prevalence of CAD in atrophic gastritis (5.8% *vs* 2.8%). Torisu *et al*[47] had shown an association between increased pulse wave velocity, a preclinical marker of atherosclerosis with atrophic gastritis. Apart from hyper-homocysteinaemia, other mechanisms are reduced ghrelin levels and induction of chronic pro-inflammatory cascade resulting into endothelial damage[46,47]. However, Bloemenkamp *et al*[48] did not support the hypothesis about *H. pylori* infection induced hyper-homocysteinemia and atherosclerosis.

H. pylori and dyslipidemia

H. pylori infection is associated with lower HDL cholesterol (HDL-C) and higher total cholesterol (TC), LDL cholesterol (LDL-C) and triglyceride levels. Higher apolipoprotein-B and lower apolipoprotein-A (apo-A) levels were also reported[11]. Murray *et al*[49] demonstrated that women with *H. pylori* infection had lower HDL-C ($P = 0.006$). Another study had also shown significantly lower HDL-C levels in

infected patients[11]. Niemelä *et al*[50] and Laurila *et al*[22] reported an increase triglyceride levels in *H. pylori* positive patients. These alterations in lipid homeostasis proved to be significant even after adjusting co-variables such as socioeconomic class, body weight, age and diabetic status[22,51]. de Luis *et al*[52] showed that eradication of *H. pylori* decreases apo-A and increases HDL-C. Other studies had also shown reduction in TC, LDL-C levels and increase in HDL-C, apo-AI and apo-AII levels following *H. pylori* eradication[53-55]. However, this association was not supported by few other authors[56-59].

H. pylori, impaired glucose metabolism and metabolic syndrome

Gillum *et al*[60] reported a significant association of *H. pylori* seropositivity with CAD in diabetic males. de Luis *et al*[51] showed that CAD and cerebrovascular diseases were significantly more seen in *H. pylori* infected diabetic patients. Yoshikawa *et al*[61] suggested that *H. pylori* seropositivity increases brachial-ankle pulse wave velocity, a marker of atherosclerosis, in patients with impaired glucose metabolism. Aydemir *et al*[62] reported that *H. pylori* positive subjects had higher homeostatic model assessment-insulin resistance (HOMA-IR) levels (2.56 ± 1.54 *vs* 1.73 ± 1.1, $P < 0.05$), a surrogate of insulin resistance, as compared to *H. pylori* negative controls. Aslan *et al*[63] had shown that paraoxanase, a marker of oxidative stress is well correlated with HOMA-IR levels and is significantly elevated in *H. pylori* positive patients. Regarding role of *H. pylori* eradication therapy in improvement of glucose tolerance, Gen *et al*[64] reported that HOMA-IR level significantly reduced after successful therapy, whereas Park *et al*[65] did not show any significant reduction. Polyzos *et al*[66] in his systematic review concluded that available evidences indicate a potential association between *H. pylori* infection and insulin resistance. Gunji *et al*[67] reported that *H. pylori* infection was significantly and independently associated with metabolic syndrome. A recent study by Ando *et al*[68] revealed that eradication of *H. pylori* increases circulating adiponectin levels and might be helpful in prevention of metabolic syndrome. Naja *et al*[69] suggested no association between *H. pylori* infection and metabolic syndrome or impaired glucose tolerance.

H. pylori, hypertension and arterial stiffness

Migneco *et al*[70] demonstrated a significant reduction in blood pressure after eradication of *H. pylori* in hypertensive subjects. The possible association of *H. pylori* with arterial stiffness was initially reported by Adachi and Yoshikawa. Adachi *et al*[71] reported that carotid pulse wave velocity was higher in seropositive subjects. Yoshikawa *et al*[61] similarly reported a higher brachial-ankle pulse wave velocity in seropositive patients with impaired glucose metabolism. The possible association of *H. pylori* and arterial stiffness tends to be more in younger subjects, whereas in the elderly arterial stiffness is more often due to aging[72]. Honda *et al*[73] demonstrated that *H. pylori* infection did not affect the age related progression of arteriosclerosis over a 4 years follow-up period.

EVIDENCE OF ASSOCIATION BETWEEN *H. PYLORI* AND ATHEROSCLEROSIS

H. pylori and CAD

Demonstration of an association between *H. pylori* and CAD is always challenging. Both conditions are more prevalent in the population, increases with age and are related to socioeconomic status. The following section reviews the evidence of *H. pylori* association with CAD.

Numerous studies have shown that CAD patients have a higher prevalence of *H. pylori* infection[74-77]. Vijayvergiya *et al*[77] demonstrated that CAD patients had higher IgG seropositivity as compared to controls (42% *vs* 23%, $P = 0.06$). Franceschi *et al*[78] found that *H. pylori* Cag-A was significantly associated with acute coronary events (OR = 1.34; 95%CI: 1.15-1.58, $P = 0.0003$). Niemelä *et al*[50] showed that the association between CAD and *H. pylori* infection was not strong. A meta-analysis revealed that there is a little association between *H. pylori* infection and stroke, but the strength of association was greater for Cag-A positive strains[79]. *H. pylori* was shown to be associated with premature CAD even in patients without conventional cardiovascular risk factors[80,81]. A number of studies had shown a negative association between *H. pylori* and CAD which include serological[82,83] and histological studies[84-86]. A negative association is even reported in long term follow-up studies[87]. The Australian Busselton health study comprising of 1612 healthy subjects demonstrated negative association between infection and CAD or stroke[88]. Danesh *et al*[89] in his meta-analysis of five prospective studies reported no significant association of *H. pylori* infection with CAD (RR = 1.13). Association of *H. pylori* infection and outcome of CAD treatment had also been studied. Schiele *et al*[90] found that *H. pylori* infection was not a risk factor for restenosis after percutaneous coronary angioplasty. Limnell *et al*[91] had shown an inverse relationship between *H. pylori* infection and coronary bypass graft occlusion. Results from Caerphilly heart disease study suggested that Cag-A seropositivity had no relations with CAD or CAD related mortality[92].

H. pylori has been associated with cardiac syndrome X, *i.e.*, angina pectoris with normal epicardial coronaries[28-30]. The proposed mechanism is chronic endothelial dysfunction. Eskandrian *et al*[28] reported a higher prevalence of *H. pylori* positivity in syndrome X patients compared to controls (95% *vs* 47.5%). Patients with syndrome X were found to be more commonly associated with *H. pylori* Cag-A positivity and elevated IL-1 and TNF-α[93]. Lanza *et al*[94] has

also described association of inflammation, infectious burden and vascular dysfunction. Assadi *et al*[30] reported 15% of patients with syndrome X had urea breath test (UBT) positivity for *H. pylori* while none of the patients with chronic stable angina or controls had UBT positivity.

H. pylori and acute myocardial infarction

H. pylori induced inflammatory reaction is possibly responsible for plaque instability and platelet aggregation in acute coronary syndrome patients. Danesh *et al*[95] demonstrated a higher prevalence of *H. pylori* infection (42% *vs* 24%, OR = 1.75) in young acute myocardial infarction (AMI) survivors. Alkout *et al*[96] showed a higher titre of *H. pylori* IgG titre in patients who died of AMI (151 ng/mL *vs* 88 ng/mL, p= 0.034). Kahan *et al*[97] reported a higher prevalence of *H. pylori* seropositivity in recent myocardial infarction patients as compared to controls (68% *vs* 53%, OR = 1.36). This remained significant even after adjusting for other CAD risk factors like age, sex, smoking and hypertension. Kinjo *et al*[98] suggested that *H. pylori* infection was significantly associated with AMI in younger patients (age < 55 years, OR = 2.7) but not in those with age of > 55 years. Frazer *et al* showed a higher prevalence of *H. pylori* infection in AMI patients compared to control (41.6% *vs* 34.5%; $P = 0.038$)[99].

Similar to CAD, negative associations is also been reported between *H. pylori* and myocardial infarction. Zhu *et al*[100] hypothesised that *H. pylori* infection could not lead to CAD or myocardial infarction. Murray *et al*[101] had shown a negative association between *H. pylori* and risk for myocardial infarction. Pellicano *et al*[102] reported a negative association between cytotoxic *H. pylori* strains and myocardial infarction, with insignificant anti-Cag-A antibody seropositivtiy between cases and controls (33.8% *vs* 26.8%).

H. pylori Cag-A positivity - Is the risk greater?

Cag-A positivity has raised a curiosity in the infectious theory of atherosclerosis. Several studies had shown a significant relationship between Cag-A strain and CAD or stroke. Carriers of Cag-A positive strains had a higher risk for stroke (OR = 2.99) and carotid plaque instability (OR = 8.42)[103]. De Bastiani *et al*[104] showed increased prevalence of Cag-A seropositivity and ischemic stroke. Rasmi *et al*[93] reported a positive relation between Cag-A seropositivity and cardiac syndrome-X. Huang *et al*[105] revealed that Cag-A positive strains enhanced atherosclerosis in CAD patients by modifying oxidised LDL levels and high sensitive C-reactive protein (hsCRP) levels. Kowalski[36] showed that Cag-A positivity was significantly associated with greater coronary artery lumen loss and restenosis after percutaneous coronary artery stenting. He also demonstrated that *H. pylori* eradication significantly attenuate reduction in coronary artery lumen after coronary artery stenting[36]. But various authors had denied the excess risk of Cag-A positive strains with atherosclerosis. Koenig *et al*[106] demonstrated a similar prevalence of Cag-A seropositivity in CAD patients and healthy subjects. Whincup *et al*[107] in his prospective study comprising of 505 patients and 1025 healthy subjects had clearly shown that there was no significant association of seropositivity with CAD. Murray *et al*[101] reported negative association between the virulent *H. pylori* Cag-A strains and acute myocardial infarction.

H. pylori and stroke

By catalysing atherosclerotic pathways, *H. pylori* infection may be a risk factor for ischemic stroke. Single infectious agent is weakly linked to stroke but cumulative chronic infectious exposures, or "infectious burden", have been associated with the risk of stroke. The adjusted hazard ratio demonstrating the risk of association between *H. pylori* and stroke was 1.13, whereas that of infectious burden and stroke was 1.39[108]. The possible mechanisms include macrophage activated plaque destabilization, increased expression of various adhesion molecules and inflammatory cytokines, localized hypercoagulability, altered gene expression, and a molecular mimicry. Markus *et al*[109] found a higher prevalence of *H. pylori* seropositivity in stroke cases compared to controls. There was an association between *H. pylori* infection and large vessel disease and lacunar stroke irrespective of other confounding factors. Another study by Grau *et al*[110] demonstrated an association between *H. pylori* seropositivity and ischemic stroke. Elkind *et al*[111] suggested that that chronic infectious burden results in increase carotid plaque thickness and stroke. A retrospective study reported higher incidence of ischemic stroke in patients with *H. pylori* infection than in non-infected group (14.8 *vs* 8.45 per 1000 person years)[112]. Diomedi *et al*[113] showed that Cag-A positive *H. pylori* infection was associated with poorer short term clinical outcomes and greater carotid intima media thickness in stroke patients. Increased risk of stroke in Cag-A positive *H. pylori* patients may be due to enhanced plaque vulnerability[103,114]. In one of the studies, the positive correlation between *H. pylori* and stroke was confounded by socioeconomic class[115]. A study on chronic bacterial infection and stroke demonstrated that elevated anti- *H. pylori* antibody was not significantly associated with ischemic stroke[116].

H. pylori and peripheral arterial disease

Studies about association of *H. pylori* infection with peripheral arterial disease (PAD) are limited. Bloemenkamp *et al*[117] demonstrated infection as a novel risk factor for PAD in young women. A case control study on infection and PAD in young women suggested that *H. pylori* infection was positively correlated with PAD only in those with high CRP levels[118]. Sawayama *et al*[119] reported a significantly higher prevalence of *H.*

pylori infection in PAD cases than in controls (79.7% *vs* 44.8%; $P < 0.01$).

CONCLUSION

Overall the association between *H. pylori* and CAD is not strong and a causal role is yet to be established. Future studies on larger scale may possibly establish a stronger link between the two. If it gets established, there can be drastic reduction in burden of CAD by managing *H. pylori* infection. Proponents of infectious theory will have a real challenge in the years to come because establishing a definite causal role of *H. pylori* in CAD will be a nightmare due to the existence of numerous confounding factors. Opponents may continue to criticise the infectious theory of CAD because of lack of strong scientific evidence.

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MINIREVIEWS

Palm oil and the heart: A review

Osaretin J Odia, Sandra Ofori, Omosivie Maduka

Osaretin J Odia, Sandra Ofori, Department of Medicine, University of Port Harcourt, Port Harcourt 500001, Nigeria
Omosivie Maduka, Department of Preventive and Social Medicine, University of Port Harcourt, Port Harcourt 500001, Nigeria
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Correspondence to: Dr. Sandra Ofori, FWACP, MSc, Department of Medicine, University of Port Harcourt, Rivers State, Port Harcourt 500001, Nigeria. sandytom77@yahoo.com
Telephone: +234-809-6598508
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Abstract

Palm oil consumption and its effects on serum lipid levels and cardiovascular disease in humans is still a subject of debate. Advocacy groups with varying agenda fuel the controversy. This update intends to identify evidence-based evaluations of the influence of palm oil on serum lipid profile and cardiovascular disease. Furthermore, it suggests a direction for future research. The sources of information were based on a PubMed, Google Scholar, African Journal online and Medline search using key words including: palm oil, palmitic acid, saturated fatty acids and heart disease. Published animal and human experiments on the association of palm oil and its constituents on the serum lipid profile and cardiovascular disease were also explored for relevant information. These papers are reviewed and the available evidence is discussed. Most of the information in mainstream literature is targeted at consumers and food companies with a view to discourage the consumption of palm oil. The main argument against the use of palm oil as an edible oil is the fact that it contains palmitic acid, which is a saturated fatty acid and by extrapolation should give rise to elevated total cholesterol and low-density lipoprotein cholesterol levels. However, there are many scientific studies, both in animals and humans that clearly show that palm oil consumption does not give rise to elevated serum cholesterol levels and that palm oil is not atherogenic. Apart from palmitic acid, palm oil consists of oleic and linoleic acids which are monounsaturated and polyunsaturated respectively. Palm oil also consists of vitamins A and E, which are powerful antioxidants. Palm oil has been scientifically shown to protect the heart and blood vessels from plaques and ischemic injuries. Palm oil consumed as a dietary fat as a part of a healthy balanced diet does not have incremental risk for cardiovascular disease. Little or no additional benefit will be obtained by replacing it with other oils rich in mono or polyunsaturated fatty acids.

Key words: Palm oil; Serum lipid profile; Heart disease; Palmitic acid; Antioxidants

Core tip: With the increase in the prevalence of cardiovascular diseases (CVD) worldwide including developing countries, increasing attention is paid to underlying risk factors. Low-density lipoprotein (LDL) cholesterol is related to CVD in a linear and continous manner and one of the strongest risk factors for CVD. Dietary saturated fat increases LDL. Palm oil contains saturated fat and has thus been touted to be "bad for the heart". However it also contains unsaturated fats and beneficial antioxidants. This review sought to clarify the role of this important source of nutrients (to a

large part of the worlds' population) in CVD.

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INTRODUCTION

In recent times, there has been a running debate mainly in mainstream literature regarding the effects of palm oil consumption on the heart especially in the development of coronary artery disease. Advocacy groups and consumer protection groups drive most of the controversy with conflicting interests and agenda[1]. For thousands of years palm oil has been a major source of cooking oil in many communities in Asia and Africa[2-5].

In 2012, the World Heart Organization listed ischaemic cardiovascular disease (CVD) as the leading cause of death worldwide[6]. The relationship between serum cholesterol and CVD risk is linear and dose dependent with a 20%-25% reduction in the risk of death from CVD and non-fatal MI as low-density lipoprotein (LDL) cholesterol decreases by 1.0 mmol/L[7]. Palm oil consists of various fatty acids and this has been of major concern in discussing the value of palm oil vis-a-vis its relationship to cardiovascular disease[4,8]. The concern about palm oil is mainly because it contains palmitic acid, which is a saturated fatty acid and by extrapolation, using the Keys-Anderson equation which proposes that dietary intake of saturated fat increases serum cholesterol, will give rise to hypercholesterolemia when used as dietary oil[8]. However the main dietary saturated fatty acids (palmitic, stearic, lauric, and myristic acids) have varying effects on serum cholesterol. Saturated fatty acids with 12 and 14 carbon atoms (lauric and myristic acids) increase all the cholesterol fractions more than palmitic acid, and palmitic acid increases all the cholesterol fractions more than stearic acid[9]. On the other hand, oleic and linoleic acids which are unsaturated fatty acids do not have an adverse effect on serum cholesterol[9]. Palm oil has almost equal parts saturated and unsaturated fatty acids. Myristic acid (1%), stearic acid (5%) and palmitic acid (44%) make up the saturated fatty acid component in addition to monounsaturated oleic acid (39%), and polyunsaturated linoleic acid (11%)[10]. Furthermore, palm oil also contains vitamin E, carotenoids and antioxidants that do, at least in theory, protect the heart and also prevent cancer[4,5,11]. This review critically evaluates the scientific literature on palm oil in order to clearly show whether or not the consumption of palm oil indeed adversely alters the serum lipid profile and increases the prevalence of heart disease.

CHEMICAL COMPOSITION OF PALM OIL

The oil palm tree belongs to the genus Elaeis. The palm fruit has a fleshy mesocarp from which palm oil is derived and a seed from which palm kernel oil is derived[10]. These are two different types of oil and this paper is concerned with the former only.

The genus Elaeis has two species: E. guineensis and E. oleifera. The former is found mainly in West Africa, particularly in Nigeria and was propagated to Malaysia, Brazil, and Indonesia by the Portuguese in the 19th century for commercial purposes. E. oleifera originated from South America and is a dwarfish plant[5].

The major constituents of palm oil are triacylglycerols (TG). The glycerol molecule is esterified with three fatty acids. During the process of palm oil extraction from the fleshy mesocarp of the fruit, triacylglycerols attract other fat-soluble cellular components. These include phosphatives, sterols, pigments, tocopherols, tocotrienols, monoglycerols, diglycerol and free fatty acids (FFAs). The fatty acids are aliphatic acids like myristic, palmitic, stearic, linoleic acid. Palm oil also contains vitamins, antioxidants and other phytonutrients[10].

EFFECT OF PALM OIL CONSUMPTION ON SERUM LIPID PROFILE AND THE HEART

Animal studies

Onyeali *et al*[3] studied the influence of a palm oil-laced diet on the plasma lipid profile of Wister albino rats. The experimental animals were given a diet supplemented with 20% palm oil for 12 wk and compared to controls that were fed standard rat feed. They estimated the serum level of total cholesterol (TC), LDL, TG and high-density lipoprotein (HDL) at intervals of 0, 4, 8, and 12 wk. They demonstrated that although in the short term (4 wk) LDL and TC levels increased, sustained intake of the palm oil diet resulted in a significant reduction of the serum TG, TC and LDL levels compared to the control diet by 12 wk. The palm oil diet had no significant effect on HDL. The authors attributed most of these beneficial effects to the high content of antioxidants and vitamin A and E in the palm oil used. Tocotrienol and tocopherol make up 70% and 30% of the vitamin E present in red palm oil respectively[10]. The tocotrienols have been suggested to inhibit HMG CoA reductase enzyme activity and thus regulate serum cholesterol levels[12]. The findings from their study were in keeping with an earlier experiment in which Sulli *et al*[13] demonstrated that the supplementation of diet with α Tocopherol and β carotene (components of palm oil) reduced plasma cholesterol in hypercholesterolemic rabbits after 8 wk. Oluba *et al*[4] in Benin City Nigeria supplemented the diets of male albino Wister rats with palm oil and studied the effect of this on peroxidation of lipids and

activity of gluthathione peroxidase in their livers[4]. They showed clearly that as compared to the rats that were fed 5% cholesterol-diets without palm oil, those that had palm oil supplementation had a significantly reduced rate of lipid peroxidation in the liver. In addition, the activity of glutathione peroxidase increased significantly in the livers of the rats who fed on the supplemented diets. They extrapolated that in atheromatous plaques, oxidative damage induced by lipids could therefore be prevented by diets containing palm oil.

In the heart, ischemic episodes induces cell damage that can be made worse by sudden reperfusion due to the release of oxygen free radicals. Palm oil has been demonstrated to attenuate this effect in animal experiments. During reperfusion in rats that were fed diets supplemented by palm oil compared to control rats that had no supplementation, Tosaki *et al*[14] demonstrated a reduction in the level of oxidatively-modified proteins as well as an attenuation of the increase in free oxygen radicals in the heart. In a similar more recent study, Narang *et al*[15] used an isolated heart model of rats to demonstrate the effect of palm olein in the diet on ischemia reperfusion injury (IRI). Three groups of Wister rats were used. Two groups received different doses of palm olein (5% and 10% respectively). The third was the control group fed a normal diet. Thirty days later, each group was divided in two and each half was made to undergo global ischaemia for twenty minutes followed by reperfusion for 40 min. Following this, the investigators demonstrated that in the rats that were given the 5% olein-supplemented diet, there was an increase in the level of antioxidants in the myocardium but the levels of thiobabituric acid and reactive substance (TBARS) did not change. This was significant when compared to the rats fed the control diet that had significant oxidative injury with no concurrent increase in anti-oxidant activity. They however failed to observe a dose-dependent effect. Their study provided further evidence of the benefit of a palm oil supplemented diet in protecting the heart from oxidative stress and tissue injury following ischaemia-reperfusion. Furthermore, Kruger *et al*[16] clearly demonstrated a reduction in ischemia reperfusion injury in rats that were fed cholesterol rich diets when supplemented with palm oil. Many other studies have confirmed this[17-19].

Although they provide some evidence for the benefits of palm oil, they do not provide evidence of the effects of using palm oil that has been heated repeatedly on serum lipid profile and oxidant-antioxidant balance. It is well known that in parts of the world where palm oil is utilized for domestic cooking, it is reheated several times especially when used as frying oil. Adam *et al*[20] studied the influence of palm oil that had been heated repeatedly (five times) on serum lipid and homocysteine levels as well as peroxidation of lipids in rats. They found that the rats that were fed the heated palm oil had significantly increased lipid peroxidation, total cholesterol and TBARS compared to controls ($P < 0.05$).

These studies are inherently limited by the fact that they were conducted in rat models, which are not generalisable to humans as rats predominantly carry their cholesterol in HDL form[10]. Moreover the natural rat-diet is not fatty acid based further limiting the extrapolation of these results to humans.

Human studies

Palm oil especially as part of an overall low-fat diet has been shown to effectively maintain total cholesterol and lipoprotein cholesterol values. Kesteloot *et al*[21] measured serum lipids and apoproteins in 542 adults living in Nigeria. The subjects used palm oil exclusively as their source of cooking oil. The researchers reported that the subjects had lower cholesterol levels compared to values obtained from black and white Americans at the time.

Peanut oil and olive oil have 52%-60% and 65%-80% of their fatty acid composition as oleic acid respectively. Oleic acid has been demonstrated in several studies to have beneficial effects on serum lipids and cardiovascular disease[22]. These oils are thus recommended as healthier options. However, palm oil has 40% oleic acid. In addition the palmitic acid it contains, has been shown to have similar effects on the serum lipid profile as oleic acid.

Zhang *et al*[23] assessed the effect of palm oil used in Chinese diets in comparison to soya bean oil, peanut oil and lard. They showed that diets containing palm oil significantly reduced the levels of cholesterol in the serum of subjects who had normal serum cholesterol levels at baseline compared to lard but comparable to the effect of the mostly polyunsaturated soybean oil. Even among those who were hypercholesterolemic, palm oil significantly reduced the TC/HDL ratio more than peanut oil as the latter reduces HDL. It is important to note however that the Chinese diet contains less animal protein and cholesterol compared to typical "western" diets. This may have influenced their results, limiting their generalisability.

Ng *et al*[24] demonstrated that the main saturated fat in palm oil, palmitic acid was comparable to oleic acid in terms of its effect on cholesterol and lipoprotein levels in serum, as well as eicosanoids. Oleic acid is the major component of olive oil that is recognized as "heart-healthy" oil[24]. They achieved this by challenging 33 subjects (whose ages ranged between 22 and 41 years) that had normal serum levels of cholesterol with a diet rich in coconut oil for four weeks. Following this, they were given diets rich in palm olein or olive oil with a subsequent crossover after 6 wk. During this time, the only oil the subjects were allowed to use was the test oil group to which they were assigned. The coconut oil containing lauric and myristic fatty acids elevated all the lipoprotein and lipid parameters in

serum significantly. During the crossover periods, the olive oil and palm olein diets did not differ significantly in their effects on all measured lipid parameters. They concluded that in healthy humans with normal serum cholesterol levels, olive oil could be substituted with palm oil without significant changes in lipid profile. Similarly Sundram conducted a cross over study that was double blinded and demonstrated that palm olein and oleic acid were similar in their ability to lower cholesterol levels in serum[25]. An Indian study by Chafoorunissa *et al*[26] reported that groundnut oil and palm olein also have similar effects on cholesterol levels. They both maintain comparatively normal serum cholesterol levels.

In a systematic review and meta-analysis of 51 human dietary intervention trials, the authors compared trials in which palm oil was substituted for diets rich in polyunsaturated fatty acids (PUFAs), stearic acid and monounsaturated fatty acids (MUFAs)[27]. Although serum lipid profile (TC, HDL and LDL cholesterol, apolipoprotein A-I and apolipoprotein B) was beneficially altered with diets containing palm oil compared to myristic and lauric acid, the same was not the case when compared to PUFAs and MUFAs. In young people and those subjects that had overall lower energy intake from fat, this latter finding was not significant. The diets rich in palm oil did not significantly change the TC/HDL or LDL/HDL cholesterol ratios. On the other hand, the palm oil rich diets significantly increased the levels of apolipoprotein A-I and HDL cholesterol and reduced the levels of TC/HDL, triacylglycerols and apolipoprotein B when compared to trans fatty acid-rich diets. They concluded that with regards to usual dietary sources of fat, palm oil was not much different except when it was substituted for trans fat where it proved beneficial. Considering that majority of global fat consumption is in the form of solid fats and the process of converting liquid oils to solid fats involves hydrogenation, which produces trans fats, palm oil has a distinct advantage; it does not require hydrogenation to turn it to solid fats. In this way solid fats made from palm oil are free from trans fats[28].

Dietary fats influence on coronary heart disease risk has traditionally been estimated from their effects on total and LDL cholesterol. Following large epidemiologic studies in the 50's and 60's saturated fats gained a bad reputation in terms of being significantly associated with cardiovascular disease especially coronary heart disease (CHD) and cardiovascular mortality[8,9,22]. Furthermore several meta-analysis and systematic reviews of randomised controlled trials and cohort studies recommended that polyunsaturated fatty acids should substitute saturated fatty acids. This was based on the supposition that this reduces the risk of CHD events and fatal CHD despite the fact that they demonstrated no direct link between saturated fatty acids and CHD death[29-31]. This informed various guideline recommendations to reduce total dietary energy intake from saturated fats in a bid to decrease the prevalence of coronary heart disease[32-34]. A recent meta-analysis has countered this theory as the authors found that a significant relationship did not exist between saturated fat intake and cardiovascular disease (coronary heart disease and stroke)[35]. In patients with established CHD, secondary prevention by means of a reduced fat or modified fat diet (in which saturated fat is substituted by mono- or poly unsaturated fat) is also recommended[34]. However, another recent meta-analysis by Schwingshackl and Hoffmann has shown that this had no significant effect on all-cause mortality and cardiovascular mortality, combined cardiovascular events and myocardial infarction[36]. Furthermore multivariate meta-regression in their study did not reveal significant relationships between changes in saturated fatty acids, monounsaturated and polyunsaturated fatty acids and risk of all-cause or cardiovascular mortality, myocardial infarction and cardiovascular events. It remains important to note however that this meta-analysis included studies that differed in various ways including the protocols of the studies resulting in some heterogeneity. In addition there was publication bias and the quality of evidence was graded as moderate.

CONCLUSION

Taking all the above into consideration, it is known that saturated fat adversely affects lipid profile and raised serum total and low-density lipoprotein cholesterol is associated with cardiovascular risk. However not all saturated fats have this adverse effect. Palmitic acid the main saturated fat in palm oil has a similar effect on lipid profile as the monounsaturated fat oleic acid that is currently recommended. In addition palm oil also contains oleic and linoleic acids, and vitamin E tocotrienols that are powerful antioxidants and inhibit cholesterol synthesis as well[37].

Therefore, in conclusion it is the opinion of the authors that palm oil consumed as a dietary fat as part of a healthy balanced diet does not have incremental risk for cardiovascular disease. Little or no additional benefit will be obtained by replacing it with other oils rich in mono or polyunsaturated fatty acids. We recognize that more longitudinal population-based studies are needed to fully characterize the impact of the consumption of diets, which utilize palm oil compared to other accepted "heart healthy" oils like olive oil on the future risk of heart disease using lipid parameters as intermediate markers of risk.

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ORIGINAL ARTICLE

Retrospective Study

Percutaneous closure of secundum type atrial septal defects: More than 5-year follow-up

Roel JR Snijder, Maarten J Suttorp, Jurriën M Ten Berg, Martijn C Post

Roel JR Snijder, Maarten J Suttorp, Jurriën M Ten Berg, Martijn C Post, Department of Cardiology, St. Antonius Hospital, 3435 CM Nieuwegein, The Netherlands
Author contributions: Snijder RJ, Suttorp MJ, Ten Berg JM and Post MC had substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; Snijder RJ drafted the article; Suttorp MJ, Ten Berg JM and Post MC revised the article critically for important intellectual content; all authors gave final approval for publication.
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Correspondence to: Martijn C Post, MD, PhD, Department of Cardiology, St. Antonius Hospital, Koekoekslaan 1, 3435 CM Nieuwegein, The Netherlands. m.post@antoniusziekenhuis.nl
Telephone: +31-88-3203000
Fax: +31-30-6034420
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Abstract

AIM: To investigate long-term efficacy of two different devices more than five years after percutaneous atrial septal defect (ASD) closure in adults.

METHODS: All patients who underwent percutaneous closure of an ASD in the St. Antonius Hospital, Nieuwegein, The Netherlands, between February 1998 and December 2006 were included. Percutaneous closure took place under general anaesthesia and transesophageal echocardiographic monitoring. Transthoracic echocardiography (TTE) was performed 24 h post-procedure to visualize the device position and to look for residual shunting using color Doppler. All complications were registered. All patients were invited for an outpatient visit and contrast TTE more than 5-years after closure. Efficacy was based on the presence of a residual right-to-left shunt (RLS), graded as minimal, moderate or severe. The presence of a residual left-to-right shunt (LRS) was diagnosed using color Doppler, and was not graded. Descriptive statistics were used for patients' characteristics. Univariate analysis was used to identify predictors for residual shunting.

RESULTS: In total, 104 patients (mean age 45.5 ± 17.1 years) underwent percutaneous ASD closure using an Amplatzer device (ASO) in 76 patients and a Cardioseal/Starflex device (CS/SF) in 28 patients. The mean follow-up was 6.4 ± 3.4 years. Device migration occurred in 4 patients of whom two cases occurred during the index hospitalization (1 ASO, 1 CS/SF). The other 2 cases of device migration occurred during the first 6 mo of follow-up (2 CS/SF). The recurrent thrombo-embolic event rate was similar in both groups: 0.4% per follow-up year. More than 12 mo post-ASD closure and latest follow-up, new-onset supraventricular tachyarrhythmia's occurred in 3.9% and 0% for the ASO and CS/SF group, respectively. The RLS rate at latest follow-up was 17.4% (minimal 10.9%, moderate 2.2%, severe 4.3%) and 45.5% (minimal 27.3%, moderate 18.2%, severe 0%) for the ASO- and CS/SF groups, respectively. There was no residual LRS in both

groups.

CONCLUSION: Percutaneous ASD closure has good long-term safety and efficacy profiles. The residual RLS rate seems to be high more than 5 years after closure, especially in the CS/SF. Residual LRS was not observed.

Key words: Percutaneous intervention; Atrial septal defect; Closure device; Right-to-left interatrial shunt; Left-to-right interatrial shunt; Echocardiography

Core tip: Several atrial septal defect (ASD) closing devices have been proven safe and effective for percutaneous ASD closure. We evaluated long-term (*i.e.*, more than 5-year of follow-up) efficacy of two different devices used in adults. Percutaneous ASD closure seems to be relatively safe using the Amplatzer device. Though, the right-to-left shunt (RLS) rate is high, a residual left-to-right shunt was absent at latest follow up. The Cardioseal/Starflex device appears to be associated with a higher complication- and residual RLS rate. The importance of a residual RLS is unclear. Therefore, long-term follow up might be necessary.

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INTRODUCTION

An atrial septal defect (ASD) is a common cardiac defect and accounts for one third of all congenital heart diseases detected in adults[1,2]. The diagnosis in adults is often made when complications of the shunt occur, such as pulmonary hypertension, heart failure, arrhythmias, or paradoxical embolism. Most of these complications might be prevented by closure.

Since the first description of the transcatheter closure device for an ASD in 1976 by King *et al*[3], percutaneous ASD closure has been practiced and described extensively. Other studies evaluated various ASD closure devices and showed good mid-term (up to 2 years) safety and efficacy profiles[4-7]. However, long-term (*i.e.*, more than 5-year after closure) safety and efficacy data on percutaneous closure devices has not been available yet. We report long-term efficacy and safety of two types of ASD closure devices.

MATERIALS AND METHODS

Population

All patients who underwent a percutaneous closure of an ASD in the St. Antonius Hospital, Nieuwegein, The Netherlands, between February 1998 and December 2006 were included in this study. All patients were invited for an outpatient visit and transthoracic echocardiography (TTE).

Closing procedure

As reported earlier, percutaneous closure took place under general anaesthesia and transesophageal echocardiographic (TEE) monitoring according to standard techniques[4]. TTE was performed 24 h post-procedure to visualize the device position and to look for residual shunting using color Doppler.

Follow-up and complications

Follow-up information was obtained at the outpatient clinic or by a telephone interview. All complications were documented, and divided into major and minor as described by Khairy *et al*[8]. Major complications included procedure related events such as haemorrhage requiring blood transfusion, occurrence of cardiac tamponade, need for procedure-related surgical intervention, massive fatal pulmonary emboli, occurrence of new thrombo-embolic events and death[8].

New-onset supraventricular tachycardia's were diagnosed by routine ECG when patients visited the outpatient clinic or when patients visited the emergency department because of symptoms.

Efficacy

The efficacy of the ASD closure was based on the presence of residual shunting using contrast TTE (cTTE) with Valsalva manoeuvre, and color Doppler. A residual right-to-left shunt (RLS) was present if microbubbles appeared in the left atrium. Opacification of the left ventricle and shunt grade were classified as minimal (maximum of 30 micro-bubbles in left ventricle), moderate (between 30 and 100 bubbles in left ventricle), and large (> 100 micro-bubbles in the left ventricle). This division was based on the maximum number of microbubbles counted in one still frame, as previously reported[9]. The presence of a left-to-right shunt (LRS) was based color Doppler imaging at the atrial septum. The LRS was not graded.

Statistical analysis

Descriptive statistics were used for patients' characteristics. Continuous variables with normal distribution are presented as mean ± SD or median with range if normal distribution was absent. Univariate analysis was used to identify predictors for residual shunting. All statistical analyses were performed using SPSS software (version 22.0 for Windows).

RESULTS

Study population

Percutaneous ASD closure was performed in 104

Table 1 Basline characteristics

Number	104
Age (yr)	45.5 ± 17.1
Female, *n* (%)	78 (75.0)
Weight (kg)	73.1 ± 15.1
Risk factors and co-morbidities (%)	
Arterial hypertension	18.4
Hypercholesterolemia	3.9
Diabetes	1.9
Smoking	18.4
CAD	4.9
History of SVT	26.2
Antithrombotic treatment, *n* (%)	
None	59 (56.7)
Aspirin	20 (19.2)
Dipyridamol	1 (1.0)
Oral anticoagulants	21 (20.2)
Unknown	3 (2.9)
Indication for closure, *n* (%)	
RV volume overload	72 (69.2)
Cryptogenic TIA/stroke	21 (20.2)
Asymptomatic	11 (10.6)
RVSP + CVP (mmHg)[1]	34.6 ± 10.5
ASD diameter (mm)[2]	18.3 ± 6.3
Follow up (yr)	6.4 ± 3.4

Data are presented as mean ± SD. [1]On transthoracic echocardiography; [2]On transesophageal echocardiography. CAD: Coronary artery disease; SVT: Supraventricular arrhythmia; TIA: Transient ischemic attack; RVSP: Right ventricular systolic pressure; CVP: Central venous pressure; ASD: Atrial septal defect.

consecutive patients (75% women; mean age, 45.5 ± 17.1 years). Baseline characteristics, risk factors, co-morbidity and indication for closure are summarized in Table 1.

Less than 12-mo follow-up: Safety and efficacy

Device implantation was initially uneventful in 102 patients (98.1%). In 76 patients (73.1%) an Amplatzer (ASO), and in 28 patients (26.9%) a Cardioseal/Starflex (CS/SF) was used for closure. In total, 4 major complications occurred within the first 6 mo. Two patients (1.9%, 1 ASO, 1 CS/SF) suffered from embolization of the device during the index hospitalization and two (1.9%, 2 CS/SF) within the first 6 mo after closure. All underwent surgical device extraction; the ASD was closed using a patch during the same operation. All patients recovered well. Procedural characteristics are shown in Table 2.

Between 6- and 12 mo, another two patients (1.9%, 2 CS/SF) underwent surgical extraction of the device and the ASD was closed with a patch during the same operation. One patient had a large residual shunt, which could not be closed with a second device. The other patient needed rhythm surgery, therefore, device extraction was performed.

Within the first 12-mo, recurrent thrombo-embolic events occurred in 1 patient (0.9%, 1 CS/SF). This 58-year-old patient suffered a transient ischemic attack (TIA). Because of a history of supraventricular tachyarrhythmia's (SVT) the patient was already using oral anticoagulation. cTTE showed no residual RLS.

Table 2 Procedural characteristics *n* (%)

Devices	
Amplatzer	76 (73.1)
Diameter, mm[1]	25 (12-38)
Cardioseal/starflex	28 (26.9)
Diameter, mm[1]	33 (20-40)
General anaesthesia	104 (100)
TEE guiding	104 (100)
In-hospital complications	
Device embolization	2 (1.9)
New-onset SVT	3 (2.9)
Allergic reaction	1 (1.0)
Fever	1 (1.0)
Groin hematoma	1 (1.0)
Tamponade	1 (1.0)
Shunt by TTE[2]	
Color Doppler	9 (11.4)
Hospital stay, d[1]	2 (2-7)

[1]Data presented as median (range); [2]Data available in 79 patients. TEE: Transesophageal echocardiography; SVT: Supraventricular tachycardia; TTE: Transthoracic echocardiography.

New-onset SVT's occurred in 6.6% of the ASO group and in 17.9% of the CS/SF group.

More than 12-mo follow-up: Efficacy and complications

Contrast TTE was performed in 57 patients (54.8%, 46 ASO and 11 CF/SF). Median follow-up time for the ASO group was 6.6 years (5.0-11.1 years) and the CS/SF group 9.9 years (6.3-13.4 years). Though, cTTE could only be performed in 57 patients, follow-up information was available in a total of 81 patients.

Long-term follow-up data could not be retrieved (interview or cTTE) in 23 patients of which 6 were surgically closed, 4 died (no device related cause was suspected) and 13 were lost to follow-up.

Contrast TTE showed a RLS shunt in eight patients (17.4%) who received an ASO. Of these, five patients (10.9%) had a minimal, one patient (2.2%) a moderate and two patients (4.3%) a severe residual shunt. Five patients who received a CS/SF device (45.5%) had a residual RLS of which three patients (27.3%) had a minimal and two patients (18.2%) a moderate residual RLS. When minimal shunts were excluded, the closure rate was 93.5% for ASO and 81.8% for the CS/SF device, respectively. There was no recurrent LRS at latest follow up. Our analyses showed no significant differences in the diameters of the ASD or the device used between the patients with or without a residual shunt. Secondly, no predictors for a right-to-left shunt at long-term follow-up could be found using univariate analysis.

Recurrent thrombo-embolic events after more than 12 mo of follow-up occurred in two patients (1.9%, 2 ASO). One 40-year-old patient suffered a cerebrovascular accident 2.5 years after ASD closure, while on aspirin because of coronary artery disease.

Table 3 Five years follow-up

	Amplatzer	Cardioseal/STARflex
5 yr follow-up available, *n* (%)	58 (76.3)	23 (82.1)
New-onset SVT		
0-1 yr	5 (6.6)	5 (17.9)
> 1 yr	3 (3.9)	0
Reoccurrence TIA/stroke		
0-1 yr	0	1 (3.6)
> 1 yr	2 (2.6)	0
TTE > 5 yr FU available, *n*	46	11
RLS		
No shunt	38 (82.6)	6 (54.5)
Minimal	5 (10.9)	3 (27.3)
Moderate	1 (2.2)	2 (18.2)
Severe	2 (4.3)	0
LRS	0	0
Follow up (yr)	6.6 (5.0-11.1)	9.9 (6.3-13.4)

SVT: Supraventricular arrhythmia; TIA: Transient ischaemic attack; TTE: Transthoracic echocardiography; FU: Follow-up; RLS: Right-to-left shunt; LRS: Left-to-right shunt.

Although there was no history of SVT or device thrombus, oral anticoagulation was initiated after this event. At long-term follow up, a minimal residual RLS was found. The other patient (48-year-old) was known with a history of multiple TIA's prior to closure and was therefore treated with Aspirin. Despite closure of the ASD and optimal medical treatment, the patient suffered from another TIA more than 5 years after ASD closure. At the long-term follow-up visit no residual shunt or thrombus formation on the device was found. This patient had no history of SVT. In total, 3 patients suffered a recurrent neurological event during a mean follow up of 6.4 years (0.5% per year follow up).

During long-term follow-up, new-onset SVT occurred in 3 patients (3.9%) who received an ASO and in none of the patients who received a CS/SF device.

Long-term residual shunt rate, recurrent thrombo-embolic event rate and new-onset SVT rate are presented in Table 3. Figure 1 shows the percentage of patients with residual right-to-left shunt at more than 5-year follow-up after percutaneous ASD closure.

A flow-chart showing the results of this study during follow-up is presented in Figure 2.

DISCUSSION

Percutaneous ASD closure has relatively good long-term safety- and efficacy profiles, especially using the ASO device. A high residual RLS was present in CS/SF. Residual LRS was not observed in either the ASO- or the CS/SF group.

Complications

Device embolization and dislocation is a well-known complication after percutaneous ASD closure[10,11]. The CS/SF is related to a relatively high embolization rate compared to the ASO[12]. In literature, embolization of the CS/SF has been described between 1.4% and 2.5% and for the ASO between 0.1% and 2.4%[7,10,13,14]. In all studies, embolization occurred during the procedure or the index hospitalization. Kefer *et al*[15] and Masura *et al*[16], described 112 and 151 patients with a mean follow-up of 5 and 6.5 years, respectively, and showed no device embolization using the ASO device.

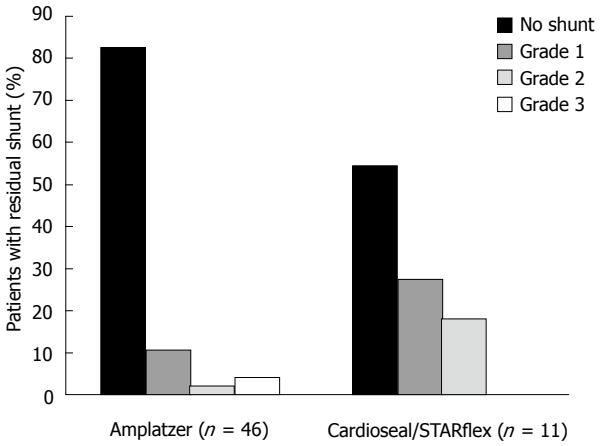

Figure 1 Percentage of patients with residual right-to-left shunt at more than 5-year follow-up after percutaneous atrial septal defect closure.

In our study 4 major complications (3.8%) occurred within the first six months after closure. Device migration occurred in 10.7% of the patients with a CS/SF device and in 1.3% using an ASO device. All devices were surgically extracted and the ASD was closed with a patch. Compared to the literature our CS/SF subgroup had a higher complication rate, while the ASO subgroup was similar. Hence, the CS/SF devices are no longer available for ASD closure. However, long-term follow-up of patients who received a CS/SF device is recommended.

Because device embolization occurred more often in patients with a Cardioseal/Startflex device, we analysed potential reasons/risk factors only for this device. Post *et al*[5] showed that the initial ASD and the device diameter were significantly higher in the patients in whom the device was embolized. However, due to the small sample size of this study it is difficult to make any conclusions.

Recurrent thrombo-embolic events

Kefer *et al*[15] described a recurrent stroke rate of 0% after percutaneous ASD closure with similar devices and follow-up time as in our study. Masura *et al*[16] described no thrombo-embolisms during the entire follow-up period. One patient (0.6% per follow-up year) with an ASO device in the study of Spies *et al*[7] suffered a thrombo-embolic event, which could not be related to a residual shunt or device related thrombus formation.

In our study, the recurrent thrombo-embolic event rate during long-term follow-up was 0.4% per

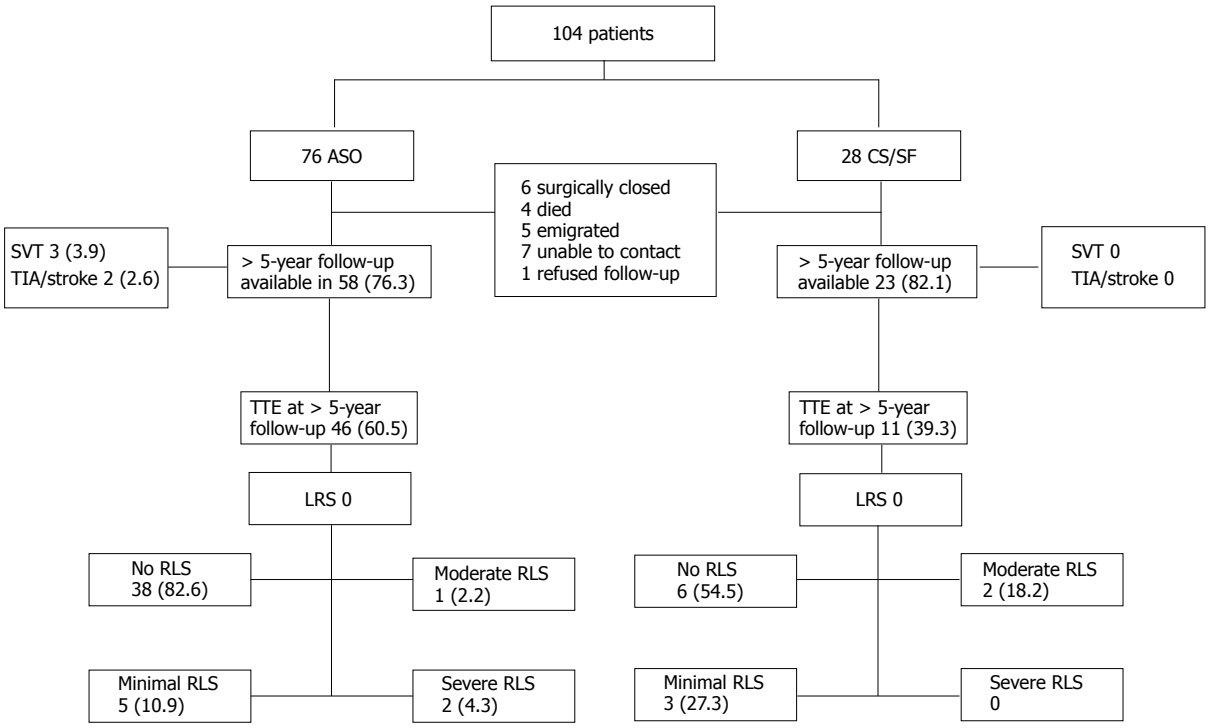

Figure 2 Flow-chart showing the results of this study during follow-up. Data is presented as number of patients (%). ASO: Amplatzer; CS/SF: Cardioseal/Starflex; SVT: Supraventricular arrhythmia; TIA: Transient ischemic attack; TTE: Transthoracic echocardiography; LRS: Left-to-right shunt; RLS: Right-to-left shunt.

follow-up year for both devices, which is similar when compared to the literature. As described above, all patients were treated with anti-platelet therapy or oral anticoagulation and a minimal residual RLS was found in only one patient.

Arrhythmias

arrhythmias early after ASO or other devices implantation are common and extensively described[10,17,18]. Masura *et al*[16] also mentioned SVT's at early follow-up, but none during long-term follow-up. Chessa *et al*[10] noted that arrhythmias are the second most common complication in their study (2.6%) early after the procedure using both the ASO and the CS/SF. Tomar *et al*[19] described peri-procedural arrhythmias but none were seen during long-term follow-up (median 56 mo). At 2-year follow-up, Spies *et al*[18] found an annual incidence of new-onset atrial fibrillation of 4.1%. Butera *et al*[13] described 274 patients (153 ASO, 121 CS/SF) and showed no arrhythmias at follow-up (respectively 16- and 24-mo).

In our study, 3.9% of the patients with an ASO device had new-onset SVT without any abnormalities during cTTE at long-term follow up.

Residual shunting

Residual LRS rates for the ASO has previously been described in several studies and ranged between 0%-12.5% at long-term follow up[13-16,19,20]. Kefer *et al*[15] described a residual LRS rate of 4%. However, only 2 patients (1.8%) with a residual LRS had received an ASO. At 3-year follow-up, Masura *et al*[16] showed no residual shunt using color-Doppler. Butera *et al*[20] described 165 patients with a residual LRS rate of 2% in patients suffering multiple ASD's. At 24-mo follow-up, a non-significant difference between the ASO and CS/SF was found (respectively 0% *vs* 4.4%). In a study by Nugent *et al*[14], 72 patients received a CS/SF device with a total residual LRS of 12.5% between 12- and 24-mo of follow-up.

Our study showed no residual LRS for both devices at more than 5-year of follow-up. However, the prevalence of a RLS is relatively high. Earlier, Luermans *et al*[12] described a residual RLS rate of 14% 3.4 years after closure in 29 patients who received a CS/SF device.

In our study, the RLS rate for the ASO was 17.4% and 45.5% for the CS/SF device more than five years after closure. When excluding the minimal shunts, the ASO had a RLS rate of 6.5% and the CS/SF of 18.2%. The importance of this relatively high rate of RLS is unclear, as the reason for closure was mainly related to the presence of a LRS. Therefore, the fact that we did not notice residual LRS is an important observation. Moreover, to assess the clinical importance of the presence of a RLS, long-term follow-up might be necessary.

The difference in RLS rate after percutaneous ASD closure might be due to the different closing mechanisms; the ASO has a "stent-like" mechanism and consists of Nitinol metal with rounded disks with a polyester fabric sewn inside the meshed disks. The CS/SF device has a "double patch" mechanism and

the fabric is directly exposed to blood[13,21]. The latter might delay the endothelialisation of the devices, which is important for complete closure of the ASD. Why endothelialisation happens in some patients better than others is unclear.

Limitations

Firstly, it is a single-centre design with a small sample-size. Secondly, we used cTTE at follow-up for residual shunt classification while the gold standard is contrast TEE. Though, we did not use contrast TEE, literature describes mostly studies where only color Doppler is used for the assessment of residual shunts. Thirdly, an independent core lab did not review the TTE's. Fourthly, the long-term follow up data was available in about 80% of patients; this might lead to an under- or overestimation.

Percutaneous closure of a secundum-type atrial septal defect seems to be safe using the ASO. Though, the RLS rate is relatively high, a residual LRS is absent more than 5-year after closure. The CS/SF appears to be associated with a relatively high complication- and residual RLS rate. Because of the unclear importance of a RLS after percutaneous ASD closure, long-term follow-up might be necessary.

COMMENTS

Background

An atrial septal defect (ASD) is a common cardiac defect and accounts for one third of all congenital heart diseases detected in adults. The diagnosis in adults is often made when complications of the shunt occur, such as pulmonary hypertension, heart failure, arrhythmias, or paradoxical embolism. Most of these complications might be prevented by closure.

Research frontiers

Since the first description of transcatheter device closure of an ASD in 1976, percutaneous closure of an ASD has been practiced and described extensively. Other studies showed the efficacy and safety of percutaneous closure of ASD's with different devices, mainly during mid-term follow up. Little is known about follow-up more than 5 years after percutaneous ASD closure in adults. We report the efficacy of ASD device closure at more than 5 years follow-up (long-term follow-up).

Innovations and breakthroughs

Previous studies showed that a percutaneous closed ASD using a Cardioseal/Startflex (CS/SF) is associated with a high residual right-to-left shunt (RLS) at mid-term follow-up. This study confirmed that a high residual RLS is still present during long-term follow-up. However, no left-to-right shunt (LRS) was present. Percutaneous closure using a Amplatzer device (ASO) has proven to be efficient at mid-term follow-up. During long-term follow-up no LRS was found. However, a relatively high RLS was present. The importance of RLS at follow-up is unclear. The safety for both devices is similar when compared to literature.

Applications

During long-term follow-up, percutaneous closure of ASD's seems to be safe using different devices, especially using the ASO device. A high residual RLS is present in CS/SF, however there was no residual LRS observed using both the ASO- and the CS/SF device.

Terminology

An ASD is an opening in the septum between the right- and left atrium. It is a congenital heart disease and therefore present at birth. An ASD can cause symptoms due to heart failure, arrhythmia's, paradoxical embolism and pulmonary hypertension. Percutaneous closure of an ASD is a relatively simple procedure where a Nitinol device is placed in the opening between the right- and left atrium. Tansthoracic ultrasound of the heart is used to check whether there is a residual opening in the atrial septum.

Peer-review

The paper by Dr. Snijder *et al* reports the experience in percutaneous closure of atrial septal defects in 104 patients using two devices. Interestingly, in a long-term follow-up a residual left-to-right shunt is absent, although the rate of a residual right-to-left shunt is relatively high.

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CASE REPORT

9.1 cm abdominal aortic aneurysm in a 69-year-old male patient

Celine Saade, Bhavi Pandya, Muhammad Raza, Mustafain Meghani, Deepak Asti, Foad Ghavami

Celine Saade, Bhavi Pandya, Muhammad Raza, Mustafain Meghani, Deepak Asti, Foad Ghavami, Department of Internal Medicine, Staten Island University Hospital, Staten Island, NY 10304, United States

Author contributions: Saade C and Pandya B were involved in drafting and analyzing the case report; Raza M, Meghani M, Asti D and Ghavami F were critically involved in interpreting and revising manuscript as well as in final approval of manuscript.
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Conflict-of-interest: None.

Correspondence to: Bhavi Pandya, MD, Department of Internal Medicine, Staten Island University Hospital, 475 Seaview Avenue, State Island, NY 10304, United States. dr.bhavipandya@gmail.com
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Abstract

We are presenting a case of one of the largest unruptured abdominal aortic aneurysm ever reported. Presented here is a rare case of a 69-year-old active smoker male with history of hypertension and incidental diagnosis of abdominal aortic aneurysm of 6.2 cm in 2003, who refused surgical intervention at the time of diagnosis with continued smoking habit and was managed medically. Patient was subsequently admitted in 2012 to the hospital due to unresponsiveness secondary to hypoglycemia along with diagnosis of massive symptomatic pulmonary embolism and non-ST elevation myocardial infarction. With the further inpatient workup along with known history of abdominal aortic aneurysm, subsequent computed tomography scan of abdomen pelvis revealed increased in size of infrarenal abdominal aortic aneurysm to 9.1 cm of without any signs of rupture. Patient was unable to undergo any surgical intervention this time because of his medical instability and was eventually passed away under hospice care.

Key words: Abdominal aortic aneurysm; Unruptured; Elderly male; Active smoking

Core tip: Regular screening of patients with abdominal aortic aneurysm with abdominal ultrasound to prevent catastrophic complication of aortic rupture and early aggressive surgical intervention when indicated.

Saade C, Pandya B, Raza M, Meghani M, Asti D, Ghavami F. 9.1 cm abdominal aortic aneurysm in a 69-year-old male patient. *World J Cardiol* 2015; 7(3): 157-160 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i3/157.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i3.157

INTRODUCTION

Abdominal aortic aneurysm is one of the most common conditions seen in elderly hypertensive male with active smoking. Aortic rupture is seen to be the

commonest catastrophic complication associated with the condition[1-3]. Purpose of our case report is to illustrate importance of regular screening and timely intervention of abdominal aortic aneurysm along with one of the largest reported image of un-ruptured aortic aneurysm.

CASE REPORT

A 69-year-old man was brought to the emergency department by emergency medical services (EMS) after being found unresponsive by his partner's mother. Initially he was detected to be hypoglycemic with blood glucose of less then 30 mg/dL, which was managed by Ⅳ dextrose administered by EMS prior to arriving at the hospital.

The patient was diagnosed 6.2 cm infra renal artery aneurysm in 2003. He also had a 3.4 cm saccular aneurysm of the descending thoracic aorta, an aneurysm of 1.9 cm in the common iliac artery, and an aneurysm of the left renal artery. At that time, he decided to receive medical treatment. His past medical history is also significant for systolic congestive heart failure (CHF) mitral regurgitation, myocardial infarction, hypertension, type 2 diabetes mellitus, peripheral vascular disease, smoking (40 packs per year), lower gastrointestinal bleeding and depression. His medications included Aspirin, Carvedilol, Digoxin, Lasix, spironolactone, Lantus insulin, Dexilant, Lorazepam, Gabapentin, and Percocet. Upon arrival to the emergency room in October 2012, the physical exam was characterized by an altered mental status, and he was barely responsive to painful stimuli. He was tachypneic and tachycardic with bilateral rhonchi and rales in respiratory exam, but had stable blood pressure. The rest of his physical exam was unremarkable.

Electrocardiogram showed sinus rhythm with premature ventricular complexes, right bundle branch block, and ST segment depression in inferior leads. Chest X-Ray revealed cardiomegaly and a right lower lobe infiltrate. Computed tomography (CT) scan of the chest with contrast revealed a segmental pulmonary embolism in the right lower lobe consistent with a small infarction, a new 1.0 cm × 0.8 cm × 1 cm marginated left upper lobe pulmonary nodule suspicious for neoplasm, a moderate centrilobular emphysema, small bilateral pleural effusions and a new filling defect within a dilated left ventricle which is suggestive of left ventricular thrombus. CT scan of the abdomen and pelvis with contrast was significant for an increase in size of the descending thoracic aortic aneurysm to 4.2 cm × 3.6 cm, an infrarenal abdominal aorta measuring 9.1 cm × 8.7 cm and right common iliac artery at about 2.4 cm × 2.6 cm. Left renal artery was stable at 3.1 cm × 2.7 cm. There was no evidence of rupture in the abdominal aortic aneurysm.

The patient had elevated troponins level possibly due to a NSTEMI. He received Ⅳ heparin to manage pulmonary embolism and possible NSTEMI. Considering his extensive medical history, poor medical management outcome and deteriorating mental condition, his family decided to place the patient in hospice care on 10/10/12. The patient died on 10/12/12.

DISCUSSION

Aortic aneurismal disease is defined as a focal dilation of the aorta with a diameter greater than 3.5 cm. Thoracic aneurysms are located above the diaphragm. The abdominal aneurysm is more common and is located below the diaphragm with a prevalence of 1.4% in the United States population age 50 to 84[2]. Aortic rupture is the most common complication of these aneurysms with a mortality rate as high as 75% for the abdominal aneurysm[3]. The profile of a patient that might have an aneurysm and eventually may benefit from a screening test would be a man between 65 and 75 with a history of smoking or a younger patient with a family history of a genetic disease associated with aneurysms[1,4]. The 2005 United States Preventive Services Task Force (USPSTF) report recommended ultrasonography as the screening test for the abdominal aneurysm with a sensitivity rate as high as 100%. Multiple risk predictors for the growth and rupture of an AAA were determined and included: continuous smoking which is the major risk factor, female gender, diastolic hypertension, maximum transverse diameter > 5.5 cm, and dyslipidemia[1,4-9]. On the other hand, other factors were found to decrease the growth rate and subsequently the risk of rupture of abdominal aortic aneurysm (AAA). The use of statins in a recent metanalysis[10] has been shown to reduce by 0.63 mm/year the growth rate of the AAA. Both macrolides and tetracyclines[11-13] were associated with a lower expansion rate of the AAA. However, propanolol use hasn't been beneficial in preventing the growth rate of AAA in a double-blind randomized study[14]. When it comes to surgical management, the threshold recognized currently is 5.5 cm or above. This was demonstrated in two trials conducted in the United Kingdom and United States[6,7]. A CT scan is warranted as part of the preoperative planning because it can help characterizing the AAA for a better surgical approach.

Our patient had both thoracic and abdominal aneurysms with the latter measuring 9.1 cm as a maximum diameter at the time of his death. To our knowledge, this is the largest unruptured AAA to be diagnosed. The patient was incidentally diagnosed to have an AAA of 6.6 cm on CT scan (Figure 1). The incidental finding of an AAA is as high as 0.5% on CT scan as reported in Al-Tahani study[15]. Even though the patient was eligible for surgical reparation of the AAA, he refused the operation knowing the risks of his decision. Between 2003 and 2012, the patient

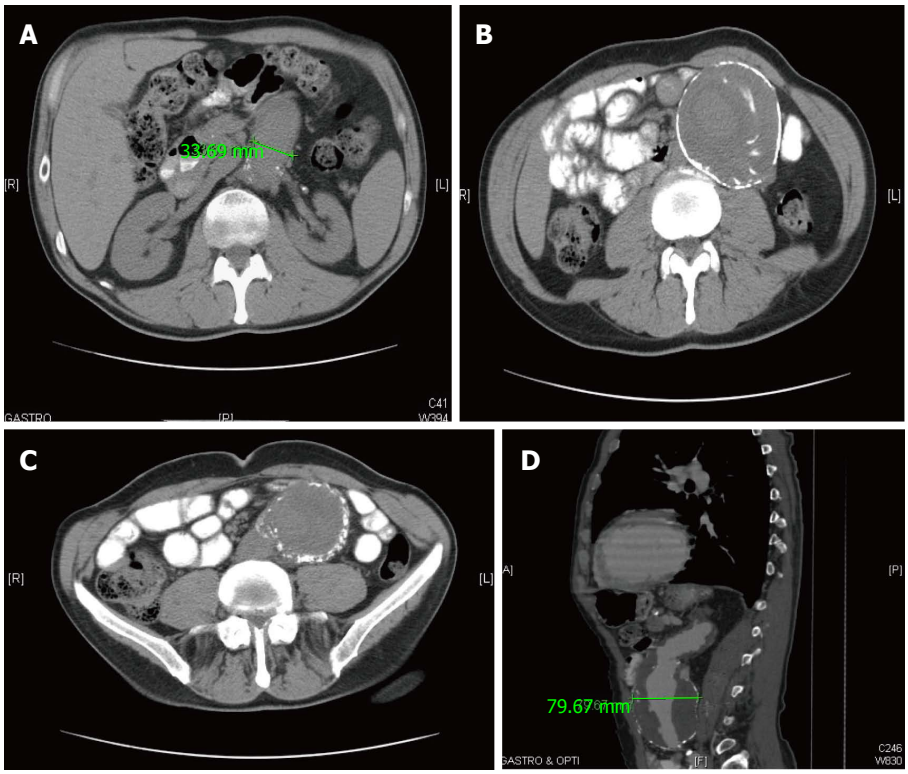

Figure 1 Computed tomography scan. A: Showing aortic abdominal aneurysm at the level of renal arteries; B: Showing massive unruptured abdominal aortic aneurysm along with loops of small intestine; C: Showing continuation of abdominal aortic aneurysm along with plaques of calcification surrounding it; D: Showing longitudinal section of contrast filled abdominal aortic aneurysm with bilateral mural thrombus.

continued to smoke and was taking medications for his hypertension and CHF. He had thus 2 major risk factors for the growth of his AAA considering that his hypertension was medically controlled. In 2012, the size of his AAA increased to 9.1 cm thus at a rate of 0.32 cm/year. At this point, the patient was inoperable because of his worsening CHF and his massive pulmonary embolism. The CT scan also showed the continuous growth of the thoracic aneurysm and the right common iliac artery aneurysm. As per Brown et al study[9], this male patient with an AAA greater than 6 cm had a 14.1% risk per year of having a ruptured AAA. The Helsinki study[16] showed that in 154 cases excluded from the surgical repair of AAA due to severe co morbidities in the patients, 43% eventually died because of a ruptured AAA. Therefore, it can be postulated that the patient had an imminent risk for AAA rupture. No autopsy was done as per the request of the family but it might be speculated that the patient's death was due to ruptured AAA especially that he had to be started on therapeutic heparin for his massive symptomatic PE.

COMMENTS

Case characteristics

A 69-year-old male presented with hypoglycemia found to have 9.1 cm × 8.7 cm unruptured AAA.

Clinical diagnosis

Patient was found to have subsequent pulmonary embolism and Non ST elevation Myocardial infarction.

Imaging diagnosis

CT scan of the abdomen and pelvis with contrast was significant for an increase in size of the descending thoracic aortic aneurysm to 4.2 cm × 3.6 cm, an infrarenal abdominal aorta measuring 9.1 cm × 8.7 cm and right common iliac artery at about 2.4 cm × 2.6 cm.

Treatment

Due to patient's medical instability, he was not a surgical candidate.

Related reports

So far reported cases in literatures are either ruptured abdominal aortic aneurysm or not significantly enlarged. Reported here is case of unruptured largest abdominal aortic aneurysm, which can be managed successfully with timely intervention.

Experiences and lessons

Regular screening of patients with abdominal aortic aneurysm with abdominal ultrasound helps prevent catastrophic complication of aortic rupture and early aggressive surgical intervention when indicated.

Peer-review

This is a well-written clinical case report regarding to the large un-ruptured abdominal aortic aneurysm. The case is interesting and illustrates importance of regular screening and timely intervention of abdominal aortic aneurysm, which will gather the great interests from the readers.

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CASE REPORT

Trauma and syncope-evidence for further sleep study? A case report

Erik Skobel, Andreas Bell, Dang Quan Nguyen, Holger Woehrle, Michael Dreher

Erik Skobel, Clinic for Cardiac and Pulmonary Rehabilitation, "An der Rosenquelle", 52077 Aachen, Germany
Andreas Bell, Department of Surgery, Marienhospital, 52077 Aachen, Germany
Dang Quan Nguyen, Department of Internal Medicine, Marienhospital, 52077 Aachen, Germany
Holger Woehrle, Sleep and Ventilation Center Blaubeuren, Respiratory Center Ulm, 89073 Ulm, Germany
Michael Dreher, Department of Cardiology, Pneumology, Angiology and Intensive Care Medicine, University Hospital, 52077 Aachen, Germany
Author contributions: Skobel E collected the data and wrote the article; Nguyen DQ collected and interpretate the patient´s clinical data; Bell A, Woehrle H and Dreher M revised the article for important clinical content.
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Correspondence to: Erik Skobel, MD, Head of Department, Clinic for Cardiac and Pulmonary Rehabilitation, "An der Rosenquelle", Kurbrunnenstraße 5, 52077 Aachen, Germany. erik.skobel@rosenquelle.de
Telephone: +49-241-6007501
Fax: +49-241-6007502
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Abstract

We report on an 83-year-old male with traumatic brain injury after syncope with a fall in the morning. He had a history of seizures, coronary artery disease and paroxysmal atrial fibrillation (AF). No medical cause for seizures and syncope was determined. During rehabilitation, the patient still complained of seizures, and also reported sleepiness and snoring. Sleep apnea diagnostics revealed obstructive sleep apnea (SA) with an apnea-hypopnoea index of 35/h, and sudden onset of tachycardia with variations of heart rate based on paroxysmal atrial fibrillation. Additional tests showed nocturnal AF which spontaneously converted to sinus rhythm mid-morning with an arrest of 5 s (sick sinus syndrome) and seizures. A DDD-pacer was implanted and no further seizures occurred. SA therapy with nasal continuous positive airway pressure was refused by the patient. Our findings suggests that screening for SA may offer the possibility to reveal causes of syncope and may introduce additional therapeutic options as arrhythmia and SA often occur together which in turn might be responsible for trauma due to syncope episodes.

Key words: Sleep apnea; Syncope; Atrial fibrillation; Trauma

Core tip: Arrhythmias and sleep apnea should be considered as relevant factors resulting in syncope and trauma in the elderly. This case report applies screening for sleep apnea to detect arrhythmia as a common cause of syncope. Screening for sleep apnea may offer the possibility of additional therapeutic options and diagnostic in trauma and syncope after performing standard diagnostics.

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INTRODUCTION

Sleep apnea (SA) has been shown to be an independent risk factor for cardiovascular diseases[1-4]. Obstructive sleep apnea (OSA) is the most prevalent type of SA. It is defined as repetitive episodes of partial or complete cessation of airflow in the upper airways during sleep. The number of people in the United States estimated to be affected by OSA is 3%-7%[5,6] and, according to US National Commission of Sleep Disorders Research, OSA contributes to 38000 cardiovascular deaths annually[7]. In Europe, a Spanish study reported that 7% of women and 15% of men aged 30-70 years had OSA, defined as an apnea-hypopnoea index (AHI) of $\geqslant$ 15/h[8].

Patients with OSA typically present with symptoms such as disruptive snoring, witnessed apneas or gasping, excessive daytime sleepiness, morning headache, sleep disturbance and cognitive dysfunction[9,10]. OSA is associated with higher prevalence of diabetes and hypertension[11,12], coronary artery disease and myocardial infarction[13], heart failure[14] and arrhythmias such as bradycardia or atrial fibrillation or flutter (AF)[15]. Traumatic brain injury (TBI) is often associated with SA and sleep disturbances[16,17]. Although the incidence of arrhythmia in the presence of SA is high[15,18,19], the influence of SA on traumatic loss of consciousness (syncope) and TBI have rarely been evaluated[20-22].

Syncope is the most common cause of TBI in the elderly and it often has an underlying cardiovascular aetiology (*e.g.*, bradycardia, tachycardia, myocardial infarction or valvular disease)[23]. The incidence of syncope is 5-11 events per 1000 person years[23]. Different forms of disease sometimes make the diagnosis difficult and different approaches may be needed. Screening for sleep apnea is not standard practice in the evaluation of syncope[20].

This case report describes a male patient with frequent syncope and seizures with SA related arrhythmia.

CASE REPORT

An 82-year-old male (BMI: 26 kg/m^2) was transferred to our rehabilitation facility after experiencing syncope 3 wk previously. He reported a history of seizures mostly in the morning hours at rest and on the day of the most recent syncope episode he fell down without warning soon after breakfast and was transferred to an emergency unit with a frontal head laceration. The patient also had a history of hypertension, dyslipidaemia, coronary artery disease (CAD) without infarction, bypass surgery (14 years ago) and paroxysmal AF. The patient was taking warfarin, ACE inhibitors, β-blockers, statins and diuretics. In the emergency department, the patient was awake without neurologic impairment. Paroxysmal AF was documented on ECG, without evidence of ischemia or myocardial infarction, and troponin testing was negative. Pulmonary embolism was ruled out. A computed tomographic (CT) scan revealed frontal cerebral haemorrhage, which was treated conservatively and warfarin therapy was stopped. Magnetic resonance (MR) imaging one week later showed that the haemorrhage was resolving.

Some further cardiac evaluation of syncope showed no abnormalities. Twenty four hours blood pressure monitoring was normal. Holter 24-h monitoring revealed sinus rhythm without bradycardia, or tachycardia, or paroxysmal AF. Two-dimensional echocardiography showed normal left ventricular function without valve disease, but no carotid sinus massage, tilting test or electrophysiological studies were performed.

At the rehabilitation facility, the patient was assessed as being well without neurologic disorders. The patient reported snoring and hypersomnia (Epworth Sleepiness Scale score of 9 points, with normal being < 5) and complained of intermediate seizures without falling. Sinus rhythm was present on 12-lead ECG.

The patient was screened for SA using 2-channel polygraphy (Figure 1), which showed intermittent nocturnal oxygen desaturations and recurrent apneas with an AHI of 35/h. Heart rate data from polygraphy revealed an increase in the morning hours (Figure 1) with arrhythmic pulse curve based on onset of paroxysmal AF during sleep apnea. A second period of 24-h Holter monitoring was performed the following day to evaluate the incidence of paroxysmal AF. Nocturnal paroxysmal AF was seen with onset in the early morning hours, which spontaneously converted to sinus rhythm mid-morning with an arrest of 5 s (sick sinus syndrome) and the patient reported a seizure at the time of the arrest (Figures 2 and 3). The patient was transferred to the cardiac department for pacer implantation (DDD) and then transferred back to the rehabilitation facility for further rehabilitation. Seizures fully resolved after pacemaker implantation and an increase in β-blocker therapy eliminated paroxysmal AF. A further performed polysomnography for SA diagnostics conformed severe OSA (Table 1).

Treatment of sleep with nasal continuous positive airway pressure (nCPAP) therapy was discussed with the patient but he refused this treatment.

DISCUSSION

In this case report, sleep apnea screening revealed the nocturnal onset of arrhythmia and facilitated further evaluation of syncope. The temporal relationship between the documented pause in the ECG and seizures in our patient means that this was highly likely to be the underlying cause of the repeated

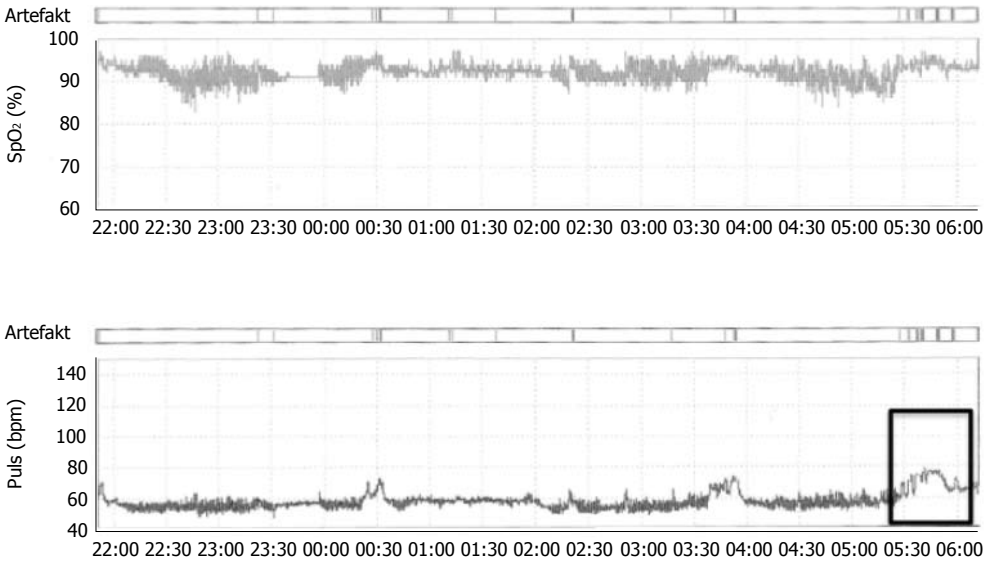

Figure 1 Overnight polygraph recording showing recurrent desaturations at night (above), increased heart rate in the morning hours (down) and arrhythmia onset due to changes in heart rate (see marker box).

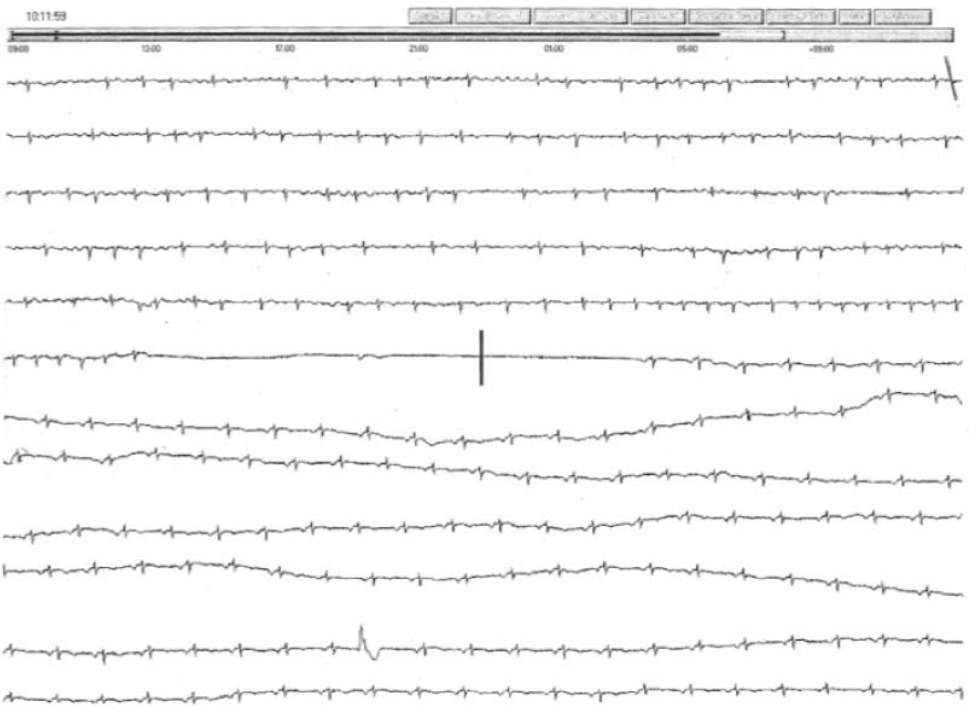

Figure 2 Twenty-hour Holter monitor recording showing atrial fibrillation in the morning with arrest of 5 s (marker) followed by sinus rhythm.

seizures and falls, and ultimately the traumatic injury he sustained. A limitation in this case report is the lack of standard diagnostic for syncope, *e.g*., carotid sinus massage, tilting test or electrophysiological studies before the rehabilitation setting.

The indication for pacing in our patient was based on syncope with trauma and documentation of sick sinus syndrome in the morning hours after onset of paroxysmal AF. After pacemaker implantation the patient had no further seizures, providing further evidence that sick sinus syndrome was the cause of syncope.

AF and nocturnal bradycardia are often triggered by sleep apnea[24]. This is based on nocturnal hypoxemia and its effects on the cardiac autonomic nerve system[25,26]. Accordingly, our patient was offered, but refused, nCPAP therapy. However, there is evidence to show that CPAP not only effectively treats SA but also reduces nocturnal arrhythmias[27,28]. Although nCPAP therapy can reduce arrhythmia burden and possibly reduce the incidence of paroxysmal AF[24], it is not clear whether nCPAP therapy can prevent

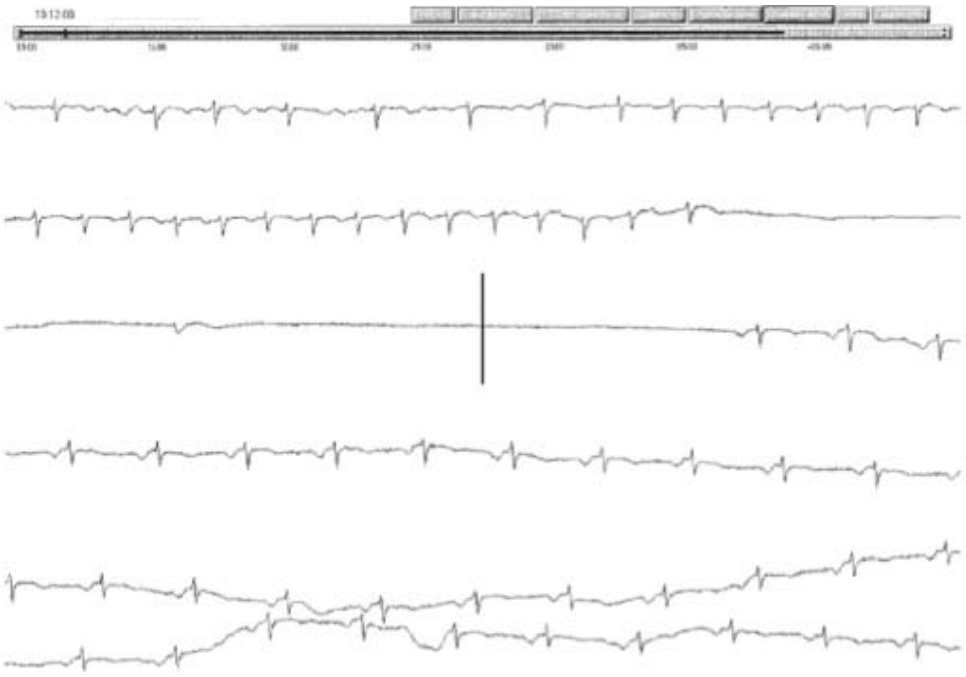

Figure 3 Twenty-hour Holter monitor recording showing atrial fibrillation in the morning with arrest of 5 s (marker) followed by sinus rhythm (enlargement of Figure 2).

Table 1 Polysomnographic data revealing obstructive sleep apnea

Polysomnographic parameter	Value
AHI (h)	33
Obstructive apnoea index (h)	31.4
Central apnoea index (h)	0
Mixed apnoea index (h)	0
Hypopnoea index (h)	1.6
Snoring, min	9
Mean oxygen saturation (%)	91
Minimum oxygen saturation (%)	85
Oxygen desaturation index (h)	18

all arrhythmias and arrests, justifying the use of a pacemaker in this case. In addition, compliance with nCPAP is necessary for the benefits of therapy to be realised, which is another justification for pacemaker implantation. However, it has been shown that patients with a pacemaker and ongoing syncope have a high incidence of SA[19], indicating that screening for SA in patients with pacemaker implement and syncope would be appropriate.

Sleep disorders are common in TBI and develop in 12%-36 % of patients[16,17,29]. For example, Verma *et al*[29] found that 50% of patients with TBI reported daytime hypersomnia and 30% were diagnosed with OSA. TBI can result in sleep/wake disturbances, sleep fragmentation, and insomnia or hypersomnia. However, the mechanism of sleep disorders in the setting of TBI is not clear. On one hand the mechanism of injury could trigger sleep disorders such as posttraumatic insomnia or hypersomnia with sleep fragmentation[30,31]. On the other hand, the underlying cause of the accident resulting in TBI may be hypersomnia as a result of nocturnal OSA or, as in this case, nocturnal arrhythmia with syncope and following TBI. Further studies are needed to evaluate the incidence of sleep-related arrhythmias and traumatic injury with the goal of determining whether screening for SA is appropriate in this setting.

Arrhythmias and SA should be considered relevant factors resulting in syncope and trauma. Screening for SA may offer the possibility of additional therapeutic options and diagnostic in trauma and syncope.

COMMENTS

Case characteristics

An 83-year-old male with traumatic brain injury after syncope of unknown origin in the morning and seizures.

Clinical diagnosis

2-channel polygraphy showed intermittent nocturnal oxygen desaturations and recurrent apneas with an apnoea-hypopnoea index (AHI) of 35/h. Heart rate data from polygraphy revealed an increase in the morning hours with arrhythmic pulse curve based on onset of atrial fibrillation (AF) during sleep apnea.

Imaging diagnosis

Nocturnal paroxysmal AF was seen with onset in the early morning hours, which spontaneously converted to sinus rhythm mid-morning with an arrest of 5 s (sick sinus syndrome) and the patient reported a seizure at the time of the arrest.

Pathological diagnosis

Diagnostic of severe obstructive sleep apnea, AHI 33/h.

Treatment

Pacer implantation (DDD). Treatment of sleep with nasal continuous positive airway pressure therapy was discussed but refused.

Related reports

Syncope is the most common cause of traumatic brain injury in the elderly and it often has an underlying cardiovascular aetiology. Arrhythmias and sleep apnea (SA) should be considered relevant factors resulting in syncope and

trauma as SA is one trigger of sudden onset of arrhythmia.

Experience and lessons

Further studies are needed to evaluate the incidence of sleep-related arrhythmias and traumatic injury with the goal of determining whether screening for SA is appropriate in this setting.

Peer-review

This manuscript reports a typical case of sick sinus syndrome in an 83-year-old male, with paroxysmal atrial fibrillation and sinus arrest, presenting clinically with syncope.

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Jin-Lei Wang, Director
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: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
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PUBLISHER
Baishideng Publishing Group Inc
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Telephone: +1-925-223-8242
Fax: +1-925-223-8243
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EDITORIAL

Percutaneous pulmonary and tricuspid valve implantations: An update

Robert Wagner, Ingo Daehnert, Philipp Lurz

Robert Wagner, Ingo Daehnert, Department of Paediatric Cardiology, University of Leipzig-Heart Center, D-04289 Leipzig, Germany
Philipp Lurz, Department of Internal Medicine/Cardiology, University of Leipzig-Heart Center, D-04289 Leipzig, Germany
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Correspondence to: Philipp Lurz, MD, PhD, Department of Internal Medicine/Cardiology, University of Leipzig-Heart Center, Struempellstrasse 39, D-04289 Leipzig, Germany. philipp.lurz@gmx.de
Telephone: +49-341-865251428
Fax: +49-341-8651461
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Abstract

The field of percutaneous valvular interventions is one of the most exciting and rapidly developing within interventional cardiology. Percutaneous procedures focusing on aortic and mitral valve replacement or interventional treatment as well as techniques of percutaneous pulmonary valve implantation have already reached worldwide clinical acceptance and routine interventional procedure status. Although techniques of percutaneous pulmonary valve implantation have been described just a decade ago, two stent-mounted complementary devices were successfully introduced and more than 3000 of these procedures have been performed worldwide. In contrast, percutaneous treatment of tricuspid valve dysfunction is still evolving on a much earlier level and has so far not reached routine interventional procedure status. Taking into account that an "interdisciplinary challenging", heterogeneous population of patients previously treated by corrective, semi-corrective or palliative surgical procedures is growing inexorably, there is a rapidly increasing need of treatment options besides redo-surgery. Therefore, the review intends to reflect on clinical expansion of percutaneous pulmonary and tricuspid valve procedures, to update on current devices, to discuss indications and patient selection criteria, to report on clinical results and finally to consider future directions.

Key words: Congenital heart disease; Right ventricular outflow tract dysfunction; Pulmonary regurgitation; Percutaneous pulmonary valve implantation; Percutaneous tricuspid valve implantation

Core tip: The field of percutaneous valve implantation/repair is rapidly developing within interventional cardiology. Percutaneous procedures focusing on aortic, mitral or pulmonary valve dysfunction have almost reached daily routine. In contrast, percutaneous treatment of tricuspid valve dysfunction is still evolving on a much earlier level. Taking into account that an "interdisciplinary challenging" population of patients previously treated by corrective, semi-corrective or palliative surgery is growing inexorably, there is an increasing need of options besides redo-surgery. This review intends to report on clinical application of pulmonary and tricuspid valve procedures. It updates on current devices, patient selection criteria, results and future directions.

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INTRODUCTION

Isolated pulmonary and tricuspid valve dysfunction, whether acquired or in the context of congenital heart disease, can be clinically asymptomatic and be tolerated for a long time[1]. In the western world, acquired primary tricuspid or pulmonary valve diseases are rare conditions and mostly related to rheumatic fever, infective endocarditis or rarities (*e.g.*, carcinoid syndrome).

For those cases with underlying congenital heart disease, dysfunction of these valves is both a primary component of many anatomical conditions and a secondary, but common consequence of several early repair strategies[2].

Increasing knowledge about potential harmful effects of chronic pulmonary artery (PA) regurgitation has made the surgical revision of the right ventricular (RV) outflow tract (RVOT) a frequently performed operation in this population[3]. Typically, most of these patients require several redo-operations during their lifetime to halt the detrimental effects of valvular dysfunction. Since techniques of percutaneous pulmonary valve implantation (PPVI) were first described by Bonhoeffer *et al*[4] more than a decade ago, the procedure has reached worldwide clinical acceptance and routine interventional procedure status. Several devices have been investigated for purposes of PPVI, but so far only the MELODY™ device (Medtronic, MN, United States) has obtained regulatory approval. Interventional procedures focusing on percutaneous tricuspid valve replacement or interventional treatment of severe tricuspid regurgitation are evolving, but yet remain at a much earlier stage and have so far not reached levels of standard procedures[5]. The authors review on clinical expansion of this revolutionary technology, discuss current indications and patient selection criteria, report on clinical results and finally consider future directions.

PPVI

Background and clinical indications

Over the last decades, advances in cardiac surgery, interventional procedures, intensive care and non-invasive imaging have led to a substantial increase in life expectancy for many patients with congenital heart disease. Therefore, an "interdisciplinary challenging", heterogeneous population of patients treated by corrective, semi-corrective or palliative surgical procedures, sometimes decades ago, is growing inexorably. For approximately 20% of these patients RVOT dysfunction caused by predominant obstruction, by predominant pulmonary regurgitation or both in combined conditions, becomes clinically evident.

Undeniably, surgical pulmonary valve replacement is the most frequent mode of redo-operation in patients with congenital heart disease[3]. Surgery for RVOT dysfunction can be performed with low morbidity and mortality[3]. However, an important drawback of this treatment is the limited lifespan of used conduits that has been reported to be around ten years[6-9]. As a consequence, the majority of patients have to undergo several open-heart procedures during their life that raise potential individual risks for a diversity of complications. To limit the need for redo-operations delaying surgery for as long as possible is the strategy of choice in any individual patient. If the necessary treatment is delayed beyond a certain point of no return, adverse RV loading conditions might lead to irreversible ventricular dysfunction, reduced exercise capacity and ultimately to an increased risk for sudden cardiac death[10-13]. Decision making on ideal timing of pulmonary valve replacement is still challenging in most cases and represents one of the most controversial issues of cardiologists who take care of children and adults with congenital heart disease[3,14,15]. RV volume thresholds on magnetic resonance imaging (MRI) have been proposed as predictors for outcome after conduit placement[14]. An RV end-systolic volume of 150-170 mL/m^2 has been reported to deserve as cut-off point above which normalisation of RV dimensions is unlikely following pulmonary valve replacement[13-17]. Nevertheless, the impact of the timing of pulmonary valve replacement on RV function, exercise performance and patient long-term survival remains undefined[13].

With the evolution of PPVI, an effective and feasible non-surgical technique was introduced. It offers a minimally invasive method which can potentially avoid open-heart surgery for RVOT dysfunction in children and adults by restoring acceptable RV loading conditions.

Since the first description of PPVI in 2000[4], more than 3000 percutaneous pulmonary valves have been implanted worldwide[18]. PPVI is performed to prolong the lifespan of RV-to-PA conduits and thereby delaying redo-operations in children and adults with congenital heart disease. Over the last decade, a marked learning curve in outcome post-PPVI could be demonstrated, with improved safety, efficacy and freedom from redo-surgery or re-intervention for pediatric or adult patients who underwent this procedure[13,19-25].

Current devices

The MELODY™ transcatheter pulmonary valve is designed of a segment of bovine jugular vein with a central valve (Figures 1 and 2) that is sewn inside an expanded platinum-iridium stent. The current carrying

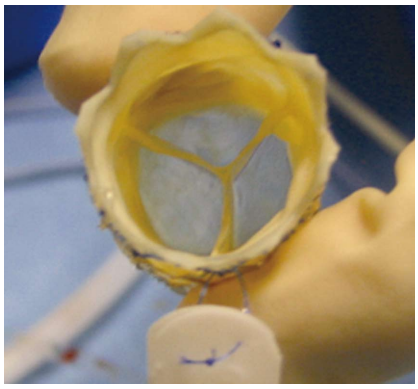

Figure 1 The MELODY™ percutaneous pulmonary valve (Medtronic, MN). The Melody device in "en face" view. Note the blue outflow line to identify the outflow end of the device (courtesy of P.Lurz).

Cheatham platinum stent (NuMED CP Stent CP8Z34) is of a length 34 mm, "crimpable" to minimum of 6 mm and re-expandable up to 22 mm. The balloon in balloon (BiB) delivery system (Ensemble™, Medtronic, MN) is commercially available with different outer balloon diameters of 18, 20 and 22 mm.

The Edwards SAPIEN™ valve (Edwards Lifesciences LLC, Irvine, CA) is radiopaque and made of a trileaflet bovine pericardial valve hand-sewn into a stainless-steel stent (length of 14 or 16 mm) (Figure 3). A sealing cuff covers the proximal part of the stent designed to prevent paravalvular leakage. Currently, the valve is commercially available in 23 and 26 mm diameter sizes and is crimped onto a designated balloon delivery system Retroflex™ Ⅲ. A 29 mm diameter valve is also available (Edwards SAPIEN™ XT). The delivery system requires either 22 Fr (SAPIEN™ 23 mm valve) or 24 Fr hydrophilic sheaths (SAPIEN™ 26 mm and SAPIEN™ XT 29 mm valve) (Figure 3). Promising improvements of design (Edwards eSheath™) offer even smaller sheath sizes.

Technical details regarding both devices for PPVI are summarized in Table 1.

Patient selection criteria

Although sophisticated MR data have been reported, the clinicians' dilemma of the right timing for treatment of RVOT dysfunction, whether predominantly caused by obstruction or regurgitation has not been solved yet[13]. According to current guidelines for the management of grown-up congenital heart disease[26], patients with RVOT obstruction should be treated if the RV to PA gradient exceeds 60 mmHg or in presence of symptoms due to RVOT obstruction regardless of RVOT gradients. Pulmonary regurgitation can be clinically asymptomatic and be tolerated for a long time[1]. When to intervene is subject to ongoing discussions. It is common sense to base the indication criteria for transcatheter or surgical treatment on a combined assessment of MR-imaging derived RV EDV and systolic function, cardiopulmonary exercise testing and the presence of atrial or ventricular dysrhythmia[13]. According to the 2010 recommendations of the ESC task force PPVI can therefore be indicated, if severe pulmonary regurgitation (as assessed on echocardiography or MR imaging) is accompanied by severe RV dilatation, severe RV dysfunction, clinical symptoms and/or impaired exercise capacity[13,26].

In 2011, the American Heart Association (AHA) stated: "It is reasonable to consider the percutaneous pulmonary valve replacement in patient with RV-to-PA conduits with moderate to severe pulmonary regurgitation or stenosis provided the patient meets inclusion/exclusion criteria for the available valve". The AHA writing committee recommended this procedure with a Class IIa evidence (Level of evidence: B)[27]. Clinical indications applicable regardless of the device used for valve implantation (MELODY™ and SAPIEN™) are summarized in Table 2.

Although there is no absolute lower age limit, an adequate body size (*e.g.*, weight > 20 kg) is required to accommodate femoral placement of the introducer[21].

Size and shape of the implantation site ("landing zone") and its anatomical relation to coronary arteries are decisive morphological criteria which have to be appropriate when considering patients as potential candidates for PPVI: In regulatory approved routine use, current MELODY™ devices are not intended for dilatations to diameters of more than 22 mm. Patients with (non-dilated) conduits between the RV and PA of 22 mm and less offer an ideal environment to perform PPVI. In contrast, native or patched RV outflow tracts after surgical repair for Tetralogy of Fallot are often enlarged (> 22 mm) and therefore do not provide a secure landing zone for MELODY™ valves[14]. In these cases (but not larger than 29 mm) the SAPIEN™ valve might be a possible alternative[28-30]. Furthermore, in our experience the RVOT shape (after prior pre-stenting) is of importance: due to its "engineered" nature of sutured pericardial tissue, optimal valved stent function in SAPIEN™ procedures is guaranteed by a circular RVOT shape. In PPVI procedures with the MELODY™ valve the RVOT shape itself appears to have less impact on valvular competence.

Coronary artery anatomy varies due to a broad spectrum of complex congenital heart defects or after surgical re-insertion into the aorta. In some cases there is relevant proximity of one or more of the relevant coronary artery branches to the main PA. This exposes patients who undergo interventions of the RVOT to the risk for fatal coronary artery obstruction due to expansion of the RVOT[31,32]. Therefore, it is essential to assess the course of proximal coronary arteries in relation to the RVOT prior to PPVI deployment. Some centers prefer MR 3-D whole heart images (Figure 4), but we recommend performing selective coronary angiography and particularly aortic root angiography and simultaneous high-pressure balloon inflation within the landing zone at the time of catheterization in all patients to rule out the risk of coronary compression

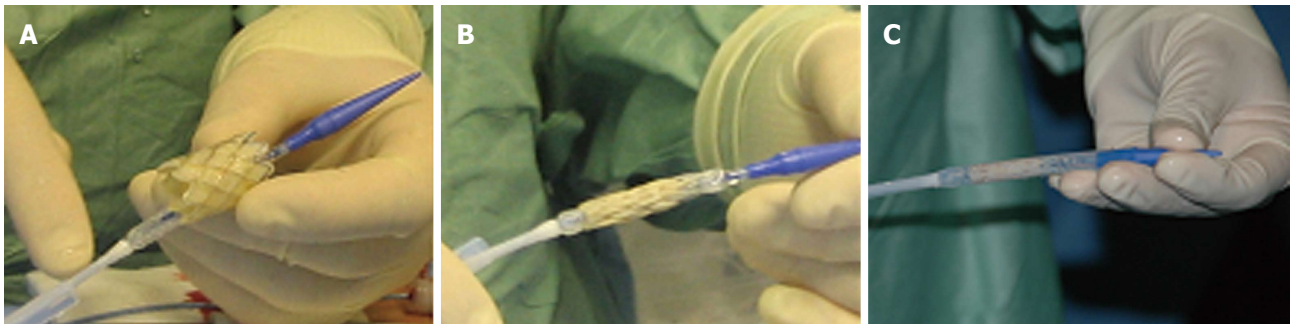

Figure 2 MELODY™ device and its delivery system. A: Uncrimped device on the delivery system with a retractable sheath; B: Crimped device before covering the device to protect it during the delivery; C: Crimped and covered device prepared for delivery (courtesy of Lurz P).

Table 1 Devices and delivery systems for percutaneous pulmonary valve implantation

	The MELODY™ transcatheter pulmonary valve	The SAPIEN™ pulmonic transcatheter heart valve
Manufactor	Medtronic Inc., MN, United States	Edwards Lifesciences LLC, Irvine, CA, United States
Regulatory approval	CE 9/2006 FDA 01/2010	CE 5/2010 FDA 10/2012
(Tissue) characteristics	Segment of bovine jugular vein with a central valve hand-sewn inside a stent	Trileaflet bovine pericardial valve hand-sewn inside a stent
Stent type	Cheatham platinum stent (NuMED CP Stent CP8Z34) Length 34 mm Expandable up to 22 mm	Stainless-steel stent Length of 14 or 16 mm
Available sizes	18, 20, 22 mm (depending on the favoured Ensemble™ delivery (system)	23, 26, (XT 29[a]) mm
Delivery system	Ensemble™ (Medtronic, MN) with balloon in balloon (BiB) deployment design	Edwards Retroflex™ Ⅲ containing a balloon catheter and a deflectable guiding catheter
Sheats for implantation	One-piece 22 Fr Teflon sheath	(18 Fr[b]) 22 Fr for 23 mm valves (19 Fr[b]) 24 Fr for 26 mm valves (16 Fr[b]) 24 Fr for 29 mm XT valves

Technical comparision of the commercially available devices for percutaneous pulmonary valve implantation: the MELODY™ device and the SAPIEN™ pulmonic transcatheter heart valve as non-surgical treatment options for RV outflow tract dysfunction ([a]"Off label-use" in pulmonary position; [b]Manufacturer's data given for the Edwards eSheath™). RV: Right ventricle.

Table 2 Clinical and morphological requirements for percutaneous pulmonary valve implantation

Clinical indications in the context of RV pressure overload/pulmonary stenosis
RV systolic pressure > 65% of systemic pressure in symptomatic patients
RV systolic pressure > 75% of systemic pressure in asymptomatic patients
Clinical indications in the context of RV volume overload/pulmonary regurgitation
Severe pulmonary regurgitation on echocardiography or MR imaging and
Severe RV dilatation > 150 mL/m^2 or the RV to LV end-diastolic ratio of > 1.7 and/or
Rapid progressiv RV dilatation and/or
Severe RV dysfunction and/or
Symptoms and/or
Sustained atrial or venticular arrhythmia and/or
Impaired exercise capacity [< 65% compared to norm peak oxygen consumption related to bodyweight (VO_2/kg)]
Morphological indications
Circumferential RV to PA conduit with dimensions ranging from 16 to 22 mm (Melody™)
Circumferential RV to PA conduit with dimension at surgical implantation of at least 18 mm but no larger than 29 mm (with some degree of conduit narrowing) (SAPIEN™)
Exclusion of risk for coronary compression

RV: Right ventricle; PA: Pulmonary artery.

(Figure 5).

To facilitate superior immediate haemodynamic results several peri-procedural interventions should be considered: (1) pre-dilatation of the landing zone to resolve relevant stenosis and facilitate positioning of the system; (2) pre-stenting of the RVOT to mark, cover and enhance the "landing zone" and avoid stent fractures; and (3) post-dilatation in any case of residual stenosis more than > 20 mmHg of invasively measured gradient[13].

The optimal timing of pre-dilatation/stenting in relation to definitive PPVI is unknown. Some centers

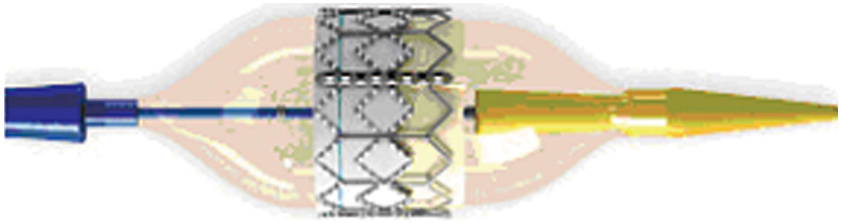

Figure 3 The Edwards SAPIEN™ pulmonic transcatheter heart valve (Edwards Lifesciences LLC, Irvine, CA). The SAPIEN™ device in lateral view mounted on its delivery system Retroflex™ III (courtesy of Edwards Lifesciences LLC).

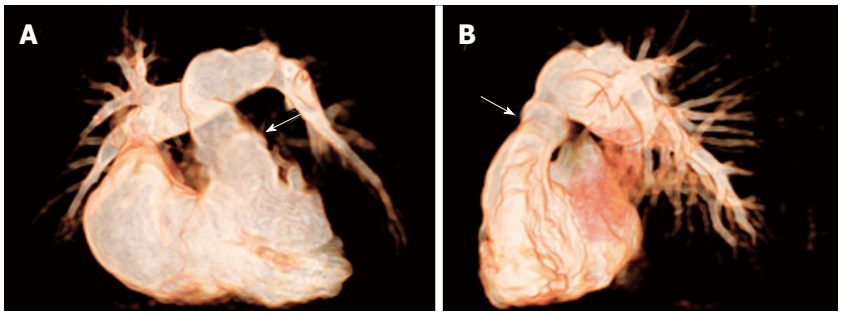

Figure 4 Non-invasive 3D whole heart imaging by magnetic resonance tomography. Non-invasive 3D whole heart imaging by magnetic resonance tomography was performed in a patient with pulmonary atresia with intact ventricular septum after repair by pulmonic homograft implantation (arrows) with RVOT dysfunction prior to PPVI (A) in a.p. and (B) in lat. view. (Courtesy of Wagner R). RVOT: Right ventricular outflow tract; PPVI: Percutaneous pulmonary valve implantation.

allow stent ingrowth for two to three months. Combined procedures as well as a two-staged procedure are valid options.

Results

Data regarding the haemodynamic outcome post PPVI reported by major reports using MELODY™ [18,19,21,24,33] and using the SAPIEN™ device[28,30] are summarized in Table 3.

Several mono- and multicenter trials consistently reported a low periprocedural complication rate of six percent using the MELODY™ pulmonic valve[19,33]. Data analysis of the "MELODY™ Registry" data distinguishes between major procedural complications [*e.g.*, homograft rupture, perforation of branch pulmonary arteries, guidewire injury, damage to tricuspid valve, device dislodgement, compression of coronary arterie(s) or obstruction of PA] in 2.7% and 11.9% of minor complications in total of 1003 MELODY™ procedures[34].

The "Early Phase 1 International Multicenter Clinical Trial" reporting on SAPIEN™ PPVI in 36 patients reported on a successful valve deployment of 97 percent, but seven patients (20.5%) experienced adverse events[30]. The major complication was device dislodgement. In none of the patients, homograft rupture occurred. All of the SAPIEN™ patients received pre-stenting (33.3%) or peri-procedural stenting procedures (66.6%).

Coronary compression due to RVOT interventions with bare metal stenting of the RVOT is a well-known and previously described complication[31]. Approximately five to six percent of all patients who are potential candidates for PPVI will have a coronary artery anatomy which bears the risk for coronary obstruction[24]. There are several reports of this potential catastrophic complication[35,36] which is strongly related to early procedural mortality[21]. Ruling out the risk for this complication represents one of the most difficult steps in pre-procedural planning for PPVI. In any case of doubt about the risk of coronary compression, we recommend to abandon the implant in either MELODY™ or SAPIEN™ valve procedures (Figure 5).

Follow-up

Short- and medium-term results of PPVI with the MELODY™ and SAPIEN™ are thought to be similar, although more data are available for the former. Long-term outcome data for both valved stent types are not yet available.

Overall mortality of PPVI during follow-up procedures was zero to five percent and seems not related to the device itself.

Failure of the device either for the MELODY™ or the SAPIEN™ could be related to malfunction of its stent or its sewn valve. Relevant dysfunction of the engrafted valve leads to pulmonary regurgitation which is rare condition that almost only occurs in the context of graft endocarditis[37-40], However, the most common reason for re-operation and re-intervention is re-stenosis of the stent portion of the device. Re-stenosis of the stent can be caused by late recoil or lost of radial strength of the device due to stent fractures. Novel data by Nordmeyer reported of a rate of 11 percent cases of stent fractures[34] representing the most common reason for re-intervention.

Overall, data are available from the major four short- and medium-term observational studies with a total of over 450 patients with one- to five-year follow-up[19,21,25,33]: freedom from valve dysfunction or re-intervention was approximately 94% at one year follow-up. Patients who did not require re-intervention had consistently mild or none pulmonic valve regurgitation at one-year follow-up. Pulmonary regurgitation decreased from median values of 16% to 27% to one to two percent. Median peak velocity over the RVOT was 1.9 to 2.7 m/s at one-year

Table 3 Haemodynamic outcome immediately post-percutaneous pulmonary valve implantation

	US Melody Valve Trial (n = 124)		London Melody experience (n = 151)		Munich/Berlin Melody experience (n = 102)		Philadelphia Melody experience (n = 104)		Early Edwards experience (n = 7)		Later Edwards experience (n = 36)	
Parameter	Pre (median)	Post (median)	Pre (mean)	Post (mean)	Pre (mean)	Post (mean)	Pre (mean)	Post (mean)	Pre (mean)	Post (mean)	Pre (mean)	Post (mean)
RV systolic pressure, mmHg	65	41[b]	63	45[b]	NA	NA	72	47[b]	NA	NA	55	42[b]
Peak RV to PA gradient, mmHg	37	12[b]	37	17[b]	37	14	39	11[b]	NA	NA	27	12[b]
RV to systemic pressure, %	0.74	0.42[b]	0.69	0.4[b]	0.62	0.3[b]	NA	NA	0.78	0.3[b]	0.6	0.4[b]

Invasively measured pressures and gradients pre and post percutaneous pulmonary valve implantation within the largest trials (n > 100) of the MELODY™[18,19,21,33] and of SAPIEN™ implants[28,30] in pulmonary position. In all studies, a profound improvement in RV to systemic pressure ratio in response to PPVI was seen ([b]$P \leq 0.001$) (all parameters expressed by medians). RV: Right ventricular; PA: Pulmonary artery. NA: Not available.

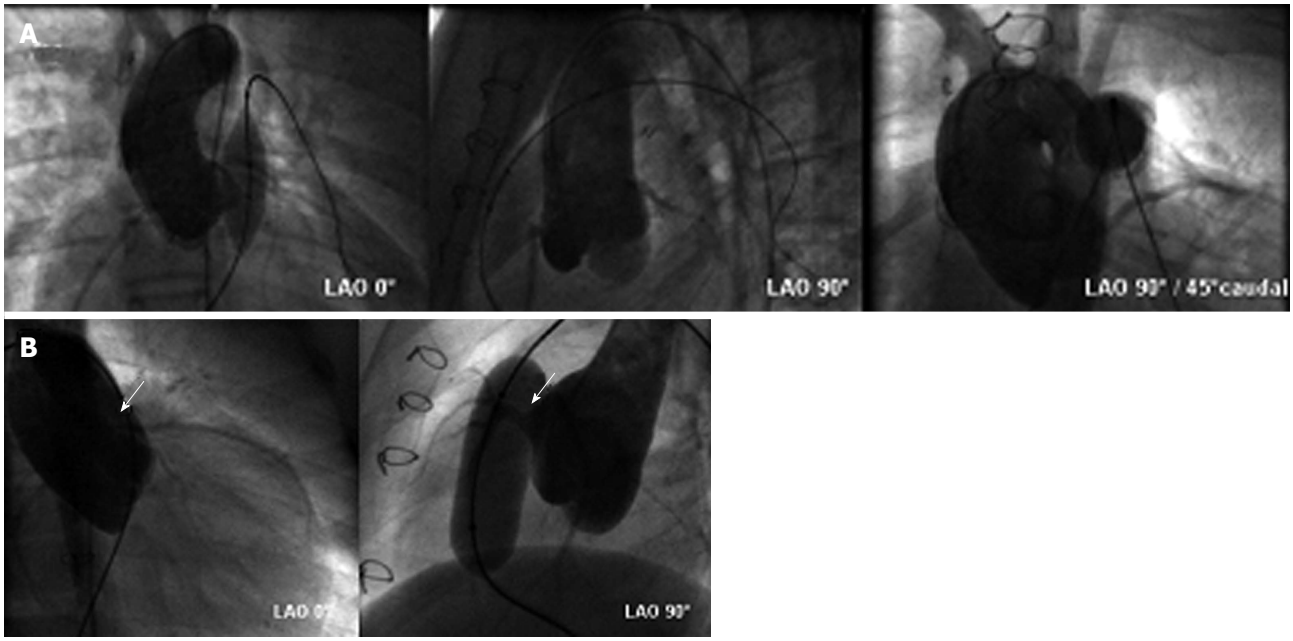

Figure 5 Assessment of risk for coronary compression. A: Aortic root angiogram and simultaneous (high-pressure) balloon inflation within the eligible landing zone is performed to rule out potential for coronary compression (courtesy of Wagner R); B: Aortic root angiogram showing compression of the left anterior descending coronary artery (arrows) during balloon inflation in the conduit. The procedure was therefore abandoned in this patient and no percutaneus pulmonary valve implantation was performed (courtesy of Wagner R).

echocardiographic follow-up. Nordmeyer recently reported preliminary but promising MELODY™ Registry data with a one-year freedom for all case events with 92.5% and 94.2% for PPVI-related events[34].

Data on device function of the SAPIEN™ valve are limited and available from smaller short- and medium-term observational studies with a total of less than 100 patients[28,30,41,42]. In the largest series (COMPASSION trial), successful valve deployment was achieved in 33 of 34 attempts[30]. Freedom from re-intervention at six-month follow-up was 97%. Haas *et al*[41] demonstrated a significant reduction of the RVOT to PA gradient, reduction in RV systolic pressure, increasing of diastolic pulmonary pressure from 6.3 to 14.5 mmHg as a sign of tremendously decreased pulmonary regurgitation with freedom from re-intervention after six months[41].

As for the MELODY™ valve, there are no comparison studies with conventional surgery regarding pulmonary valve replacement by SAPIEN™ valves.

Regarding the functional outcome, several studies have shown a marked improvement in NYHA functional class post-PPVI[18,19,33]. The improvement has been maintained consistently for the duration of follow-up, irrespective of the treated lesion (predominant stenosis *vs* predominant regurgitation)[24].

Parameters of exercise cardiopulmonary function such as peak oxygen consumption related to body weight (VO_2/kg), ventilatory efficiency and anaerobic oxygen consumption have been assessed in several studies addressing PPVI[20,43-45]. Only patients with a predominant stenotic lesion showed an improvement in peak VO_2/kg. Assuming that significant RVOT

obstruction may limit the increase of cardiac output in exercise testing resolving of the obstructive lesion at least partially reverses limited exercise capacity in these patients[45,46]. Recently, we reported on the ability to recovery from exercise as described by VO_2 and VCO_2 decay after maximal exercise. Recovery form exercise after PPVI improves in both groups (predominant stenosis *vs* predominant regurgitation). These findings could explain the symptomatic improvement observed in patients with predominant regurgitation despite the lack of increased maximal exercise capacity and might have implications for how we judge procedural success[47].

Right and left ventricular function and calculation of great vessel blood flow analyzed by functional and morphologic MR imaging performed prior to and within one month after PPVI has shown mixed data regarding changes in RV ejection fraction following PPVI, with some studies finding no change[21,33] and others reporting on improvements in the acute or short term[23,43,44,46]. Importantly, PPVI also results in improved left ventricular filling[13,44,48].

Extended indications and future directions

Many patients are not ideal candidates for PPVI procedures due to their small physical size, limited vascular access or, most important, due to the size and shape of the RVOT[49]. The majority of patients suffering from dysfunction of the RVOT have enlargement of the patched RVOT as part of the initial surgical repair strategy[24]. This unmet clinical challenge led to the development of novel approaches to treat RVOT dysfunction using existing interventional pulmonic valve technology. A small case series reveals an approach to implant or post-dilate the MELODY™ valve using 24 mm balloons. This practice does not compromise function of the engrafted valve and may effectively broaden the pool of eligible patients[24,50].

However, RVOT dysfunction with predominant regurgitation and marked dilatation are not be eligible to this approach. Treatment strategies, *e.g.*, MELODY™ valve implantation into the branch pulmonary arteries[51,52] or a "jailing" procedure of the pulmonary bifurcation by implanting a bare metal stent across the main pulmonary into a pulmonary branch have been described as potential options[53].

A hybrid approach combining intra-operative PPVI with simultaneous conduit down-sizing[54] or direct exposure of the RV or RVOT (*e.g.*, after failed percutaneous attempt, "bailout" procedure) have also proven to be feasible[55].

Innovative (experimental) technologies, *e.g.*, the self-expanding Medtronic Native Outflow Tract device[56], infundibular reducer devices[57] or newer low-profile pulmonary valves such as the Colibri Heart Valve (Colibri Heart Valve, LLC, CO, United States) indicate future treatment alternatives and hopefully will offer a non-surgical treatment to a much broader patient population[58].

PERCUTANEOUS TRICUSPID VALVE IMPLANTATION

Background and clinical indications

Primary tricuspid valve disease is rare: the underlying etiology can be of either congenital (Ebstein, tricuspid valve dysplasia) or of acquired nature (*e.g.*, rheumatic, endocarditis or carcinoid disease). RV volume and/or pressure overload, left heart failure or mitral valve dysfunction can result in secondary RV enlargement, geometric distortion and tricuspid annular dilation. These circumstances can promote concomitant tricuspid regurgitation, thus called functional tricuspid regurgitation (80 percent of all cases)[59,60]. Patients with tricuspid regurgitation may be asymptomatic for prolonged periods. Surgical treatment is often reserved for advanced stages of tricuspid disease when dysfunction, particularly in patients with congestive heart failure, has led to symptomatic right heart failure[61]. For that reason, patients undergoing tricuspid repair or replacement procedures tend to be at higher risk with poorer outcome[61]. European and American guidelines on surgical management of valvular heart disease were updated in 2012[62] and confirmed in 2014[60]. The level of indication was raised to Class I and IIa for most situations of functional tricuspid regurgitation[62]. A transcatheter approach for tricuspid valve repair or replacement seems to be desirable and beneficial to his high-risk population but is still a long way ahead[2].

Patient selection criteria (in selected series)

Van Garsse *et al*[63] reported on the first "Percutaneous Transcatheter Valve-in-Valve Implantation in stenosed Tricuspid Valve Bioprosthesis" in 2011 amongst other case reports with small patient numbers[64-66].

A multicenter series by Roberts *et al*[67] enrolled 15 patients with failing tricuspid prostheses of whom ten underwent implantation into various failing bioprosthetic valves after careful (echocardiographic) confirmation of a suitable anchor point that allows safe positioning of the stented-valve. Median NYHA class was III and all patients were considered to be "high risk" for conventional surgery for tricuspid prothesis failure. The primary lesion was predominant stenosis (mean gradient > 5 mmHg, mean inflow gradient 12.9 mmHg), although a few had significant regurgitation.

Recently, Cullen *et al*[5] has reported a single-center series on transvenous Melody "Valve-in-Valve" implantation for bioprosthetic valve dysfunction that enrolled ten "high risk" patients with failing tricuspid prosthesis among others. Patients were considered candidates for the interventional procedure if they had significant bioprosthetic tricuspid valve dysfunction (either stenosis, regurgitation, or both) with co-morbid

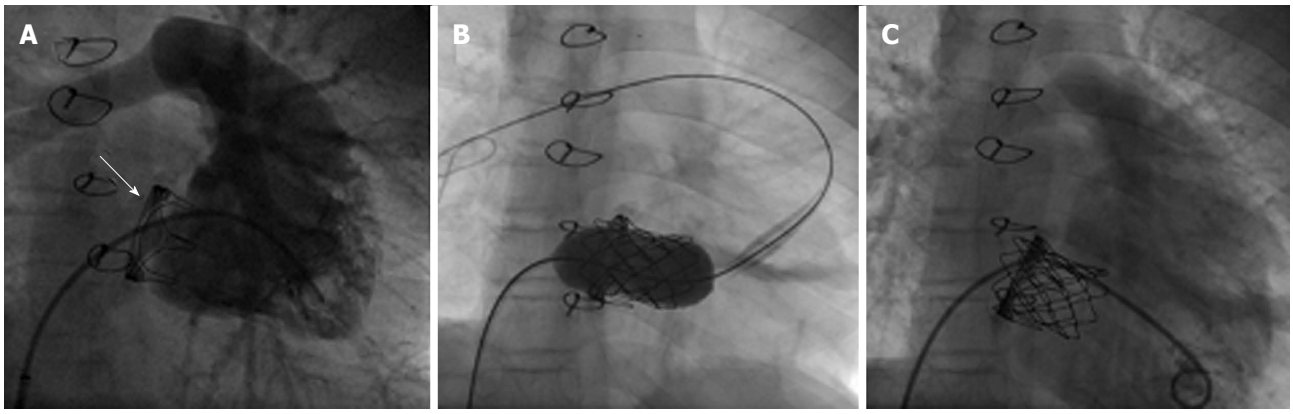

Figure 6 MELODY™ device for "Valve-in-Valve" implantation in tricuspid position. MELODY™ valve implantation procedure in a patient with severe stenosis of a prosthetic biological tricuspid valve (arrow): fluroscopy reveals (A) a RV angiogram in systoly prior to implantation (B) guidewire in the right PA and stable device position after inflating of both balloons of the BiB-delivery system and (C) RV angiogram showing stent position within the biological prosthesis and relative to the ventricle. There is no tricuspid regurgitation. (Courtesy of Wagner R). RV: Right ventricular; PA: Pulmonary artery.

conditions which would preclude surgery. Median NYHA class was Ⅲ with seven of the ten tricuspid patients suffering from moderate or worse tricuspid valve regurgitation with a mean inflow gradient 10 ± 4.3 mmHg.

After all, patient selection criteria for percutaneous tricuspid valve replacement are yet based on (very) limited data. Principally, if a percutaneous approach seems to be an option of treatment in clinical practice, the clinical indication for "Valve-in-Valve" implantation should be based on the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) or the American College of Cardiology/American Heart Association guidelines for tricuspid valve surgery. The percutaneous approach should be reserved particularly for those cases considered to be high-risk cases for conventional surgery[68].

Devices for tricuspid valve implantation

Two percutaneous devices have been described for transcatheter valve implantation in failing bioprosthetic valves so far. These are the Edwards SAPIEN™ and its iterations and the Medtronic MELODY™ valve as described previously (Figure 6).

So far, none of them have been approved or certified to be delivered in tricuspid position. Therefore, implantation of these devices in tricuspid position is off-label-use.

Results

In both series procedural success with device deployment was achieved in all of the tricuspid patients[5,67]. There were no early periprocedural complications in the Cullen series. Procedural complications occurred in one of Roberts' patients (atrioventricular block requiring a pacemaker). Another patient suffered endocarditis eight weeks post-procedural. In both series mean tricuspid gradient decreased significantly (drop to 5.6 ± 2.5 mmHg in Cullen's patients, drop to 3.9 mmHg in Roberts' series). The level of regurgitation revealed to be mild or none in all but one case of the Cullen's series.

Follow-up and outcome

Mean follow-up was nine months in the series by Roberts but with 41 d (range 11 to 209) shorter and more heterogeneous in Cullen's patients. As reported by Roberts *et al*[67] functional class improved in 12 of the treated patients. Nine of the patients sustained the good interventional result nine months after implantation with one percutaneous valve-in-valve which had to be replaced. Cullen's group observed a 30-d readmission rate with three out of ten in the tricuspid patients. And NYHA functional class improvement in nine of the ten treated patients.

Extended indications and future directions

Several groups selected similar patients to demonstrate the feasibility of percutaneous deployment of stent-mounted valves (SAPIEN, SAPIEN™ XT or MELODY™) into the venous system (inferior and/or superior vena cava). The focus is not on the tricuspid regurgitation itself, but rather on its hemodynamic disturbance. These procedures are therefore called "Caval-valve Implantation"[69-72].

Although, a number of animal studies examined the experimental feasibility of percutaneous valve implantation into a native tricuspid valve[2,73,74], Kefer *et al*[75] recently demonstrated feasibility of SAPIEN™ valve implantation into a "native" tricuspid annulus after failed repair without bioprothesis but mixed tricuspid disease.

SUMMARY AND PERSPECTIVE

The aim of PPVI is to prolong the lifespan of surgically placed conduits. The prolonged conduit lifespan, and hence delayed surgery, should limit the number of needed open chest redo-operations over the

patients' total lifespan in cases of congenital and acquired heart disease that indicated the implantation of pulmonic conduits. This sophisticated strategy potentially improves these patients' life expectancy. Pulmonary valve replacement with stent-mounted with stent-mounted valves containing xenograft materials represents the derived valves represents the non-surgical treatment of choice in patients with dysfunction of the RVOT. Although indications continue to extend even to patients with "native", but dysfunctional RV outflow tracts, the diameter of the proposed implantation site limits the feasibility in a relevant number of patients. Even though significant improvement has been achieved in early and late outcomes after PPVI, the risk of stent fractures and graft rupture have not yet been sufficiently explored. Further research is necessary to avoid these complications.

Besides, the extending use of PPVI, the percutaneous approach to tricuspid valve replacement has briefly moved beyond its experimental character. It has been shown to be feasible, but should mainly be reserved for high-risk patients with conditions that preclude surgery.

In conclusion, evolution of the interventional treatment of dysfunctional valves/RVOTs can only be achieved by continuous creative thinking and encouraged teamworking of cardiologists, surgeons, specialists in imaging and bio-medical engineers.

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EDITORIAL

Practical update on imaging and transcatheter aortic valve implantation

Gisela Feltes, Iván J Núñez-Gil

Gisela Feltes, Iván J Núñez-Gil, Cardiovascular Unit, Centro Médico Paris, 28023 Pozuelo, Spain
Iván J Núñez-Gil, Interventional Cardiology, Hospital Clínico San Carlos, 28040 Madrid, Spain
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Correspondence to: Iván J Núñez-Gil, MD, PhD, FESC, Interventional Cardiology, Hospital Clínico San Carlos, Prof. Martín Lagos S/N, 28040 Madrid, Spain. ibnsky@yahoo.es
Telephone: +34-91-3307265
Fax: +34-91-3300000
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Abstract

After very rapid advances in the development of the technique and devices, transcatheter aortic valve implantation (named TAVI or TAVR), is today a reality that is here to stay. It has become the minimally-invasive treatment option for high-risk and non-surgical patients with severe symptomatic aortic stenosis. Requiring the participation of a multidisciplinary team for its implementation, cardiac imaging plays an important role. From pre-assessment to determine the suitability of the patient, the access site, the type of device, to the guidance during the procedure, and ultimately the long term monitoring of the patient. Correct selection of the patient and device, correct placement of the stent-valve and early detection of complications are of paramount importance for procedural success and for patient outcome. Each technique has advantages and disadvantages, being the cardiologist who will determine the best approach according to the type of patient and the expertise of the center in each one of them. This article summarizes the last contributions of the most common used imaging techniques, in each step of the procedure.

Key words: TAVI; TAVR; Echocardiography; Multislice tomography; Cardiac magnetic resonance; Aortic stenosis

Core tip: Cardiac imaging is of crucial importance in the whole process of transcatheter aortic valve implantation, from initial evaluation, intraprocedural guidance and post implantation evaluation and early detection of complications. Multiple techniques are available for this, and as the rapid development of new devices and equipments, the greater the necessity of being aware of these advances. We provide current data and tips for this purpose. This is the reason of this work.

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INTRODUCTION

Since its beginnings 20 years ago, with first implantations in animals, transcatheter aortic valve implantation (TAVI or TAVR) has evolved substantially[1].

With the advancement of cardiovascular imaging, the application of the most innovative techniques acts jointly to obtain the best clinical results. At present, TAVI is a serious alternative treatment for inoperable or high risk patients with aortic stenosis (AS). In addition, is expected to expand quickly to other subgroups (intermediate risk and aortic regurgitation), since trial results are encouraging compared with medical treatment and cardiac surgery.

Several bioprosthesis types are available, being by far the most commonly used the self-expandable porcine Medtronic CoreValve (Medtronic Inc, Minneapolis, MN, United States), available in the sizes 23 mm, 26 mm, 29 mm, and 31 mm, and the balloon-expandable Edwards Sapien XT bovine valve (Edwards Lifesciences Inc, Irvine, CA, United States), available in multiple sizes: 20 mm, 23 mm, 26 mm, and 29 mm. Both models recently introduced their latest valve generations: Corevalve Evolut R and the Sapien 3, with several advantages and thinner sheaths-introducers (up to 14F). There are now being marketed other valves with different delivery systems, like for instance the Direct Flow valve, the Jena Valve or the Lotus valve, between others.

The preferred implantation route is usually transfemoral. If this is not possible because of patient characteristics, both valve types can be implanted via the subclavian artery, and the Edwards Sapien valve can be implanted *via* a transapical access.

Here we review the contribution of imaging techniques to the whole process of selection of patients and prosthesis, intraprocedural guidance and evaluation of deployment and complications.

PRE-IMPLANTATION EVALUATION

Aortic stenosis severity

Invasive cardiac catheterization used to be the standard for quantification of AS, but nowadays echocardiography is used for diagnostic purposes and in its replacement. Transthoracic echocardiography (TTE) allows evaluation of a calcified valve with restricted leaflet opening and quantification of peak and mean aortic valve (AV) gradient by applying the simplified Bernoulli equation ($\Delta p = 4v2$) to the maximal velocity recorded through the AV by continuous wave Doppler. Severe aortic stenosis is defined as a peak velocity > 4.0 m/s (peak gradient of 64 mmHg), a mean gradient > 40 mmHg, or valve area (AVA) < 1.0 cm^2 (0.6 cm^2/m^2) whith normal left ventricular (LV) systolic function[2]. In cases of low gradient with small area, a dobutamine stress study (maximum dose 20 mcg/kg per minute), may be helpful to determine if the valve is truly severely stenotic, when the maximum jet velocity rises over 4 m/s with the dobutamine-induced increase in stroke volume, whereas the AVA remains less than 1.0 cm[2]. The AS is only mild to moderate in severity if stroke volume increases but there is a small rise in gradient (and therefore the valve area increases greatly), and thus other causes are the origin of LV dysfunction[3].

Annular size

Particularly in the candidates selected for TAVI, other parameters than the severity of the stenosis and the ejection fraction must be evaluated previously to the intervention.

The most important aspect of anatomical screening includes assessment of the arterial vasculature and aortic valvar complex [left ventricular outflow tract (LVOT), aortic annulus, sinus of Valsalva, sinutubular junction and ascending aorta]. All this data will guide physicians to choose the most appropriate access route (subclavian, transfemoral, transaortic or apical) and transcatheter valve size, and it will help to be alert in the detection of potential complications during the procedure[4].

An annular size accurate evaluation is of utmost importance. Underestimation of its dimension could lead to selection and deployment of a smaller valve, with possible complications like paravalvular regurgitation, poor hemodynamics, valve migration and embolism. Overestimation of annular size and deployment of a larger valve can lead to incomplete unfolding (with the consequence of valvular and paravalvular regurgitation) or annular rupture. TAVIs are designed to be utilized in slightly smaller annuli than the prosthesis size[5]. The annular size and the correspondent prosthesis are listed in Table 1.

Aortic annulus can be evaluated using various techniques. Echocardiography is extensively available, repeatable, and easy to perform even taking into account that transesophageal echocardiography (TEE) is semi-invasive and usually requires sedation[6] (Figure 1). We use the sagital plane obtained from a 2-dimensional (2D) parasternal long axis image (TTE) or a mid-esophageal long axis (TEE) image among 120° and 140°, during early systole, measuring from the right coronary cusp to the left noncoronary commissure. To obtain measurements in the coronal and sagittal planes three-dimensional (3D) reconstructions and biplane imaging can be performed.

Multislice computed tomography (MSCT) can give appropriate measurements and may provide important additional information like the anatomy of the coronary arteries, the aortic valve anatomy and area, the plane of the valve and the amount and distribution of calcifications, but iodine injection and radiation are relative limitations. It is important to remember that the aortic annulus is not only a complex 3-dimensional structure, but also that its shape is oval and not circular in the vast majority of patients, as it was demonstrated in previous MSCT studies[7]. The aortic annulus plane is acquired by a reconstruction using two orthogonal planes, the short and long axis of the virtual basal ring, and measurements are taken from systolic phase reconstructions from 20% to 45% of the R-R interval. MSCT multiplanar reconstructions

Table 1 The annular size and the correspondent prosthesis

Prosthesis type	Prosthesis size (mm)	Annular size (mm)	Introducer profile (F)	Minimun vessel diameter (mm)
Edwards Sapien	23	18-22	22	⩾ 7
	26	21-25	24	⩾ 8
Edwards Sapien XT	20	16-19		
	23	18-22	16	⩾ 6
	26	21-25	18	⩾ 6.5
	29	24-27	20	⩾ 7
Edwards Sapien 3	23	18-22	14	⩾ 5.5
	26	21-25	14	⩾ 5.5
	29	24-28	16	⩾ 6
CoreValve	23	18-20	18	⩾ 6
	26	20-23	18	⩾ 6
	29	23-26	18	⩾ 6
	31	26-29	18	⩾ 6
CoreValve evolut R	23	18-20	12	⩾ 6

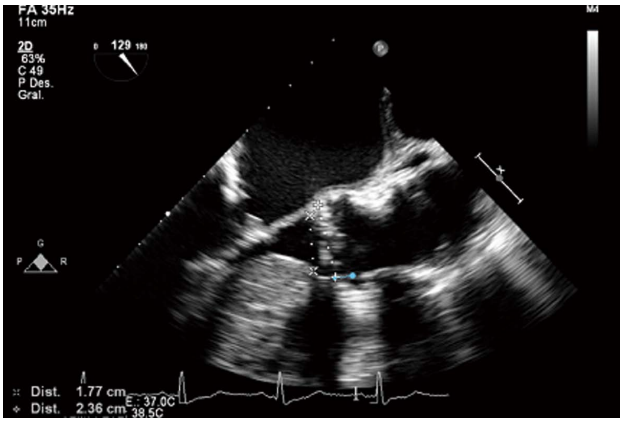

Figure 1 Transesophageal echocardiography 2D at 129° in long axis view in systole with measurements of the left ventricular outflow tract and aortic annulus. The measurement in blue is the distance to the rigth coronary artery.

(MPRs) can provide coronal, sagittal and axial images of the aortic root, with accurate measuraments, almost always underestimated by 2D echocardiography.

On the other hand, cardiac magnetic resonance (CMR) permits an anatomic and functional evaluation of the aortic valve and aortic root, with most sequences in 2D and the selection of the imaging plane during the examination. However, whole heart, echogated 3D CMR with contrast allows obtaining images for multiplanar reconstruction and demonstrates the oval shape of the annulus with minimal and maximal diameters.

Every technique has its advantages and disadvantages. The annulus size is in general 1 mm smaller by TTE than by TEE, and the TEE measurement is 1 to 1.5 mm smaller than MSCT measurements[8,9]. Echocardiography (TTE or TEE) still is the most used technique to assess the aortic annulus, nevertheless, with the acquisition of more data in the near future, MSCT will probably become the first imaging modality to do this.

Aortic anatomy

The characteristics of the aortic valve, like the number of cusps, grade of calcification, thickness and mobility are important to predict the procedure success. Congenital or acquired bicuspid aortic valve stenosis were initially considered a contraindication for TAVI, nevertheless, several successful case reports have been documented and today is considered a relative issue[10-12]. It is frequently not easy to examine cusp anatomy in the severely calcified valves, but in these cases, MSCT or review of old echocardiograms may permit better evaluation of the underlying anatomy. With echocardiography important calcification might cause acoustic shadowing, so MSCT is nowadays the technique of choice in evaluating severity and showing the location of aortic cusp calcification. CMR is not a good choice because of the signal void caused by calcium. Large aortic valve calcifications raise the risk of gaps between the external face of the prosthesis and the patient's native valve, allowing paravalvular regurgitation leaks. Also, the asymmetry, severity and the prosthesis "landing zone" calcification, may produce differences in the tension-force across the valve, with the consequent asymmetric deployment of the device and increased risk of obstruction of the coronary arteries ostium; and important sinotubular junction calcifications may cause limitation during balloon dilatation at the aortic end and may produce ventricular displacement of the prosthesis at the moment of unfolding[13].

Another important issue to consider is the distance from the annulus to the coronary ostia, in order to avoid its compromise during the valve deployment. The distance to the right coronary ostia is easily determined with TEE (Figure 1), but not to the left coronary ostia, which requires 3D TEE (Figure 2). MSCT provides a more comprehensive assessment, showing an average annular-right coronary artery distance of 13.6 ± 2.8 mm and annular-left coronary artery distance of 13.4 ± 3.2 mm[5]. The distance between the aortic valve annular plane and the coronary ostia should be at least of ⩾ 10-11 mm for both type of prosthesis.

Aorta evaluation

Evaluation of the aortic root and tubular portion of the aorta is as well essential, specially when using Core Valve, because its length is greater compared with regular valves, ranging from 52 mm (31-mm valve) to 55 mm (26-mm valve, *i.e.*,). It is advised that the dimensions of the tubular aorta measured at 45 mm above the annulus be 40 mm for the 26-mm valve and 43 mm for the 29-mm and 31-mm CoreValve prosthesis. MSCT can provide an excellent reconstruction and evaluation of the aortic sinus diameter, sinotubular junction, ascending and descending aorta and its iliofemoral branches (Figure 3). This is very important for the selection of the vascular

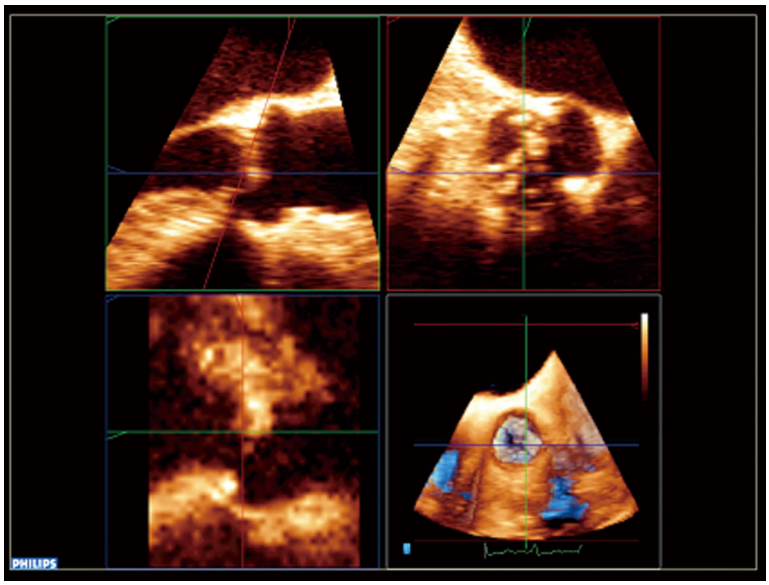

Figure 2 Transesophageal echocardiography 3D with multiplanar projection showing the measurements at annulus level.

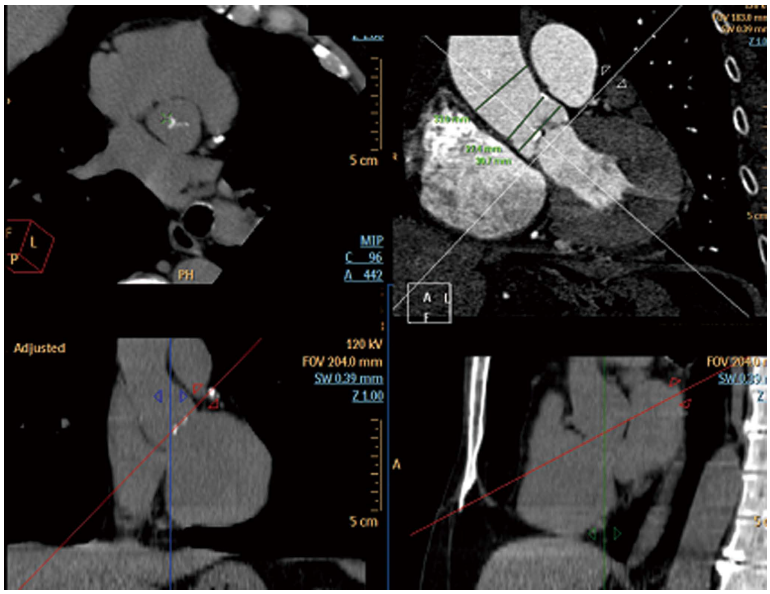

Figure 3 Computed tomography with multiplanar reconstruction showing measurements at different levels of the aorta.

access, because its complications rates range from 5% to 25%[14], and are associated with a striking increase in early mortality risk. Its evaluation often begins with conventional angiography, but it proportionates a very limited data. MSCT provides many reconstructions, as 3D volume rendered imaging, curved multiplanar reformats, and maximum intensity projection images allowing evaluation of vessel size, minimal luminal diameter, calcification severity, plaque burden, vessel tortuosity and identification of high risk characteristics like dissections and complex atheroma[15] (Figure 4). Calcification of less than 180º or eccentric calcification usually do not cause procedural trouble like would nearly circumferential and luminal calcification do. Vascular complications and 30 d mortality can be predicted with the use of a sheath/femoral artery ratio of 1.05 or higher[16]. It is remarkable that the existence of significant aneurysmal dilatation in ascending aorta is a contraindication for the use of CoreValve.

Like MSCT, CMR can give exhaustive evaluation of the aortic valve, annulus, aortic root, coronary ostia, course of the thoracoabdominal aorta and luminal caliber of the iliofemoral branches, and LV function, with the advantage of not using ionizing radiation (Figure 5). Also, free-breathing noncontrast navigator-gated 3D whole-heart acquisition can be acquired, similar to the volumetric acquisition of CT[17].

Other issues

Calcification in other areas, as dense calcification in the intertrigonal area rises the risk of paravalvular aortic regurgitation (AR) due to asymmetric unfolding of the valve[18]. The angle between the aorta and the LV and proximal septal hypertrophy are essential issues to consider when planning the procedure. A very prominent proximal septum is an important consideration to have in mind during the placement of the valve because can cause valve repositioning when stopping the pacing run[15]. LV function must be evaluated in order to minimize the number of pacing

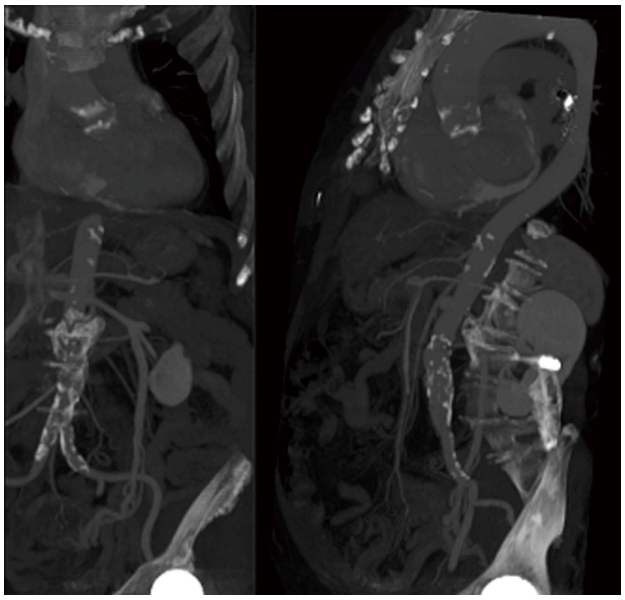

Figure 4 Computed tomography images showing aortic anatomy and calcifications, previous to transcatheter aortic valve implantation procedure.

runs to prevent hemodynamic compromise in those with severe dysfunction. The degree of baseline aortic regurgitation is also very important, since balloon inflation might aggravate regurgitation and cause hemodynamic deterioration.

In conclusion, echocardiography (in all modalities, TTE, TEE, 2D and 3D), MSCT and CMR can be used to make a pre-implantation evaluation, but it is very important to remind that the imaging technique used might influence TAVI size selection and strategy.

INTRAPROCEDURE GUIDANCE

During the procedure, fluoroscopy and angiography are the principal techniques used to guide device placement (Figure 6). However, they involve several shortcomings, for example: (1) radiation use; (2) restricted 2D visualizations with scarce soft-tissue contrast, sometimes preventing early identification of complications (cardiac tamponade, *etc.*); and (3) the reiterated use of nephrotoxic contrast media to observe the aortic annulus and coronary ostia during the procedure.

Nevertheless, other imaging modalities, like TEE, may overcome the lower soft tissue contrast resolution of fluoroscopy and do not use radiation or contrast. Specially 3D-TEE permits a good visualization of the guide wire path and allows a good assessment of the prosthesis position on the balloon, with respect to the native valve annulus and other structures. Using mid-esophagus long axis view is possible to observe the guide wire across the aortic valve in its delivery, retrograde (transaortic, transubclavian, transfemoral) or anterograde (transapical)[13]. For the adequate position of the prosthesis, TEE can be very useful. In

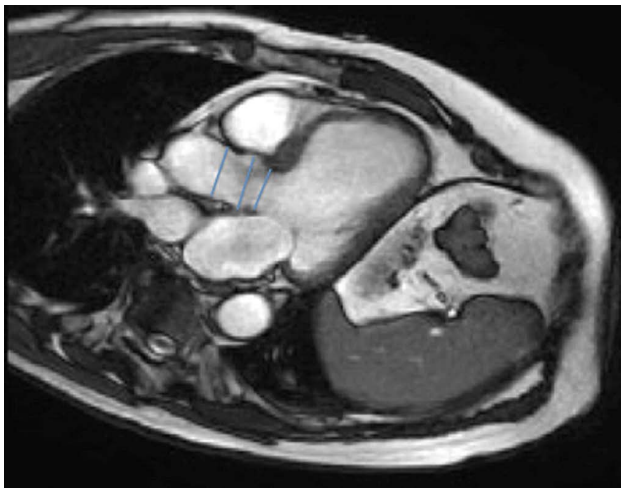

Figure 5 T1 weighted cardiac magnetic resonance image depicting aorta measurements.

the case of Sapien valve, about half of the prosthesis should be below and above the aortic annulus, and when using CoreValve, the nitinol stent must be well within the borders of the calcified native annulus. With 3D TEE it is feasible to observe ortogonal planes simultaneously, aortic valve's views in long and short-axis in realtime, very helpfull for every step of the procedure (aortic valve pass, balloon inflation and prosthesis unfolding).

Intracardiac echocardiography has also been used for TAVI guidance, but imaging abilities are worse than with TEE[19].

In patients with preserved LV systolic function, it was described an improvement in diastolic function, evaluated minutes after deployment with TEE, evaluated through E wave deceleration time, E wave velocity and isovolumetric relaxation time[20].

Immediately after deployment, it is very important to discard complications, for example AR, the most common one. This must be performed rapidly and in multiple echocardiographic views to permit possible reballooning or up delivery of a second prosthesis if the AR is severe and cannot be controlled in another way. It is of utmost importance to differentiate between paravalvular and valvular regurgitation (Figures 7 and 8). Because of irregular calcification in the native valve, small paravalvular leaks are usually observed due to gaps between the annulus and the device, particularly at the commissural areas.

In the case of paravalvular jets, the recommendations indicate that the rate of the circumference of the annulus occupied by the jet offers a guide to severity: < 10% mild, 10%-29% moderate, and ⩾ 30% severe[21]. In a work of our group, we found that the vena contracta planimetry on 3D TTE was better correlated with AR volume than vena contracta width on 2D TTE [Kendall's τ = 0.82 (P < 0.001) *vs* 0.66 (P < 0.001)]. The areas under the receiver operating characteristic curves were 0.96 for vena contracta

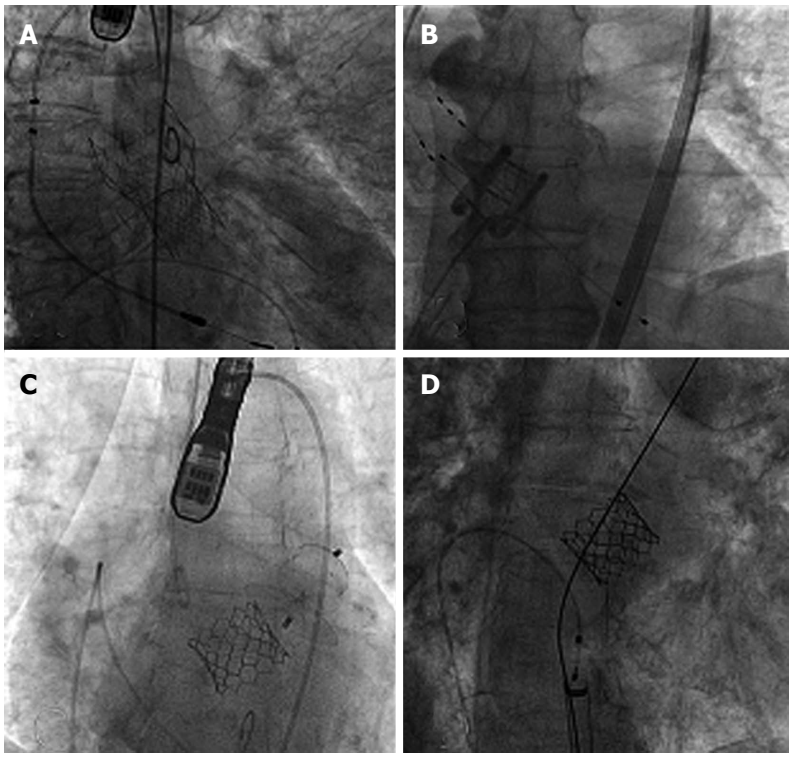

Figure 6 Fluoroscopy images showing different prosthesis models. A: CoreValve evolut R; B: Direct Flow valve; C: Edward Sapien 3; D: Edward Sapien XT.

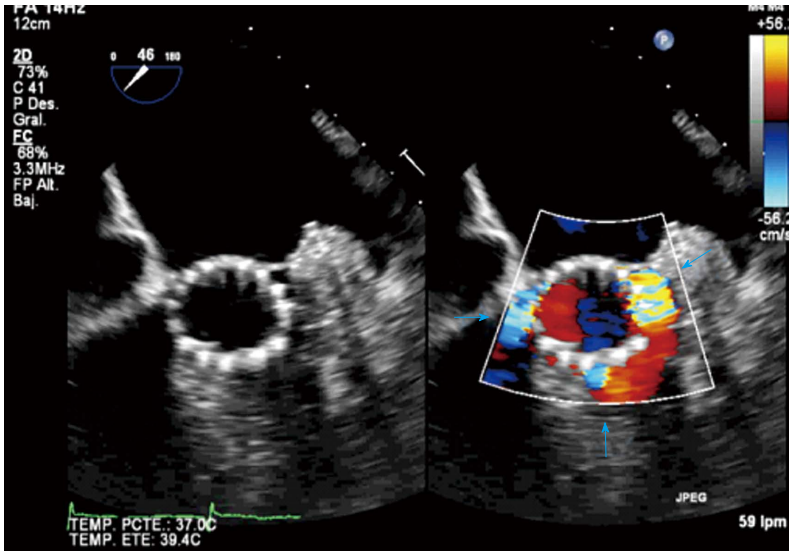

Figure 7 Transesophageal echocardiography at 46° without and with color Doppler showing 3 paravalvular leaks (arrows) after valve implantation.

planimetry and 0.35 for vena contracta width[22]. To avoid paravalvular leak, is required that the covered portion of the prosthesis must be well-apposed to the host valve and interleaflet triangles and the ventricular border of the device just under the hinge points of the AV. It is also common the presence of mild central valvular regurgitation that frequently resolves with removal of the guidewire; otherwise, it must be assessed looking for underexpansion of the prosthesis (Figure 9). Significant valvular AR is generally due to AV harm in the course of the procedure, too large a device for a little annulus with consequent valve distortion, or severe calcification of the patient's AV producing deformation of the frame of the valve[23].

Obstruction of a coronary artery by the prosthesis or displaced calcium is another possible complication. This situation can be seen as regional hypokinesis, best evaluated from the transgastric view and, if it is possible, assessing the coronary arteries flow. If there is LVOT obstruction, this may cause hypotension because of rapid drop in afterload. Other causes must also be considered, like severe mitral regurgitation, pericardial tamponade, displacement of the device, air embolism, right ventricule perforation by a pacemaker lead, aortic dissection and vascular access bleeding.

Other imaging techniques in vogue at the moment are fusion imaging modalities, like C-arm computed tomography with valve landmark detection and automatic aorta segmentation, that tries to make simpler the procedure using 3D over fluoroscopic

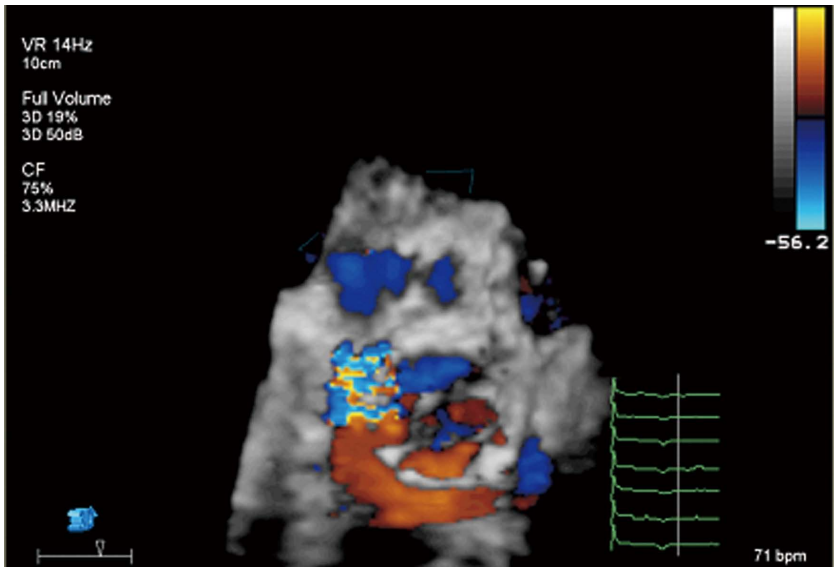

Figure 8 Transesophageal echocardiography 3D full volume showing the exact position of an important paravalvular leak.

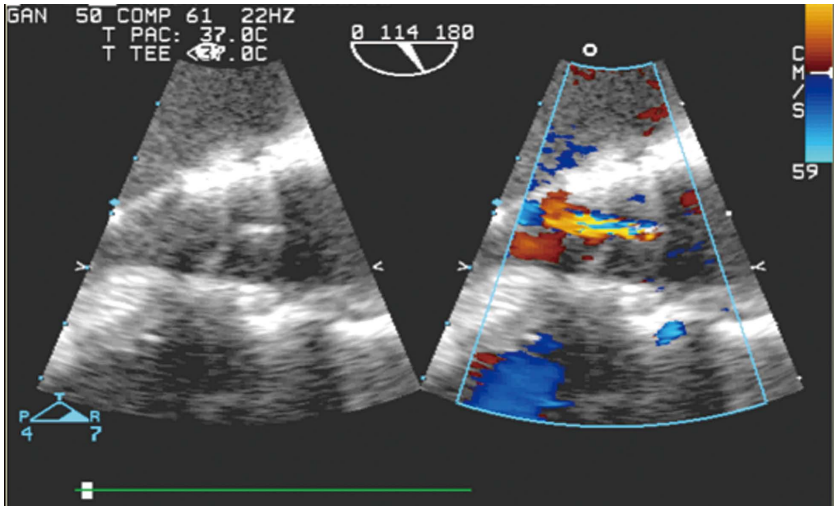

Figure 9 Transesophageal echocardiography at 114° without and with color Doppler depicting the presence of central aortic regurgitation due to underexpansion of the prosthesis.

imaging[24]. CMR capacities are real-time image without restricted scan plane orientation and incomparable soft-tissue contrast, with concomitant display of the prosthesis; and it seems convenient over X-ray angiography and fluoroscopy during all of complete procedure. It allows on-line monitoring cardiac performance, instant recognition of vascular and cardiac harm, and real-time orientation for axial placement and unfolding of the device[25].

POST-IMPLANTATION ASSESSMENT

After TAVI implantation, the imaging evaluation will contribute to determine the valve hemodynamic condition, like effective valve area and gradients; the presence and quantity of valvular and paravalvular regurgitation; the effect of the procedure in ventricular function, hypertrophy, *etc*. and the recognition of long term problems like device migration, endocarditis, ventricular perforation, mitral valve impingement and thrombus formation.

Echocardiography remains the technique of choice for this, because of its wide availability, avoidance of ionizing radiation, together with real time hemodynamic and structural assessment. MSCT and CMR can also provide and excellent anatomic detail and detection of complications like pseudoaneurysm of the root or the apex. Postprocedural evaluation of remaining AR by CMR might have a possible part in TAVI patients[26]. Nevertheless, CMR is a time-intensive method, and this could be an important factor, especially in older patients. In cases of renal disfunction the advantages for the use of gadolinium must be greater than the risks of nephrogenic systemic fibrosis[27]. Also CMR is not warranted in patients with defibrillators, pacemakers or intracranial aneurysm clips, even though the prosthesis used now are CMR compatible.

LV function and hemodynamics

Echocardiography is most common used tool to evaluate the changes described after TAVI, like reductions in LV mass[28], recovery in EF[29], amelioration in diastolic function, and reduction of mitral regurgitation[30].

Although, CMR evaluation of LV mass provides a greatly precise calculation[31].

Valve area and gradients

Determination of mean and peak transvalvular pressure gradients and the calculation of effective orifice area are easily obtained with continuous wave Doppler with TTE, not forgetting suprasternal notch and right parasternal windows to confirm that maximum gradients are catched. CoreValve and Edward Sapien valves have very good flow features with mean gradients of 10-15 mmHg, with relatively stable gradients[32] or little rise in mean transvalvular gradient (3.8%/year) and a low decrease in valve area (0.06 cm^2/year)[33].

Aortic regurgitation

Follow-up echocardiograms should recognize the existence, position, and severity of valvular and paravalvular AR, using all the possible views in case of eccentrical jets. Paravalvular regurgitation is generally produced by imperfect device apposition to the host annulus because of to remaining calcium, undersized prosthesis, or too low position of the valve[34], so imaging in multiple planes is necessary. Valvular and paravalvular AR affect LV hemodynamic condition, determined by raised volume burden, consequently affecting chamber dilatation, LV performance, and progress to pulmonary hypertension, so total AR should routinely be calculated combining information from color and spectral Doppler. Three-dimensional TTE allows quantification of AR with greater accuracy than 2D TTE. CMR might be the technique of election in cases of severe AR, or disagreement in gradients with echocardiography[35].

Although it is always difficult to predict the future, TAVI seems a truly promising therapeutic alternative with settled indications nowadays and expanding indications (intermediate risk, aortic regurgitation, valve in valve[36], *etc.*). What seems clear is that cardiovascular imaging will be needed in this field in order to achieve all its potential objetives.

To sum up, although at the begining multiple test and measurements were required, as experience grows, patients and devices selection are improving, with a more rational imaging algorithm, based in local expertise and availability.

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EDITORIAL

Helicobacter pylori vs coronary heart disease - searching for connections

Magdalena Chmiela, Adrian Gajewski, Karolina Rudnicka

Magdalena Chmiela, Adrian Gajewski, Karolina Rudnicka, Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Łódź, 90-236 Łódź, Poland

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Correspondence to: Magdalena Chmiela, Professor, Chief of Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Łódź, Banacha 12/16, 90-236 Łódź, Poland. chmiela@biol.uni.lodz.pl
Telephone: +48-42-6354541
Fax: +48-42-6655818

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Abstract

In this review, we discussed the findings and concepts underlying the potential role of *Helicobacter pylori* (*H. pylori*) infections in the initiation, development or persistence of atherosclerosis and coronary heart disease (CHD). This Gram-negative bacterium was described by Marshall and Warren in 1984. The majority of infected subjects carries and transmits *H. pylori* with no symptoms; however, in some individuals these bacteria may cause peptic ulcers, and even gastric cancers. The widespread prevalence of *H. pylori* infections and the fact that frequently they remain asymptomatic may suggest that, similarly to intestinal microflora, *H. pylori* may deliver antigens that stimulate not only local, but also systemic inflammatory response. Recently, possible association between *H. pylori* infection and extragastric disorders has been suggested. Knowledge on the etiology of atherosclerosis together with current findings in the area of *H. pylori* infections constitute the background for the newly proposed hypothesis that those two processes may be related. Many research studies confirm the indirect association between the prevalence of *H. pylori* and the occurrence of CHD. According to majority of findings the involvement of *H. pylori* in this process is based on the chronic inflammation which might facilitate the CHD-related pathologies. It needs to be elucidated, if the infection initiates or just accelerates the formation of atheromatous plaque.

Key words: *Helicobacter pylori*; Coronary heart disease; Inflammation; Microbiota; Lipopolysaccharide

Core tip: *Helicobacter pylori* (*H. pylori*) is a Gram-negative spiral bacterium which colonizes gastric mucosa of nearly half of human population. A characteristic feature of *H. pylori* infection is an excessive inflammatory response. The majority of *H. pylori* infections remain asymptomatic. However, still it leads to the development of histological gastritis with the recruitment of immune cells. About 10% of infected subjects develop symptomatic gastritis, erosions or peptic ulcer. Gastric cancer is the most severe consequence of *H. pylori* infection. Recently, a possible association between chronic infections with *H. pylori* and extragastric disorders - including coronary heart disease, has been intensively investigated. Here we have revised recent studies confirming or excluding possible

connections between chronic bacterial infections and the occurrence of coronary heart disease (CHD) within different populations, especially in the context of *H. pylori* infections. We have also presented various study approaches investigating direct and indirect interplay between *H. pylori*-driven consequences and CHD development to clarify already gained knowledge and suggest future directions. Considering the significance of already conducted research studies, the involvement of *H. pylori* infection in the process of CHD development is highly probably, however, still a lot need to be done to clarify whether this association is direct (with the involvement of *H. pylori* antigens and products) or indirect (with the involvement of inflammatory-related molecules accelerating/initiating CHD development).

Chmiela M, Gajewski A, Rudnicka K. *Helicobacter pylori vs* coronary heart disease - searching for connections. *World J Cardiol* 2015; 7(4): 187-203 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i4/187.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i4.187

INTRODUCTION

Since classic risk factors do not explain all cases of coronary heart disease (CHD) the concept that atherogenesis may have infectious background should be considered. The role of virus and bacterial pathogens including *Helicobacter pylori* (*H. pylori*) are now considered as factors implicated in the development of CHD. Chronic infections may influence the course of CHD *via* different mechanisms such as chronic inflammatory reactions, an autoimmune processes and modification of classic CHD risk factors. The pioneer finding of Mendall and co-workers, published in 1994, showed that CHD patients have elevated levels of serum anti-*H. pylori* antibodies. Following this finding, some authors confirm and some exclude the existence of this connection. Still there is no consensus on the role of *H. pylori* in either causation or progression of CHD. In order to describe the involvement of *H. pylori* in the development of CHD, it is necessary to find the largest number of reliable research studies confirming this relationship.

PATHOGENESIS OF CHD

CHD is one of the most severe chronic diseases of the coronary vessels - an important health and social problem - often life-threatening. It occurs due to endothelial dysfunction within the vessels, accompanied by an increased blood pressure, remodeling of vascular wall, local inflammation, platelet aggregation and blood clotting. These disorders promote the formation of atheromatous plaque, which is often unstable and subsequently ruptures. This might impair the blood flow leading to vascular blockage or myocardial infarction. Classic risk factors of CHD include cigarette smoking, hypertension, elevated levels of total cholesterol, triglycerides and low density lipoproteins (LDL) *vs* decreased high density lipoproteins (HDL) fraction, diabetes mellitus, as well as raised homocysteine and coagulation factors. Predisposing factors that increase the probability of CHD development are obesity, lack of physical activity, previous incidents of CHD in relatives, male gender, low socioeconomic status, as well as ethnic and behavioral factors that[1,2].

CHD is a group of symptoms resulting from chronic malnutrition and hypoxia of myocardial cells which is accompanied by oppression, burning, feeling the burden, discomfort and chest choking. These disorders are a consequence of atherosclerosis, which histologically is characterized by the accumulation of macrophages (MØ), LDL fractions, foam cells derived from macrophages filled with oxidized (ox) LDL and extracellular cholesterol complexes deposited within the vessels. On the inner surface of the vessel, lipid deposits are formed, which are gradually surrounded by a connective tissue and undergo fibrosis[3,4]. According to the statistics of World Health Organization (WHO), ischemia associated with atheromatous plaque is the main reason for CHD development, 70% of heart failure cases and 80% of sudden cardiac deaths. The natural history of atherosclerosis suggests that lesions in the arteries may occur already in the uterus or in early childhood. However, clinical manifestations of atherosclerosis are associated with the presence of atherosclerotic plaques, which in men usually develops after the age of 50 and in women postmenopausally[1].

DYSFUNCTION OF VASCULAR ENDOTHELIUM AS AN INITIATOR OF ATHEROMATOUS PLAQUE FORMATION

The interior of blood vessels is covered with a single layer of adjacent endothelial cells (size 0.2-0.3 mm) attached to the basal membrane and extracellular matrix molecules through integrin adhesion molecules[3]. Endothelium contacts with smooth muscle cells through gap junctions, which are permeable to the electric current, ions and low molecular weight compounds. Human vascular endothelium is a barrier that separates blood containing clotting proteins, platelets and inflammatory cells, from connective tissue and muscle layers of the blood vessel wall. The balance between the internal and external environment of the vessel depends on mechanical, chemical and immune reactions occurring within endothelial cells[1]. The endothelium is affected by physical pressure of blood flow (hemodynamic forces), various soluble substances and immune cells. Endothelium delivers many effector substances such as vasolidation and vasoconstrictioning factors (determining the proper tension of the vessel wall), cytokines and adhesion molecules (responsible for interactions with blood

cells and the development of the inflammatory response), factors involved in blood coagulation and fibrinolysis. All together the endothelium plays a role in the maintenance of the vascular homeostasis which is determined by its large mass, distribution and the ability to receive and respond to signals from external environment (hemodynamic and chemical stimuli, pO_2), by changing the expression of various active substances and proteins[1,5]. The endothelium expresses structures that are necessary for adhesion, migration, activation and diapedesis of immune cells and platelets, which allows for the development of inflammatory response[6,7]. These are mostly adhesion molecules (selectins) such as: P-selectin (platelet), E-selectin (endothelial) and L-selectin (leukocyte) and immunoglobulin-derived adressins, including: intracellular adhesion molecules (ICAM)-1 and -2, vascular cell adhesion molecule 1 (VCAM-1), platelet endothelial cell adhesion molecule 1, and macrophage chemotactic protein-1 (MCP-1). If endothelium is damaged it loses its functional integrity and homeostasis which initiates the occurrence of multiple lesions[5,8]. This dysfunction is usually leads to increased tension, vascular wall remodeling, vascular inflammation, increased platelet adhesion and aggregation. These processes contribute to the development of atherosclerosis or destabilization of existing atherosclerotic plaques[2].

CHD AS AN INFLAMMATORY PROCESS

In the late 90s we believed that the atherosclerotic process is a response to a mechanical trauma, resulting in the loss of endothelial cell lining in the vessels. Since the majority of CHD symptoms are induced by both local and systemic inflammatory responses, recently the attention is focused on the role of inflammation in the development of atherosclerosis[9-12]. Inflammatory markers, such as C-reactive protein (CRP) have been found to be higher in CHD patients than in controls, similarly to the concentration of interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α) in plasma and supernatants of immune cells stimulated *in vitro* with bacterial lipopolysaccharide (LPS). Increased expression of E-selectin, L-selectin and P-selectin as well as higher expression of VCAM-1 and ICAM-1 was also noted in CHD cases[9,13]. It is difficult to identify factors that initiate cascade of inflammation and plaque formation. However, it is clear that endothelial dysfunction and raised cholesterol play a major role in the inflammation. Cholesterol contributes to the localization of atherosclerotic lesions, preferentially in the sites where it leads to the activation of endothelial NF-κB signal transduction pathway[14]. The inflammatory response is characterized by the influx of MØ and monocytes to the endothelium, with the latter being transformed first into MØ and subsequently to foam cells prior ingestion of oxLDL. Protein components of the LDL particles are processed by macrophages and dendritic cells and presented to T cells in the context of class Ⅱ major histocompatibility complex[15]. Activated MØ and other inflammatory cells release chemokines that stimulate the migration of smooth muscle cells which together with foam cells, form a fibrous cap. This process is facilitated by interferon gamma (IFN-γ) and TNF-α secreted by T helper (Th)-1 lymphocytes, as well IL-12 produced by macrophages and foam cells[16]. The latter undergo apoptosis, and together with cholesterol crystals form lipid plaque cover[13,17]. It has been revealed that atherosclerotic lesions are associated with the increased reactivity of immune cells. The injured tissue releases IL-33 which alarms the immune system, induces expression of adhesion molecules and attracts Th2 lymphocytes delivering IL-4-considered anti-inflammatory cytokine[18-20]. However, a growing body of evidence indicates that IL-4 may play a role in atherosclerosis through induction of inflammatory responses, such as upregulation of VCAM-1 and MCP-1[21]. The main population of cells in newly formed atherosclerotic lesions are T lymphocytes, while in chronic lesions this proportion is reversed towards MØ that initiate immune processes by presenting antigens to T cells and the production of cytokines and chemokines[1,15,16,22].

MØ and neutrophils contains granules where myeloperoxidase and metalloproteinase are stored - the inflammatory markers correlated with a risk of atherosclerosis[23,24]. Myeloperoxidase contributes to leukocyte migration and the accumulation of foam cells. Indirectly it is involved in endothelial dysfunction and the induction of apoptosis with a consequence of plaque rupture and its destabilization. Due to the this, occurrence of vascular tissue factor is released and the activation of the blood coagulation cascade take place. Myeloperoxidase reduces the availability of endothelial nitric oxide and inhibits its diastolic and anti-inflammatory function. Moreover, it is involved in the oxidative modification of LDL to its atherogenous form, recognized by MØ receptors[3,10]. Prominent inflammation markers, activated by myeloperoxidase are delivered by macrophage-derived metalloproteinases (MMPs), hydrolyzing the components of extracellular matrix such as elastin and collagen, leading to the destabilization of atherosclerotic plaque. Metalloproteinases are also involved in the lipid peroxidation process and accelerated consumption of nitric oxide[22]. CRP belonging to the group of acute phase proteins which raises during infection or tissue damage, is an important marker of inflammation and is considered as an indicator of coronary events associated with endothelial damage. The upregulation of CRP is correlated with the elevation of IL-6, TNF-α, obesity and insulin resistance, which may indicate a link between chronic inflammation and endothelial dysfunction[12]. It has also been shown that CRP is more accurate marker of coronary events than the LDL cholesterol. This was based on the observation that women with the highest levels of CRP and low LDL

were more susceptible to acute coronary insufficiency compared with those with high LDL and low CRP levels[25].

INFECTIOUS RISK FACTORS OF CHD

Classic risk factors do not explain all cases of CHD. Many data indicate that atherogenesis may be associated with chronic infections, accompanied by a long-term persistent inflammation[26-30]. Compelling evidence supports also the concept that gut microbiota actively promotes weight gain as well as fat accumulation, and indirectly sustains a condition of low-grade inflammation, thus escalating the risk of CHD[31-33]. The occurrence of microbiota favors not only intestinal but also the systemic exposure to the LPSs of Gram-negative bacteria. This microbiome-derived compound can cause a condition called "metabolic endotoxemia" characterized by low-grade inflammation, insulin resistance, and augmented cardiovascular risk. LPS is a powerful trigger for the cells of the innate immunity[34]. Variety of immune cells (monocytes, macrophages, Kupfer cells, and preadipocytes) and non-immune cells (adipocytes, hepatocytes, and endothelial cells) express Toll like receptor (TLR) 4 complex recognizing bacterial LPS[35]. Upon binding to TLR, it induces the release of proinflammatory molecules that interferes with metabolic paths of glucose and insulin, promotes development of the atherosclerotic plaque, and favors progression of fatty liver diseases[36,37].

Chronic infections may influence the development of CHD *via* various mechanisms such as chronic inflammatory reactions, an autoimmune responses and the modifications of classic risk factors for CHD[26,38]. They may pose direct effect on the vessel wall by inducing foam cell formation[39]. Therefore, *Herpes simplex* and Hepatitis C viruses as well as bacteria such as *Chlamydophila pneumoniae, Mycoplasma pneumoniae, Porphyromonas gingivalis, Streptococcus mutans* and *H. pylori* have been considered as factors involved in the development of CHD[40-45]. It has also been suggested, that *Ch. pneumoniae* promotes atherogenesis by inducing the synthesis of MCP-1, IL-8 and ICAM-1 in endothelial cells[44]. Among various pathogens possibly involved in atherogenesis *H. pylori* is particularly interesting, since it induces chronic long-term infection within gastric epithelium which leads not only to local but also systemic inflammation[45-48].

H. PYLORI A VERSATILE PATHOGEN

H. pylori is a Gram-negative bacterium demonstrating the affinity to gastric epithelial cells and perfect adaptation to the acidic environment of the stomach. In the majority of infected patients the interplay between *H. pylori* and the host cells are transformed into some sort of long lasting homeostasis. The majority of infected individuals (80%-90%) carry and transmit *H. pylori* with no symptoms, however, in some patients these bacteria induce pathological changes like gastroduodenal ulcers, as well as gastric cancers[49]. *H. pylori* are acquired early in life, and if not successfully treated persist for lifetime[50]. It is believed that the history and adaptation of *H. pylori* is associated with the evolution and migration of *Homo sapiens*. This bacterium has evolved to successfully colonize the hostile environment of the human stomach in the face of innate and adaptive immune responses[51]. In some ways, *H. pylori* resemble commensal bacteria. Contrary to this assumption, stays the fact that *H. pylori* expresses virulence factors with unquestionable pathogenic properties. For these reasons *H. pylori* infections should be monitored since, even if asymptomatic, they may cause systemic complications[52,53].

The interactions between *H. pylori* and gastric tissue cells determines the establishment and development of the disease[54]. Colonization of gastric epithelial cells by *H. pylori via* bacterial adhesins is followed by the occurrence of the acute phase of inflammation accompanied by the infiltration of gastric mucosa with granulocytes and MØ. *H. pylori* survives inside epithelial cells, also temporarily in MØ or in other niches within gastric tissues[55]. When infection becomes persistent, acute phase becomes chronic and is accompanied by an infiltration of lymphocytes. Inflammation is necessary for the proper recognition and elimination of infectious agents and tissue healing. But in case of *H. pylori* the inflammatory reaction is excessive and results in the development of pathological processes in gastric epithelium such as erosions, ulcers, modifications in the cells phenotype, their excessive proliferation as well as secretion of proinflammatory cytokines[56-58]. *H. pylori* possess an abundant composition of antigens[59]. Urease and vacuolating cytotoxin (VacA) stimulate inflammatory responses by damaging gastric epithelial cells, whereas cytotoxin-associated gene A (CagA) antigen, when introduced into the host cells through secretion system Ⅳ, evokes structural and functional changes. Also soluble forms of CagA may influence the activity of host gastric epithelial cells stimulating them to secrete IL-8 with chemotactic properties[60-62]. It inhibits proliferation of lymphocytes[63] and enhances expansion of gastric epithelial cells[64]. *H. pylori* modulates the activity of immune cells *via* different mechanisms such as molecular mimicry, antigen variation and immunomodulation of nonspecific and specific adaptive responses[65,66]. Some antigens of *H. pylori* enhance, while others inhibit the activity of immune cells. The first group includes surface lectins whereas CagA, VacA and LPS represents the second group[63,67-70]. *H. pylori* LPS shares some features common with human tissues. These are Lewis (Le) determinants: Le^X, Le^Y, Le^{XY} present in the O-specific chain of *H. pylori* LPS and on the surface of host cells: erythrocytes, granulocytes, monocytes, epithelial and vascular endothelial cells. In consequence, *H. pylori*

can impair its recognition by host immune cells and pose a risk of autoreactive antibody production[71]. *H. pylori* LPS of Le^{XY} type impairs phagocytic activity of granulocytes, cytotoxic activity of NK cells and lymphocyte proliferation[68,70]. It binds with dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and may interfere with the development of specific immune response[72].

Recently, possible association between *H. pylori* infection and extragastric disorders, including iron deficiency anemia, chronic idiopathic thrombocytopenic purpura, growth retardation, diabetes mellitus and CHD is being considered[73]. Also, an inverse correlation between *H. pylori* prevalence and an increase in allergies, has been suggested. However, since the understanding of *H. pylori*-related pathologies continues to evolve, the idea that *H. pylori* might confer benefit to humans generates serious controversy. Postulated role of *H. pylori* in the pathogenesis of extragastric disorders is based on the following facts: (1) local inflammation induced by these pathogens has also systemic effects; (2) *H. pylori* infection induces chronic low grade process lasting for decades; and (3) persistent infection induces immune responses, which may have local and remote consequences.

The widespread prevalence of *H. pylori* infection and the fact that they are frequently asymptomatic may suggest that, similarly to intestinal microflora, *H. pylori* can be a source of antigenic components that stimulate not only local, but also systemic inflammatory response. Possibly *H. pylori* together with intestinal microbiota may enhance a risk of cardiovascular disorders, probably through a mechanism that involves an increased exposure to bacterial products translocated from the gut to the circulation[74,75]. Both *H. pylori* proteins and LPS demonstrate pro-inflammatory properties. Considering the role of *H. pylori* LPS as a proinflammatory compound, the different structure of its lipid A is taken into account[76,77]. This component of *H. pylori* LPS determines its diminished proinflammatory properties in comparison to other bacterial LPSs discussed in previous review[75]. Moreover the impact of Le determinants on the severity of *H. pylori* induced-inflammation has also been investigated. For instance, it has been shown that *H. pylori* LPS with or without Le^{XY} determinants exhibits different effectiveness in stimulating the secretion of proinflammatory cytokines: IL-8 and TNF-α[78].

Recent knowledge on the pathoetiology of atherosclerosis together with current findings in the area of *H. pylori* infections constitute the background for the newly proposed hypothesis that those two processes may be related. To describe the involvement of *H. pylori* infection in the development of atherosclerosis, multiple study approaches have been undertaken. To discover a significance of *H. pylori* compounds, in the modulation of cell barrier function and its contribution to CHD development complex studies have to be undertaken. The understanding of subsequent stages of *H. pylori* infections and the processes induced on the level of cellular barriers: gastrointestinal epithelium, vascular endothelium and the cells of innate immunity seem to be crucial.

Local chronic inflammation induced by *H. pylori* in the gastric epithelium, may be reflected on the periphery by the appearance of acute phase proteins and cytokines produced by immune cells and particular tissues[58,59,79]. These soluble systemic inflammatory markers may enhance the development of lesions within vascular endothelium. Also, it cannot be excluded that certain *H. pylori* components crossing the epithelial barrier in the stomach or intestines can have a direct influence on the vascular endothelial cells as well as circulating immune cells maintaining their constant activation (Figure 1). So far, it has been shown that *H. pylori* vacuolating toxin and urease contribute to the intercellular tight junction degradation[80]. If so, bacterial agents penetrating lamina propria may interact with immune cells or even enter the circulation. Although *H. pylori* colonize particularly the gastric epithelium its antigens are translocated to a deeper parts of gastrointestinal tract where they may be easily detected in feces[81]. In the jejunum components of *H. pylori* affect the expression of surface molecules, secretion of cytokines, epithelial permeability and its barrier function. Probably in Peyer's patches *H. pylori* antigens initiate specific adaptive immunity and from this site could be spread into the circulation[79,82]. It has been hypothesized that *H. pylori* antigens may affect vascular endothelium by direct interactions with endothelium, indirectly in a form bound with leukocytes or as complexes with LDL/oxLDL fractions - classic risk factors of CHD[75]. The vascular endothelium can also be affected by *H. pylori* - driven cytokines and chemokines[57,78,83].

In order to evaluate the involvement of *H. pylori* infection in the development of CHD, it is necessary to find the largest number of research studies and possible connections confirming this relationship. The search for such connections should combine serological, biochemical, immunological as well as molecular markers. Serological and molecular studies on the material derived from patients with clinically confirmed CHD can provide markers helpful in defining individual susceptibility to chronic infections and extensive inflammation, predisposing to CHD. These cellular and molecular study approaches would describe the background of *H. pylori*-driven proinflammatory mechanisms directed towards epithelial and endothelial barrier functions, and innate immune cells, which would help to define their role in the atherogenesis.

H. PYLORI VS CHD - CURRENT STATE

Serological studies

The role of *H. pylori* infection in the development of CHD was suggested by Mendall *et al*[84] in 1994, where he observed for the first time the elevation of anti-*H.*

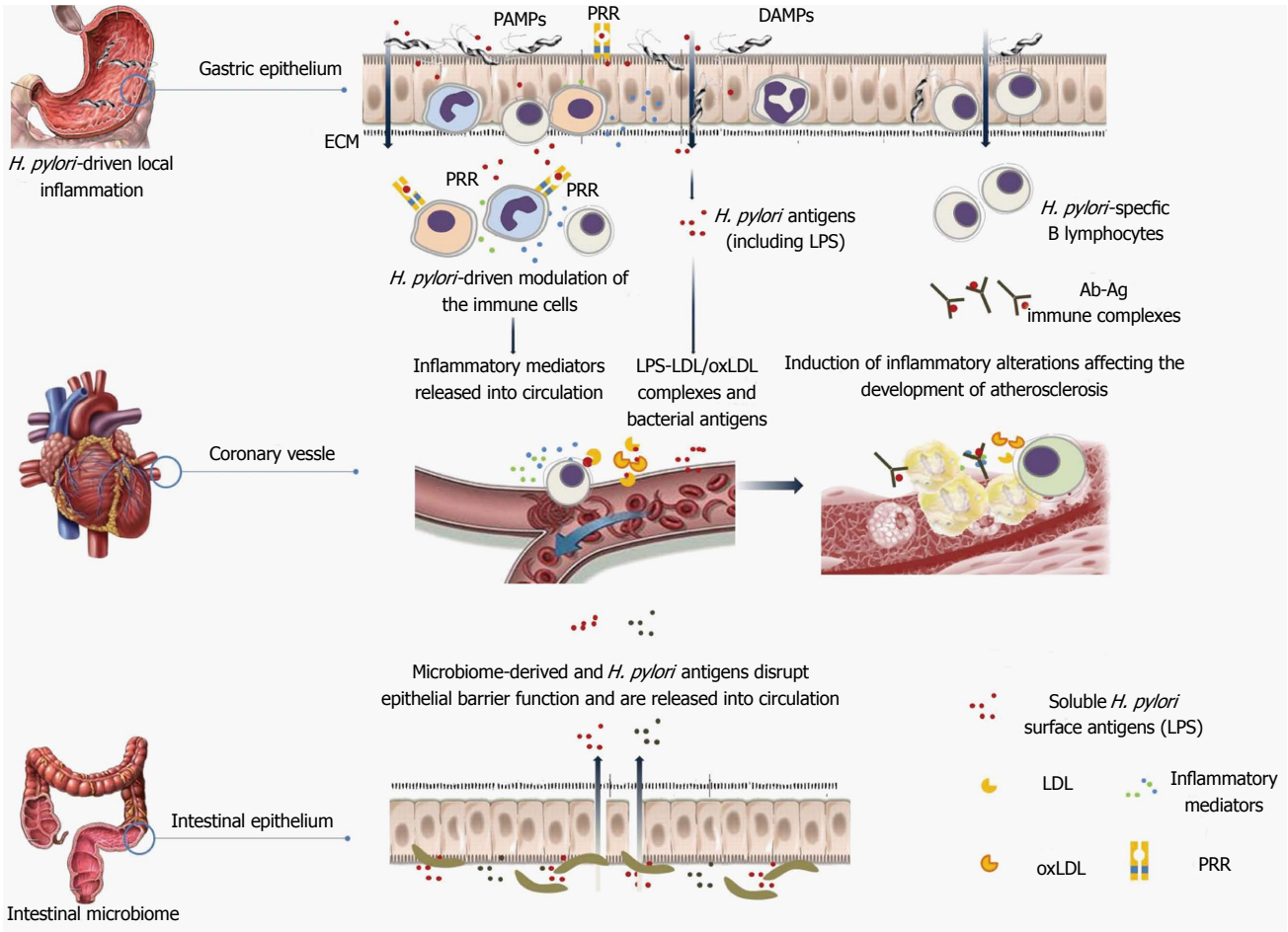

Figure 1 A possible link between local inflammation induced by *Helicobacter pylori* on surface of the gastric epithelium and the inflammatory response within vascular endothelium. *H. pylori*: *Helicobacter pylori*; LDL: Low density lipoproteins; LPS: Lipopolysaccharide; PRR: Pattern recognition receptor; PAMPs: Pathogen associated molecular patterns; DAMPs: Damage associated molecular patterns.

pylori antibodies in the sera of CHD cases[84]. Following this pioneer finding, some authors made confirmed this association in several serological studies[85-88]. Searching for that connection other groups concentrated on the evaluation of bacteriological, biochemical, inflammatory and epidemiological parameters related with CHD and *H. pylori* infection (Table 1). The *H. pylori* seropositivity in CHD group varied from 40% up to 90%. Several studies also supported the association between CagA$^+$ *H. pylori* infection and CHD prevalence. This relation is probably based on the increased levels of trombin - Factor Ⅶ and the prothrombin subunits: F1 + 2 or through the stimulation of low-grade persistent inflammatory response in CHD cases infected with *H. pylori* CagA$^+$ strains[88-92]. However several authors obtained contrary data[93,94]. The findings coming from other studies showed no increase in the production of anti-*H. pylori* antibodies in CHD patients.

Thus, still there is no consensus on the role of *H. pylori* infections in either causation or progression of CHD[95-98]. Possible reasons of these controversies may result from differences in: (1) magnitude of the study groups; (2) exclusion/inclusion criteria used in study groups selection; (3) the usage of serological tests for the *H. pylori* diagnostics; and (4) approaches for data analysis and statistical tests or insufficient knowledge on possible mechanisms involved. However, a new approach suggesting a role of gut microbiota in the development of chronic diseases prompts to continue the research[32]. Particularly stimulating are the results of research conducted in ethnic groups with low incidence of classic risk factors for CHD, and high prevalence of *H. pylori* infection. Recently published data[99] showed that high levels of anti-*H. pylori* IgG were significantly associated with the increased risk for CHD in a group of Central Africans. After adjusting with classic risk factors of CHD, *H. pylori* infection was found to be the only independent predictor of carotid plaque and stroke incidence in this group. Also Sealy-Jefferson *et al*[100] (2013) showed that the exposure to *H. pylori* in Mexican Americans may constitute a risk factor for stroke. Taking into consideration the increased prevalence of *H. pylori* in this population, the infection itself may contribute to the ethnic differences in stroke risk. It has also been suggested that *H. pylori* seroprevalence may influence long term prognosis for patients with unstable angina[101,102]. This finding is supported by several studies where genomic material (16S rRNA) of *H. pylori* was identified in the coronary

Table 1 Major results of the clinical and basic research studies on the relationship between coronary heart disease and *Helicobacter pylori* infection

Study characteristic	Ref.
Serological parameters	
Higher prevalence and concentrations of anti-*H. pylori* antibodies in CHD *vs* non-CHD individuals	[84-88]
Association between *H. pylori* CagA positive infections and CHD; exposure of endothelial and smooth muscle components within atherosclerotic plaques to the anti-CagA antibodies	[88-92]
Autoimmunity hypothesis: the presence of the immune complexes $Le^{X/Y}$-anti-$Le^{X/Y}$ IgG in CHD patients infected with *H. pylori*	[47,127,134]
Bacteriological parameters	
Detection of *H. pylori* genomic material (16S rRNA) in the coronary arteries and atheromatous plaques from patients with cardiologic disorders	[43,91,103-105]
Presence of viable *H. pylori* bacteria in atherogenic plaques	[106]
Biochemical parameters	
Association of *H. pylori* infection with the increased biochemical and inflammatory parameters of CHD as well as coronary lumen reduction	[85,92,107-109]
Higher prevalence of LDL-hiperchlesterolemia, HDL-hypocholesterolemia and elevated levels of CRP in *H. pylori* infected than uninfected individuals	[110,127-129]
Lower activity of serum paraxonase-1 (a major anti-atherogenous component of HDL) and higher carotid-intima media thickness (one of the surrogate marker of atherosclerosis) in *H. pylori* positive in comparison to negative subjects	[108]
Positive correlation between raised LBP levels and the severity of CHD with co-existing *H. pylori* infection. The escalation of inflammatory process occurring *via* Toll-like receptors and LPS-LDL complexes	[127]
Increased levels of homocysteine in *H. pylori* infected individuals caused by malabsorption of vitamine B12 and foliate from diet, leading to obesity-related resistance to insulin	[48,130,131]
Inflammation and inflammation-related parameters	
Increased concentrations of IL-6, IL-8, TNF-α, plasminogen, activator inhibitor type-1, and von Willebrand factor in CHD patients infected with *H. pylori*	[3,38,69,83]
High levels of fibrinogen, a marker of systemic inflammation – putative link between *H. pylori* infections and pathophysiology of CHD	[133]
Recruitment of immune cells to the infectious foci and survival of *H. pylori* within the endothelium due to interaction of *H. pylori* LPS Le determinants with E- and L-selectins	[38,136]
Stimulation of Th1 lymphocytes to produce cytokines by *H. pylori* HspB	[47, 127,134]
Epidemiological studies	
Higher risk of CHD in ethnic groups of Central Africans and Mexican Americans with increased prevalence of *H. pylori* infections	[100-102]
Genetic susceptibility to infections and predisposition to strong inflammatory response	[140,145-146]

CagA: Cytotoxin-associated gene A; CHD: Coronary heart disease; CRP: C-reactive protein; Ig: Immunoglobulines; HDL: High density lipoprotein; Hsp: Heat shock protein; LBP: Lipopolysaccharide binding protein; IL: Interleukin; LDL: Low density lipoprotein; Le: Lewis; TNF: Tumor necrosis factor.

arteries and atheromatous plaques from patients with cardiologic disorders including myocardial infarction and coronary artery disease - suggesting the direct involvement of *H. pylori* in CHD pathogenesis[43,91,103-105]. Some authors postulate the presence of viable *H. pylori* in atherogenic plaques supporting their results by the culture of bacteria on solid media[106].

Inflammatory markers

It has been epidemiologically reported that *H. pylori* infections are associated with the changes in biochemical and inflammatory parameters as well as coronary lumen reduction[85,92,107-109]. In both *H. pylori* infected and CHD patients local inflammation occurring in gastric mucosa or in blood vessels, respectively turns into a chronic phase, which leads to a constitute presence of an inflammation-inducing agents. Increased concentrations of systemic inflammatory markers, both in patients with atherosclerosis and *H. pylori* infected individuals are usually considered a symptom or a result of a local inflammation. However, it has been claimed that systemic inflammation might be a cause and not a result of a local inflammatory reaction within atherosclerotic lesions[110]. Inflammation occurring in both, CHD and *H. pylori* infected individuals is determined by innate immune mechanisms with a participation of cell receptors called "alarmins". They recognize conservative structures of infectious agents - pathogen associated molecular patterns (PAMPs) as well as host endogenous ligands - damage associated molecular patterns (DAMPs) appearing on MØ, dendritic cells (DC) and natural killer (NK) cells, as well as on epithelial and endothelial cells. It is supposed that the activation of immune or epithelial cells *via* pattern recognition receptors (PRRs) may be a reason for subacute inflammation in chronic diseases including CHD[11,69,111,112]. Local inflammation results with increased cytokine levels including IL–6 and TNF-α. Both stimulate the liver to produce acute phase proteins such as CRP, lipopolysaccharide binding protein (LBP) and MMP including MMP-9. Since, acute phase proteins are ligands for PRRs, they enhance the primary inflammation. However, chronic *H. pylori* infection leads to an excessive activation of inflammatory cells and a release of active radicals into the environment. This, due to oxidative stress, leads to tissue damage and apoptosis, therefore providing endogenous DAPMs such as heat shock protein (Hsp) 70, galectin-1, IL-1α, IL-33, mitochondrial damage motifs (mtDNA) and high mobility group box1 protein. Their probable role is a maintenance of inflammation, stimulation of tissue healing within the gastric ulcer

niche, or removing damaged cells from the ischaemic niche, in the vascular endothelium. Mitochondrial DAMPs may increase endothelial permeability through neutrophil dependent and independent pathways[113]. Also specific microRNA expression is associated with the inflammatory response to damaged cells with possible deleterious implications[114]. Prolonged exposure to PAMPs and DAMPs is an apparent reason for a transformation of a local inflammation into a chronic form. The damage of vascular endothelium results in an increased production of reactive oxygen species and inactivation of nitric oxide, which has an anti-atherosclerotic properties. These changes lead to the activation of nuclear transcription factor NF-κB and result with a transformation of endothelium to a proinflammatory phenotype characterized by an increased expression of adhesins and chemokines, including MCP-1 and IL-8, with chemotactic activity towards inflammatory cells[1,14,22]. Proinflammatory phenotype of vascular endothelium exhibits an increased expression of PRR receptors including Toll-like receptors *e.g.*, TLR4, CD14 and TLR2 recognizing bacterial LPS. The enhanced expression of these receptors also occurs on MØ accumulated in the atherosclerotic plaques[6,115,116].

Signaling pathways involving PRR receptors

In recent considerations recognizing CHD as an inflammatory disease, much attention has been paid to the role of signaling pathways involving PRR receptors present on MØ, DC and NK cells as well as endothelial and smooth muscle cells. There are different classes of PRR, including scavenger receptors, and the TLRs. Their role in the pathogenesis of CHD is still unclear and the results obtained in this issue vary greatly[112,117]. Toll-like receptors have been identified as molecules belonging to primary innate immunity. The studies on TLR4 and TLR2 knockout mice confirmed pro-atherogenous effect of TLR4/TLR2 signaling induction[118,119]. Although the expression of TLR2 and TLR4 on endothelial cells in normal arteries is rather low, it was found to be increased in the endothelium from atherosclerosis lesions[112]. Certain studies made an attempt to find a link between the susceptibility to CHD and TLR polymorphisms. Two single nucleotide polymorphisms of TLR4 - Asp299Gly and Thr399Ile were suspected to impair TLR signaling in response to LPS, in carriers of these alleles. It was suggested that both alleles were associated with the protection from carotid artery atherogenesis and the reduction of myocardial infarction risk up to 30%, in carriers of the Asp299Gly polymorphism[112,116,120]. Several TLR types: 1, 2, 4 and 5 are expressed in atherosclerotic plaques by resident cells and leukocytes that migrate into the arterial wall. The upregulation of TLR4 on MØ induced by proatherogenic oxidized LDL suggests that TLRs may provide a potential pathophysiological link between lipids, infection, inflammation and atherosclerosis[115]. The oxidized lipids may also serve as endogenous ligands of TLR2 and TLR4[121]. The study by Talreja *et al*[122] (2004) showed that mast cell-derived histamine up-regulates TLR4 and TLR2 expression on the host cells and by this enhances their sensitivity to cell wall components of Gram-positive and Gram-negative bacteria[122] - with Hsp and LPS considered as potential mediators linking bacterial infections with atherosclerosis. Moreover, it was shown that standard *E. coli* LPS induces the overexpression of TLR4, NF-κB, ICAM-1, VCAM-1 and the endothelial growth factor (VEGF), as well as the production of nitric oxide and IL-8[14,123].

The escalation of inflammatory process occurring in atherosclerosis does not exclude the participation of *H. pylori* LPS, which has low endotoxic activity, however, its proinflammatory potential is preserved. It stimulates MØ to secrete TNF-α, that inhibits lipoprotein lipase activity. This implies an increase in triglycerides and lower HDL cholesterol levels[124]. The recognition of *H. pylori* LPS by the immune cells and its interaction with vascular endothelium are not well understood. In the context of the correlation between the CHD incidence and *H. pylori* infection the interactions of LPS with TLR4 and TLR2 are taken into consideration, especially in regard to the variability of Le determinants in *H. pylori* LPS. It has been shown that *H. pylori* LPS without Le determinants (Le^{X-Y-}) stimulates monocytes to produce lower concentrations of IL-8 and TNF-α than the LPS of Le^{X+Y+} type. Cytokine production induced by the latter type was inhibited by anti–CD14 and anti–LBP antibodies which confirms the involvement of both Le determinants and lipid A in those interactions[78].

H. pylori LPS exhibits weaker activity than the LPS of *E. coli* and express antagonistic properties towards TLR4. Current data do not rule out a role of TLR2 in the signaling induced by LPS of non-enterobacterial origin and its cooperation with TLR4[36]. It was shown that low stimulation of the TLR4 signaling by bacterial LPS may induce the expression of TLR2 in endothelial cells, probably *via* NADPH oxidase released by neutrophils[125]. Chronic *H. pylori* infection favors the formation of LPS-LDL complexes, directly or with the involvement of LBP. Such complexes, when deposited in the vascular endothelium, may enhance proinflammatory atherosclerotic processes[126]. It was shown that the presence of LBP is required for the LPS-dependent activation of intracellular TLR4 in endothelial cells. LBP acts as a catalyst of this process by the translocation of serum sCD14-LPS complexes into the cells[111]. In this context, the positive correlation between raised LBP and the severity of CHD with co-existing *H. pylori* infection seems to be of great importance[127]. It is also possible that *H. pylori* LPS contributes to CHD due to its anti-phagocytic, anti-cytotoxic and anti-proliferative properties, towards phagocytes, NK cells and lymphocytes respectively[68-70].

The expression of TLR4 and TLR2 is intensified in the inflamed endothelium. Recent data indicate that

the binding of *E. coli* LPS with TLR4 may increase the permeability of the vascular epithelium[36]. Any kind of endothelial dysfunction, including a reduction of cell integrity may result in inflammatory cascade. The involvement of TLRs in the development of atherosclerosis is associated with the ability of those receptors to bind ox-LDL, which initiate atherogenesis. Binding of such complexes induces a cascade of signals that activate the transcription factor NF-κB and results in the upregulation of inflammasome components such as cytokines and acute phase proteins[14]. In the context of atherosclerosis the key NF-κB-dependent proteins include inflammatory cytokines: IL-1β and TNF-α, chemokines: IL-8, MCP-1 and MMPs hydrolyzing the extracellular matrix[1]. The role of IL–1β in the development of CHD is associated with the stimulation of endothelial cells to produce IL–6, fibrinogen, CRP and adhesins resulting in a activation of signal cascade leading to the destabilization of atherosclerotic plaques[8].

Acute phase response, lipid metabolism, homocysteine and fibrinogen related mechanisms

Significant association of *H. pylori* infection with LDL-hipercholesterolemia, HDL-hypocholesterolemia and elevated levels of CRP was found. This indicates a possible impact of chronic infection on a lipid metabolism, which is associated with the increased CHD risk[110,128,129]. It was also noted that seropositive patients with unstable angina develop diabetes more frequently than seronegative individuals. *H. pylori* infection increases obesity-related resistance to insulin causing malabsorption of vitamin B12 and foliate from diet, ultimately leading to an increase in circulating homocysteine levels[48,130,131]. Since raised homocysteine may disturb the function of vascular endothelium it might be implicated in the coronary slow flow phenomenon. However, there are also suggestions that homocysteine is a marker rather than a cause of CHD[132]. In *H. pylori* positive subjects the activity of serum paraoxonase-1 (a major anti-atherogenous component of HDL) was lower while carotid-intima media thickness (one of the surrogate marker of atherosclerosis) was higher[108]. The sera of *H. pylori* infected subjects contain increased concentrations of inflammatory cytokines, particularly IL-6, IL-8 and TNF-α, plasminogen, activator inhibitor type-1, and von Willebrand factor - a sensitive indicator of atherosclerosis and a predictive factor of acute coronary syndrome[133]. Certain studies also showed that high levels of fibrinogen, a marker of systemic inflammation can constitute a probable link between *H. pylori* infections and CHD pathophysiology[47]. The putative mechanism of this association might involve *H. pylori*-driven stimulation of mononuclear cells to produce a tissue-factor-like pro-coagulant that, converts fibrinogen to fibrin though the extrinsic blood coagulation pathway. Fibrinogen also stimulates macrophage chemokine secretion through TLR4, promoting immune surveillance at sites of inflammation[134]. However, there are also contradictory results and hypotheses that the occurrence of CHD is positively associated with age and lower social class[135]. It would be of great importance to check, whether *H. pylori* eradication is associated with the decrease in the level of the above markers and lower CHD incidence. To date, anti-*H. pylori* eradication therapy confirmed only some suggestions. Mean coronary artery lumen loss in patients undergoing percutaneous transluminal coronary angioplasty (PTCA) with stent and anti-*H. pylori* eradication therapy was significantly smaller compared to PTCA and placebo treated group. Similarly, cytokines such as TNF-α, IL-1β and IL-8 were significantly lower in plasma of PTCA patients after *H. pylori* eradication, while there were no changes in plasma lipids, homocysteine and clotting factors[85].

Autoimmunity hypothesis

Bacterial pathogens, including *H. pylori* might contribute to CHD pathogenesis. This approach is supported by the fact that CHD is starting to be considered as an autoimmune inflammatory process. The antigenic structures of infectious agents can induce the expansion of potentially autoreactive T and B cells, or B cells producing antibodies cross-reacting with host tissues. This phenomenon is defined as antigenic mimicry. For instance *H. pylori* HspB (60 kDa) might be implicated in CHD pathogenesis *via* stimulation of Th1 lymphocytes to secrete IFN-γ and IL-12 or activation of MØ to express metalloproteinases and adhesins[38]. Antigenic mimicry as a cause of inflammation in CHD is also related to Le determinants. In human tissues Le antigens serve as ligands for endothelial (E and P–selectin) and leukocytes (L–selectin) adhesins. This interaction drives cell migration into the inflammatory milieu and plays an important role in the accumulation of immune cells in peripheral lymph nodes. It was shown that *H. pylori* bearing Le antigens in their LPSs are able to bind E- and L-selectins. This linkage enables the recruitment of immune cells to the infectious foci and may promote survival of *H. pylori* within the endothelium[136]. The activity of *H. pylori* LPS is also manifested by the activation of monocytes, MØ and secretion of proinflammatory cytokines: IL-1, IL-6 and IL-8[69]. *H. pylori* strains bearing Le^X or Le^A attract circulating lymphocytes that express L–selectin. It was shown that *H. pylori* expressing Le determinants induce higher colonization rates and more excessive infiltration of gastric mucosa with neutrophils and lymphocytes - a phenomenon also observed in individuals infected with *H. pylori* expressing Le^X determinants[137]. Due to the ongoing inflammation the endothelial and smooth muscle components within atherosclerotic plaque might be revealed and exposed to the anti-CagA antibodies. The formation of such immune complexes facilitates the risk for further damage of the endothelium caused by lytic complex of complement proteins[46].

INDIVIDUAL SUSCEPTIBILITY TO INFECTION AND INFLAMMATION IN RESPECT TO THE DEVELOPMENT OF CHD

The risk for cardiovascular diseases might also be considered on the genetic level-determining the susceptibility to CHD development related to inflammatory process[138]. For example, one of the explanations for elevated levels of CRP in CHD patients might lay in chronic, bacterial or viral infection. However, since viral as well *H. pylori* and *Ch. pneumoniae* infections, are very common, it is believed that an individual susceptibility to infections and accompanying inflammation could explain the role of infectious agents in the course of CHD. This individual predisposition to persistent infections and chronic inflammatory response can be determined, to some extent, by the Le antigens, receptors for PAMPs and proinflammatory cytokines. It is believed that Lewis antigens can play a key role in shaping the individual susceptibility to CHD development: by directing the adverse effects of infection and excessive inflammatory response[41]. There are also clear examples of protection against infectious diseases (particularly to *H. pylori*, norovirus, and *Vibrio cholerae*) based on polymorphisms in genes encoding and regulating the expression of ABH blood group and Lewis antigens[139]. There are two types of Lewis antigens in humans: Le a and Le b. Their expression depend on genes located on chromosome 1 encoding fucosyltransferases: *FUT2* and *FUT3*. Depending on the genotype, and thus the expression of one or both Le antigens, in the Caucasian population, there are three dominating phenotypes: Le^{a+b-}, Le^{a-b+}, Le^{a-b-}, and Le^{a+b+} which occurs very rarely. Le antigens expressed on cell surface and released in body fluids are associated with the susceptibility to infections especially related to the mucus layer, such as those caused by *H. pylori*[140]. It is assumed that Le antigens promote adhesion-dependent infections[141]. There are speculations on the link between the Le^{a-b-} phenotype and several disorders constituting a risk factors for CHD development, with examples such as insulin resistant diabetes, elevated levels of clotting factor VIII and von Willebrand factor. This phenotype is considered a genetic marker for the risk for CHD development[142,143]. It is also believed that the polymorphism in *FUT3* associated with the presence of point mutations 59T > G, 202T > C, 314 C > T, 1067 T > A, may determine the individual susceptibility to infections and the development of atherosclerotic lesions and strong inflammatory response[144].

The polymorphism of inflammation-related genes, may indirectly contribute to the development of CHD, and the dynamics of the disease. Such a possibility appears especially when the mutations accumulate in several genes related with inflammatory response. Thus, while searching for the relationship between *H. pylori* infections and their role in the development of CHD, mutations in the genes encoding TLR4/CD14 receptors (binding LPS), and IL–1β should be taken into consideration[145]. IL-1β acts as a stimulating mediator of IL-6, fibrinogen, CRP or adhesive molecules expression by endothelial cells within a cascade leading to the development and destabilization of the atherosclerotic plaques. In regard to IL-1β the most frequently considered gene mutations are: -511C > T and -31C > T[145,146]. It was showed that carriers of the two relatively frequent variants of *IL-1β* gene at -31 and -511 positions, *i.e.*, -31 TT and -511 CC, are at a higher risk of developing CHD requiring surgical treatment or two-stage percutaneous angioplasty. In patients prone to the development of atherosclerosis, polymorphism of *IL-1β* gene cluster may be associated with the extent and dynamics of lesions in the coronary arteries[146]. For gene encoding TLR4: Asp299Gly and Thr399Ile and for *CD14* gene: 159C > T mutations are considered to play a role in CHD and chronic infections[112,116]. Patients carrying Asp299Gly, a common variant of the *TLR4* gene presented reduced prevalence of angiographic artery disease and low levels of CRP. This common variant of the TLR4 gene, probably attenuates receptor signalling and diminishes inflammatory response to Gram-negative pathogens[147].

Since neither infection nor the activation of TLR4/TLR2 is sufficient to induce atherosclerosis in animal models[148], it is rather unlikely that microbes and/or TLRs signaling play a causative role in this disease. Instead, it is thought that they may be important as associates of silent disease. For instance, microbial components such as LPS or lipoteichoic acid released during acute infection or exacerbation of chronic infection might activate plaque cells. It has been suggested that such local "echos" of infections could lead to increased local production of cytokines and initiate plaque activation and rupture. The expression of TLRs in plaques suggests a pathway through which such an echo effect could occur[6]. Because *H. pylori* infection is located in the stomach, the question arises why the possible inflammation should only be transferred to the heart blood vessels and not to other vessels of the body? Various activities of the immune cells are mediated by endothelial cells, which form specialized microcirculatory networks used by the immune cells under both physiological and pathological circumstances. Endothelial cells represent a highly heterogenous population of cells with the ability to interact with and modulate the function of immune cells[149]. Atherosclerotic lesions occur at distinct sites within the arterial tree, such as branches, bifurcations, and curvatures, where they cause characteristic alterations in the blood flow, including decreased shear stress and increased turbulence. The nature of the flow appears to determine whether lesions occur at these vascular sites. The low-shear hypothesis of

atherosclerosis has been validated[150]. Decreasing shear stress at branches, bifurcations, and curvatures results in endothelial activation, adhesion molecule expression, and greater monocyte transmigration. It has been shown that atherosclerotic lesions appear first at lesion-prone sites, where activated endothelium expresses specific molecules, which favors the recruitment of monocytes and T cells. For instance, it has been hypothesized that the regiospecitifity of atherosclerotic lesions might be determined by the lower expression of TLR2 molecules[111]. The localization of atherosclerotic lesions could be also related to the local overexpression of NF-κB/IκB pathways[14].

FUTURE RESEARCH PERSPECTIVES

To describe the role of *H. pylori* in the initiation, acceleration or the development of CHD a few fundamental questions need to be addressed. It needs to be elucidated, whether viable *H. pylori* or bacterial compounds are able to break the single layer of epithelial cells and have unimpeded access to the systemic circulation. Also, it is not clear, whether classic risk factors such as hypercholesterolemia may act synergistically with *H. pylori* or their compounds to destabilize or disrupt gastric epithelial barrier function. It is also interesting whether CHD as systemic disease can lead independently to the disruption of gastric epithelial barrier function. The use of well-defined cell lines which mimic the *in vivo* conditions and exclude the naturally occurring phenotypic variations or the influence of external agents will enable to clarify the relationship between *H. pylori* as effective colonizer of gastric mucosa and inflammatory response. Methodology of culturing the cells using trans-well systems can help to examine whether *H. pylori* antigens alone or in combination with classic CHD risk factors interfere with the integrity of gastric epithelial and endothelial cells, cytotoxicity, the cell cycle, chemokines as well as cytokines and cell signaling. Microfluidic culture systems enable to explain if *H. pylori* compounds might be delivered to the inflammatory sites within vascular endothelium and interact with both endothelial and the immune cells[151].

Since, *H. pylori* infection has been defined as class I gastric carcinogen and many epidemiological studies demonstrated positive correlation between serum lipids and the risk of gastrointestinal malignancies, it is tempting to evaluate the prevalence of malignancies in CHD patients infected with *H. pylori*. Although it has been shown that *H. pylori* infection is related with increased LDL level, the association between abnormal concentrations of serum lipid components, the infection with *H. pylori* and the risk of gastrointestinal cancer is unknown[152].

CHD patients are recommended for antitrombocythic therapy with aspirin, which can be beneficial to individuals who already have experienced a heart attack, stroke, angina or peripheral vascular disease, or have had certain procedures such as angiography or bypass. However, aspirin can be prescribed to prevent heart disease and stroke in same individuals who have not previously experienced these events. The United States Preventive Services Task Force recommends that men with no history of heart disease or stroke aged 45-79 years should use aspirin to prevent myocardial infractions and that woman with no history of heart disease or stroke aged 55-79 should use aspirin to prevent stroke[153]. On the other hand, NSAIDs such as aspirin is positively correlated with the incidence of gastrointestinal tract disorders. Such damage can take a form of mucosal erosions or ulcers. NSAIDs can stimulate leukocytes, particularly neutrophils, such that they adhere to the vascular endothelium within the gastrointestinal microcirculation. Moreover NSAIDs impair the rapid restitution that occurs through cell migration following damage to the superficial epithelium of the stomach, reduce rates of epithelial turnover and thus impair the healing process. It is necessary to elucidate, whether ulcers are more likely to develop in long-term NSAIDs users who have mucosal erosions or in individuals infected with *H. pylori*, or both – and what is the role of NSAIDs on the course of CHD and *H. pylori*-related pathologies[153,154].

Proinflammatory agents released directly due to damage induced by *H. pylori* or indirectly by neutrophils recruited to the site of infection break the epithelial barrier. An initial effect of *H. pylori* infection is amplified significantly and impairs the proper action of cellular barrier. The question is whether inflammatory mediators generated in the stomach can reach and harm distant tissues, leading to systemic disorders related with CHD.

Tissue inflammation, cell injury or death result in the release of molecules that are endogenous PRR ligands. DAMPs stimulates cells to produce acute phase cytokines and activates other inflammatory compounds. Depending on the affected tissue, various stromal cells, including epithelial and endothelial cells, may function as sentinels for detection of DAMPs, which felicitate neutrophil recruitment. It was hypothesized that IL-33 may have protective effects during atherosclerosis by inducing a Th1-to-Th2 switch of immune responses[19]. However, many questions regarding the role of specific DAMPs during *H. pylori* infections and cardiovascular diseases remain to be solved.

Since initial moment of *H. pylori* infection is almost impossible to identify, little is known about the natural history and kinetics of infection and immune responses. There is an urgent need to establish and optimize the animal model mimicking human immune system, sensitive for *H. pylori* infection and CHD development. Immunologic similarities between guinea pigs and humans in regard to: leukocyte antigens, complement system, antigen presenting molecules, patterns of IFN-γ, IL-8, IL-12 release, as well as their receptors

suggest that this animal model may be suitable for studies on the relation between *H. pylori* infection and the development of its extragastric consequences. Antigen-specific lymphocyte proliferation has been found a suitable marker of immune response in guinea pigs with sustained *H. pylori* infection. Recently guinea pigs were successfully used to show the role of endotoxemia in the myocardial injury and sepsis-associated dysfunction[42,72,155].

It is believed that an individual susceptibility to infections and accompanying inflammation could help to explain the role of infectious agents in the course of CHD. Using the samples from patients with clinically confirmed CHD infected or not with *H. pylori* in comparison with control group it is necessary to look for cellular and molecular markers which may determine an individual susceptibility to chronic infections and extensive inflammation, predisposing to CHD.

These cellular and molecular studies would help to understand the role of *H. pylori* infections in the pathogenesis of CHD. Describing the background of *H. pylori* - driven proinflammatory mechanisms directed towards epithelial and possibly endothelial barrier, would help to allocate their role in the process of atherogenesis. In case of proven causative role of this bacterium in the pathogenesis of CHD, its eradication will be important for diminishing one of CHD infectious risk factors.

CONCLUSION

CHD, one of the most severe chronic diseases of the coronary vessels is a multifactorial disorder. Since classic risk factors do not explain all cases of CHD it has been suggested that chronic infections and even commensal microorganisms may affect the development or maintenance of CHD. Among various pathogens possibly involved in atherogenesis *H. pylori* is particularly interesting, since it induces chronic long-term infection within gastric epithelium which leads to not only local but systemic inflammation. Recent knowledge on the pathogenesis of atherosclerosis together with current findings in the field of *H. pylori* related diseases constitute the background for the newly proposed hypothesis that those two processes may be related.

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MINIREVIEWS

Autonomic and endocrine control of cardiovascular function

Richard Gordan, Judith K Gwathmey, Lai-Hua Xie

Richard Gordan, Judith K Gwathmey, Lai-Hua Xie, Department of Cell Biology and Molecular Medicine, Rutgers-New Jersey Medical School, Newark, NJ 07101, United States
Lai-Hua Xie, School of Pharmacology, Xinxiang Medical University, Xinxiang 453000, Henan Province, China
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Correspondence to: Lai-Hua Xie, PhD, FAHA, Associate Professor, Department of Cell Biology and Molecular Medicine, Rutgers-New Jersey Medical School, 185 South Orange Ave, MSB-G609, Newark, NJ 07101,
United States. xiela@njms.rutgers.edu
Telephone: +1-973-9722411
Fax:+1-973-9727489
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Abstract

The function of the heart is to contract and pump oxygenated blood to the body and deoxygenated blood to the lungs. To achieve this goal, a normal human heart must beat regularly and continuously for one's entire life. Heartbeats originate from the rhythmic pacing discharge from the sinoatrial (SA) node within the heart itself. In the absence of extrinsic neural or hormonal influences, the SA node pacing rate would be about 100 beats per minute. Heart rate and cardiac output, however, must vary in response to the needs of the body's cells for oxygen and nutrients under varying conditions. In order to respond rapidly to the changing requirements of the body's tissues, the heart rate and contractility are regulated by the nervous system, hormones, and other factors. Here we review how the cardiovascular system is controlled and influenced by not only a unique intrinsic system, but is also heavily influenced by the autonomic nervous system as well as the endocrine system.

Key words: Heart; Cardiovascular function; Autonomic nervous system; Endocrine system; Regulation

Core tip: The function of the heart is to contract and pump oxygenated blood to the body and deoxygenated blood to the lungs. To achieve this goal, a normal human heart must contract regularly and continuously, and respond to the changing requirements of the body's tissues. Here we review how the cardiovascular system is controlled and influenced by not only a unique intrinsic system, but is also heavily influenced by the autonomic nervous system as well as the endocrine system.

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INTRODUCTION

The cardiovascular system is a closed system connecting a pump to blood vessels (*i.e.*, arteries, capillaries, veins). The heart serves as the pump that moves blood through blood vessels thereby providing the needed oxygen and nutrients to the body. The

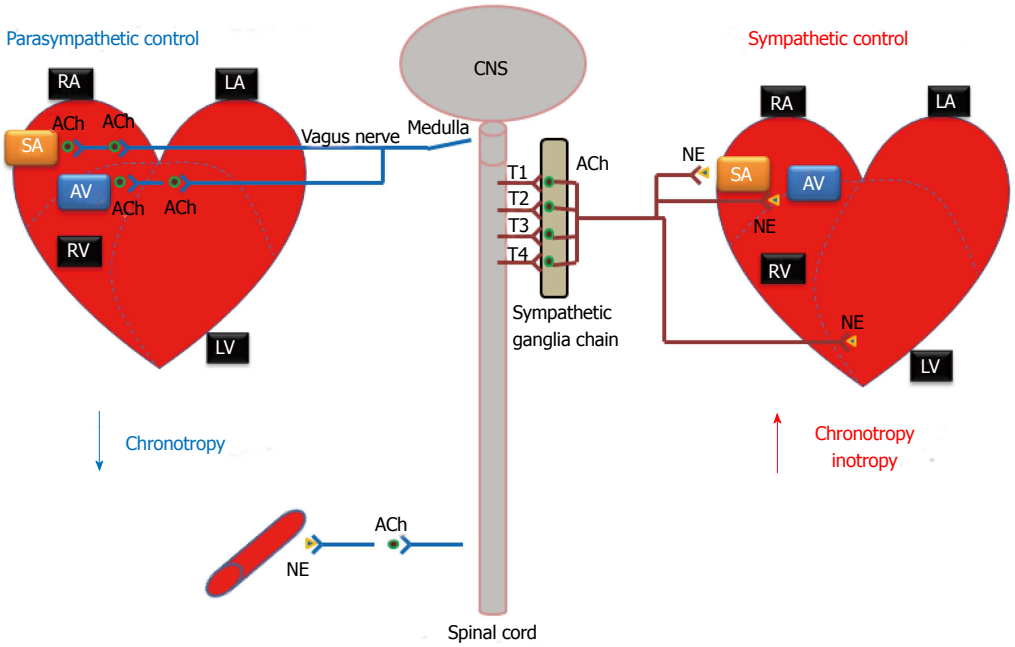

Figure 1 Autonomic nervous system regulation of the heart function. The autonomic nervous system affects the rate and force of heart contractions. CNS: Central nervous system; RA: Right atria; LA: Left atria; RV: Right ventricle; LV: Left ventricle; SA: Sino-atrial node; AV: Atrioventricular node; NE: Norepinephrine; ACh: Acetylcholine.

heart consists of four chambers: right atrium, right ventricle, left atrium and left ventricle. The right atrium receives oxygen-poor blood from the systemic veins; this blood then moves across the tricuspid valve to the right ventricle. From the right ventricle the de-oxygenated blood is pumped pass semilunar valves out through the pulmonary arteries to the lungs. In the lungs, the blood becomes oxygenated and returns to the left atrium *via* the pulmonary veins. This oxygen-rich blood next moves across the mitral valve to the left ventricle and is pumped out across semilunar valves to the systemic arteries and to body tissues. To achieve this goal, a normal human heart must beat regularly and continuously for one's entire life. Autorhythmic cardiac cells initiate and distribute impulses (action potentials) throughout the heart. The intrinsic conduction system coordinates heart electrical activity. This electrical activity in the heart corresponds to electrocardiogram (ECG) wave tracings. On a normal ECG recording, the P wave reflects atrial depolarization followed by atrial contraction. The QRS wave reflects ventricular depolarization followed by ventricular contraction and the T wave reflects ventricular repolarization and ventricular relaxation.

In the intrinsic conduction system, heartbeats originate from the rhythmic pacing discharge from the sinoatrial node (SA node) within the heart itself. The SA node, located in the right atrium, is a part of the intrinsic conduction (or nervous) system found in the heart. This conduction system in order of rate of depolarization starts with the SA node or pacemaker and results in atrial depolarization and atrial contraction, the internodal pathway, the AV node (where the impulse is delayed), AV bundle, the left and right branches of the bundle of His and lastly the Purkinje fibers, both of which result in ventricular depolarization and contraction. All of the components of the intrinsic conduction system contain autorhythmic cells that spontaneously depolarize. In the absence of extrinsic neural or hormonal influences, the SA node pacing rate would be about 100 beats per minute (bpm). The heart rate and cardiac output, however, must vary in response to the needs of the body's cells for oxygen and nutrients under varying conditions. In order to respond rapidly to changing requirements of the body's tissues, the heart rate and contractility are regulated by the autonomic nervous system, hormones, and other factors.

AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system (ANS) is the component of the peripheral nervous system that controls cardiac muscle contraction, visceral activities, and glandular functions of the body. Specifically the ANS can regulate heart rate, blood pressure, rate of respiration, body temperature, sweating, gastrointestinal motility and secretion, as well as other visceral activities that maintain homeostasis[1-4]. The ANS functions continuously without conscious effort. The ANS, however, is controlled by centers located in the spinal cord, brain stem, and hypothalamus.

The ANS has two interacting systems: the sympathetic and parasympathetic systems. As illustrated in Figure 1, sympathetic and parasympathetic neurons exert antagonistic effects on the heart. The sympathetic

system prepares the body for energy expenditure, emergency or stressful situations, *i.e.,* fight or flight. Conversely, the parasympathetic system is most active under restful conditions. The parasympathetic counteracts the sympathetic system after a stressful event and restores the body to a restful state. The sympathetic nervous system releases norepinephrine (NE) while the parasympathetic nervous system releases acetylcholine (ACh). Sympathetic stimulation increases heart rate and myocardial contractility. During exercise, emotional excitement, or under various pathological conditions (*e.g.,* heart failure)[5], the sympathetic nervous system is activated. The stimulation of the sympathetic nervous system causes pupil dilatation, bronchiole dilatation, blood vessel constriction, sweat secretion, inhibits peristalsis, increases renin secretion by the kidneys, as well as can induce reproductive organ contraction and secretion. In contrast, parasympathetic stimulation decreases heart rate and constricts the pupils. It also increases secretion of the eye glands, increases peristalsis, increases secretion of salivary and pancreatic glands, and constricts bronchioles. Most organs receive innervations from both systems, which usually exert opposing actions. However, this is not always the case. Some systems do not have a response to parasympathetic stimulation. For example, most blood vessels lack parasympathetic innervations and their diameter is regulated by sympathetic nervous system input, so that they have a constant state of sympathetic tone. It is a decrease in sympathetic stimulation or tone that allows vasodilatation. During rest, sleep, or emotional tranquility, the parasympathetic nervous system predominates and controls the heart rate at a resting rate of 60-75 bpm. At any given time, the effect of the ANS on the heart is the net balance between the opposing actions of the sympathetic and parasympathetic systems.

Unlike the somatic nervous system, where a single neuron originating in the spinal cord typically connects the central nervous system and a skeletal muscle *via* a neuromuscular junction, both sympathetic and parasympathetic pathways are composed of a two-neuron chain: a preganglionic neuron and a postganglionic neuron. The neurotransmitter between the preganglionic and postganglionic neurons is acetylcholine, the same as that in neuromuscular junctions. Messages from these systems are conveyed as electrical impulses that travel along axons and cross synaptic clefts (using chemical neurotransmitter).

In the sympathetic system (thoracolumbar division), these nerves originate from the thoracolumbar region of the spinal cord (T1-L2) and radiate out towards the target organs. In contrast, the nerves of the parasympathetic system originate within the midbrain, pons and medulla oblongata of the brain stem and part of these fibers originate in the sacral region (S2-S4 sacral spinal nerves) of the spinal cord. While sympathetic nerves utilize a short preganglionic neuron followed by a relatively long postganglionic neuron, parasympathetic nerves (*e.g.,* the vagus nerve, which carries about 75 percent of all parasympathetic fibers) have a much longer preganglionic neuron, followed by a short postganglionic neuron.

Cardiac sympathetic nervous system

The sympathetic nervous system is the component of the ANS that is responsible for controlling the human body's reaction to situations of stress or emergency (otherwise known as the "fight-or-flight" response), while the parasympathetic nervous system is generally responsible for basal organ system function.

Cardiac sympathetic preganglionic nerves (typically all myelinated) emerge from the upper thoracic segments of the spinal cord (T1-T4). After traveling a short distance, preganglionic fibers leave the spinal nerves through branches called white rami and enter sympathetic ganglia. The cardiac sympathetic neurons form the sympathetic chain ganglia located along the side of the viscera column (*i.e.,* paravertebral ganglia). These ganglia comprise the sympatheric trunks with their connecting fibers. The postganglionic fibers, extend to the viscera, such as the heart. In general, sympathetic preganglionic neurons are shorter than sympathetic postganglionic neurons (Figure 1).

Sympathetic neurotransmitters: Neurotransmitters are chemical substances released into the synaptic cleft from nerve terminals in response to action potentials. They transmit signals from a neuron to a target cell across a synapse, *e.g.,* acetylcholine for neuromuscular junctions. While the preganglionic neurons of both the sympathetic and parasympathetic system secret acetylcholine (ACh) which is why they are referred to as cholinergic, the majority of the postganglionic endings of the sympathetic nervous system release NE, which resembles epinephrine (*i.e.,* adrenalin). Thus, these sympathetic postganglionic fibers are commonly called adrenergic fibers.

Sympathetic receptors: There are two types of adrenergic receptors: β and α. In the cardiovascular system there are β_1, β_2, α_1, and α_2 adrenergic receptors (Table 1).

β_1 adrenergic receptors are expressed in the heart (in the SA node, AV node, and on atrial and ventricular cardiomyocytes). The activation of β_1 receptors increases heart rate (*via* the SA node), increases contractility as result of increased intracellular calcium concentrations and increased calcium release by the sarcoplasmic reticulum (SR), and increased AV node conduction velocity. Additionally, activation of this receptor also induces renin release by the kidneys to help maintain blood pressure, plasma sodium levels and blood volume.

β_2 adrenergic receptors are mainly expressed in vascular smooth muscle, skeletal muscle, and in

Table 1 Sympathetic and parasympathetic receptors and their functions in the heart and vessels

	Heart				Vessels	
	Receptor	Function			Receptor	Function
		Inotropy	Chronotropy	Dromotropy		
Norepinephrine	α_1	+	+	+	α_1	Vasoconstriction
	β_1	+	+	+	β_1	Vasoconstriction
	β_2	+	+	+	β_2	Vasodilation
Acetylcholine	M_2	-	-	-	M_2	Vasodilation

the coronary circulation. Their activation elicits vasodilatation, which, in turn increases blood perfusion to target organs (especially the liver, heart, and skeletal muscle). These receptors are not innervated and thus are primarily stimulated by circulating epinephrine. There are also some low expressions of β_2 receptors in cardiomyocytes.

α_1 adrenergic receptors are expressed in vascular smooth muscle proximal to sympathetic nerve terminals. Their activation elicits vasoconstriction. There are also some low expressions of α_1 receptors in cardiomyocytes.

α_2 adrenergic receptors are expressed in vascular smooth muscle distal from sympathetic nerve terminals. Their activation also elicits vasoconstriction.

Sympathetic nervous system control and heart function: Stimulation by the sympathetic nervous system results in the following effects on the heart (Table 1): Positive chronotropic effect (increase in heart rate): The sinoatrial (SA) node is the predominate pacemaker of the heart. It is located within the upper posterior wall of the right atrium, and is responsible for maintaining a sinus rhythm of between 60 and 100 beats per minute. This rate is constantly being affected by innervations from both the sympathetic and parasympathetic nervous systems. Stimulation by the sympathetic system nerves results in an increase of heart rate, as occurs during the "fight-or-flight" response.

Positive inotropic effect (increase of contractility): Myocardial contractility represents the ability of the heart to produce force during contraction. It is determined by the incremental degrees of binding between myosin (thick) and actin (thin) filaments, which in turn depends on the concentration of calcium ions (Ca^{2+}) in the cytosol of the cardiomyocyte. Stimulation by the sympathetic nervous system causes an elevation in intracellular (Ca^{2+}) and thus an increase in contraction of both the atria and ventricles.

Positive dromotropic effect (enhancement of conduction): Stimulation by the sympathetic nervous system also enhances the conductivity of the electrical signal. For example, it increases AV conduction velocity.

Parasympathetic nervous system

As previously mentioned, the parasympathetic nervous system is responsible for the unconscious regulation of the body's systems, most notably, salivation, lacrimation, urination, digestion, and defecation (acronym SLUDD). Importantly, the parasympathetic nervous system plays an antagonistic role in regulating heart function.

The parasympathetic system has preganglionic neurons (craniosacral division) that arise from neurons in the mid-brain, pons and medulla oblongata. The cell bodies of parasympathetic preganglionic neurons are located in the homologous motor nuclei of the cranial nerves. Parasympathetic preganglionic fibers associated with parts of the head are carried by the oculomotor, facial, and glossopharygeal nerves. The fibers that innervate organs of the thorax and upper abdomen are parts of the vagus nerve which as previously mentioned carries approximately 75% of all parasympathetic nerve fibers passing to the heart, the lungs, the stomach, and many other visceral organs. Preganglionic fibers arising from the sacral region of the spinal cord make up parts of S2-S4 sacral spinal nerves and carry impulses to viscera in the pelvic cavity. The short postganglionic neurons reside near effector organs, *e.g.,* lacrimal gland, salivary glands, heart, trachea, lung, liver, gallbladder, spleen, pancreas, intestines, kidney, and urinary bladder, *etc*. Unlike the sympathetic system, most parasympathetic preganglionic fibers reach the target organs and form the peripheral ganglia in the wall of the organ. The preganglionic fibers synapse within the ganglion, and then short postganglionic fibers leave the ganglia to the target organ. Thus, in the parasympathetic system, preganglionic neurons are generally longer than postganglionic neurons (Figure 1).

Parasympathetic neurotransmitters: Acetylcholine is the predominant neurotransmitter from the parasympathetic nervous system, in both the preganglionic and postganglionic neurons. Although excitatory in skeletal muscle by binding to nicotinic receptors and inducing the opening of ligand gated sodium channels, acetylcholine inhibits the contraction of cardiomyocytes by activating muscarinic receptors (M_2). These parasympathetic postganglionic fibers are commonly called cholinergic fibers because they secrete acetylcholine at their nerve endings.

Acetylcholine is synthesized by choline acetlytransferase in cholinergic neurons by combining choline and acetyl-COA molecules. Once assembled in synaptic vesicles near the end of the axon, the entry of calcium causes the vesicles to fuse with the membrane of the

neuron and to release acetylcholine into the synaptic cleft (the space between the neuron and post-synaptic membrane or effector cell). Acetylcholine diffuses across the synaptic cleft and binds to receptors on the post-synaptic membrane increasing the permeability to sodium causing depolarization of the membrane and propagation of the impulse. This chemical transmission is much slower than the electrical "all or none" transmission of the action potential seen in the intrinsic nervous system of the heart. To regulate the function of these neurons (and thus, the muscles they control), acetylcholinesterase is present in the synaptic cleft. It serves to hydrolyze the acetylcholine molecule by breaking it down into choline and acetate, which are then both taken back up by the neuron, to be again synthesized into acetylcholine.

Parasympathetic receptors: The parasympathetic postganglionic fibers are cholinergic. Acetylcholine can bind to two types of cholinergic receptors called nicotinic receptors and muscarinic receptors. Muscarinic receptors are located in the membranes of effector cells at the end of postganglionic parasympathetic nerves and at the ends of cholinergic sympathetic fibers. Responses from these receptors are excitatory and relatively slow. The nicotinic receptors are located at synapses between pre- and post-ganglionic neurons of the sympathetic and parasympathetic pathways. Nicotinic receptors in contrast to muscarinic receptors produce rapid, excitatory responses. Neuromuscular junctions found in skeletal muscle fibers are nicotinic.

In relation to the cardiovascular system the parasympathetic nervous system has two different kinds of muscarinic receptors: the M_2 and M_3 receptors (Table 1).

The M_2 receptors are expressed in the heart; abundant in nodal and atrial tissue, but sparse in the ventricles. The binding of acetylcholine to M_2 receptors serves to slow heart rate till it reaches normal sinus rhythm. This is achieved by slowing the rate of depolarization, as well as by reducing the conduction velocity through the atrioventricular node. Additionally, the activation of M_2 receptors reduces the contractility of atrial cardiomyocytes, thus reducing, in part, the overall cardiac output of the heart as a result of reduced atrial kick, smaller stroke volume, and slower heart rate. Cardiac output is determined by heart rate and stroke volume (CO = HR x SV).

The M_3 receptors are mainly expressed in vascular endothelium. The predominate effect of M_3 receptor activation is dilatation of the vessels, by stimulating nitric oxide production by vascular endothelial cells[6]. M_3 receptors impact afterload and vascular resistance which can again alter cardiac output and blood pressure.

Parasympathetic nervous system control and heart function: As mentioned earlier, parasympathetic activity produces effects that are, in general, opposite to those of sympathetic activation. However, in contrast to sympathetic activity, the parasympathetic nervous system has little effect on myocardial contractility.

Negative chronotropic effect (decrease in heart rate): The vagus nerve directly innervates the sinoatrial node; when activated, it serves to lower the heart rate, thus exhibiting a negative chronotropic effect.

Negative inotropic effect (decrease in myocardial contractility): Currently, it is a matter of debate whether parasympathetic stimulation can exhibit negative inotropic effects, as the vagus nerve does not directly innervate cardiomyocytes other than that of the sinoatrial and atrioventricular nodes, however, recent *in vivo* studies may suggest otherwise, at least in the atrium.

Negative dromotropic effect (decrease conduction velocity): Stimulation of the parasympathetic system serves to inhibit AV node conduction velocity.

Cellular signal transduction

Most sympathetic and parasympathetic receptors are known to be G protein-coupled receptors (GPCRs). In the heart, the G-protein-cAMP-PKA signaling pathway mediates the catecholaminergic control on heart rate and contractility.

Signaling pathway of sympathetic stimulation: The sympathetic stimulation-induced effects in the heart result from activation of β_1-adrenoceptors, which are GPCRs (Figure 2). The sympathetic neurotransmitter NE (as well as other catecholamines) bind to β_1 receptors and activate stimulatory G proteins (G_s) by causing a conformational change within the G_s, so that the disassociated α_s subunit can then bind to and activate adenylyl cyclase (AC). The activation of this enzyme then catalyzes the conversion of ATP into cyclic adenosine monophosphate (cAMP). This second messenger may then activate a myriad of other pathways, ion channels, transcription factors, or enzymes. With regards to the cardiovascular system, the most important enzyme that cAMP activates is protein kinase A (PKA). PKA, which in turn, phosphorylates multiple target proteins, such as L-type Ca channels (LTCC), the SR Ca handling protein phospholamban, and contractile machinery such as troponin C, I and T. Additionally, cAMP binds directly to ion channels responsible for the funny current (I_f), thus increasing the heart rate[7].

Signaling pathway of parasympathetic stimulation: The parasympathetic stimulation-induced effects in the heart result from activation of muscarinic (M_2) receptors, which are also GPCRs by acetylcholine (Figure 2). The parasympathetic neurotransmitter ACh binds to M_2 receptors thereby activating inhibitory G proteins (G_i) by causing a conformational change within the G_i subunit, so that the disassociated α_i

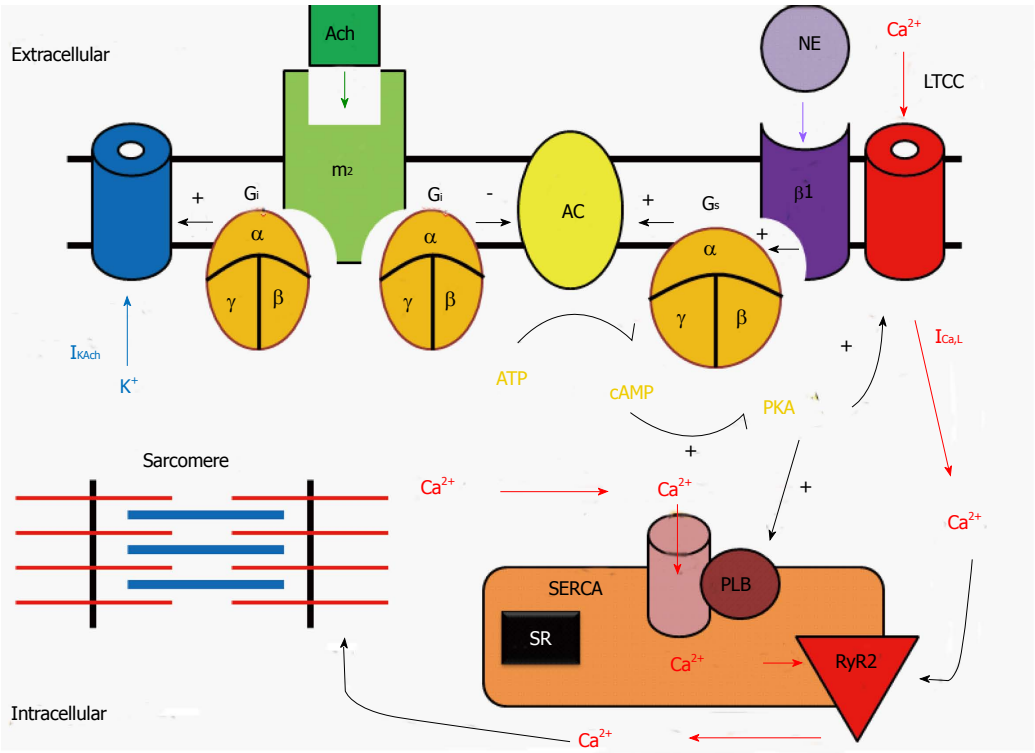

Figure 2 Signal transduction systems for β-adrenergic receptor and muscarinic-receptor stimulations in a cardiac myocyte. NE: Norepinephrine; β1: Beta1-adrenergic receptor; G_s: Stimulatory G-protein: Ach: Acetylcholine; m_2: Type-2 muscarinic receptors; G_i: Inhibitory G-protein; AC: Adenylate cyclase; PKA: Protein kinase A; $I_{Ca,L}$: L-type Ca channel; RyR2: Ryanodine receptor 2; SERCA: Sarcoplasmic reticulum Ca^{2+}-ATPase2a; PLB: Phospholamban.

subunit can then bind to and inhibits AC. Since M_2 receptors are negatively coupled to AC and thus reduce cAMP formation, M_2 receptors act to inhibit PKA activity and exert an opposite effect on ion channels, Ca^{2+} handling proteins, and contractile machinery, compared to sympathetic stimulation.

Autorhythmic cells: Regulation of pacemaking current and heart rate: The funny current (I_f) is thought to be the pace making current in the SA node. It is a non-selective cation channel that can inwardly conduct both sodium and potassium ions. As the membrane potential becomes increasingly hyperpolarized during phase 3 and 4 of the action potential, I_f increases inward potassium and sodium currents, which causes the phase 4 diastolic depolarization. I_f channels are activated by direct binding of cAMP[7].

In addition to the funny current, one of the other driving mechanisms behind the automaticity of the pacemaking cells within the SA node is the calcium clock[8]. As the SR fills with calcium, the probability of spontaneous calcium release increases; in contrast, when the SR calcium stores are depleted, the probability of spontaneous calcium release is reduced. Increased Ca^{2+} entry also increases automaticity because of the effect of $[Ca^{2+}]_i$ on the transient inward current carried by the sodium-calcium exchange current (I_{NCX}). When these pacemaking mechanisms depolarize the resting membrane potential and reach the threshold voltage, which induces the opening of L-type Ca channel (LTCC), an action potential is fired.

On the other hand, M_2 receptor stimulation opens muscarinic potassium channels (K_{ACh})[9]. These channels are opened by M_2 receptors binding to ACh and produce a hyperpolarizing current that opposes the inward pacemaker current. Therefore, the parasympathetic stimulation increases outward K^+ permeability, slowing the heart rate and reducing automaticity.

Cardiomyocytes: Regulation of cellular Ca^{2+} handling and cardiac contraction: Excitation-contraction coupling in cardiomyocytes is dependent on calcium-induced calcium release, whereby an action potential initiates an increase in cellular calcium through opening of the LTCC on the cellular membrane. This creates a positive feedback loop by activating the ryanodine receptors of the SR causing the release of an even greater amount of calcium. This calcium then binds to troponin C, moving the tropomyosin complex off the actin active site, so that the myosin head can bind to the actin filament. Hydrolysis of ATP then causes the myosin head to pull the actin filament toward the center of the sarcomere. Free intracellular calcium is then resequestered into the SR *via* the SR ATPase pump (SERCA), or is pumped from the cell *via* the sodium-calcium exchanger on the cellular membrane. Finally, the troponin complex returns the actin filament to its binding sites to tropomyosin.

Sympathetic stimulation leads to the elevation

of cAMP levels and the activation of PKA, which phosphorylates the α_1 subunits of the LTCCs. This increases the opening probability of LTCCs and the inward Ca^{2+} current, and thus enhances the force of cardiac contraction. In addition, PKA phosphorylates phospholamban, thus relieving its inhibition of SERCA, which in turn facilitates Ca^{2+} uptake by the SR and increases the amount of Ca^{2+} (*i.e.,* SR Ca^{2+} content) available for release by the action potential. Furthermore, activation of β_1-adrenoceptors also increases the Ca^{2+} sensitivity of the contractile machinery, mediated by phosphorylation of troponin C. Taken together, the net result of sympathetic stimulation is to elevate cardiac function and steepen both contraction and relaxation.

Since M_2 receptors are negatively coupled to AC and thus reduce cAMP formation, they act to decrease the open probability of LTCCs and reduce Ca^{2+} current. In opposition to sympathetic stimulation, parasympathetic stimulation reduces the activity of Ca^{2+} handling proteins in cardiomyocytes.

Autonomic regulation of vascular function: In contrast to the heart, most vessels (arteries and veins) only receive sympathetic innervation, while capillaries receive no innervation. These sympathetic nerve fibers tonically release norepinephrine, which activates α_1-adrenergic and β_2-adrenergic receptors on blood vessels thereby providing basal vascular tone. Since there is greater α_1-adrenergic than β_2-adrenergic receptor distribution in the arteries, activation of sympathetic nerves causes vasoconstriction and increases the systemic vascular resistance primarily *via* α_1 receptor activation. On the other hand, modified sympathetic nerve endings in the adrenal medulla release circulating epinephrine, which also binds to α_1 and β_2-adrenergic receptors in vessels. However, β-adrenergic receptors show greater affinity for epinephrine than for norepinephrine. Therefore, circulating epinephrine at low concentrations activates only β_1-adrenergic (mainly in the heart) and β_2-adrenergic (mainly in vessels) receptors, which increase cardiac output and cause vasodilation, respectively. It should be noted that vessels at different locations may react differently to sympathetic stimulation. For example, during the "fight or flight" response the sympathetic nervous system causes vasodilation in skeletal muscle, but vasoconstriction in the skin.

Cardiovascular reflexes and the regulation of blood pressure

In the human body, the ANS is organized as functional reflex arcs (Figure 3). Sensory signals from receptors distributed in certain parts of the body are relayed *via* afferent autonomic pathways to the central nervous system (*i.e.,* spinal cord, brain stem, or hypothalamus), the impulses are then integrated and transmitted *via* efferent pathways to the visceral organs to control their activities. The following reflexes play major roles in regulating cardiovascular functions.

Baroreceptor reflex: Baroreceptors located within the aortic arch and the carotid sinuses detect increases in blood pressure. These mechanoreceptors are activated when distended, and subsequently send action potentials to the rostral ventrolateral medulla (RVLM; located in the medulla oblongata of the brainstem) which further propagates signals, through the autonomic nervous system, adjusting total peripheral resistance through vasodilatation (sympathetic inhibition), and reducing cardiac output through negative inotropic and chronotropic regulation of the heart (parasympathetic activation). Conversely, baroreceptors within the venae cavae and pulmonary veins are activated when blood pressure drops. This feedback results in the release of antidiuretic hormone from cell bodies in the hypothalamus into the bloodstream from the nerve endings in the posterior lobe of the pituitary gland. The renin-angiotensin-aldosterone system is also activated. The subsequent increase in blood plasma volume then results in increased blood pressure. The final baroreceptor reflex involves the stretch receptors located within the atria; like the mechanoreceptors in the aortic arch and carotid sinuses, the receptors are activated when distended (as the atria become filled with blood), however, unlike the other mechanoreceptors, upon activation, the receptors in the atria increase the heart rate through increased sympathetic activation (first to the medulla, then subsequently to the SA node), thus increasing cardiac output and alleviating the increased blood volume-caused pressure in the atria[10].

Chemoreceptor reflex: Peripheral chemoreceptors located in the carotid and aortic bodies monitor oxygen and carbon dioxide content as well as the pH of the blood. Central chemoreceptors are located on the ventrolateral medullary surface in the central nervous system and are sensitive to the surrounding pH and CO_2 levels. During hypovolemia or severe blood loss, blood oxygen content drops and/or pH is decreased (more acidic), and levels of carbon dioxide are likely increased, action potentials are sent along the glossopharyngeal or vagus nerves (the former for the carotid receptors, the latter for the aortic) to the medullary center, where parasympathetic stimulation is decreased, resulting in an increase in heart rate (and thus an increase in gas exchange as well as respiration). Additionally, sympathetic stimulation is increased, resulting in further increases to heart rate, as well as stroke volume, which in turn results in an even greater restoration of cardiac output.

Cardiovascular autonomic dysfunction and heart rate variability: It has been known that sympathetic stress/dominance occurs during heart failure or after myocardial infarction, and may trigger

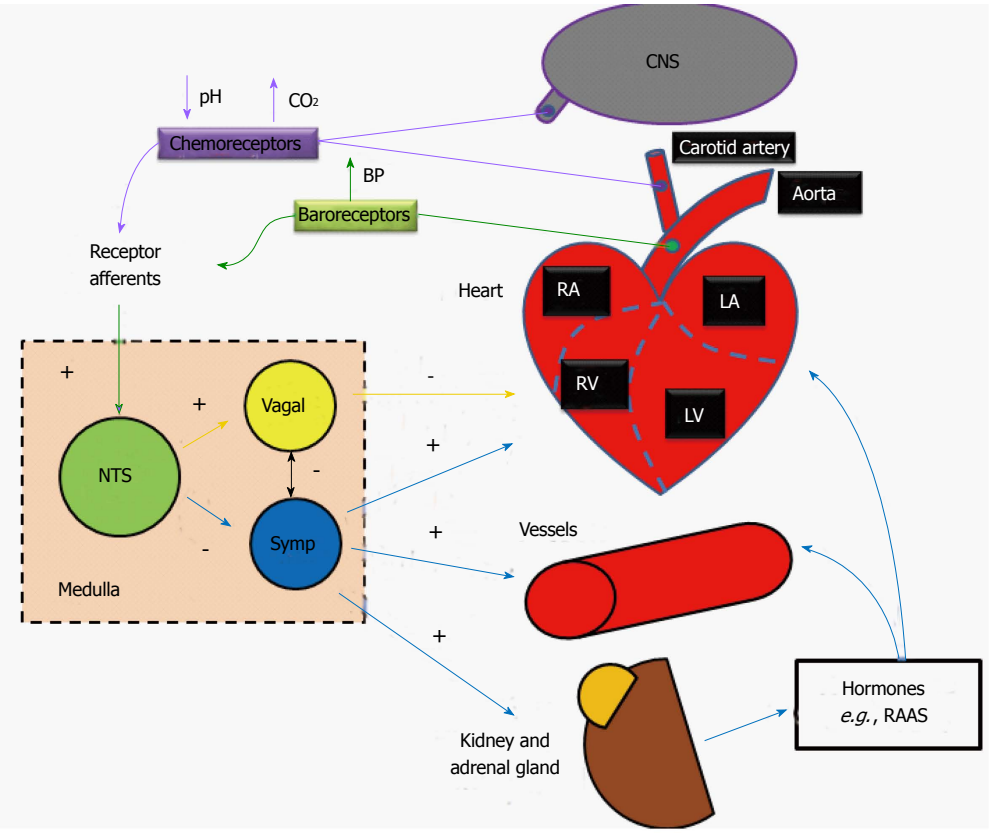

Figure 3 Schematic of cardiovascular reflexes and their influences on heart and vessels functions. NTS: *Nucleus tractussolitarii*; Symp: Sympathetic; CNS: Central nervous system; RAAS: Renin-angiotensin-aldosterone system.

lethal arrhythmias. This sympathovagal imbalance is reflected by reduced heart rate variability (HRV). HRV is determined from ECG and has currently been used clinically as both a diagnostic as well as a prognostic factor for assessing cardiovascular autonomic dysfunction including cardiac autonomic neuropathy. Please refer a recent review article for specific HRV indicators and their interpretations[11].

ENDOCRINE/PARACRINE REFLEXES AND THE REGULATION OF BLOOD PRESSURE REGULATION

In addition to the ANS, cardiovascular function is also influenced by numerous endocrine hormones. Released from the adrenal gland, epinephrine and dopamine (and ultimately, norepinephrine) are all involved in the initiation of the "fight-or-flight" response, while vasopressin, renin, angiotensin, aldosterone, and atrial-natriuretic peptide are all involved in water reabsorption for the purpose of blood pressure regulation.

Adrenal medulla (epinephrine)

An important exception to the usual arrangement in sympathetic fibers is the set of preganglionic fibers that pass through the sympathetic ganglia and extend to the medulla of the adrenal glands. These fibers terminate on special hormone secreting cells, *i.e.*, chromaffin cells, that release norepinephrine (20%) and epinephrine (80%) when stimulated. Epinephrine and norepinephrine are the two main catecholamines that can activate or deactivate sympathetic receptors within the cardiovascular system. Another neurotransmitter dopamine that has limited actions in the autonomic nervous system may excite or inhibit depending on the receptors. Dopamine can be converted into norepinephrine and thus can increase heart rate and blood pressure. Epinephrine is produced (from phenylalanine and tyrosine) and released from chromaffin cells in the adrenal medulla of the adrenal glands. It can stimulate all major adrenergic receptors, including α_1, α_2, β_1, and β_2 receptors. Epinephrine at low concentrations is β_2-selective, producing vasodilatation, while at high concentrations it also stimulates α_1, α_2, and β_1 receptors, producing vasoconstriction (mediated by α_1 and α_2 receptors), and increases heart rate and contractility (mediated by β_1 receptor). Blood pressure is regulated through a system of vasoconstriction and vasodilatation (*i.e.*, vascular resistance). The change in vessel resistance is proportional to the length (L) of the vessel and the viscosity (η) of the blood and inversely proportional to the radius of the vessel to the fourth power (r^4). It is clear from this relationship that vessel diameter controlled by the sympathetic nervous system can have a tremendous impact on

blood pressure regulation *via* small changes in vessel diameter.

$$R \propto \frac{\eta.L}{r^4}$$

Importantly, epinephrine serves to initiate the fight or flight response system by boosting the oxygen and glucose supplies to the brain and skeletal muscle through increased cardiac output and vasodilatation.

Posterior pituitary gland

Vasopressin (antidiuretic hormone) is released during hypovolemic shock as a homeostatic attempt to increase blood pressure and maintain organ perfusion. Vasopressin serves to regulate water retention and vasoconstriction. Vasopressin is produced and released from the parvocellular neurosecretory neurons. It is synthesized in the hypothalamus, and then stored in the posterior pituitary gland, until it is secreted in response to a reduction in plasma volume, an increase in plasma osmolarity, or an increase in cholecystokinin[12]. Within the kidney, vasopressin causes water retention by increasing water permeability of the distal tubule and collecting duct cells, by inserting Aquaporin-2 channels, thus resulting in the inner medullary collecting duct becoming more permeable to urea. Within the cardiovascular system, vasopressin is a vasoconstrictor which increases arterial blood pressure. An increase in blood volume results in increased cardiac output and improved cardiovascular function.

Kidney

There are three hormones produced in the kidneys: calcitriol, thrombopoietin and renin. Of these three, only renin is involved in cardiovascular reflexes and the regulation of blood pressure. Calcitrol works in conjunction with parathyroid hormone to increase the absorption of calcium and phosphate from the gastrointestinal tract[13]. Abnormal calcium metabolism in the cardiovascular system can result in medial arterial calcification and increased vascular stiffness, plaque formation and rupture. Thrombopoietin is made by the proximal convoluted tubule cells, and is responsible for stimulating the production of megakaryocytes of the bone marrow to eventually produce platelets[14]. Low numbers of platelets can lead to hemorrhage and anemic states. Anemia is known to result in high output heart failure.

In the kidney renin is released from the juxtaglomeruler cells, and activates the renin-angiotensin system. The renin-angiotensin-aldosterone system can play both physiological and pathological roles in the cardiovascular system. Angiotensin is known to be involved in heart failure. A main stay in the treatment of heart failure is the use of angiotensin converting enzyme inhibitors.

Renin-angiotensin-aldosterone system: The renin-angiotensin-aldosterone system serves to regulate blood pressure and fluid balance during for example instances of hypovolemia or blood loss. There are three mechanisms by which this system can be activated: baroreceptors with the carotid sinus can detect decreases in blood pressure, a decrease in sodium chloride concentration and/or a decreased rate of blood flow through the macula densa. Once a decrease in blood volume is detected, renin is released by the kidney and cleaves angiotensinogen (produced in the liver) into angiotensin Ⅰ. Angiotensin Ⅰ is further converted to angiotensin Ⅱ by the angiotensin converting enzyme (which is produced in the capillary beds of the lungs). Angiotensin Ⅱ then acts upon the proximal tubules to increase sodium reabsorption, thus helping to retain water while maintaining the glomerular filtration rate and blood pressure. It also serves to constrict the renal arteries, as well as the afferent and efferent arterioles. Through contraction of the mesangial cells, it can also decrease the filtration rate of the kidneys. Angiotensin Ⅱ also increases the sensitivity to tubuloglomerular feedback by increasing the afferent arterioles responsiveness in the macula densa. It can also reduce medullary blood flow. Finally, it causes the adrenal cortex to release aldosterone, which causes sodium retention and potassium excretion.

Angiotensin Ⅱ has three major effects on the cardiovascular system: it is a potent vasoconstrictor, causing a direct increase in systemic blood pressure; it also exhibits prothrombotic effects, stimulating platelet aggregation and causing the production of PAI-1 and PAI-2[15]; finally, it acts as a Gq stimulator when released in an autocrine-paracrine fashion from cardiomyocytes, causing cell growth through protein kinase C during myocardial hypertrophy.

Hormones released by the heart

There are two major hormones produced by the heart. The first, atrial-natriuretic peptide (ANP), is produced by atrial cardiomyocytes, and serves to reduce blood pressure through several mechanisms.

ANP is produced, stored, and released by atrial myocytes (while also being produced in the ventricles, brain, suprarenal glands, and renal glands). There are five major causes for ANP release: distention of the atria, β-adrenergic stimulation, hypernatremia, increases in angiotensin Ⅱ, and increases in endothelin[16]. Upon the vasculature, atrial-natriuretic peptide blocks catecholamines, while in the heart, it inhibits hypertrophy by blocking norepinephrine-stimulated protein synthesis. It is also believed to exhibit cardioprotective properties related to its ability to block cardiac fibrosis following ischemia-reperfusion injuries[17].

The other major hormone, brain-natriuretic peptide

(BNP), is produced by ventricular cardiomyocytes, and works in a similar fashion to ANP. BNP is secreted by the ventricles of the heart in response to excessive stretching of ventricular myocytes and its level is typically increased in patients with left ventricular dysfunction. Therefore, clinically BNP levels are used to monitor heart function. Elevated levels of BNP are thought to be indicative of poor left ventricular function and heart failure.

Additional hormones that may impact cardiovascular function

Endothelin-1: Endothelin-1 is a potent vasoconstrictor that is produced by endothelial cells. There are four endothelin receptors, which are mainly expressed in vascular smooth muscles, each with varying actions upon activation. Activation of ET_A results in smooth muscle vasoconstriction; ET_B causes the release of nitric oxide from endothelial cells, thus resulting in vasodilatation; while activation of ET_{B2} causes vasoconstriction. ET_A receptors also function like G-protein coupled receptors in ventricular cardiomyocytes[18,19]. The effects of ET_C activation are currently unknown[20]. Endothelin-1 may play a role in cardiac hypertrophy *via* intracellular alkalinization.

Thyroxin: Thyroxin (T_4) is a hormone produced by the follicular cells of the thyroid gland. While it acts on nearly every cell type within the human body, one of its most important functions is to increase the effect of epinephrine. Through this permissive relationship, thyroxin increases the number of β_1 receptors and is thus indirectly responsible for increasing cardiac output (in both an inotropic and chronotropic manner) and increasing respiration rates. It is directly responsible for increasing basal metabolic rates by increasing protein and carbohydrate metabolism[21]. Clinical increases in thyroxin are associated with the occurrence of atrial fibrillation, a common cardiac arrhythmia. Elevated heart rates from thyroxin induced atrial fibrillation or other arrhythmias can result in myocardial decompensation and heart failure if not returned to normal sinus rhythm.

CONCLUSION

In conclusion, the heart is not simply an isolated actor. The cardiovascular system responds to not only acute but also chronic changes in blood pressure and homeostasis. Body homeostasis and survival are therefore the main functions of the cardiovascular system. Factors actively influencing the cardiovascular system range from the central nervous system including the brain and spinal cord to the peripheral nervous system with fibers being transported through spinal nerves to the glands, *e.g.*, adrenals, vasculature and even to the urinary system (kidneys). The cardiovascular system is controlled and influenced by not only a unique intrinsic conduction system, but is also heavily influenced by the autonomic nervous system as well as the endocrine system.

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ORIGINAL ARTICLE

Prospective Study

Effective treatment of depression improves post-myocardial infarction survival

Soudabeh Khojasteh Banankhah, Erika Friedmann, Sue Thomas

Soudabeh Khojasteh Banankhah, Erika Friedmann, Sue Thomas, School of Nursing, University of Maryland, Baltimore, MD 21201, United States

Author contributions: Banankhah SK contributed to the conception of the study; Banankhah SK and Thomas S designed; Friedmann E contributed to acquisition of data; Banankhah SK and Friedmann E analyzed and interpreted the data; Banankhah SK wrote the paper; Banankhah SK, Friedmann E and Thomas S revised the manuscript for final submission.

Ethics approval: The study was reviewed and approved by the University of Maryland Institutional Review Board.

Clinical trial registration: This study is registered at http://clinicaltrial.gov/ct2/show/NCT00000557?term=ENRICHD&rank=1, The clinical Trials.gov Identifier: NCT00000557.

Informed consent: This study is a secondary data analysis using data from the ENRICHD randomized trial and received in a de-identified format.

Conflict-of-interest: The authors have no relationships with industry to report and have no conflict of interest including financial, personal, political interest in this study.

Data sharing: Technical appendix and statistical code are available from the corresponding author at sbana001@umaryland.edu. The ENRICHD limited use data set is available from NIH by application through BIOLINCC at https://biolincc.nhlbi.nih.gov/home/

Correspondence to: Soudabeh Khojasteh Banankhah, CRNP, CRNA, PhD, School of Nursing, University of Maryland, 655 W. Lombard St., Room 316, Baltimore, MD 21201, United States. sbanank1@jhmi.edu
Telephone: +1- 410-7060659

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Abstract

AIM: To examine the contribution of treatment resistant depression (TRD) to mortality in depressed post-myocardial infarction (MI) patients independent of biological and social predictors.

METHODS: This secondary analysis study utilizes the Enhancing Recovery in Coronary Heart Disease (ENRICHD) clinical trial data. From 1834 depressed patients in the ENRICHD study, there were 770 depressed post-MI patients who were treated for depression. In this study, TRD is defined as having a less than 50% reduction in Hamilton Depression (HAM-D) score from baseline and a HAM-D score of greater than 10 in 6 mo after depression treatment began. Cox regression analysis was used to examine the independent contributions of TRD to mortality after controlling for the biological and social predictors.

RESULTS: TRD occurred in 13.4% (n = 103) of the 770 patients treated for depression. Patients with TRD were significantly younger in age (P = 0.04) (mean = 57.0 years, SD = 11.7) than those without TRD (mean = 59.2 years, SD = 12.0). There was a significantly higher percentage of females with TRD (57.3%) compared to females without TRD (47.4%) [χ^2 (1) = 4.65, P = 0.031]. There were significantly more current smokers with TRD (44.7%) than without TRD (33.0%) [χ^2 (1) = 7.34, P = 0.007]. There were no significant differences in diabetes (P = 0.120), history of heart failure (P = 0.258), prior MI (P = 0.524), and prior stroke (P = 0.180) between patients with TRD and those without TRD. Mortality was 13% (n = 13) in patients with TRD and 7% (n = 49) in patients without TRD, with a mean follow-up of 29 mo (18 mo minimum and maximum of 4.5 years). TRD was a significant independent predictor of mortality (HR =

1.995; 95%CI: 1.011-3.938, *P* = 0.046) after controlling for age (HR = 1.036; 95%CI: 1.011-1.061, *P* = 0.004), diabetes (HR = 2.912; 95%CI: 1.638-5.180, *P* < 0.001), heart failure (HR = 2.736; 95%CI: 1.551-4.827, *P* = 0.001), and smoking (HR = 0.502; 95%CI: 0.228-1.105, *P* = 0.087).

CONCLUSION: The analysis of TRD in the ENRICHD study shows that the effective treatment of depression reduced mortality in depressed post-MI patients. It is important to monitor the effectiveness of depression treatment and change treatments if necessary to reduce depression and improve cardiac outcomes in depressed post-MI patients.

Key words: Depression treatment; Post-myocardial infarction; Mortality; Anti-depressant; Cognitive behavioral therapy

Core tip: Treatment resistant depression (TRD) was associated with increased mortality in post-myocardial infarction (MI) patients with depression. Conversely, effective treatment of depression with cognitive behavioral therapy with or without medication decreased mortality in post-MI patients who were depressed. Since TRD post-MI patients are at higher risk for mortality, closer follow-up and more aggressive treatment for depression and risk factor modification is needed to improve patient outcome. It is important to monitor the effectiveness of depression treatment and change treatments if necessary to reduce depression and improve cardiac outcomes in post-MI patients with depression.

Banankhah SK, Friedmann E, Thomas S. Effective treatment of depression improves post-myocardial infarction survival. *World J Cardiol* 2015; 7(4): 215-223 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i4/215.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i4.215

INTRODUCTION

Depression predicts morbidity and mortality in patients after myocardial infarction (MI)[1-5]. Depression in post-MI patients is associated with increased mortality. A meta-analysis of 29 studies with an average of 16 mo follow-up and a total of 16889 patients revealed that post-MI depression is associated with more than doubling in odds of all cause mortality (OR = 2.25, 95%CI: 1.73-2.93; *P* < 0.001)[6]. Prevalence of depression is about 20% in patients with MI, compared to 5% in the general population[7,8]. Depression predicts a poorer prognosis and lower functional status in post-MI patients[4].

Treating depression in depressed post-MI patients should improve their long-term prognosis; however, in randomized clinical trials treating depression in depressed post-MI patients, did not improve their survival[9-12].

Cognitive behavioral therapy, plus adjunctive sertraline treatment in the case of insufficient response, did not improve mortality or nonfatal re-infarction with a mean 29 mo follow-up in post-MI patients with depression and/or low perceived social support (LPSS) enrolled in the Enhancing Recovery in Coronary Heart Disease (ENRICHD) clinical trial[9]. There was no difference in event-free survival between the intervention and usual care groups (75.5% *vs* 74.7%). The intervention resulted in a temporary reduction in depression, which was present at 6 mo but disappeared by 30 mo after randomization[9]. Similarly, depression treatment did not improve cardiac event-free survival (treatment group 86.2% *vs* usual care group 87.3%) during the 18 mo of follow-up in the Myocardial Infarction and Depression-Intervention Trial (MIND-IT)[11]. Antidepressant medication (sertraline) for depressed patients with heart disease (*n* = 369) resulted in a slight, but non-significant reduction in recurrent MI and death after an average of 30 mo of follow-up (RR = 0.77; 95%CI: 0.51-1.16) in the Sertraline Antidepressant Heart Attack Randomized Trial (SADHART)[10]. The recent 8-year follow-up of the MIND-IT trial that evaluated the effects of antidepressant treatment in depressed post-MI patients revealed that the treatment group's mortality was not reduced when compared usual care group[12]. However, patients who actually received treatment for depression, regardless of group assignment, had an improved mortality (HR = 0.52, 95%CI: 0.28-0.97).

Secondary analyses of data from subgroups within clinical trials hint that the effectiveness of the treatment of depression might be a factor in whether treatment improves health outcomes. In an on-treatment secondary analysis of the 1834 depressed patients from the ENRICHD intervention and control groups showed significantly lower risk of recurrent MI and death in patients taking selective serotonin reuptake inhibitors (SSRIs) from both the usual care and treatment group. During an average of 29 mo of follow-up, 26% of patients who did not receive antidepressants died or had a recurrent MI *vs* 21.5% of patients on antidepressant therapy. Use of SSRIs was associated with significant reduction in risk of death or nonfatal MI (HR = 0.72; 95%CI: 0.44-1.22) and of all-cause mortality (HR = 0.73; 95%CI: 0.34-1.38)[13].

In patients with at least 1 prior episode of major depression in the ENRICD intervention group whose depression did not improve over the first 6 mo had higher late mortality (unimproved 21.2% *vs* improved 10.4%) and were more likely to be taking antidepressant medication and have a higher body mass index at enrollment than patients whose

depression symptoms improved. In the usual care arm of ENRICHD, improvement in depression was not related to mortality[2].

In a 7-year follow-up analysis of SADHART, patients whose major depression did not respond to medication experienced 2.39 times as much mortality (28.4%) as those whose depression was treated effectively (15.6%)[14].

The ENRICHD study did not find that randomized treatment of depression in depressed post-MI patients decreased mortality or morbidity. Even with extensive effort to treat their depression, many patients in the intervention arm did not improve their depression symptoms after 6 mo of treatment. Instead, they seem to have a treatment refractory depression that was resistance to current available therapy. This finding and other subsequent analysis of ENRICHD studies left health professionals without clear direction for the treatment of depression in post-MI patients.

Clinical studies show that not all depressed patients respond adequately to treatment. Fava *et al*[15] meta-analysis of 36 clinical trials demonstrated that about 50% of depressed patients have an adequate response to antidepressant therapy, 15% had partial response, and 20%-35% did not respond to depression treatment. Patients who do not respond adequately labeled as treatment resistant. Depression was considered treatment resistant when at least 2 trials of different antidepressant therapy with adequate dose/duration/compliance failed to produce a significant clinical improvement in depression symptoms[16].

Patients with treatment resistant depression (TRD) in previous studies may have masked the effect of reducing depression on improvement of survival of depressed post-MI patients. In a cohort study of 4037 depression post-MI patients, 12.1% of the patients had treatment-resistant depression and they were 1.71 times as likely to die than treated patients[17].

The current study focuses on TRD and compares survival of patients with TRD to survival of patients who responded to treatment of depression.

MATERIALS AND METHODS

Research design

This secondary data analysis uses longitudinal data from the ENRICHD randomized clinical trial. The limited use de-identified data set was obtained from the National Heart, Lung, and Blood Institute (NHLBI) after University of Maryland full IRB review.

Data source

ENRICHD was the first randomized multi-center study to examine the effect of psychosocial intervention on survival in patients who were depressed and/or had LSSP after an MI. This study was sponsored by the NHLBI, and recruitment started in October 1996 and ended in October 1999. Patients were enrolled in the ENRICHD study within 28 d of an acute MI.

All patients with an acute MI admitted to 1 of the 73 participating hospitals were screened for acute MI eligibility including MI documented by cardiac enzymes and by chest pain with typical ST-T changes or new Q waves. Complete ENRICHD inclusion and exclusion criteria are published elsewhere[9]. After informed consent was obtained, patients were screened for depression and/or LPSS. If either depression or LPSS was present, patients were randomly assigned to the intervention or usual care arm. Participants had follow-up examinations at 6 and 18 mo and annually thereafter. The primary end-point of the study was the occurrence of re-infarction or all cause mortality[9].

ENRICHD intervention

Cognitive behavioral therapy (CBT) was utilized as the standard of the ENRICHD intervention[18]. Intervention group patients with scores higher than 24 on the Hamilton rating scale for depression (HAM-D) or who had < 50% reduction in BDI score after 5 wk were referred to study psychiatrists for pharmacotherapy consideration. If there were no contraindications, sertraline hydrochloride was used as the drug of choice. The maximum CBT duration was 6 mo[9].

Patients in the usual care arm of the study received only the care provided by their primary care providers, which was standard medical care for post-MI patients. Patients in both groups received health education regarding cardiovascular disease and its management and both groups received standard medical treatment as practiced in that institution.

ENRICHD measures

Baseline assessment in ENRICHD included demographics, cardiovascular health history, risk factors, current medications, detailed medical record documentation of the course of treatment for the acute MI, an electrocardiogram, the Depression Interview and Structured Hamilton (DISH), beck depression inventory (BDI), and ENRICHD social support inventory (ESSI).

The DISH is a semi-structured interview developed for ENRICHD trial and was used for screening and diagnosing depression[19] using principles and criteria from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-Ⅳ)[20]. The DISH incorporated material from the 17-item version of the Hamilton Rating Scale for Depression (HAM-D)[21], the Structured Interview Guide for the Hamilton Depression Scale (SIGH-D)[22], the Diagnostic Interview Schedule (DIS)[23], and the modified versions of the DIS[24]. The DISH depression severity score was based on the 17-item HAM-D. The first 9 items on HAMD-D are 5 point Likert format ranging from 0 (absent) to 4 (severe). The last 8 items are 3 point Likert format ranging from 0 (absent) to 2 (clearly present). The possible score ranges from 0 to 50. Scores of > 10 indicates the presence of depression symptoms. Hig-

her total scores indicate more depression symptom severity. The concurrent validity of DISH was evaluated using the Pearson correlation coefficient between 17-item HAM-D embedded in the DISH and BDI scores that resulted in a correlation of 0.76 ($P < 0.001$)[19]. The DISH was administered at the screening phase of the ENRICHD study and at 6 mo follow-up.

The BDI[25] was used in the ENRICHD study to evaluate baseline depression status and assess progress during treatment and follow-up. The BDI is the most widely use depression instrument in clinical and research settings. The BDI is considered 1 of the best methods to assess presence and severity of depression. It is easy to use and only takes 5 min to complete by the patient or provider. The BDI is a 21-item inventory, and each item is rated on a 0 to 3 scale with a total score ranging from 0 to 64. BDI scores > 10 indicate depression, scores of 10-15 mild depression, 16-23 moderate depression, and 24-64 severe depression. Concurrent validity of BDI was established by comparing BID to HAM-D that positively correlated (r = 0.71, $n = 87$). This indicates an acceptable concurrent validity of BDI. The BDI was administered to all participants at baseline, 6, 12, 18 mo and annually thereafter.

The ESSI was used in the ENRICHD study to assess perceived social support at baseline and durng treatment and follow-up. It was developed for ENRICHD study to measure functional social support. The ESSI was used as a screening tool to determine patients' eligibility for ENRICHD based on low social support, and to assess changes in patients' social support following treatment. The ESSI is a 7-item inventory, and item 1 to 6 is rated on a 1 to 5 scale, which indicates none to all respectively. Item 7 is not rated on numeric scale. The total score ranges from 6 to 30 with lower scores indicating LPSS. A score < 3 on 2 or more items and a total score < 18, or a score of 2 on 2 items without regard to total score indicated LPSS which was the criteria for inclusion in ENRICHD trial[26]. In this study, 3 variables derived from ESSI instrument that were: live alone, perceived social support, and social isolation. Live alone was the seventh question on the ESSI questionnaire ("do you live alone?") that was separated and transferred into the study dataset. Perceived social support was the total score on the ESSI and social isolation was dichotomous value based on scoring the ESSI. Social isolation was defined as meeting the ENRICHD criteria for LPSS. The reliability of ESSI was estimated by using test-retest reliability that showed no significant differences in mean scores administered 1 mo apart ($P = 0.98$). The internal reliability was measured with the use of Cronbach's alpha that revealed an alpha of 0.88, which indicates a high internal consistency. The intra-class correlation coefficient was 0.94, reflecting excellent reproducibility[26]. The ESSI was administered to all participants at baseline, 6, 12, 18 mo and annually thereafter.

Sample

The study sample consists of the depressed patients in either arm of ENRICHD who received treatment for depression and completed the DISH at 6 mo. Treatment was defined as any combination of CBT and/or antidepressant medication. TRD was defined as < 50% improvement of 6-mo HAM-D score from baseline in patients with 6-mo HAM-D > 10. The outcome variable in this study was all cause mortality. All deaths were documented and verified by the ENRICHD investigators.

Statistical analysis

Data analysis: Normality of distributions of continuous variables was examined; no variables had extreme values that needed transformation[27]. Missing data was < 5% thus there was not a need to examine patterns of missing data[27]. All inferential tests were conducted at the 0.05 level of significance. Statistical procedures were performed using SPSS version 20.0 software.

Cox regression analyses was used to examine the hypothesis. The proportional hazards assumption of Cox regression was confirmed. The contribution of each biological (age, female gender, minority status, and presence of diabetes, history of heart failure, hypertension, prior MI, prior stroke, and current smoker), social (lives alone, perceived social support, social isolation), and psychological (baseline depression symptoms and TRD) predictor to mortality was initially examined in individual Cox regression analyses. Age was centered on 34 years, the lowest age in the study for ease of interpretation. The reference group (0) for dichotomous predictors was the un-named category. A combined model was constructed with biological, psychological, and social factors that predicted mortality at $P < 0.20$ in the individual analyses. Based on prior research showing differences between men and women in reporting depression[28,29], the interaction of TRD and female gender was added to the model. Similarly, based on prior research showing differences between minorities and non-minorities in reporting depression[30], the interaction of TRD and minorities status was added to the model. Neither of the interaction were significant predictors of mortality so they were not included in the final parsimonious Cox regression combined model. Biostatistician reviewed the statistical methods of this study.

Sample description: A total of 770 ENRICHD participants were depressed at the study entry and received depression treatment (Table 1). These participants received cognitive behavior treatment (CBT) ($n = 469$, 60.9%), or antidepressant medication ($n = 85$, 11%), or both ($n = 216$, 28.1%). The sample included 365 (47%) women and 239 (31%) minority participants.

Table 1 Summary of demographic biological, social, and psychological characteristics of depressed post-myocardial infarction patients treated for depression and comparison of those with treatment resistant and non treatment resistant depression (n = 770) n (%)

Characteristics	Treated Depressed Post-MI[1]	TRD n = 103[1]	Non TRD n = 667[1]	
Demographics and biological characteristics				
Age, mean (SD), yr	59.2 (12.0)	57.0 (11.7)	59.5 (12.0)	t = -2.07, P = 0.04
Gender, female	365 (47.4)	59 (57.3)	306 (45.9)	χ^2 (1) = 4.65, P = 0.031
Ethnicity, minority	239 (31.0)	32 (31.1)	207 (31.0)	χ^2 (1) = 0.0, P = 0.995
Education:				χ^2 (2) = 3.76, P = 0.153
Basic or no HS degree	187 (24.3)	25 (24.3)	162 (24.3)	
HS without college degree	419 (54.4)	64 (62.1)	355 (53.2)	
Advanced education	146 (19.0)	13 (12.6)	133 (19.9)	
Body mass index, mean (SD)	29.2 (6.1)	29.7 (6.3)	29.2 (6.1)	t = 0.77, P = 0.444
Diabetes	264 (34.3)	42 (40.8)	222 (33.3)	χ^2 (1) = 2.41, P = 0.120
History of heart failure	101 (13.1)	17 (16.5)	84 (12.6)	χ^2 (1) = 1.28, P = 0.258
Hypertension	458 (59.5)	70 (68.0)	388 (58.2)	χ^2 (1) = 4.56, P = 0.033
Prior MI	187 (24.3)	22 (21.4)	165 (24.7)	χ^2 (1) = 0.41, P = 0.524
Prior stroke	70 (9.1)	13 (12.9)	57 (8.7)	χ^2 (1) = 1.80, P = 0.180
Current smoker	254 (33.0)	46 (44.7)	208 (31.2)	χ^2 (1) = 7.34, P = 0.007
Social characteristics				
Live alone	322 (41.8)	46 (44.7)	276 (41.4)	χ^2 (1) = 0.32, P = 0.570
Social support	356 (46.2)	54 (52.4)	302 (45.3)	χ^2 (1) = 1.84, P = 0.176
Psychological characteristics				
Depression				χ^2 (2) = 16.7, P > 0.001
Major depression	398 (51.7)	69 (67.0)	329 (49.3)	
Minor depression	348 (45.2)	28 (27.2)	320 (48.0)	
Dysthymia	24 (3.1)	6 (5.8)	18 (2.7)	
Psychosocial measures				
Baseline Depression Symptom Severity (BDI), mean (SD)	17.7 (7.8)	22.4 (9.3)	17.0 (7.4)	t = 6.62, P < 0.001
Baseline Perceived Social Support (ESSI), mean (SD)	24.5 (6.4)	22.9 (7.0)	24.7 (6.3)	t = -2.74, P = 0.006
Baseline Depression Symptom (HAM-D), mean (SD)	17.9 (6.3)	20.9 (6.5)	17.4 (6.1)	t = 5.07, P < 0.001

[1]Except where mean (SD) is noted. BDI: Beck Depression Inventory; ESSI: ENRICHD Social Support Inventory; HAM-D: Hamilton Depression Rating Scale; HS: High school; TRD: Treatment resistant depression; MI: Myocardial infarction.

Ages ranged from 34 to 85 with a mean of 59 (SD = 12) years. Treatment resistant depression was present in 103 (13.4%) of the patients who were treated for depression.

Biostatistics

The statistical methods of this study were reviewed by Erika Friedmann from University of Maryland.

RESULTS

Baseline characteristics of patients with and without TRD were compared (Table 1). Patients with TRD were significantly younger (P = 0.04), more likely to be female (P = 0.031), be hypertensive (P = 0.033), and have lower perceived social support (P = 0.006) than those without TRD. Major depression was significantly more common (P < 0.001) and baseline depression symptom severity assessed with the BDI was significantly worse (P < 0.001) among patients who experienced TRD.

Of the 770 participants, there were 62 (8%) deaths with an average follow-up of 29 mo. All cause mortality tended to be higher in the patients with TRD (13/103: 13%) than those whose depression responded to treatment (49/667: 7%) (χ^2 = 3.35, P = 0.05).

In univariate Cox regression analysis (Table 2), age (P < 0.001), female gender (P = 0.005), diabetes (P < 0.001), history of heart failure (P < 0.001), hypertension (P = 0.002), prior MI (P < 0.001), prior stroke (P < 0.001), current smoker (P = 0.002) and live alone (P = 0.032) were significant predictors of mortality. Minority status (P = 0.943), perceived social support (P = 0.279), social isolation (P = 0.446), and baseline depression symptoms (P = 0.978) did not predict mortality.

In the combined simultaneous Cox regression model, TRD (P = 0.046), age (P = 0.004), diabetes (P < 0.001), and history of heart failure (P = 0.001) were significant independent predictors of mortality; current smoker tended to predict mortality (P = 0.087) (Table 3).

When the interaction between female gender and TRD and minority status and TRD were each added to the model, the interactions were not significant (P = 0.467, P =0.87, respectively). Neither interaction was added to the final model. In the final model TRD contributed significantly to mortality (P = 0.046) after controlling for age, diabetes, heart failure, and currently smoking. Patients with TRD had approximately double the risk of all cause mortality compared with patients without TRD (95%CI: 1.011-3.938). Other significant independent predictors of mortality were age, diabetes and heart failure.

This study showed a significant improvement in survival in depressed post-MI patients whose depre-

Table 2 Results of separate Cox regression analyses to examine the contributions of each biological, social and psychological predictor to mortality among treated post- myocardial infarction patients with depression (*n* = 770)

	B	SE	Wald	Sig.	HR	95%CI
Biological factors						
Age[1]	0.048	0.011	19.205	< 0.001	1.05	1.027-1.073
Female gender	0.741	0.266	7.791	0.005	2.098	1.247-3.531
Minority status	0.020	0.272	0.005	0.943	1.020	0.599-1.737
Diabetes	1.448	0.272	28.401	< 0.001	4.255	2.498-7.247
Heart failure	1.495	0.275	29.507	< 0.001	4.461	2.601-7.652
Hypertension	1.002	0.322	9.675	0.002	2.723	1.449-5.120
Prior MI	0.961	0.261	13.533	< 0.001	2.616	1.567-4.365
Prior stroke	1.165	0.306	14.496	< 0.001	3.207	1.760-5.843
Current smoker	-1.128	0.361	9.781	0.002	0.324	0.160-0.656
Social factors						
Live alone	0.552	0.258	4.591	0.032	1.737	1.048-2.880
Perceived social support	-0.046	0.028	2.768	0.096	0.955	0.904-1.008
Social isolation	-0.197	0.258	0.582	0.446	0.822	0.496-1.361
Psychological factor						
Baseline depression symptom severity	< 0.001	0.016	0.001	0.978	1.000	0.968-1.032
Treatment resistant depression	0.534	0.312	2.927	0.087	1.705	0.925-3.143

[1]Age centered on the lowest age of 34 years. For dichotomous variables reference group is the absence of the named predictor. MI: Myocardial infarction.

ssion was effectively treated (Figure 1). Among treated depressed post-MI patients, TRD was a significant independent predictor of mortality after controlling for biological and social factors.

DISCUSSION

Not all depressed patients respond adequately to treatment, and their depression does not improve significantly with current depression treatments. These depressed patients are treatment resistant[13]. Recent studies do not show improved mortality of acute myocardial infarction patients after treatment for depression[8-12]. One explanation of this may be that previous studies of post-MI patients included a subgroup of patients with TRD who may have impacted the results. The presence of the TRD subgroup may cause a failure in the study's ability to show improved survival in the intervention group.

The purpose of this study was to examine the differences in mortality between patients with TRD and those without TRD. The significant predictors for mortality among depressed post-MI patients whose depression was treated were; age, diabetes, and history of heart failure. The presence of TRD significantly predicted mortality after controlling for these other factors (HR = 1.995). TRD was associated with an increased risk of mortality when compared to non-TRD patients. This finding is consistence with the 7-year follow-up analysis of the Sertraline Antidepressant Heart Attack Randomized Trial (SADHART) [10]. Initial findings of SADHEART revealed a non-significant reduction in re-current MI and death (RR = 0.77; 95%CI: 0.51-1.16) in treated depressed individuals. At that time, they did not examine the difference between survival in those individuals who responded and not respondent to depression treatment. Subsequently, the 7-year follow-up analysis of the SADHART trial found that patients with major depression who responded to treatment with sertraline had a reduction in mortality *vs* non-responders (15.6% *vs* 28.4%; HR = 2.39)[14]. The current study extends this finding from post MI patients with major depression to less psychologically distressed patients, namely post MI patients with dysthymia through major depression. Further the current study began treatment with cognitive behavioral therapy and added medication if depression was not responsive. This study is also consistent with Scherrer *et al*'s[17] cohort study's finding that post-MI patients with TRD had increased mortality. The criteria for TRD in that study were that: the patient received (1) electroconvulsive therapy, (2) a monoamine oxidase inhibitor, or (3) two or more antidepressant at the same time. In contrast, the current study uses depression measurement tools to identify post-MI patients with TRD.

This re-analysis of the ENRICHD study data clearly shows that successful treatment of depression decreases mortality in post-MI patients. It also demonstrates that current depression treatments are not effective in treating all depressed post-MI patients. According to this study, over 13% of treated patients still suffered from depression at the end the treatment. This is consistent with the Scherre study, which showed 12.1% of post-MI patients experienced TRD. Perhaps a treatment that is more effective in alleviating depression would improve survival in post-MI patients. Until more effective treatments are developed, closer follow-up of depression symptoms, aggressive treatment of depression, actively treating other modifiable risk factors, and modifying risky health behaviors may lead to a better survival in post-MI patients with TRD.

Strengths and limitations

This study was a secondary data analysis of an existing trial. This places limitation to this study regarding

Table 3 Final model: Results of Cox regression analysis to examine the independent contributions of biological, social, and psychological predictors and treatment resistant depression to all cause mortality among post-myocardial infarction patients treated for depression (n = 770)

	B	SE	Wald	Sig.	HR	95%CI
Biological factors						
Age[1]	0.035	0.012	8.280	0.004	1.036	1.011-1.061
Diabetes	1.069	0.294	13.243	0.000	2.912	1.638-5.180
Heart failure	1.007	0.290	12.089	0.001	2.736	1.551-4.827
Current smoker	-0.690	0.403	2.932	0.087	0.502	0.228-1.105
Social factors						
None						
Psychological factor						
Treatment resistant depression	0.691	0.347	3.967	0.046	1.995	1.011-3.938

[1]Age centered on the lowest age of 34 years. For dichotomous variables, reference group is the absence of the named predictor.

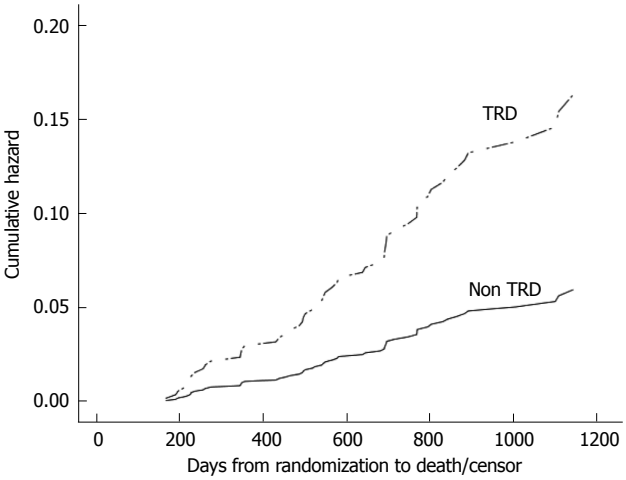

Figure 1 Hazard function for treatment resistant depression and non-treatment resistant depression participants to mortality after myocardial infarction. TRD: Treatment resistant depression.

control over the variable definition, measurement, data collection and other crucial aspects of the study design. With this study, we were not able to establish causality of the contributions of TRD for mortality. The study findings were only able to provide evidence of the associations between the variables. Despite the limitations of this study, the strengths outweigh the limitations of the study. Using a large existing database, this study has the advantage of providing a representative sample. Women and minorities were well represented in the ENRICHD data. ENRICHD included detailed questionnaire development, data collection procedures, data management, and quality control measures. The use of ENRICHD dataset provided an opportunity to use high quality dataset without the high cost and effort of obtaining this type and amount of data.

Implications for practice and future research

The analysis of TRD in ENRICHD shows that treatment of depression in the ENRICHD sample was effective in reducing mortality in those whose depression was effectively reduced by cognitive behavioral therapy and/or anti-depressant medication. By separating out those who were depressed and whose treatment was effective it was possible to evaluate the contribution of effective treatment of depression to mortality in post-MI patients. The findings of this study provided evidence that depressed patients with TRD have more than double the risk of mortality when compared with patients whose depression is effectively treated. This sub-group of depressed patients with TRD did not improve their depression symptoms with their current depression treatment. Closer follow-ups of depression symptoms, aggressive treatment of depression, aggressively treating other modifiable risk factors, and modifying risky health behaviors may lead to a better chance of survival in TRD post-MI patients.

As shown in this current study, depression is a risk factor for mortality among TRD post-MI patients. Yet the question remains how to best treat it. Future research is needed to address the development of effective treatment for depression. Both ENRICHD and SADHART trials reported small effect size using current depression interventions, this suggest a need to increase efficacy of current interventions for depressed post-MI patients. The results of the present study accentuate the need for future research for drug development and effective interventions to alleviate depression symptoms in order to improve cardiac outcomes.

The potential mechanisms linking depression and impaired cardiovascular prognosis are still poorly understood and remain an area that is in need of more research. Future studies are needed to give insight and provide evidence that will direct us toward a future in which we, health providers, are able to help and improve survival in post-MI patients who have TRD.

This study provided evidence that TRD is associated with increased mortality in post-MI patients who are depressed. Conversely, effective treatment of depression with cognitive behavioral therapy without or with medication decreased mortality in post-MI patients who were depressed. Since, TRD post-MI patients are at higher risk for mortality, closer follow-up and more aggressive treatment for depression and risk factor modification is needed to improve patients' outcomes. This may lead to an integrated treatment strategy that may decrease risk of mortality in post-MI patients. It is important to monitor the effectiveness of depression treatment and change treatments if necessary to reduce depression and improve cardiac outcomes in post-MI patients who are depressed.

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limited access dataset obtained from the NHLBI Biologic Specimen and Data Repository Information Coordinating Center and does not necessarily reflect the opinions or views of the ENRICHD Study or the NHLBI. The authors thank the investigators, coordinators, staff, and patients of the ENRICHD Study.

COMMENTS

Background

Depression increases mortality after myocardial infarction (MI). Clinical trials have not demonstrated a reduction in mortality with depression treatment. Depression treatment is not effective in a substantial proportion of the depressed patients. The presence of treatment resistant depression (TRD) may explain why previous clinical trials did not demonstrate decreased mortality when depression was treated.

Research frontiers

Recent clinical studies show that not all depressed patients respond adequately to treatment, and their depression does not improve significantly with current depression treatments. These patients have TRD. Presently, there is no study that shows improved outcome of depressed patients with acute MI by treating depression. This can be explained by the presence of TRD in some post-MI patients, which would impact the results of the study. The presence of TRD may cause a failure in the study's ability to show improved survival in the intervention group.

Innovations and breakthroughs

This study considers the presence of TRD in depressed post-MI patients and its influence on prior analysis. Treatment resistant depression is a possible explanation for why previous clinical trials did not demonstrate decreased mortality when depression was treated. The result of this study reveals that TRD significantly predicts mortality after controlling for biological and social factors. Among depression treated post MI patients, TRD was associated with an increased risk of mortality when compared to non-TRD patients. Depressed patients with TRD have double the risk of mortality when compared with patients whose depression is effectively treated.

Applications

This study provides new evidence that depressed patients with TRD did not improve their mortality with current depression treatment, and they are in desperate need for more efficacious depression treatment. Closer follow-ups of depression symptoms, aggressive treatment of other modifiable risk factors, and modifying risky health behaviors may lead to a better chance of survival in depressed post-MI patients. This knowledge may assist primary care providers regarding clinical decision making that provides a better treatment options for patients.

Terminology

TRD is a term describing patients with depression symptoms that do not improve after treatment is completed. In other words, the total hamilton depression (HAM-D) score does not decrease more than 50% from baseline with a total HAM-D score above 10 after completing depression treatment.

Peer-review

Authors showed that effective treatment of depression improved survival of patients after AMI. This paper is well described, and includes important clinical findings.

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CASE REPORT

Limitations of multimodality imaging in the diagnosis of pannus formation in prosthetic aortic valve and review of the literature

Juan Bautista Soumoulou, Tomás Francisco Cianciulli, Andrea Zappi, Alberto Cozzarin, María Cristina Saccheri, Jorge Alberto Lax, Robert Guidoin, Ze Zhang

Juan Bautista Soumoulou, Tomás Francisco Cianciulli, Alberto Cozzarin, María Cristina Saccheri, Jorge Alberto Lax, Department of Cardiology, Hospital "Dr. Cosme Argerich", Ciudad Autónoma de Buenos Aires C1155AHD, Argentina
Tomás Francisco Cianciulli, Researcher of the Ministry of Health, Government of the City of Buenos Aires, Argentina C1035AAN, Argentina
Andrea Zappi, Department of Pathology, Hospital "Dr. Cosme Argerich", Ciudad Autónoma de Buenos Aires C1155AHD, Argentina
Robert Guidoin, Ze Zhang, Department of Surgery and Radiology, Laval University and Québec Biomaterials Institute, CHUQ, Quebec G1K 7P4, Canada
Author contributions: Cianciulli TF and Cozzarin A attended the patient; Soumoulou JB and Saccheri MC prepared the manuscript and figures; Zappi A performed pathology examination; Lax JA performed the echocardiographic images and participated in the manuscript description; Guidoin R and Zhang Z participated in the design and review of the manuscript; all authors read and approved the final manuscript.
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Correspondence to: Tomás Francisco Cianciulli, MD, FACC, Director Echocardiography Laboratory, Department of Cardiology, Hospital "Dr. Cosme Argerich", Pi y Margall 750, Ciudad Autónoma de Buenos Aires C1155AHD, Argentina. tcianciulli@gmail.com
Telephone: +54-11-41210879
Fax: +54-11-48015510

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Abstract

Pannus formation is a rare complication and occurs almost exclusively in mechanical prosthetic valves. It consists of fibrous tissue that covers the surface of the prosthesis either concentrically or eccentrically, resulting in valve dysfunction. The pathophysiology seems to be associated to a chronic inflammatory process that explains the late and insidious clinical presentation. This diagnosis should be considered in patients with high transvalvular gradients on transthoracic echo, and workup should be completed with fluoroscopy and transesophageal echocardiography. Treatment is always surgical and recurrence is rare. We present a case of pannus formation in a prosthetic aortic valve and a review of the literature regarding this disorder.

Key words: Pannus formation; Prosthetic aortic valve; Fluoroscopy; Transthoracic echocardiography; Transesophageal echocardiography

Core tip: Pannus is an infrequent complication that mainly affects mechanical prosthetic valves. Its diagnosis requires clinical suspicion and the association of fluoroscopy, transthoracic and transesophageal echocardiography. The case presented is a characteristic example of pannus, given its clinical presentation

(progressive dyspnea), the steps followed to reach diagnosis and the surgical resolution. Suspecting this disorder and making an accurate diagnosis is of paramount importance, to implement adequate treatment and to avoid prolonging the natural course of the disease and its repercussion on the left ventricle and the quality of life of affected patients.

Soumoulou JB, Cianciulli TF, Zappi A, Cozzarin A, Saccheri MC, Lax JA, Guidoin R, Zhang Z. Limitations of multimodality imaging in the diagnosis of pannus formation in prosthetic aortic valve and review of the literature. *World J Cardiol* 2015; 7(4): 224-229 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i4/224.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i4.224

INTRODUCTION

Mechanical prosthetic valve dysfunction caused by pannus or thrombosis is an unusual but serious complication of heart valve replacement. Thrombotic complications are most common early postoperatively, whereas pannus occurs later, especially in bileaflet valves in the aortic position. Pannus formation consists of fibrous tissue usually covering the circumference of a prosthetic valve, and causing valve dysfunction[1]. The incidence of this rare complication is 1.6%-2% in the different series published[2,3] and occurs almost exclusively in mechanical prostheses. Its most frequent location varies according to the authors, but in most series prostheses in the aortic position were affected more often[4] than those in mitral position[3,5].

CASE REPORT

The patient is a 55-year-old man with multiple cardiovascular risk factors (type 2 diabetes, hypertension, past history of smoking, obesity, dyslipidemia, and family history of cardiovascular disease) and intermittent claudication at 200 meters. Also, in 1998 he underwent aortic valve replacement with a mechanical #23 St. Jude valve, and coronary artery bypass grafting (CABG) with three grafts (left internal mammary artery to the left anterior descending artery and saphenous vein graft to the right coronary artery and circumflex coronary artery). In 2009 he began experiencing dyspnea in FC Ⅱ [New York Heart Association (NYHA) classification], that progressed to FC IV. Upon clinical consultation, a meso-telesystolic murmur radiating to the neck was detected, as well as a pulsus tardus et parvus and an apical beat in the anterior axillary line. No other relevant findings were reported.

His chest X-ray showed a cardiothoracic index slightly above 0.5, an old posterior infarction (R/S > 1 in lead V2) was seen on ECG, and routine laboratory results were within normal values. Transthoracic echocardiography (TTE) and two-dimensional transesophageal echocardiography (TEE) showed a bileaflet mechanical prosthesis in aortic position with normal opening of both discs (Figure 1A), with severely increased mechanical aortic valve gradients (peak instantaneous gradient: 97 mmHg, mean gradient: 58 mmHg) and decreased effective prosthetic area (0.67 cm^2). No detectable image suggestive of pannus or thrombus was seen in the left ventricular (LV) outflow tract. There was infero-posterior akinesis and mild LV dysfunction (EF: 40%). Fluoroscopy revealed normal opening of both tilting discs (Figure 1B). Cardiac multiple detector computed tomography (MDCT) did not showed any soft tissue mass on the ventricular side of the prosthetic aortic. Coronary angiography showed a severe lesion in the venous graft to the right coronary artery, without significant lesions in the other grafts or native arteries.

In spite of absence of any tissue mass on the ventricular side of the prosthetic aortic and absence of limitation of motion of the tilting disc to allow suggest pannus, given the clinical suspicion of prosthetic valve obstruction due to pannus formation, the decision was made to replace the aortic prosthesis with a new mechanical valve (ATS # 23) and perform CABG (venous graft to the right coronary artery); there were no postoperative complications. On pathological examination (Figure 2), the explanted specimen exhibited fibrous tissue with a smooth, annular surface, in contact with the ventricular surface of the mechanical prosthesis, consistent with pannus. Histological examination confirmed the diagnosis (Figure 3).

DISCUSSION

Pannus formation consists of fibrous tissue usually covering the circumference of a prosthetic valve, and causing valve dysfunction[1]. The incidence of this rare complication is 1.6%-2% in the different series published[2,3] and occurs almost exclusively in mechanical prostheses. Its most frequent location varies according to the authors, but in most series (Table 1) prostheses in the aortic position were affected more often[4] than those in mitral position[3,5].

Pathology studies of valves explanted due to pannus formation have shown that it consists of fibrous tissue ingrowth, with a generally smooth surface and a ring-like shape covering the valve surface. Pannus formation may be an isolated finding or associated to various degrees of thrombosis[2,5,6]. According to the type of growth, pannus may be classified as concentric or eccentric[7,8], the latter being more frequent[5]. However, the morphology of pannus could be associated to the type of prosthetic valve affected, which would explain the higher frequency of the eccentric type on single-disc valves[5], while the concentric type is more common in bi-leaflet valves[2].

On histological examination, pannus consists of a structure of collagen fibers interspersed with small

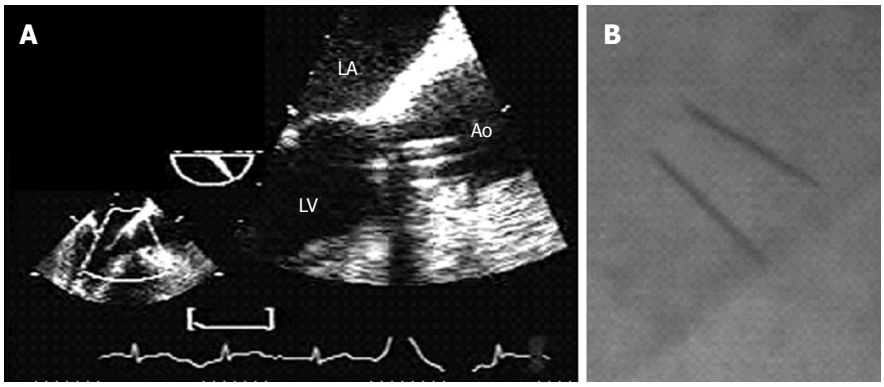

Figure 1 Transesophageal echo and fluoroscopy. A: Transesophageal Echo: 3-chamber view at 129°, with zoom in the LV outflow tract, showing the prosthetic valve with its parallel discs; B: Fluoroscopy showing the almost parallel tilting discs. Both exams confirm an adequate prosthetic valve opening. LA: Left atrium; LV: Left ventricle; Ao: Aorta.

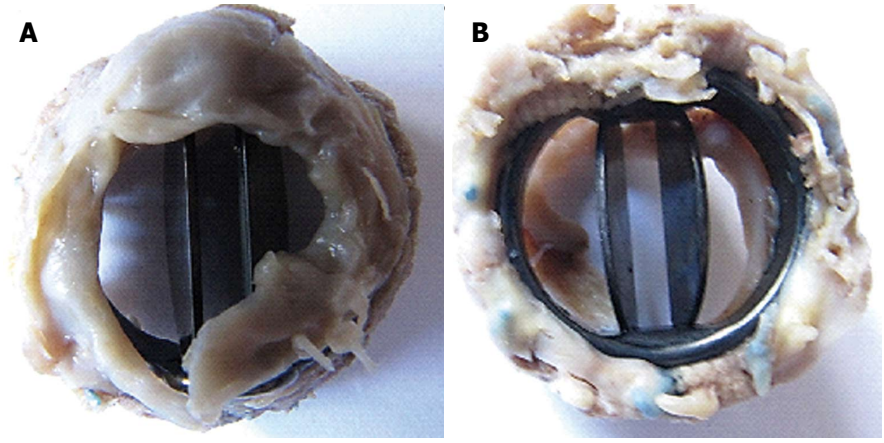

Figure 2 Explanted prosthetic valve. A concentric pannus formation is seen, obstructing the effective prosthetic orifice. A: Ventricular side; B: Arterial side.

Table 1 Characteristics of the different series that assessed the presence of pannus *n* (%)

Ref.	Year	Total number/No. of re-interventions	Pannus (*n*)	Location of pannus			Type of valve affected		Time to re-intervention (mo)	Follow-up (yr)
				Aortic	Mitral	Combined	Biologic	Mechanical		
Vitale *et al*[5]	1997	1878/87	66	0 (0)	66 (100)	0 (0)	0 (0)	66 (100)	51.5 ± 41.4[1]	0.26-20.1
Deviri *et al*[9]	1998	ND/100	51	ND	ND	ND	0 (0)	51 (100)	48 (1.5-144)[1]	13
Barbetseas *et al*[4]	1998	ND/23	10	7 (70)	3 (30)	0 (0)	0 (0)	23 (100)	178 ± 52[2]	ND
Rizzoli *et al*[3]	1999	2680/334	44	13 (30)	27 (61)	4 (9)	0 (0)	44 (100)	156[3]	27
Girard *et al*[18]	2001	ND/92	27	27 (100)	0 (0)	0 (0)	1 (4)	26 (96)	Vmec 156 ± 98[3] Vbio 84 ± 48[3]	ND
Roudaut *et al*[10]	2003	17250/126	26	ND	ND	ND	0 (0)	26 (100)	ND	23
Teshima *et al*[2]	2003	615/12	12	12 (100)	0 (0)	0 (0)	0 (0)	12 (100)	83 ± 52[2]	19
Toker *et al*[19]	2006	63	45	ND	ND	ND	0 (0)	45 (100)	58.9 ± 56.1[1]	ND

[1]Patients with a diagnosis of obstructive prosthetic dysfunction; [2]Patients with a diagnosis of pannus alone; [3]Patients with obstructive or non-obstructive prosthetic dysfunction. ND: No data; Vmec: Mechanical valve; Vbio: Biologic valve.

vessels and capillaries surrounded by giant cells, especially around and over suture stitches[5]. Pannus can be systematically divided into three layers and one core[2]. From the surface in contact with blood flow, the three layers towards the prosthetic material are: the lumen (which consists of endothelial cells, is found in the surface of the pannus), the internal lamina media (is composed of myofibroblasts) and the external lamina media (is composed of collagen and elastic fibers).

The core is located between the prosthetic tissue and the pannus, and consists mainly of a chronic inflammatory infiltrate comprising macrophages, lymphocytes, giant cells, plasmocytes and mastocytes[2].

The pathophysiology of this disorder is not yet completely understood. After implantation of a prosthetic valve, two inflammatory events occur. The first involves replacement of the damaged myocardium around the valve ring by a scar formed by nonspecialized connective tissue. The second event involves a foreign body-like inflammatory response to the presence of the prosthetic material. Prolonged exposure to the non-degradable prosthetic material is a persistent stimulus for inflammatory cells such as macrophages (which cluster as giant cells) and for proliferation of fibroblasts; both phenomena are characteristically seen in chronic inflammation. The presence of giant cells should be construed as a severe reaction, in which the foreign material is not well

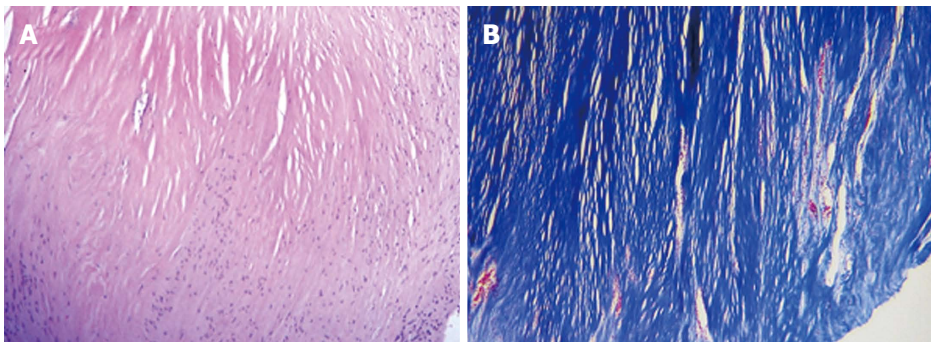

Figure 3 Histology of the pannus. Structure of collagen fibers, interspersed with small vessels and capillaries, surrounded by giant cells. A: Hematoxylin and Eosin staining; B: Masson's Trichrome staining.

tolerated and hence is a target for phagocytosis. This explanation allows to infer that the presence of pannus in only one valve surface represents an early stage of a chronic progressive inflammatory disorder[5].

The clinical presentation of pannus is variable; in most cases signs and symptoms of the disease occur as a consequence of prosthetic valve obstruction. The most common symptom is dyspnea, which may occur as a manifestation of obstruction in valves implanted both in aortic and in mitral valve position. Other less common clinical presentations are: low cardiac output syndrome, shock, embolization, chest pain, absence of a valve click on auscultation, exercise intolerance, cardiorespiratory arrest, and in many cases, patients may be asymptomatic[4,9,10]. In a study[4] that assessed the clinical characteristics of patients with pannus formation *vs* patients with thrombosis, the first group had a longer delay in appearance of symptoms, a longer duration of symptoms at the time of re-intervention, a greater time interval between the first and second surgical intervention and a higher rate of adherence to anticoagulation treatment.

The imaging techniques that are used most often for the diagnosis of this disorder are fluoroscopy, TTE and/or TEE HYPERLINK\l "Cia05"[11], although currently other techniques offer promising results, such as three-dimensional echocardiography and multislice Angio-CT[12-14].

For mechanical prostheses, the use of fluoroscopy is simple and allows to clearly identify the valve ring, cage, ball, the tilting disc/discs and the opening and closure angles; however, in biologic valves its use remains limited[1,11,15-17]. Specifically, in the case of pannus formation, fluoroscopy allows to detect absent motion of the disc/s; a frequent finding in pannus as well as in prosthetic valve thrombosis, albeit more frequent in the latter. In patients with normal leaflet motion, in whom high gradients are found and possible causes are prosthesis-patient mismatch, pannus or thrombosis, the echocardiographic findings shall define the final diagnosis[11]. In a study by Girard *et al*[18] that assessed 16 patients before their second aortic valve replacement, 63% of patients with a post-operative diagnosis of pannus had an abnormal fluoroscopy.

Pannus is suspected in patients who exhibit high gradients on echocardiography. Once structural failure and patient-prosthesis mismatch are ruled out, the only two differential diagnoses that remain to be defined are: pannus or thrombus. Since the advent of thrombolytics as an option for the treatment of valve thrombosis, making an accurate diagnosis has become of utmost importance, since such patients could benefit from the use of thrombolytics, and thus avoid the need for surgical reintervention. Although TTE is most useful in the initial approach to the diagnosis of pannus and thrombus, its usefulness to assess disc/s motion or the etiology of valve obstruction remains limited. However, where TTE fails, TEE appears as a more sensitive and specific method at the time of assessing the etiology of prosthetic valve obstruction. Thus, TEE has allowed to determine certain characteristics associated with pannus, such as: preserved prosthetic disc motion and evidence of a hyper-reflective mass of decreased length and motion, associated to the prosthetic valve[4]. Currently available diagnostic tools including TTE and 2D-TEE are insufficient to detect pannus formation, and detection rate is so poor that a preoperative diagnosis is almost impossible. Real-time three-dimensional transesophageal echocardiography may provide data to the diagnosis of pannus formation.

The treatment of pannus formation is surgical re-intervention to perform a new valve replacement. Occasionally, when pannus does not make contact with the prosthetic ring, the fibrotic tissue could be resected without replacing the prosthetic valve, but certain authors suggest that this surgical option is associated to a greater recurrence of pannus formation. All series agree in that the time to re-intervention is prolonged (Table 1). During follow-up of 63 patients with an obstructed mitral or aortic prosthetic valve, or both (pannus in 71.4% of cases), of whom 100% underwent valve replacement, in-hospital mortality was 20.6%. The main cause of death was low cardiac output syndrome and the only predictor of high mortality on multivariate analysis was LV systolic impairment[19]. In the series by Vitale *et al*[5] which

included 87 patients with obstructive mitral disease, of whom 75.8% had pannus either alone or associated to thrombus, 100% of patients underwent valve replacement (mechanical valve in 88.8%, biologic valve in 11.8%) with a 30-d mortality of 12.5%.

Recurrence is a finding of low prevalence and high mortality, and occurred predominantly in patients who underwent pannus resection without valve replacement[5,10].

Pannus is an infrequent complication that mainly affects mechanical prosthetic valves. Its diagnosis requires clinical suspicion and the association of fluoroscopy + TTE/TEE. Currently, the treatment of choice is a new valve replacement and prognosis depends mainly on LV function. The case presented is a characteristic example of pannus, given its clinical presentation (progressive dyspnea), the steps followed to reach diagnosis and the surgical resolution. Suspecting this disorder and making an accurate diagnosis is of paramount importance, to implement adequate treatment and to avoid prolonging the natural course of the disease and its repercussion on the LV and the quality of life of affected patients.

COMMENTS

Case characteristics

A 55-year-old man with a mechanical aortic prosthetic valve presented with clinical suspicion of prosthetic valve obstruction due to pannus formation.

Clinical diagnosis

The patient began experiencing dyspnea in FC II (NYHA) that progressed to FC IV. A meso-telesystolic murmur radiating to the neck was detected, as well as a pulsus tardus et parvus, with severely increased mechanical aortic valve gradients and decreased effective prosthetic area. No detectable image suggestive of pannus or thrombus was seen in the left ventricular outflow tract in multimodality imaging.

Differential diagnosis

Mechanical prosthetic valve dysfunction caused by pannus or thrombosis.

Imaging diagnosis

Fluoroscopy, transthoracic echocardiography, two-dimensional transesophageal echordiography and cardiac multiple detector computed tomography, failed to diagnose pannus formation.

Treatment

Given the clinical suspicion of prosthetic valve obstruction due to pannus formation, the decision was made to replace the aortic prosthesis with a new mechanical valve (ATS # 23). On pathological examination, the explanted specimen exhibited fibrous tissue with a smooth, annular surface, in contact with the ventricular surface of the mechanical prosthesis, consistent with pannus. Histological examination confirmed the diagnosis.

Related reports

The case presented is a characteristic example of pannus, given its clinical presentation (progressive dyspnea), the steps followed to reach diagnosis and the surgical resolution.

Experiences and lessons

Multimodality imaging in the diagnosis of pannus formation may have limitations. Suspecting this disorder and making an accurate diagnosis is of paramount importance, to implement adequate treatment and to avoid prolonging the natural course of the disease and its repercussion on the left ventricle and the quality of life of affected patients.

Peer-review

Authors have made good and fluent review of pannus formation and presented a clinical case ignored by transesophageal echocardiography and fluoroscopy.

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WJC covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of cardiology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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EDITORIAL OFFICE
Jin-Lei Wang, Director
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: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
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PUBLISHER
Baishideng Publishing Group Inc
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Telephone: +1-925-223-8242
Fax: +1-925-223-8243
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EDITORIAL

Pulmonary hypertension in hereditary haemorrhagic telangiectasia

Veronique MM Vorselaars, Sebastiaan Velthuis, Repke J Snijder, Jan Albert Vos, Johannes J Mager, Martijn C Post

Veronique MM Vorselaars, Sebastiaan Velthuis, Martijn C Post, Department of Cardiology, St. Antonius Hospital, 3435 CM Nieuwegein, The Netherlands
Repke J Snijder, Johannes J Mager, Department of Pulmonology, St. Antonius Hospital, 3435 CM Nieuwegein, The Netherlands
Jan Albert Vos, Department of Interventional Radiology, St. Antonius Hospital, 3435 CM Nieuwegein, The Netherlands
Author contributions: Vorselaars VMM contributed to drafting the article; Vorselaars VMM, Velthuis S, Snijder RJ, Vos JA, Mager JJ and Post MC contributed to conception and design and final approval of the version to be published; Velthuis S, Snijder RJ, Vos JA, Mager JJ, Post MC contributed to revision of the article.
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Correspondence to: Veronique MM Vorselaars, MD, Department of Cardiology, St. Antonius Hospital, Koekoekslaan 1, 3435 CM Nieuwegein, The Netherlands. m.post@antoniusziekenhuis.nl
Telephone: +31-88-3201228
Fax: +31-30-6092274
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Abstract

Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant inherited disorder characterised by vascular malformations in predominantly the brain, liver and lungs. Pulmonary hypertension (PH) is increasingly recognised as a severe complication of HHT. PH may be categorised into two distinct types in patients with HHT. Post-capillary PH most often results from a high pulmonary blood flow that accompanies the high cardiac output state associated with liver arteriovenous malformations. Less frequently, the HHT-related gene mutations in ENG or ACVRL1 appear to predispose patients with HHT to develop pre-capillary pulmonary arterial hypertension. Differentiation between both forms of PH by right heart catheterisation is essential, since both entities are associated with severe morbidity and mortality with different treatment options. Therefore all HHT patients should be referred to an HHT centre.

Key words: Hereditary haemorrhagic telangiectasia; High cardiac output; Pulmonary arterial hypertension; ENG; ACRVL1; Pulmonary hypertension

Core tip: Pulmonary hypertension (PH) is increasingly recognised as a severe complication of hereditary haemorrhagic telangiectasia (HHT), but the true prevalence of PH in HHT is not known. Post-capillary PH most often results from the high cardiac output associated with hepatic arteriovenous malformations. More rarely the HHT gene mutations (ACRVL1 or ENG) result in pre-capillary pulmonary arterial hypertension (PAH). Differentiation between post-capillary PH en pre-capillary PAH can be done by right heart catheterisation, and is of importance since both entities are associated with severe morbidity and mortality and have different options for treatments.

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HEREDITARY HAEMORRHAGIC TELANGIECTASIA

Hereditary haemorrhagic telangiectasia (HHT), also known as Rendu-Osler-Weber syndrome, is an autosomal dominant inherited disorder with late onset penetrance (nearly 97% at the age of 60 years) characterised by vascular malformations with an estimated prevalence of 1:5000 individuals[1,2]. The abnormal vascular structures in HHT range from small telangiectasia of the skin and mucosal membranes to arteriovenous malformations (AVMs) in predominantly the brain, liver and lungs[3,4].

Genetics and pathogenesis

HHT consist of two main subtypes, HHT type 1 and HHT type 2, which results from mutations in the ENG gene on chromosome 9, encoding the protein endoglin and from mutations in the activin receptor-like kinase (ACVRL1) gene on chromosome 12, encoding the protein ALK-1 respectively[5,6]. A third disease-causing mutation has been found in the *SMAD4* gene, causing a combination of the juvenile polyposis syndrome and HHT[7] . Most HHT families have a unique mutation and many types of mutations have been described.

The exact pathogenesis of HHT is still unclear. However, hypoxia or local hemodynamic changes could act as a possible trigger promoting tissue inflammation or endothelial cell injury[8,9]. Both endoglin, ALK-1 and SMAD4 proteins are endothelial receptors of the transforming growth factor β (TGF-β) superfamily. All three proteins cooperate in the TGF-β/ALK-1 signalling pathway, which is involved in angiogenesis. In HHT, most vessels are normal, but the mutations in ACVRL1 and ENG result in abnormal angiogenetic responses and lead to the formation of abnormal arteriovenous connections, ranging from small telangiectases that bleed easily, to large arteriovenous malformations, that can occur in every organ, but especially in the lungs, liver and brain[10,11].

Diagnosis

The clinical diagnosis can be based on the four Curaçao criteria[1], which consist of: (1) Spontaneous, recurrent epistaxis; (2) Multiple telangiectasia at characteristic sites (lips, oral cavity, fingers, nose); (3) Visceral lesions (gastrointestinal telangiectasia, pulmonary, hepatic, cerebral or spinal AVMs); and (4) A first degree relative with HHT.

Three criteria suffice for a definitive diagnosis of HHT, two criteria are considered as "possible" HHT, and one or no criterion makes the diagnosis "unlikely". The positive predictive value for a definite clinical diagnosis and the negative predictive value for an unlikely diagnosis are excellent (100% and 97.7% respectively), when compared with DNA testing[12]. However, HHT has an age dependent penetrance and the clinical presentation varies among patients[1]. Therefore genetic testing has emerged as an important tool to help make the diagnosis in paediatric patients and younger adults with a "possible" clinical diagnosis[12].

PULMONARY HYPERTENSION

Pulmonary hypertension (PH) is a haemodynamic and pathophysiological condition defined as an increase in mean pulmonary arterial pressure (mPAP) of equal to or more than 25 mmHg as assessed by right heart catheterisation (RHC)[13]. PH is a progressive disease of many origins, affecting more than 100 million people world wide[14]. The elevated pressure in the pulmonary circulation can lead to various symptoms including limited exercise capacity and dyspnoea on exertion. The chronic elevated pressure may ultimately result in right-sided heart failure and premature death[13].

Depending on the origin, PH can be divided into two main groups; pre- and post capillary PH. Patients with pre-capillary PH are characterised by a mPAP $\geqslant$ 25 mmHg, pulmonary artery wedge pressure (PAWP) $\leqslant$ 15 mmHg, and elevated pulmonary vascular resistance (PVR) (> 3 Wood units)[15]. Pre-capillary PH can be further divided in different clinical groups, based on pathophysiological mechanisms, clinical presentation and therapeutic options (Table 1)[13].

Transthoracic echocardiography is the cornerstone for screening in all patients suspected of PH. Typically, a dilatation of the right ventricle with septal flattening (also called D-sign) and an increase in right ventricular systolic pressure (RVSP) (sum of right ventricle-right atrium pressure gradient and estimated pressure in the right atrium based on the dimension and collapse of the inferior caval vein) (Figure 1)[13,16].

PH and hereditary haemorrhagic telangiectasia

PH is increasingly recognised as an important complication of HHT. HHT associated PH can occur by several mechanisms. Most often, post-capillary PH may develop as a consequence of a hyperkinetic state resulting in heart failure associated with high cardiac output (CO) due to hepatic arteriovenous malformations (HAVMs) (Figure 2)[17], while less frequently, precapillary PH can be related to pulmonary arterial hypertension (PAH) characterised by remodeling of small pulmonary arteries with broadly similar histologic lesions than observed in idiopathic PAH[17]. The HHT-related gene mutations (ENG or ACVRL1) appear to predispose for the development of PAH[18-22]. Various studies found a high estimated prevalence of PH in HHT when screening with echocardiography[23,24]. An elevated RVSP on echocardiography was found in 9 (20.5%) out of 44 HHT patients (22 ACVRL1, 3 ENG, 19 unknown mutation), in 7 out of these 9 subjects an *ACVRL1* gene was found[23]. Sopeña *et al*[24] found a high estimated prevalence of PH (31%) in 29 hospitalised patients with HHT with a mean estimated RVSP of 73 ± 17.0 mmHg measured with echocardiography. HAVMs were

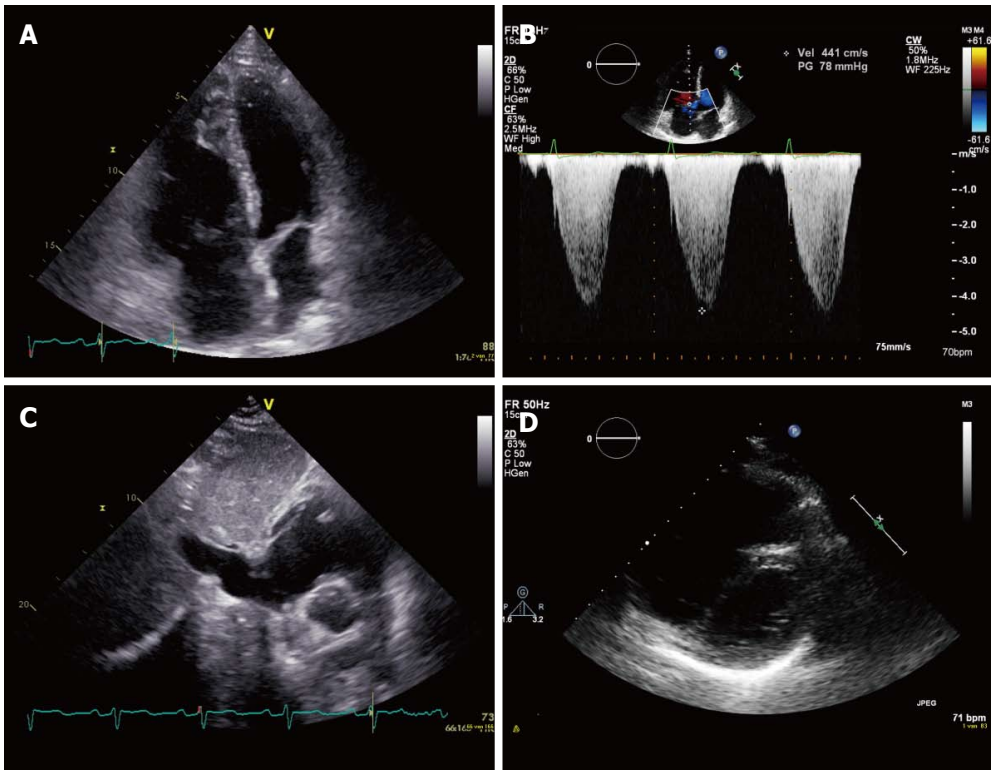

Figure 1 Characteristic echocardiogram of a patient with pulmonary hypertension. A: Apical 4-chamber view showing dilatation of the right ventricle; B: Apical 4-chamber view with Doppler signal (continuous wave) showing an increased right ventricular- right atrial pressure gradient (4.4 m/s); C: Subcostal view showing dilatation of the inferior caval vein corresponding with an increased pressure in the right atrium; D: Parasternal short axis view showing flattening of the interventricular septum (D-sign) and dilatation of the right ventricle.

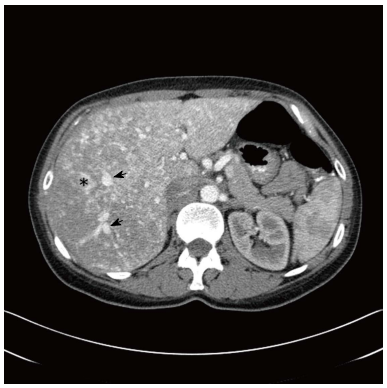

Figure 2 Hepatic arteriovenous malformations. Computed tomography with contrast in arterial fase showing extensive filling of the hepatic veins (arrows), and diffuse hepatic arteriovenous malformations (asterix).

documented in 67% of these patients. However, large observational studies including consecutive HHT patients are lacking.

Since the treatment strategies differ between post-capillary high-output PH and pre-capillary PAH, it is important to differentiate between these two different entities. RHC is the gold standard for making the diagnosis of both high-output PH and PAH[13,17,25].

In PAH, the mPAP is usually higher with an increase in PVR and transpulmonary gradient due to arteriopathy. Most often a normal or decreased CO and PAWP is seen. In high-output PH on the other hand, there is only a moderate increase in mPAP, with a normal PVR, elevated PAWP and most importantly, an increased CO (Table 2)[13,17].

High output PH

High-output heart failure is the most common initial presentation of HAVMs in HHT. Liver involvement is present in 32%-78% of the HHT patients and is predominantly seen in HHT type 2[1,26-28]. The presence of symptoms is directly associated with significant morbidity and mortality and therefore, screening for liver AVMs with Doppler ultrasound is warranted in all patients who are symptomatic or have abnormal liver enzymes[1,25]. In the majority of cases, only small telangiectasia are seen, which do not lead to symptoms. However, large HAVMs exist in typically three different and often concurrent types of intrahepatic shunting; from the hepatic arteries to hepatic veins, from the hepatic arteries to portal veins, and from the portal veins to hepatic veins[17,25]. These hepatic shunts can lead to high-output cardiac failure, portal hypertension, biliary ischaemia or encephalopathy with a wide range of symptoms[25]. Overall, symptoms due to HAVMs occur in 8% of HHT patients and predominantly in females[29]. Symptoms of high-output cardiac failure usually develop in females between 50 and 70 years of age and are characterised by dyspnoea on exertion,

Table 1 Updated classification of pulmonary hypertension

Pulmonary arterial hypertension
Idiopathic PAH
Hereditable PAH
BMPR2
ALK1, endoglin (with or without hereditary haemorrhagic telangiectasia), SMAD9, CAV1, KCNK3
Unknown
Drug and toxin induced
Associated with:
Connective tissue diseases
HIV infection
Portal hypertension
Congenital heart diseases
Schistosomiasis
Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis
Persistent pulmonary hypertension of the newborn
Pulmonary hypertension due to left heart disease
Left ventricular systolic dysfunction
Left ventricular diastolic dysfunction
Valvular disease
Congenital/acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies
Pulmonary hypertension due to lung diseases and/or hypoxia
Chronic obstructive pulmonary disease
Interstitial lung disease
Other pulmonary diseases with mixed restrictive and obstructive pattern
Sleep-disordered breathing
Alveolar hypoventilation disorders
Chronic exposure to high altitude
Developmental abnormalities
Chronic thromboembolic pulmonary hypertension
PH with unclear and/or multifactorial mechanisms
Haematological disorders: chronic haemolytic anaemia, myeloproliferative disorders, splenectomy
Systemic disorders: sarcoidosis, pulmonary histiocytosis, lymphangioleiomyomatosis
Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
Others: tumoural obstruction, fibrosing mediastinitis, chronic renal failure, segmental PH

PH: Pulmonary hypertension; BMPR2: Bone morphogenetic protein receptor, type 2; CAV1: Caveolin-1; HIV: Human immunodeficiency virus. Adapted from Simonneau *et al*[46], with permission of the publisher.

fatigue, orthopnoea, ascites and/or oedema[17,29].

Pathophysiology of high output PH: Exercise testing in healthy persons revealed that an increase in CO leads to an elevation in pulmonary artery pressure (increase in mPAP up to 0.5 to 3.0 mmHg/L per minute)[30].

In patients with HAVMs, shunting of blood from the hepatic arteries and/or portal veins to the hepatic veins results in a hyperdynamic state, in which the CO can be elevated two-to-three fold[31]. Besides this cause of high cardiac output, severe epistaxis or gastro-intestinal bleeding in patients with HHT may lead to anaemia with a compensatory increase in CO as well.

In HHT, a multifactorial cascade will eventually lead to high-output cardiac failure. At first, the increase in CO will be compensated by dilatation of the pulmonary arteries and thereby pulmonary pressure will still be maintained. An increase in left atrial (LA) pressure will predispose patients for atrial fibrillation (due to enlargement of the LA) and diastolic dysfunction of the left ventricle. Increased LA pressure and impaired pulmonary vasodilatation will eventually result in PH. The combination of volume and pressure overload leads to right ventricular (RV) dilatation, decreased systolic function of the RV and subsequent right heart failure. Severe bleeding (*e.g.*, epistaxis or gastro-intestinal bleeding) may trigger the cascade because of the subsequent increase in CO[17,29,31].

Table 2 Haemodynamics in pulmonary hypertension associated with hereditary haemorrhagic telangiectasia

	High output PH	PAH
mPAP (mmHg)	+	++
PAWP (mmHg)	=/+	=
PVR (Wood units)	=	++
CO (L/min)	++	-

PH: Pulmonary hypertension; PAH: Pulmonary arterial hypertension; mPAP: Mean pulmonary artery pressure; PAWP: Pulmonary artery wedge pressure; PVR: Pulmonary vascular resistance; CO: Cardiac output. +: Increase; =: Normal; -: Decrease. Adapted from Faughnan *et al*[17], with permission of the publisher.

Treatment of high output PH: The first-line treatment of PH associated with a high-output state consists of intensive medical treatment including salt restriction and diuretics, correction of anaemia, antihypertensive and antiarrhythmic agents and digoxin if necessary[9]. In patients refractory to medical-therapy, liver transplantation is the best option, with a 5-year survival of 83% in a series of 40 patients[29]. However, a high post-operative morbidity is seen[25,32].

Recently, Dupuis-Girod *et al*[33] treated 25 patients with severe HAVMs and a high CO [median cardiac index (CI) 5.1 L/min per square meters (range 4.1-6.2 L/min per square meters)] with bevacizumab, a vascular endothelial growth factor inhibitor. This treatment resulted in a significant decrease in CO [median CI at 6 mo 4.1 L/min per square meters (range 3.0-5.1 L/min per square meters)], normalisation of the pulmonary pressure in 5 out of 8 patients with PH at baseline and clinical improvement of dyspnoea[33]. Other invasive treatments such as surgical hepatic artery ligation or transcatheter therapeutic embolisation of the hepatic artery are associated with a high morbidity and mortality and therefore not recommended[1,17].

PAH

PAH is a clinical condition characterised by the presence of pre-capillary PH due to arteriopathy with media hypertrophy and intima proliferation. It is increasingly recognised as a severe complication of HHT.

There have been a few case series that describe the association between PAH and HHT, however these series all included patients with PH in which HHT

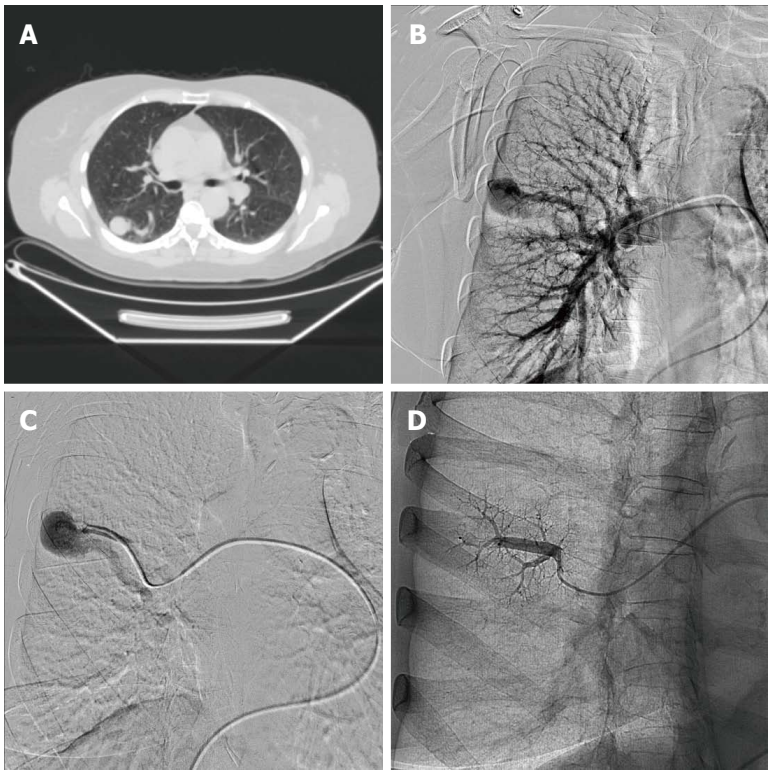

Figure 3 Pulmonary arteriovenous malformations. A: Computed tomography of the chest with large pulmonary arteriovenous malformation (PAVM) in the lower lobe of the right lung; B: Pulmonary angiogram of the PAVM in the same patient; C: Selective pulmonary angiogram of the PAVM in the apex of the lower lobe of the right lung; D: Repeat angiogram after transcatheter embolisation of the PAVM with a vascular plug.

symptoms were also present[18-22].

Pathophysiology and genetics of PAH: In 2001, it was demonstrated for the first time that different mutations in ACVRL1 predispose patients for the development of PAH[18]. This was confirmed in a few case series describing the presence of PAH in patients with an *ACVRL1* mutation and clinical features of HHT[19,21,22]. Trembath *et al*[18] described that mutations in *ACVRL1* may lead to both occlusion of the pulmonary arteries together with vascular dilatation, manifested as AVMs in HHT. Although less frequently, ENG mutations have also been identified in patients with both HHT and PAH, suggesting a less potent association between endoglin and PAH[18,19]. Mutations in the bone morphogenic protein receptor type 2 gene, which is another gene encoding the endothelial surface protein components of the TGF-β receptor that is detected in approximately 70% of the patients with hereditable PAH, were not found in HHT associated PAH[34].

Prognosis: The clinical outcomes of patients with PAH caused by an ACVRL1 mutation have been analysed in 32 patients and compared to other PAH patients without this mutation. PAH caused by an *ACVRL1* mutation was found in significantly younger patients (mean age 21.8 ± 16.7 years) and had a significantly shorter survival, despite similar therapy[34]. No data exist about the prognosis of patients with *PAH* and *ENG* mutations. The overall prognosis of PAH in general ranges from 6 mo to several years based on the underlying disease[13].

It is noteworthy that *ACVRL1* mutation carriers may develop severe PAH without any clinical evidence of HHT because of the early development of PAH in these patients and the late-onset penetrance of ACVRL1 mutation for HHT manifestations[34].

Treatment of PAH in HHT: No systematic evidence exists for treatment of HHT patients with PAH. It seems rational to treat patients according to the guidelines for PAH, with PAH-specific medication and supporting therapy (diuretics, oxygen, and digoxin)[13].

Today there are three different groups of PAH specific medication; endothelin receptor antagonists (ERA), phosphodiesterase inhibitors (PD5I) and prostacyclins. There are two case-reports that describe successful treatment of PAH in HHT patients with the ERA bosentan. After treatment, improvement of symptoms, exercise capacity and laboratory findings and a decrease in mPAP were found[35,36].

There are no reports describing the treatment with other PAH specific medication in patients with PAH and HHT. Since there was no response to acute vasodilator challenge in 32 patients with HHT and PAH, there is probably no role for the use of calcium channel blockers in this population[17,34]. And due to an increase in bleeding complications regular treatment with oral anticoagulation is not advised[1]. However, based on recent literature, treatment with anticoagulation could be considered on a case by case basis[37].

Pulmonary arteriovenous malformations in PH: The coexistence of PH and pulmonary arteriovenous malformations (PAVMs) has specific clinical and therapeutic implications. PAVMs are low-resistance, high-flow abnormal vascular structures that bypass the

normal capillary filter and thereby result in permanent pulmonary right-to-left shunting (Figure 3A-C)[38-40]. Paradoxical embolisation through these PAVMs can lead to severe neurological complications, such as a stroke or brain abscess[1,40]. Contrast echocardiography is the screening test of choice (sensitivity up to 98.6%), with a direct relationship between shunt grade and prevalence of cerebral manifestations in patients screened for HHT[40-42]. To avoid neurologic and bleeding complications, PAVMs can be treated with transcatheter embolisation with coils or plugs (Figure 3D)[1,43]. It may be expected that closing this low resistance system will result in a rise in mPAP. Measuring the pulmonary pressure before and after embolisation of PAVMs in 43 patients, Shovlin *et al*[44] found no significant found no significant increase in mPAP after embolisation, even in patients with pre-existing mild to moderate PH.

A possible explanation is a decrease in CO after embolisation which has a greater effect on the PVR than occlusion of the PAVMs. This fall in CO immediately after PAVM closure was recently described in 29 HHT patients by Vorselaars *et al*[45]. Furthermore, PAVM-related hypoxemia can induce vasoconstriction with a concomitant increase in PVR. Both studies described an increase in saturation after embolisation which may result in a decrease in pulmonary vasoconstriction and thereby PVR[44,45]. One case report described a fatal rupture of a PAVM in a patients with severe PAH. Although patients with severe PH were excluded from the above studies, it would be prudent to consider that the higher the mPAP and PVR at baseline and the larger the PAVM, the greater likelihood of worsening PH after embolisation[44].

Further research and recommendations

Although a number of studies described patients with PH and HHT, no data are available about the exact prevalence of PH in the overall HHT population. Most studies used a small sample size of highly selected patients and data from RHC are lacking. Therefore we recommend to perform a systematic screening to reveal the true prevalence of both forms of PH with their different aetiologies in a HHT population.

Because of the non-specific symptoms and potentially fatal prognosis, all HHT patients should be referred to an HHT centre of excellence.

CONCLUSION

PH is increasingly recognised as a severe complication of HHT, but the true prevalence of PH in HHT is still unknown. PH in HHT is mostly post-capillary in origin and results from high cardiac output due to HAVMs and anaemia. Rarely ACRVL-1 or ENG mutations results in pre-capillary PAH. Differentiation between both forms of PH in HHT by RHC is essential, since both entities are associated with severe morbidity and mortality with different specific treatment options. Therefore all HHT patients should be referred to an HHT centre.

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FIELD OF VISION

Insights into cardio-oncology: Polypharmacology of quinazoline-based α1-adrenoceptor antagonists

Salvatore Patanè

Salvatore Patanè, Cardiologia Ospedale San Vincenzo, Taormina (Me) Azienda Sanitaria Provinciale di Messina, 98039 Taormina, Messina, Italy
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Correspondence to: Salvatore Patanè, MD, Cardiologia Ospedale San Vincenzo, Taormina (Me) Azienda Sanitaria Provinciale di Messina, Contrada Sirina, 98039 Taormina, Messina, Italy. patane-@libero.it
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Abstract

New uses of cardiovascular drugs with proven experience are emerging, including for treating cancer. Quinazoline is a compound made up of two fused six member simple aromatic rings, benzene and pyrimidine rings, with several biological effects. Cardiologists first used quinazoline-based α1-adrenoceptor antagonists prazosin, doxazosin, and terazosin; currently available data support their use as safe, well tolerated, and effective add-on therapy in uncontrolled hypertension with additional favourable metabolic effects. Recent findings highlight the anticancer effects of quinazoline-based α1-adrenoceptor antagonists, indicating that they may have a significant role in uncontrolled hypertensive cancer patients without signs of ischemia.

Key words: Cardio-oncology; Multidrug resistance; Prazosin; Terazosin; Doxazosin

Core tip: New uses of cardiovascular drugs with proven experience and without high cost have been emerging, including to have anticancer abilities by targeting human ether-a-go-go-related gene K(+) channels, epidermal growth factor receptors, vascular endothelial growth factor receptors, as well as to overcome cancer multidrug resistance. Quinazoline-based α1-adrenoceptor antagonists (doxazosin, prazosin, and terazosin) exhibit anticancer abilities and emerging findings indicate that these drugs may have a significant role in uncontrolled hypertensive cancer patients without signs of ischemia.

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INTRODUCTION

Despite the tremendous efforts, the medicine field has not yet come to absolute conclusions in oncology and the emerging scenario of the onco-cardiovascular patients is emerging[1]. New targeted anticancer therapies have not proven to be free from cardiovascular side effects while old anticancer therapies have shown delayed serious consequences in long-term cancer survivors[1,2]. Moreover, the heavy burden of concomitant problems and diseases requires changes in setting to prevent serious diseases such as infective endocarditis or in

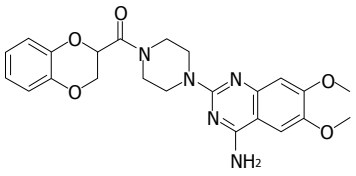	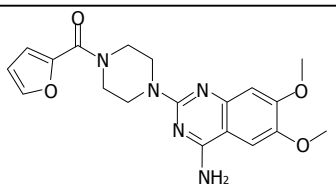	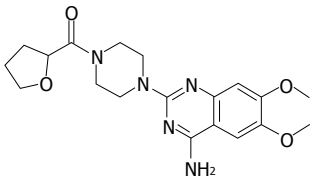
Doxazosin	Prazosin	Terazosin
Quinazoline-based	Quinazoline-based	Quinazoline-based
α_1-adrenoceptor antagonist	α_1-adrenoceptor antagonist	α_1-adrenoceptor antagonist
Antihypertensive effect	Antihypertensive effect	Antihypertensive effect
HERG ligand	HERG ligand	HERG ligand
EGFR inhibition	EGFR inhibition	
Anti-angiogenic activity	Anti-angiogenic activity	
Cancer cell growth inhibition		Cancer cell growth inhibition
Apoptosis induction	Apoptosis induction	Apoptosis induction
Anoikis induction		Anoikis induction
	Cell autophagy induction	Cell autophagy induction
Role in MDR	Role in MDR	Weaker or no effect in MDR
Akt inhibition	Cdk 1 inactivation	G_1 phase cell cycle arrest
Androgen receptor downregulation	DNA damage stress induction	p27KIP1 up-regulation
Bax expression upregulation	G_2 checkpoint arrest	Proteasome activity downregulation
Caspase-3 activity increase	Mitochondria-mediated apoptosis induction	Ubiquitinated protein accumulation
EphA2 agonism	p53-mediated mechanism	
FGFR-2 antagonism		
Focal adhesion kinase reduction		
HIF-1α inhibition		
MAPK activation decrease		
mTOR inhibition		
p27 downregulation prevention		
PDK1 inhibition		
PKB/Akt activation inhibition		
PI3K inhibition		
Rho kinase-Ⅱ activation decrease		
Soluble guanylate cyclaseα decrease		
TGF-β and IκB activation		
Tubulin-polymerization-enhancing activity		

Figure 1 Structure and polypharmacology of quinazoline-based α1-adrenoceptor antagonists doxazosin, prazosin and terazosin in cardio-oncology. MDR: Multidrug resistance; EGFR: Epidermal growth factor receptor; FGFR-2: Fibroblast growth factor receptor-2; HIF-1α: Hypoxia-inducible factor 1α; mTOR: Mammalian target of rapamycin; PDK1: 3-phosphoinositide-dependent protein kinase 1; TGF: Transforming growth factor.

perioperative oncosurgery[3-12]. The progress in cancer biology and treatment has led to a new frontier: the cardio-oncology[1-27]. New uses of cardiovascular drugs with proven experience have been emerging[1,4,5-9,27-32], including to have anticancer abilities by targeting human ether-a-go-go-related gene K(+) (HERG) channels[5], epidermal growth factor (EGF) receptors[9], vascular endothelial growth factor (VEGF) receptors, as well as to overcome cancer multidrug resistance (MDR)[4,26,29,33,34]. These old cardiovascular drugs do not have high cost, however, there was a lack of noninferiority randomized, controlled trials[33], comparing them with new anticancer therapies.

Quinazoline is a compound made up of two fused six member simple aromatic rings, benzene and pyrimidine rings[35]. The search for quinazoline-based substances as cardiovascular agents begun after pharmacological identification of quinazoline compounds having a glycine amide or β-alanine amide residue in the 3rd position that display a hypotensive activity. Other quinazoline derivatives have also demonstrated significant anticancer activities[26,35-39] and new molecules have been synthesized as gefitinib, erlotinib, afatinib, and lapatinib[26,36]. Cardiologists first used quinazoline-based α_1-adrenoceptor antagonists, including prazosin, doxazosin, and terazosin[26] (Figure 1). Currently available data have supported the use of these antagonists as safe, well tolerated, and effective add-on therapy in uncontrolled hypertension with additional favorable metabolic effects[37] and without association with an increased risk of heart failure[26,37-39]. New data suggest that adverse cardiac outcome of doxazosin is only among patients with moderate-to-severe ischemia on myocardial perfusion imaging[26,40]. Furthermore, it has been reported that the β-plus α_1-blocker pretreatment (propranolol + prazosin) has led to better severity reduction of postresuscitation myocardial tissue injury and myocardial dysfunction with better neurologic function and prolonged duration of survival than propranolol treatment alone[41]. This latter finding will require certainly further evaluation.

Research has suggested several anticancer mechanisms of doxazosin, including upregulation of Bax expression, transforming growth factor (TGF)-β and IκB activation[42], focal adhesion kinase reduction[43],

inhibition of protein kinase B/Akt activation[44], and death receptor mediated apoptosis induction[45,46]. Doxazosin is known to be a HERG ligand, EGFR inhibitor[47], VEGF-mediated angiogenic response antagonist[48], and fibroblast growth factor receptor-2 antagonist[48,49]. Several signalling pathways are also inhibited from doxazosin VEGF antagonism including PI3K, Akt, 3-phosphoinositide-dependent protein kinase 1, mammalian target of rapamycin, and hypoxia-inducible factor 1α[49]. In addition, doxazosin is also an agonist of receptor tyrosine kinase triggering ephrin type-A receptor 2 internalization which, in turn, suppresses haptotactic and chemotactic migration of prostate cancer, breast cancer, and glioma cells[26,50]. Notably, a tubulin polymerization-enhancing activity of doxazosin has been found[51]. A doxazosin derivative, DZ-50, impairs tumour growth and metastasis *via* anoikis[52]; similarly, doxazosin induces changes in morphology consistent with anoikis in both benign and cancerous prostatic cells and increased caspase-3 activity[43]. Moreover, doxazosin significantly decreases benign prostatic hyperplasia-induced mitogen-activated protein kinase kinase and Rho kinase-Ⅱ activation and decreases expression of soluble guanylate cyclasea[53] also leading to prostate cancer cell growth inhibition[54] Doxazosin also downregulates expression of androgen receptor[54], prevents p27 downregulation[55] and may partly reverse P-glycoprotein/MDR1-mediated cancer multidrug resistance (CMDR) and the transport of anticancer drugs[56].

Terazosin, another quinazoline-based antihypertensive α_1-adrenoceptor antagonist[57], is also an HERG ligand[58], a cancer cell growth inhibitor[59], and an apoptosis and anoikis inductor[58,60]. Terazosin induces cell death which is associated with G_1 phase cell cycle arrest, upregulation of cyclin-dependent kinase inhibitor 1B (p27KIP1)[60], accumulation of ubiquitinated proteins and downregulation of proteasome activity[46]. Terazosin seems to have weaker or no effects regarding CMDR[55].

Prazosin, another quinazoline-based and anti-hypertensive α_1-adrenoceptor antagonist[60], is also an HERG ligand[58] and EGFR inhibitor[61]. Prazosin induces autophagic cell death *via* a p53-mediated mechanism[62] and cell apoptosis through the induction of DNA damage stress, leading to cyclin-dependent kinase 1 inactivation and G_2 checkpoint arrest triggering mitochondria-mediated apoptosis induction[62]. In addition, prazosin exhibits an anti-angiogenic activity[63] and its role in MDR modulation has also been suggested[55,64]. These emerging findings indicate that the quinazoline-based antihypertensive α_1-adrenoceptor antagonists may have a significant role in uncontrolled hypertensive cancer patients without signs of ischemia[26,29,38,40].

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REVIEW

Recent advances in the diagnosis and treatment of acute myocardial infarction

Koushik Reddy, Asma Khaliq, Robert J Henning

Koushik Reddy, Asma Khaliq, Robert J Henning, Department of Medicine, James A Haley Veterans Administration Hospital and the University of South Florida College of Medicine, Tampa, FL 33612, United States

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Correspondence to: Robert J Henning, MD, Department of Medicine, James A Haley Veterans Administration Hospital and the University of South Florida College of Medicine, 13000 Bruce B Downs Blvd, Tampa, FL 33612, United States. robert.henning@va.gov
Telephone: +1-813-9785873

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Abstract

The Third Universal Definition of Myocardial Infarction (MI) requires cardiac myocyte necrosis with an increase and/or a decrease in a patient's plasma of cardiac troponin (cTn) with at least one cTn measurement greater than the 99th percentile of the upper normal reference limit during: (1) symptoms of myocardial ischemia; (2) new significant electrocardiogram (ECG) ST-segment/T-wave changes or left bundle branch block; (3) the development of pathological ECG Q waves; (4) new loss of viable myocardium or regional wall motion abnormality identified by an imaging procedure; or (5) identification of intracoronary thrombus by angiography or autopsy. Myocardial infarction, when diagnosed, is now classified into five types. Detection of a rise and a fall of troponin are essential to the diagnosis of acute MI. However, high sensitivity troponin assays can increase the sensitivity but decrease the specificity of MI diagnosis. The ECG remains a cornerstone in the diagnosis of MI and should be frequently repeated, especially if the initial ECG is not diagnostic of MI.

There have been significant advances in adjunctive pharmacotherapy, procedural techniques and stent technology in the treatment of patients with MIs. The routine use of antiplatelet agents such as clopidogrel, prasugrel or ticagrelor, in addition to aspirin, reduces patient morbidity and mortality. Percutaneous coronary intervention (PCI) in a timely manner is the primary treatment of patients with acute ST segment elevation MI. Drug eluting coronary stents are safe and beneficial with primary coronary intervention. Treatment with direct thrombin inhibitors during PCI is non-inferior to unfractionated heparin and glycoprotein Ⅱb/Ⅲa receptor antagonists and is associated with a significant reduction in bleeding. The intra-coronary use of a glycoprotein Ⅱb/Ⅲa antagonist can reduce infarct size. Pre- and post-conditioning techniques can provide additional cardioprotection. However, the incidence and mortality due to MI continues to be high despite all these recent advances. The initial ten year experience with autologous human bone marrow mononuclear cells (BMCs) in patients with MI showed modest but significant increases in left ventricular (LV) ejection fraction, decreases in LV end-systolic volume and reductions in MI size. These studies established that the intramyocardial or intracoronary administration of stem cells is safe. However, many of these studies consisted of small numbers of patients who were not randomized to BMCs or placebo. The recent LateTime, Time, and Swiss Multicenter Trials in patients

with MI did not demonstrate significant improvement in patient LV ejection fraction with BMCs in comparison with placebo. Possible explanations include the early use of PCI in these patients, heterogeneous BMC populations which died prematurely from patients with chronic ischemic disease, red blood cell contamination which decreases BMC renewal, and heparin which decreases BMC migration. In contrast, cardiac stem cells from the right atrial appendage and ventricular septum and apex in the SCIPIO and CADUCEUS Trials appear to reduce patient MI size and increase viable myocardium. Additional clinical studies with cardiac stem cells are in progress.

Key words: Myocardial necrosis; Type 1-5 myocardial infarctions; Troponin assays; Percutaneous coronary intervention; Fibrinolytic therapy; Thienopyridines; Cardioprotection; Bone marrow stem cells; Cardiac stem cells

Core tip: The Third Universal Definition of myocardial infarction (MI) combines clinical symptoms, cardiac biomarkers and electrocardiogram (ECG) changes. Small amounts of myocardial necrosis may occur with heart failure, renal failure, myocarditis, arrhythmias, pulmonary embolism or uneventful percutaneous or surgical coronary revascularization and should be termed myocardial injury. High sensitivity troponin assays increase the sensitivity but decrease the specificity of MI diagnosis. The ECG remains a cornerstone of MI diagnosis. Primary percutaneous coronary intervention in a timely manner is the primary treatment of patients with acute ST segment elevation MI. Antiplatelet agents (clopidogrel, prasugrel or ticagrelor), in addition to aspirin, reduce patient MI morbidity and mortality. The recent LateTime, Time, and Swiss Multicenter Trials of bone marrow stem cells in MI treatment did not demonstrate significant improvement in patient LV ejection fraction in comparison with placebo. In contrast, cardiac stem cells from the right atrial appendage or ventricular septum/apex in the SCIPIO and CADUCEUS Trials reduced patient MI size and increased viable myocardium. Studies with cardiac stem cells are continuing.

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DEFINITION OF MYOCARDIAL INFARCTION

The Third Universal Definition of myocardial infarction (MI) expert consensus document was published in October 2012 by the global Myocardial Infarction Task Force[1]. The definition of MI requires cardiac myocyte necrosis with an increase and/or a decrease in plasma of cardiac troponin (cTn). At least one cTn measurement should be greater than the 99th percentile normal reference limit during: (1) symptoms of myocardial ischemia; (2) new (or presumably new) significant ECG ST-segment/T-wave changes or left bundle branch block; (3) the development of pathological electrocardiographic (ECG) Q waves; (4) new loss of viable myocardium or regional wall motion abnormality identified by an imaging procedure; or (5) identification of intracoronary thrombus by angiography or autopsy.

Cardiac troponin (I or T) has high myocardial tissue specificity as well as high clinical sensitivity because cTn T and I are essential contractile components of myocardial cells and are expressed almost exclusively in the myocardium. Release of cardiac troponin from the myocardium can result from normal turnover of myocardial cells, myocyte apoptosis, myocyte release of troponin degradation products, increased myocyte wall permeability and bleb formation, or myocyte necrosis[1].

Myocardial necrosis due to myocardial ischemia is defined as myocardial infarction[2]. Detection of a rise and a fall of troponin, expressed in ng/L or pg/mL, is essential to the diagnosis of acute MI[3,4]. Blood samples for the measurement of cTn should be drawn during the initial patient assessment and repeated 3-6 h later. Subsequent additional blood samples are required if further ischemic episodes occur, or when the timing of the initial symptoms onset is unclear[5]. The demonstration of a rise and fall in troponin measurements is extremely important in the differentiation of acute from chronic elevations in cTn concentrations that can be associated with structural heart disease such as patients with left ventricular hypertrophy (LVH), renal failure and heart failure (Table 1)[6].

The ECG remains a cornerstone in the diagnosis of MI and should be acquired and interpreted within 10 min after patient presentation[7]. Since ECG changes of MI can be transient, ECGs should be acquired at 15-30 min intervals, especially if the initial ECG is equivocal. Wide spread and profound ST-T changes are associated with greater degrees of myocardial ischemia. The extent and severity of coronary stenosis, collateral coronary circulation and prior myocardial necrosis impact on the ECG manifestations of myocardial ischemia[8]. Prior ECGs, when available, should be compared with current tracings. Mimickers of ECG changes of MI such as acute pericarditis, LVH, left bundle branch block (LBBB), Brugada syndrome, stress cardiomyopathy, and early repolarization patterns should be considered in the differential diagnosis[9].

Electrocardiographic ST-T wave criteria for the diagnosis of acute myocardial ischemia is listed in Table 2. The J point is used to determine the magnitude of the ST-segment shift. "Contiguous leads" refers to lead groups such as anterior leads (V1-V6), inferior leads

Table 1 Causes of troponin elevation

System	Causes of troponin elevation
Cardiovascular	Acute aortic dissection
	Arrhythmia
	Medical ICU patients
	Hypotension
	Heart failure
	Apical ballooning syndrome
	Cardiac inflammation
	Endocarditis, myocarditis, pericarditis
	Hypertension
	Infiltrative disease
	Amyloidosis, sarcoidosis, hemochromatosis, scleroderma
	Left ventricular hypertrophy
Myocardial injury	Blunt chest trauma
	Cardiac surgeries
	Cardiac procedures
	Ablation, cardioversion, percutaneous intervention
	Chemotherapy
	Hypersensitivity drug reactions
	Envenomation
Respiratory	Acute PE
	ARDS
Infectious/immune	Sepsis/SIRS
	Viral illness
	Thrombotic thrombocytopenic purpura
Gastrointestinal	Severe GI bleeding
Nervous system	Acute stroke
	Ischemic stroke
	Hemorrhagic stroke
	Head trauma
Renal	Chronic kidney disease
Endocrine	Diabetes
	Hypothyroidism
Musculoskeletal	Rhabdomyolysis
Integumentary	Extensive skin burns
Inherited	Neurofibromatosis
	Duchenne muscular dystrophy
	Klippel-Feil syndrome
Others	Endurance exercise
	Environmental exposure
	Carbon monoxide, hydrogen sulfide

GI: Gastrointestinal; ICU: Intensive care unit; PE: Pulmonary embolus; ARDS: Acute respiratory distress syndrome; SIRS: Systemic inflammatory response syndrome.

(Ⅱ, Ⅲ, aVF) or lateral/apical leads (Ⅰ, aVL).

Supplemental leads such as V3R and V4R, in the third and fourth right intercostal spaces, indicate the electrical activity in the free wall of the right ventricle and V7-V9 indicate the electrical activity in the inferobasal left ventricular wall. In patients with inferior and right ventricular infarction, ST segments are often elevated ⩾ 0.05 mV in V3R and V4R. In addition, ST elevation of ⩾ 0.05 mV ST in leads V7-V9 (V7 at the left posterior axillary line, V8 at the left mid-scapular line, and V9 at the left paraspinal border), supports the diagnosis of inferobasal MI due to left circumflex coronary artery occlusion. ST depression in leads V1-V3 also may be suggestive of inferobasal myocardial ischemia (posterior infarction), especially when the terminal T wave is positive[10-12].

Table 2 Electrocardiogram manifestations of acute myocardial ischemia (in absence of left ventricular hypertrophy and left bundle branch block)

ST elevation
New ST elevation at the J point in two contiguous leads with the cut-points:
⩾ 0.1 mV in all leads other than leads V2–V3 where the following cut points apply: ⩾ 0.2 mV in men ⩾ 40 yr; ⩾ 0.25 mV in men < 40 yr, or ⩾ 0.15 mV in women
ST depression and T wave changes
New horizontal or down-sloping ST depression ⩾ 0.05 mV in two contiguous
leads and/or T inversion ⩾ 0.1 mV in two contiguous leads with prominent R wave or R/S ratio > 1

ST segment elevation of > 0.5 mV is observed in lead aVR in acute left main coronary artery (LMCA) obstruction and proximal left anterior descending coronary artery (LAD) obstruction proximal to the first major septal branch. The ST elevation in aVR is more pronounced than in V1 in patients with acute LMCA occlusion. This pattern occurred in 88% of the patients with acute occlusion of LMCA group in one study[10,13]. Types of MI, five types of MI are based on pathological, clinical and prognostic differences (Table 3).

DIFFERENTIATING BETWEEN SPONTANEOUS TYPE 1 AND ISCHEMIC IMBALANCE TYPE 2 MYOCARDIAL INFARCTION

Differentiation between type 2 and type 1 MI is challenging and needs careful clinical assessment. It is very important that the differentiation be made whether the myocardial injury is likely to be due to plaque rupture (type 1 MI), or whether it is due to an imbalance in myocardial oxygen supply or demand (type 2 MI), because the management of these two conditions is very different. While, the treatment of type 1 MI primarily includes antithrombotic therapy and/or revascularization, as clinically appropriate, the management of type 2 MI is more varied because several different mechanisms may be responsible for pathogenesis ischemic imbalance. In critically ill patients or in patients with major (non-cardiac) surgery, biomarker elevation may be caused by the direct toxic effects of endogenous or exogenous high circulating catecholamines, coronary vasospasm and/or endothelial dysfunction or fixed coronary atherosclerosis and demand-supply mismatch (Figure 1). For example, a post-operative patient with hypotension and troponin elevation due to hypovolemia or acute blood loss, requires treatment with intravascular volume replacement, including blood transfusion. In certain instances, troponin elevation due to ischemic demand may unmask severe coronary artery disease (CAD) by increasing myocardial oxygen demand in the presence of fixed coronary stenosis. Consequently once the

Table 3 Third universal classification of myocardial infarction

Type 1: Spontaneous MI Spontaneous MI due to atherosclerotic plaque rupture, ulceration, fissuring, erosion, or dissection with resulting intraluminal thrombus in one or more of the coronary arteries leading to decreased myocardial blood flow or distal platelet emboli with ensuing myocyte necrosis. The patient may have underlying severe CAD, non-obstructive coronary disease or no CAD
Type 2: MI secondary to an ischemic imbalance Myocardial injury with necrosis occurs due to conditions other than CAD that contribute to an imbalance between myocardial oxygen supply and/or demand such as coronary endothelial dysfunction, coronary artery spasm, coronary embolism, tachycardia-bradycardia arrhythmias, anemia, respiratory failure, hypotension, and hypertension
Type 3: MI resulting in death when biomarker values are unavailable Cardiac death with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes or new LBBB, but death occurs before blood samples can be obtained, before cardiac troponins biomarkers rise, or when cardiac biomarkers were not collected
Type 4A: MI related to percutaneous coronary intervention MI associated with PCI is defined by elevation of cTn values greater than five times the 99th percentile upper normal reference limit (URL) in patients with normal baseline values (< 99th percentile URL) or a rise of cTn values by > 20% if the baseline troponins are elevated and are stable or falling. In addition one of the following criterion are required: (1) symptoms suggestive of myocardial ischemia; (2) new ischemic ECG changes or new LBBB; (3) angiographic loss of patency of a major coronary artery or a side branch or persistent slow- or no coronary flow or coronary embolization; or (4) demonstration with imaging of a new loss of viable myocardium or new regional wall motion abnormality
Type 4B: MI related to stent thrombosis MI associated with stent thrombosis detected by coronary angiography or autopsy in the presence of myocardial ischemia with a rise and/or fall of troponin biomarkers. One troponin measurement should be above the 99th percentile UR
Type 4C: MI related to restenosis MI associated with restenosis defined as ⩾ 50% stenosis or a complex lesion demonstrated at coronary angiography after (1) initial successful stent deployment; or (2) dilatation of a coronary artery stenosis with balloon angioplasty. These coronary angiographic changes should be associated with an increase and/or decrease of cTn values > 99th percentile URL and no other significant obstructive CAD
Type 5: MI related to coronary artery bypass grafting MI associated with CABG is defined by elevation of cardiac troponins greater than ten times the 99th percentile URL in patients with normal baseline cTn values (< 99th percentile URL). In addition, one of the following should be present: (1) new pathological Q waves or new LBBB; or (2) angiographic documented new graft or new native coronary artery occlusion; or (3) new loss of viable myocardium or new regional wall motion abnormality as shown by an imaging modality

Adapted from Thygesen *et al*[14]. MI: Myocardial infarction; CAD: Coronary artery disease; PCI: Percutaneous coronary intervention; cTn: Cardiac troponin; CABG: Coronary artery bypass grafting; LBBB: Left bundle branch block.

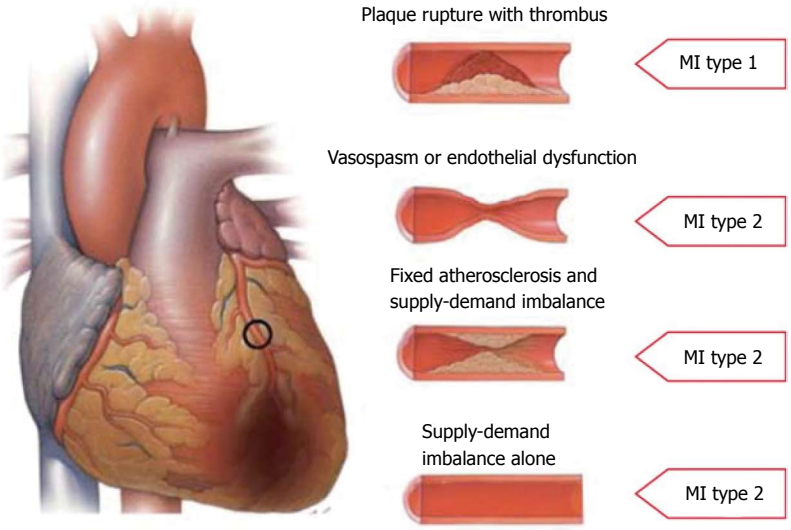

Figure 1 Type 1 and type 2 myocardial infarctions.

patient recovers from the acute illness, a stress test for inducible ischemia or coronary angiography can be helpful.

MYOCARDIAL INFARCTION DUE TO RE-VASCULARIZATION PROCEDURES

The 2007 universal MI definition required the presence of cardiac biomarkers greater than three times the 99th percentile of the upper normal range limit (URL) without requirements for associated ischemic changes or complications from angiographic procedures. This resulted in approximately 15% of patients undergoing PCI being diagnosed with an AMI[15,16]. In the 2012 definition of MI, there is a more strict definition of type 4a MI[1]. Percutaneous coronary intervention related MI is defined by cTn elevation greater than *five times* 99th percentile within 48 h after the procedure with: (1) symptoms suggestive of myocardial ischemia; or

(2) new ischemic ECG changes; or (3) angiographic findings consistent with a procedural complication with loss of a major artery or side coronary artery branch, decreased coronary flow, or coronary embolization; or (4) demonstration of new loss of viable myocardium or new regional wall motion abnormality. The occurrence of procedure-related myocardial cell injury with necrosis can be detected by measurements of cardiac troponin before the procedure, 3-6 h after the procedure and, optionally, re-measurement 12 h thereafter. An increasing cTn can only be interpreted as a procedure-related myocardial injury if the pre-procedural cTn value is $\leqslant 99^{th}$ percentile URL or if the troponin measurements are stable or falling. If the pre-procedural troponin is increased but is either stable or falling, an increase in cTn levels of > 20% is used to characterize a PCI-related MI.

The relationship between troponin increases after revascularization and mortality is controversial. The evidence for the association between biomarkers and mortality has evolved over the last 15 years. Studies have suggested a stronger association with the post-PCI MB fraction of creatine kinase (CK-MB) and subsequent cardiovascular events than with cTn elevation[15,17]. The level of CK-MB measurements varied from three to ten times the URL in these studies. When analyzed in categories of incrementally increasing biomarker elevations, most contemporary PCI studies have reported associations between periprocedural myonecrosis and mortality only for very large patient infarctions[17]. Only pre-procedure cTn elevations are correlated with subsequent mortality[18,19]. Consequently, in patients with baseline troponin elevation prior to PCI, the diagnostic accuracy of using the definition of post-PCI MI is limited.

With the application of the 2007 universal definition of post CABG MI (type 5), 42% to 82% of cardiac surgical patients had cardiac biomarker elevation greater than five times the URL[20], but only 4% to 7% had electrocardiographic evidence required for post-CABG MI[21]. Elevation of cardiac biomarker values after CABG can occur due to myocardial trauma, with dissection of the coronary arteries, manipulation of the heart, inadequate cardiac protection, reperfusion injury, or graft failure. Any increase in cardiac biomarker values > 99^{th} percentile URL is defined as myocardial injury. The new criteria for type 5 MI in patients with CABG requires an increase in biomarkers > 10 × 99^{th} percentile URL from a normal baseline during the first 48 h after surgery, plus new electrocardiographic Q waves or new LBBB, angiographic documentation of new graft or new native coronary artery occlusion, or imaging evidence of new regional wall motion abnormality or new loss of viable myocardium. The 2012 global MI task force emphasized that the threshold for diagnosing MI is more robust for on-pump CABG. The existing criteria for the universal definition of myocardial infarction should be used for diagnosing MI in patients who are more than 48 h after cardiac surgery[1].

The Society for Cardiovascular Angiography and Interventions has published an expert consensus document that defines clinically relevant myocardial infarction after revascularization (Table 4)[14].

REINFARCTION/RECURRENT MI

The term "reinfarction" is used for an acute MI that occurs within 28 d of a MI. If the cTn concentration is elevated, but stable or decreasing at the time of suspected reinfarction, the diagnosis of reinfarction requires a 20% or greater increase in the cTn measurement. If the initial cTn concentration is normal at the time of suspected reinfarction, the criteria for new acute MI apply[1,22].

TROPONIN ELEVATION IN HEART FAILURE

Based on the type of assay used, a range of elevated cTn values, indicative of myocardial injury with necrosis, may be seen in patients with a heart failure (HF) syndrome[23]. In stable heart failure patients, the median concentration of hs-cTnT is 12 ng/L, which is very close to the 99^{th} percentile URL of 14 ng/L for this assay[24]. Hence, using hs-cTn assays, cTn concentrations may be measured in nearly all patients with HF. Many HF patients exceed the 99^{th} percentile URL, especially those patients with severe decompensated HF syndrome[25]. While type 1 MI is an important cause of acutely decompensated heart failure, other mechanism(s) leading to troponin elevation in HF syndromes such as supply-demand inequity (type 2 MI) should be considered. Non-coronary triggers, such as anemia, cellular necrosis, apoptosis, or autophagy in the context of wall stress may cause troponin release in HF, as can the toxic effects of circulating neurohormones, toxins, inflammation, and infiltrative processes. Nonetheless, in patients with HF, troponin elevation independent of its mechanism, is strongly predictive of an adverse outcome and should not be ignored[25].

HIGH SENSITIVITY TROPONIN ASSAYS

Highly sensitive assays for cTnT and cTnI are available and are widely used in many parts of the world, although they are not generally used at the present time in the United States[26]. Two criteria should be met for high sensitivity troponin assays (hs-Tn). First, the coefficient of variation at the 99^{th} percentile value should be $\leqslant$ 10%. Second, the assay should be able to measure cTn concentrations below the 99^{th} percentile in $\geqslant$ 95% of normal individuals[27]. Compared with standard cTn assays, the hs-cTn assays have improved sensitivity and discrimination for MI, particularly in the first 3 to 6 h after symptom onset[28]. These advantages are somewhat offset by a decrease in specificity for MI[28-30] and concerns regarding the broad application of these tests, especially in populations with

Table 4 Proposed definition of clinically relevant myocardial infarction after both percutaneous coronary intervention and coronary artery bypass grafting procedures

In patients with normal baseline CK-MB	The peak CK-MB measured within 48 h of the procedure rises to ≥ 10 × the local laboratory ULN, or to ≥ 5 × ULN with new pathologic Q-waves in ≥ 2 contiguous leads or new persistent LBBB, OR in the absence of CK-MB measurements and a normal baseline cTn, a cTn (I or T) level measured within 48 h of the PCI rises to ≥ 70 × the local laboratory ULN, or ≥ 35 × ULN with new pathologic Q-waves in ≥ 2 contiguous leads or new persistent LBBB
In patients with elevated baseline CK-MB (or cTn) in whom the biomarker levels are stable or falling	The CK-MB (or cTn) rises by an absolute increment equal to those levels recommended above from the most recent pre-procedure level
In patients with elevated CK-MB (or cTn) in whom the biomarker levels have not been shown to be stable or falling	The CK-MB (or cTn) rises by an absolute increment equal to those levels recommended above plus new ST-segment elevation or depression plus signs consistent with a clinically relevant MI, such as new onset or worsening heart failure or sustained hypotension

ULN: Upper limit of normal; MI: Myocardial infarction; cTn: Cardiac troponin.

a low MI prevalence.

There is controversy regarding the metrics that should be used with hs-cTn assays for the diagnosis of AMI. In this regard, attempts have been made to define in these assays the optimal value for relative change or deltas in hs-cTn concentrations. Higher deltas increase specificity while lower ones improve sensitivity. The potential for analytical interferences with hs-cTn assays is greater than with conventional assays. Examples include reductions in hs-cTnT concentrations due to hemolysis and autoantibodies or increases due to heterophilic antibodies[31]. Studies suggest that an absolute increase of hs-cTnT values, *i.e.*, > 7 ng/L over 2 h, is superior to relative percentage changes from the baseline in the diagnosis of MI[32-34].

According to the recent guideline for the management of patients with acute coronary syndromes, blood samples for high-sensitivity cardiac troponin measurements should be obtained at presentation and 3 h after admission[35]. Measurements of hs-cTn should be repeated 6 h after admission in patients in whom the 3 h values are unchanged but in whom the clinical suspicion of MI is still high[36].

Distinguishing between type 1 and type 2 MI is challenging with high sensitivity troponin measurements. As troponin assay sensitivity increases, the frequency of possible type 2 MI increases and the distinction from type 1 MI becomes more complicated. Moreover, the diagnostic accuracy of a baseline measurement of hs-cTn for presence of AMI in patients with renal insufficiency is poor[37]. Nevertheless, elevated hs-cTns have important prognostic implications and patients require additional evaluations because a high cTnT level is associated with all-cause and cardiovascular mortality and with incident heart failure in 3 population based studies[30].

TREATMENT OF ACUTE MYOCARDIAL INFARCTION

The incidence of ST segment myocardial infarction (STEMI) has gradually declined over the past decade. However it still accounts for 25%-40% of all acute coronary syndrome related hospitalizations in the United States[37]. Moreover, the incidence of acute myocardial infarction is increasing in the developing countries[38]. Heart disease is expected to be the leading cause of death in the developing world by the year 2020. With changing dietary and personal habits, the prevalence of smoking, hypertension, diabetes, obesity and metabolic syndrome are increasing in areas of the world with large populations such as India[39], China[40] and South America[41]. Advances made in the area of medical therapy and coronary interventions have resulted in a significant decrease in the mortality rates. Current in-hospital and one year mortality are in the order of 5%-6% and 7%-18% respectively[42,43]. During the course of last three decades, there have been significant advances in our understanding of the pathophysiology and treatment of STEMI. In addition to these scientific advances, substantial progress has been made in the areas of public awareness and guideline driven clinical practice[44]. This has led to a gradual decline in STEMI related mortality and improved patient related outcomes. However, there continues to be significant difference in the 30-d mortality rates based on the geographic region[45], age[46,47], gender[48] and race[49]. In addition, individuals with diabetes and chronic renal insufficiency continue to have high rates of mortality[50-52]. In the recent INFUSE-AMI (Intracoronary Abciximab and Aspiration Thrombectomy During Primary PCI for Anterior STEMI) trial[53], diabetics compared to non-diabetics, had higher incidence of stent thrombosis at 30 d (4.3% *vs* 0.8%, P = 0.03) and higher rates of major cardiovascular and cerebrovascular events at 1 year (16.5% *vs* 8.0%, P = 0.04). It has been shown that patients with end-stage renal disease frequently do not receive guideline based therapies. In one registry, it has been shown that only 45% of eligible patients on dialysis received coronary reperfusion therapy, and only 70% of patients received aspirin on admission for coronary syndromes. In-hospital mortality rate from myocardial infarction is 21.3% in those on dialysis and 11.7% in those with renal disease but not on dialysis[54].

Approximately 7% of the eligible patients with myocardial infarctions do not receive reperfusion therapy[55]. There is evidence suggesting that reperfusion therapy offers benefit in the elderly. However, age is

the strongest predictor associated with an individual not receiving reperfusion therapy[56]. Programs that focus on patient education, systematic organization of STEMI programs and standardization of clinical practice result in improved care of all groups of patients and minimize disparities[57,58].

One of the most important components of STEMI management is getting the patients in a time efficient manner to a hospital that is capable of administering reperfusion therapies such as fibrinolytic therapy and primary percutaneous coronary intervention. Although approximately 98% of the United States population is within the reach of 911 based emergency medical service systems, patients with STEMI do not routinely utilize the system[59]. System based delays have been shown to increase STEMI related morbidity and mortality[60-63]. Hence increased community awareness and preparedness is important. In addition, regional STEMI centers with organized protocols, system based time-to-treatment goals and quality improvement programs must be established. Such efforts minimize delays and lower morbidity and mortality in STEMI patients[64,65]. In a study by Sørensen *et al*[66], where 759 consecutive STEMI patients were divided into a group with pre-hospital diagnosis and direct referral to a primary PCI center *vs* a group without pre-hospital diagnosis. Pre-hospital diagnosis and direct referral resulted in shorter system delay (92 min *vs* 153 min, $P < 0.001$).

CORONARY REPERFUSION STRATEGIES

Fibrinolytic therapy (FT) and Primary Percutaneous Coronary Intervention (P-PCI) are the two currently available modalities of reperfusion therapies. Both of these options are extensively studied. P-PCI, when performed in a timely manner at a high patient volume center is superior to FT. However, P-PCI is not universally available[67]. Delays in door-to-balloon times (D2B) are associated with increased mortality[68]. Adherence to D2B goal of < 90 min lowers mortality[69,70]. Although P-PCI is superior to FT, emphasis should be placed on timely administration of some form of reperfusion therapy rather than the mode of treatment[71].

For patients who present to a P-PCI capable hospital, the door to balloon time should not exceed 90 min. When patients present to a hospital that is not capable of P-PCI, factors such as time of onset of symptoms, risk of bleeding, presence of acute heart failure or shock, risk of mechanical complications, time-to-transfer to a P-PCI capable hospital should be taken into consideration. In patients who present within less than 1-2 h of onset of symptoms, immediate FT may be advantageous even if the transfer times are short[72].

ROLE OF PRE-HOSPITAL FIBRINOLYTIC THERAPY

Multiple trials have shown the safety and efficacy of pre-hospital FT[73-76]. This approach reduces the time to treatment by approximately 60 min and decreases mortality by 17%[77]. Similar findings were also seen in the pooled analysis of two other trials[78]. The Swedish and the French (USIC) registries showed that pre-hospital FT can be administered safely and results in reduces mortality[79,80]. At this time, pre-hospital use of FT is not commonly used in the United States but is used frequently in Western Europe and England.

STEMI PATIENTS WITH OUT-OF-HOSPITAL CARDIAC ARREST

Approximately 70% of CAD related deaths present as cardiac arrest prior to presenting to a hospital[81]. Less than a quarter of patients presenting with sudden cardiac arrest have ventricular tachycardia or ventricular fibrillation that can be electrically converted to normal sinus rhythm[82]. Of the 60% patients who are resuscitated by emergency response teams, the median survival rate to hospital discharge is 7.9%[83]. In patients with STEMI who present with sudden cardiac arrest, timely defibrillation and hypothermia have been shown to increase survival. For every minute delay in defibrillation, there is 7% to 10% drop in survival[83,84]. Increasing access to and use of defibrillators in public places has resulted in an increase in the number of patients that are neurologically intact after sudden cardiac arrest[84-86]. In patients with out-of hospital cardiac arrest, hypothermia with temperatures between 32 ℃ to 34 ℃ increases survival. In a study of 77 patients[87], hypothermia (with the core body temperature reduced to 33 degrees C within 2 h after the return of spontaneous circulation and maintained at that temperature for 12 h) compared to normal temperature increased the survival rates 26% to 49% $P = 0.0046$. In another study, survival was shown to be improved with hypothermia[88]. In patients with out-of hospital cardiac arrest in the setting of STEMI, hypothermia should be initiated as soon as possible.

FIBRINOLYTIC THERAPY

When P-PCI is not available, FT is an alternative. It reduced mortality and morbidity when carefully administered within 12 h of symptom onset[89-94]. The usefulness of FT in patients presenting greater than 12 h from the onset of symptoms is not well established[95-98]. Fibrin specific agents such as tenecteplase, retaplase and alteplase are preferred. Tenecteplase is the most fibrin specific. None of the fibrin specific agents are antigenic. Patency rates of the infarct related artery with fibrin specific agents are approximately 85%[99-103]. Streptokinase is a non-fibrin-specific agent and can cause antigenic reactions. Infarct related artery patency rate with streptokinase is 60%-70%[104]. When the delay from first medical contact to primary PCI is > 120 min, FT is indicated if the time of onset of symptoms is < 12 h.

ADJUNCTIVE PHARMACOTHERAPY WITH FIBRINOLYTIC THERAPY

The role of Aspirin and Clopidogrel with fibrinolytic therapy is well established[105-107]. Aspirin and Clopidogrel should be given prior to the administration of fibrinolytic agent. Dual antiplatelelt therapy should be continued for at least one year[107]. The data on using newer antiplatelet agents like Prasugrel and Ticagrelor as an adjunct to thrombolytic therapy for fibrinolysis is not yet well established.

In addition to antiplatelet therapy, the use of adjunctive anticoagulants is supported when fibrinolytic agents are used for STEMI[108]. Unfractionated heparin, Enoxaparin and Fondaparinux can be used. However, low molecular weight heparins (LMWH) should be avoided in patients with impaired renal function (Creatinine Clearance < 30 mL/min)[109].

FAILED FIBRINOLYTIC THERAPY

Ongoing chest pain, lack of > 50% ST segment resolution and the absence of reperfusion arrhythmias at 60-90 min after the administration of fibrinolytics is considered failure of treatment. These parameters predict TIMI flow < 3 in the infarct artery[110]. In patients who don't respond to (FT), "rescue" PCI has been shown to be beneficial. In the Rapid Early Action for Coronary Treatment Trail[111]. The primary components endpoint of death, reinfarction, stroke, or severe HF at 6 mo, was lower among patients randomized to rescue PCI compared to conservative care or repeat fibrinolysis (event-free survival rate: 84.6% *vs* 70.1% *vs* 68.7%, $P = 0.004$). This was due to reduction in reinfarction. There was no significant survival benefit. Minor bleeding was significantly higher among patients randomized to rescue PCI. However, there were no differences in major bleeding among the conservative therapy, repeat fibrinolysis or, rescue PCI groups. Similar findings of improved event free survival were reported in the Middlesbrough Early Revascularization to Limit Infarction trail. However, higher rates of stroke and periprocedural bleeding were associated with rescue PCI[112,113]. In patients with ongoing symptoms, lack of signs reperfusion, significant hypotension, severe CHF, cardiogenic shock, ECG evidence of large area of myocardium at risk, the benefit of early PCI justifies the risk of bleeding. Conservative treatment might be reasonable in a patient with improving symptoms and a limited inferior infarction despite the persistence of ST elevation.

PATIENTS PRESENTING WITH CARDIOGENIC SHOCK

In the SHOCK trial[114], 302 patient with STEMI with shock were randomized to medical stabilization ($n = 150$) group, which included thrombolysis (63% of patients), intra-aortic balloon counterpulsation (86%), and subsequent revascularization (25%), or to an early revascularization group ($n = 152$). The primary endpoint of survival at 1 year was 46.7% for patients in the early revascularization group compared with 33.6% in the initial medical stabilization group (absolute difference in survival, 13.2%; $P < 0.03$). In a prespecified subgroup analyses, only age (< 75 years *vs* ⩾ 75 years) interacted significantly ($P < 0.03$) with treatment. The benefit was seen only in patients younger than 75 years (51.6% survival in early revascularization group *vs* 33.3% in initial medical stabilization group). The benefit of early revascularization was apparent across a wide time window, extending up to 54 h after MI and 18 h after shock onset. Based on this data, STEMI patients who present with acute cardiogenic shock should undergo emergency cardiac catheterization and revascularization. This is especially true for patients younger than 75 years.

ROUTINE EARLY ANGIOGRAPHY AFTER SUCCESSFUL FIBRINOLYTIC THERAPY

In the Grup de Analisis de la Cardiopatia Isquemica Aguda trial[115], 500 patients with STEMI that were treated with recombinant tissue plasminogen activator were randomly assigned to angiography and coronary intervention if indicated within 24 h of thrombolysis, or to an ischemia-guided conservative approach. The primary endpoint of combined rate of death, reinfarction, or revascularization at 12 mo occurred in 9% of the angiography and intervention group compared to 21% in the conservative group ($P = 0.0008$). There was a trend towards reduced rates of death or reinfarction (7% *vs* 12%, $P = 0.07$). There were no differences in major bleeding or vascular complications.

In the Trial of Routine Angioplasty and Stenting after Fibrinolysis to Enhance Reperfusion in Acute Myocardial Infarction[116], 1059 high-risk patients who had a STEMI received FT at centers not capable of performing P-PCI were randomized to either standard treatment (including rescue PCI, if required, or delayed angiography) or immediate transfer to another hospital and PCI within 6 h after fibrinolysis. At 30 d, the primary composite endpoint of death, reinfarction, recurrent ischemia, new or worsening congestive heart failure, or cardiogenic shock occurred in 11.0% of PCI and in 17.2% of the patients assigned to standard treatment ($P = 0.004$). There was no evidence of increased major bleeding with the early invasive strategy.

In the Norwegian Study on District Treatment of ST-Elevation Myocardial Infarction trial[117] 266 patients with acute STEMI living in rural areas, where the transfer time to P-PCI are greater than 90 min, were initially treated with the combination of tenecteplase, aspirin, enoxaparin, and clopidogrel and were randomized to immediate transfer for P-PCI or to standard man-

agement in the local hospitals with early transfer, only if indicated for rescue or clinical deterioration. The primary outcome of composite of death, reinfarction, stroke, or new ischemia at 12 mo occurred in 21% *vs* 27% in the early invasive group and the conservative treatment group respectively (P = 0.19). Although this study failed to demonstrate a statistically significant difference between the 2 treatment groups in the incidence of the primary composite endpoint, the incidence of death, recurrent MI, or stroke was significantly lower in the immediate-transfer group. The risk reduction was similar to that reported for high-risk patients in the Trial of Routine Angioplasty and Stenting after Fibrinolysis to Enhance Reperfusion in Acute Myocardial Infarction.

In a meta-analysis by Borgia *et al*[118] that included 2961 patients from 7 trials, early PCI after successful fibrinolysis reduced the rate of re-infarction (P = 0.003), the combined endpoint death/re-infarction (P = 0.004) and recurrent ischemia (P < 0.001) at 30-d. There was no evidence of an increase in patient major bleeding or stroke.

In the recent Strategic Reperfusion Early After Myocardial Infarction trial[119], 1892 patients with STEMI who presented within 3 h of symptom onset who were unable to undergo primary PCI within 1 h, were randomly assigned to undergo either primary PCI or fibrinolytic therapy with bolus tenecteplase (half dose in patients ⩾ 75 years of age), clopidogrel, and enoxaparin before transport to a P-PCI capable hospital. Emergency coronary angiography was performed only if fibrinolysis failed, which occurred in 36.3% of the patients; otherwise, angiography was performed 6 to 24 h after randomization. The primary composite end point of death, shock, congestive heart failure, or reinfarction within 30 d occurred in 12.4% of the patients in the fibrinolysis group and in 14.3% in the primary PCI group (P = 0.21). In patients who did not undergo primary PCI within one hour of medical contact, pre-hospital fibrinolysis with coronary angiography with a median time = 17 h resulted in effective reperfusion. The incidence of intracranial bleeding was higher with FT when compared to PCI (1.0% *vs* 0.2% P = 0.04).

Based on these studies, in STEMI patient who are treated successfully with FT, cardiac catheterization can be considered as part of a routine pharmacoinvasive or ischemia-guided approach > 24 h after administration of FT. Very early cardiac catheterization and PCI within 2-3 h after the administration of (FT) increases the risk of bleeding. Very early (< 2-3 h) invasive approach should be utilized for patients who require rescue PCI.

FACILITATED PCI

Fibrinolytic agents use as adjunct to primary PCI has been studied. This approach is called facilitated PCI. Full dose or half dose of a fibrinolytic agent is administered with or without glycoprotein Ⅱb/Ⅲa (GP Ⅱb/Ⅲa) inhibitor prior to planned PCI. This approach is based on the assumption that pre PCI pharmacotherapy will facilitate higher and faster rates of reperfusion.

The Assessment of the Safety and Efficacy of a New Treatment Strategy for Acute Myocardial Infarction trail[120] was stopped prematurely because of an increased mortality associated with facilitated PCI. In the Facilitated Intervention with Enhanced Reperfusion Speed to Stop Events trial[121], patients were randomized to primary PCI or facilitated PCI with abciximab or facilitated PCI with half-dose reteplase and full-dose abciximab. Although the rates of death, heart failure, and ischemic outcome at 90 d for all three groups were similar, there was increased rate of major bleeding with the facilitated strategies. Because of these findings, facilitated PCI is currently not advised.

PRIMARY PCI

Timely reperfusion with primary PCI (P-PCI) by experienced operators at an experienced center is superior to FT. Compared to FT, P-PCI results in higher rates of infarct related artery patency, higher rates of TIMI 3 flow and lower rates of complications such as recurrent ischemia, reinfarction, emergency repeat revascularization procedures, intracranial hemorrhage (ICH), and death. However, P-PCI is associated with increased rates of access site bleeding complications[122]. In addition, PCI can result in the "no reflow" phenomenon where the myocardial perfusion is inadequate despite restoration of TIMI 3 epicardial flow in the infarct related artery. No reflow phenomenon is due to a combination of endothelial injury, edema, atheroembolization, vasospasm, and myocyte reperfusion injury and inflammation[123]. Occurance of no reflow is associated with with increased mortality[123]. Multiple treatment strategies that included the use of GP Ⅱb/Ⅲa antagonists, nitroprusside, verapamil, adenosine, nicorandil, pexelizumab have not shown promising results[123].

The benefits of P-PCI over FT are time sensitive. Door to balloon (D2B) times greater than 90-120 min can eliminate the benefits of P-PCI over FT[124]. Over the past ten years, there has been a significant reduction in the median D2B times. Although approximately 80% of the United States population lives within one hour from a P-PCI capable hospital, the majority of patients in the rural areas do not have access to such facilities. A significant increase in the number of PCI capable hospitals from 2001 to 2006 result in minimal increase in the overall patient access to such facilities[125,126]. One of the strategies to make P-PCI more accessible is to allow hospitals without onsite cardiac surgery facilities to perform PCI procedures. The Cardiovascular Patient Outcomes Research Team trail[127] showed that primary PCI can be performed safely and rapidly at hospitals without cardiac surgery back-up. Other strategies include bypassing the non PCI hospital and transferring the patients to a primary PCI capable hospital where the care and transfer protocols are standardized. These strategies have been shown to extend P-PCI to more

patients and result in better patient outcomes[127-129].

DELAYED PRESENTATION

In patients presenting more than 12 h after the onset of symptoms, cardiac catheterization and PCI should be considered in the setting of ongoing chest pain, cardiogenic shock, acute severe heart failure, or spontaneous or provoked myocardial ischemia.

In the Occluded Artery Trial[130], 2166 patients with occluded infarct related arteries who presented 3-28 d after myocardial infarction and had ejection fractions less than 50% were randomized to PCI *vs* conservative medical therapy. The 4-year cumulative primary event rate was 17.2% in the PCI group and 15.6% in the medical therapy group (P = 0.20). However, patients with high risk features such as New York Heart Association class Ⅲ-Ⅳ, rest angina, high risk stress test, left main or three vessel diseases were excluded from the trial. The trail showed that routine PCI did not reduce the occurrence of death, reinfarction, or heart failure, and there was a trend toward excess reinfarction during 4 years of follow-up in stable patients with occlusion of the infarct-related artery 3 to 28 d after myocardial infarction. Based on this data, in patients who present more than 12 h after their symptom onset and are clinically stable, routine cardiac catheterization and PCI are not advised.

PCI OF THE NON-INFARCT RELATED ARTERY

In patients presenting with STEMI, multivessel coronary artery disease is frequently seen and is associated with poor outcomes[131]. PCI of a non-infarct related artery prior to discharge from the hospital, at a time that is separate from the index STEMI related PCI, is indicated if there is evidence of spontaneous myocardial ischemia. However, this practice is largely based on non-randomized cohort studies[132-134]. The role of fractional flow reserve (FFR) at the time of STEMI, to evaluate the functional significance of a non-infarct related artery is not well established. In a small study by Ntalianis *et al*[135], FFR was useful in evaluating the functional significance of a non-culprit coronary lesion.

In the recent Preventive Angioplasty in Acute Myocardial Infarction trail[136], 465 patients with acute STEMI who were undergoing primary PCI were randomly assigned to either preventive PCI defined as immediate PCI of any lesion with > 50% stenosis or no preventive PCI. The trial was stopped early by the data safety monitoring board. In an intention to treat analysis, the primary composite endpoint of death from cardiac causes, nonfatal myocardial infarction, or refractory angina occurred in 9% of the preventive PCI arm and 23% of the non-preventive PCI arm, respectively (P = 0.001). It should be noted that the trail excluded patients with concomitant disease in the left anterior descending and left circumflex arteries, patients with > 50% stenosis of the left main artery, patients with prior CABG and patient with a non-culprit artery with a chronic total occlusion.

At this time, PCI of a non-infarct related artery should be performed prior to hospital discharge if the patient has evidence of spontaneous or provocable myocardial ischemia.

PCI TECHNIQUE BASED STRATEGIES

During the past decade, we have seen significant advances in the field of interventional cardiology as it relates to the management of acute myocardial infarction. Some of the frequently debated issues include access site (radial *vs* femoral), routine use of aspiration thrombectomy, and bare-metal *vs* drug eluting stents.

Access site

Transradial PCI had gained widespread acceptance and is now used routinely for elective angioplasty. Major advantages with transradial approach include reductions in bleeding complications and length of hospitalizations and improved quality of life. Given these advantages, transradial PCI during STEMI has been extensively studied. Multiple randomized trials and a large meta-analysis showed that transradial primary PCI is associated with significant reduction in access site complications. In the Radial *vs* femoral access for coronary angiography and intervention in patients with acute coronary syndromes trial[137] 7021 patients with STEMI were randomly assigned to radial *vs* femoral access sites. The primary endpoint of death, myocardial infarction, stroke, or non-CABG-related major bleeding at 30 d occurred in 3.7% and 4.0% of the radial access and femoral access patients respectively (P = 0.5). In a pre-specified subgroup analysis, non-CABG-related major bleeding at 30 d occurred in 24 patients in the radial group compared with 33 patients in the femoral group (P = 0.23). At 30 d, 42 of 3507 patients in the radial group had large hematomas compared with 106 of 3514 in the femoral group (P < 0.0001). In the Radial *vs* Femoral Randomized Investigation in ST Elevation Acute Coronary Syndrome[138,139] trial, 1001 STEMI patients undergoing primary/rescue percutaneous coronary intervention were randomized to the radial or femoral approach. The primary endpoint of cardiac death, stroke, myocardial infarction, target lesion revascularization, and bleeding at 30 d occurred in 13.6% in the radial artery group and 21.0% in the femoral artery group (P = 0.003). Radial access was associated with significantly lower rates of cardiac mortality (5.2% *vs* 9.2%, P = 0.020), bleeding (7.8% *vs* 12.2%, P = 0.026), and shorter hospital stay. In the STEMI-RADIAL trail[103] 2959 patients undergoing primary PCI within 12 h of onset of symptoms were randomized to radial *vs* femoral approach. The primary

endpoint of access site complications and bleeding occurred in 7.2% of the femoral *vs* 1.4% of the radial group (80% relative risk reduction, $P = 0.001$). Radial and femoral approaches are both safe and effective for PCI. Lower rates of local vascular complications may be a reason to use the radial access approach. There is some concern about longer D2B times and increased radiaton exposure with radial artery access. This is mostly limited to low volumes centers and operators[140,141]. Data from the randomized control trials suggests that D2B times and the cumulative radiation dose are minimally increased with radial artery catheterization. The impact of the radial artery approach on patient mortality remains unclear at this time as the reported studies are underpowered to evaluate this end-point.

Adjunctive thrombectomy

A vast majority of patients with STEMI have large thrombus burden. It seems intuitive that thrombectomy may improve epicardial coronary flow, prevent distal embolization, reduce microvascular obstruction and the no-reflow phenomenon. However, trials that have used mechanical thrombectomy have been largely negative without any improvement in myocardial blush grade, final infarct size and overall left ventricular ejection fraction[142-144]. Recently there has been renewed interest in aspiration thrombectomy. In the Thrombus Aspiration during Percutaneous coronary intervention in Acute myocardial infarction Study[145], 1071 patients were randomly assigned to the thrombus-aspiration group or the conventional-PCI group before undergoing coronary angiography. The primary end point of myocardial blush grade of 0 or 1 occurred in 17.1% of the patients in the thrombus-aspiration group and in 26.3% in the conventional-PCI group ($P < 0.001$). At one year follow up, cardiac death occurred in 3.6% of the patients in the thrombus aspiration group and 6.7% in the conventional PCI group ($P = 0.020$). In the Impact of Thrombectomy With Export Catheter in Infarct-Related Artery During Primary Percutaneous Coronary Intervention trail[146], 155 STEMI patients were randomly assigned to standard percutaneous coronary intervention PCI ($n = 87$) or aspiration thrombectomy guided PCI ($n = 88$). The primary end points of myocardial blush grade $\geqslant 2$ and the rate of 90-min ST-segment resolution > 70% occurred more frequently in the thrombectomy guided PCI group (88% *vs* 60%, $P = 0.001$; and 64% *vs* 39%, $P = 0.001$). In a meta-analysis conducted by Bavry *et al*[147], total of 30 studies with 6415 patients were included, a weighted mean follow-up of 5.0 mo showed that the morality was 3.2% for the adjunctive thrombectomy group *vs* 3.7% for conventional PCI. In an by Kumbhani *et al*[148] data from clinical trials that randomized AMI patients to aspiration (18 trials, $n = 3936$) or mechanical thrombectomy (7 trials, $n = 1598$) before PCI compared with conventional PCI alone was analyzed. It showed that at a weighted mean clinical follow-up period of 6 mo major adverse cardiac events (RR = 0.76; 95%CI: 0.63-0.92; $P = 0.006$) and all-cause mortality (RR = 0.71; 95%CI: 0.51-0.99; $P = 0.049$) were significantly reduced with aspiration thrombectomy. ST-segment resolution at 60 min (RR = 1.31; 95%CI: 1.16-1.48; $P < 0.0001$) and Thrombolysis In Myocardial Infarction blush grade 3 post-procedure (RR = 1.37; 95%CI: 1.19-1.59; $P < 0.0001$) were both improved with aspiration thrombectomy.

In the recently published TASTE[149] (The Thrombus Aspiration in ST-Elevation Myocardial Infarction in Scandinavia) trial, aspiration thrombectomy did not show reduction in 30 d mortality. The event rate was 2.8% in the aspiration arm *vs* 3.0% in the routine PCI arm ($P = 0.63$). This study has some significant limitations. The treating physician was aware of the group assignment. Event adjudication and review of coronary angiograms was not done in a blinded manner. Despite these limitations, the TASTE trial does suggest that routine use of aspiration thrombectomy, may not be beneficial in reducing mortality. Larger studies are needed to see if aspiration thrombectomy offers mortality benefit. The ongoing TOTAL A Trial of Routine Aspiration Thrombectomy With Percutaneous Coronary Intervention (PCI) *vs* PCI Alone in Patients With ST-Segment Elevation Myocardial Infarction (STEMI) Undergoing Primary PCI trial may answer some of the questions.

TYPE OF STENTS IN THE SETTING OF PRIMARY PCI

It is now a routine practice to use coronary stents during primary PCI. Compared to balloon angioplasty, primary PCI with bare metal stents (BMS) has been shown to reduce the rates of reinfarction and target vessel revascularization. However, this does not translate into a reduction in mortality[150]. Drug eluting stents (DES) are currently being used for both elective and primary PCI. DES when compared with BMS significantly reduces restenosis rates and the need for reintervention but does not definitively reduce rates of death[151]. First generation DES such as Taxus and Cypher, when compared to BMS, can increase the risk of very late stent thrombosis[152]. Newer generation DES such as Xience, Promus and Endeavour, when compared to BMS do not increase the risk of acute or late stent thrombosis. Cobalt-chromium based everolimus eluting stents have the lowest reported rates of stent thrombosis[153]. In the Xience or Vision Stents for Management of Angina in the Elderly trail[154], second generation, everolimus eluting DES were safely used in the elderly without increasing the risk of bleeding. Patients who are taking oral anticoagulation and present with a STEMI pose a significant challenge. Triple therapy significantly increases the risk of bleeding. In the What is the

Optimal antiplatelet and anticoagulant therapy in patients with oral anticoagulation and coronary stenting trial[155], the group receiving warfarin plus clopidogrel had lower bleeding complications compared with the group receiving warfarin, clopidogrel and aspirin. Although the rate of stent thrombosis was not increased, this trial was not powered to evaluate the risk of stent thrombosis.

Given the advantages of marked reduction in the rates of restenosis, target vessel and target lesion revascularization and very low rates of late stent thrombosis, second generation DES should be the preferred choice during primary PCI. However, that decision should be made on a case to case basis. Factors such as bleeding risk, other indications for systemic oral anticoagulants, socioeconomic status, compliance, need for surgical procedures during the following one year should be considered. If these factors are a concern, DES implantation should be avoided. There still remain gaps in our understanding of routine use of DES in the elderly and patients who are on oral anticoagulants. Further research is need in these areas.

ADJUNCTIVE PHARMACOTHERAPY BASED STRATEGIES

In recent years, there has been extensive research done in the area of adjunctive pharmaco-therapy. As a result, we now have multiple antithrombotic and anti-platelet agents that have been shown to reduce major adverse cardiac events in the setting of STEMI.

Unfractionated heparin (UFH) is time tested and the most familiar of all the agents. It is used frequently. When titrated to appropriate activated clotting times of 250-300 s, it is an acceptable strategy. Low molecular weight heparins (LMWN) such as Enoxaparin and Fondaparinux are not well studied in the setting of STEMI. In the STEMI Treated With Primary Angioplasty and Intravenous Lovenox or Unfractionated Heparin trial[156] 901 patients were randomized to treatment with enoxaparin (n = 450) or unfractionated heparin (n = 460). The composite primary endpoint of 30-d incidence of death, complication of myocardial infarction, procedure failure, or major bleeding occurred in 126 (28%) patients after anticoagulation with enoxaparin *vs* 155 (34%) patients on unfractionated heparin (P = 0.06). Data from this trail suggests that enoxaparin can be safely and effectively used in patients with STEMI. In the OASIS-6 trial[157] death or reinfarction at 30 d was significantly reduced from 11.2% in the control group to 9.7% patients in the fondaparinux group (P = 0.008). However, fondaparinux was associated with higher rates of catheter thrombosis. At this time, fondaparinux in not used as an anticoagulant in the setting of primary PCI.

The role of Bivalirduin in the setting of STEMI treated with primary PCI was tested in the Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction trial[158]. Three thousand six hundred and two patients with ST-segment elevation myocardial infarction presenting within 12 h after the onset of symptoms and were undergoing primary PCI, were randomly assigned to treatment with heparin plus a glycoprotein Ⅱb/Ⅲa inhibitor or to treatment with bivalirudin alone. Primary end points of major bleeding and combined adverse clinical events, including death, reinfarction, target-vessel revascularization for ischemia, and stroke within 30 d occurred in 22% of the heparin plus glycoprotein Ⅱb/Ⅲa inhibitor group *vs* 9.2% in the bivalirudin group (P = 0.005). The risk of acute stent thrombosis within 24 h in the bivalirudin group increased, but no significant increase was present by 30 d. This was most likely secondary to a combination of adenosine diphosphate–induced platelet activation before maximal thienopyridine blockade of the platelet P_2Y_{12} receptor or by residual thrombin activity after the discontinuation of bivalirudin. Based on the results from the HORIZONS-AMI trail, it is a reasonable approach to use bivalurudin in patients with STEMI who are undergoing primary PCI. This approach may provide long term survival benefit by lowering the rate of bleeding complications.

ADJUNCTIVE ANTIPLATELET THERAPY

Aspirin

An initial single dose of 325 mg of Aspirin should be administered as early as possible. This should be followed by a maintenance dose of 81 mg once daily. Higher doses of Aspirin for maintenance therapy have shown to increase the risk of bleeding. In the Committee members of the Clopidogrel and Aspirin Optimal Dose Usage to Reduce Recurrent Events-Seventh Organization to Assess Strategies in Ischemic Syndromes trial[159] 25086 patients with an acute coronary syndrome who underwent cardiac catheterizatoin were randomized to either double-dose clopidogrel (a 600-mg loading dose on day 1, followed by 150 mg daily for 6 d and 75 mg daily thereafter) or standard-dose clopidogrel (a 300-mg loading dose and 75 mg daily thereafter) and either higher-dose aspirin (300 to 325 mg daily) or lower-dose aspirin (75 to 100 mg daily). The primary outcome of cardiovascular death, myocardial infarction, or stroke at 30 d was not different between higher-dose and lower-dose aspirin (4.2% *vs* 4.4%, P = 0.61) or major bleeding (2.3% *vs* 2.3%, P = 0.90).

Clopidogrel

The importance of at least 12 mo of dual antiplatelet therapy with aspirin and clopidogrel in the setting of ACS with and without PCI has been well established based on the data from the Clopidogrel in Unstable Angina to Prevent Recurrent Event (CURE)[160] and PCI-CURE[161] trials. A 600 mg loading dose of clopidogrel offers rapid platelet inhibition compared to 300 mg dose[162]. In the CURRENT-OASIS 7 trail[159] the

primary outcome of cardiovascular death, myocardial infarction, or stroke at 30 d occurred in 4.2% of the double-dose clopidogrel group *vs* 4.4% in the standard-dose clopidogrel (P = 0.30). Major bleeding occurred in 2.5% of the double-dose group and in 2.0% in the standard-dose group patients (P = 0.01). Rates of stent thrombosis was lower in the double dose group (1.6% *vs* 2.3% P = 0.001). Incidence of major bleeding was 2.5% in the double dose group *vs* 2.1% in the standard dose group (P = 0.001). Clopidogrel 600 mg loading dose followed by 75 mg once daily for at least one year should be considered for all patients with acute coronary syndromes.

One common clinical concern with the use of clopidogrel is the variable therapeutic response. This is secondary to multiple factors such as diabetes, obesity, polymorphisms in enteric ABCB 1 and hepatic cytochrome P450 (CYP450) enzymes (CYP2C19*2) and drug interaction that interferes with the metabolism of clopidogrel. Nearly 30% of patients have a reduced functional allele of CYP2C19*2. This has been shown to be associated with decreased levels of the active metabolite of clopidogrel, suboptimal platelet inhibition and increased rates of major adverse cardiac events and stent thrombosis[162-165]. Based on this data, the United States Food and Drug Administration made changes to clopidogrel's prescribing information noting the potential impact of CYP2C19 genotype on clopidogrel's bioavailability and clinical response. However, in a study by Mega *et al*[166] homozygotes and heterozygotes for loss of functional allele had similar rates of primary efficacy outcomes. At this time routine testing for CYP2C19*2 polymorphisms is not indicated. Further studies are needed to fully understand the clinical risk associated with these polymorphisms and to develop effective treatment strategies.

Proton-pump inhibitors, such as omeprazole, have been shown to interfere with clopidogrel metabolism resulting in decreased antiplatelet effect[167]. However, this does not lead to worse clinical outcomes[168]. At this time there is no strong evidence to avoid concomitant use of PPIs, when clinically indicated, in patients receiving clopidogrel.

Prasugrel

Prasugrel is a thienopyridine class of drug that competively antagonizes the P2Y12 receptor. Similar to Clopidogrel, it is also a pro drug that requires biologic conversion to an active metabolites. In the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel trial[169] 13608 patients with moderate-to-high-risk acute coronary syndromes treated with early invasive approach were randomly assigned to prasugrel, with a 60-mg loading dose and a 10-mg daily maintenance dose, or clopidogrel, with a 300-mg loading dose and a 75-mg daily maintenance dose, for 6 to 15 mo. The primary efficacy end-point of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke occurred in 12.1% of patients receiving clopidogrel and 9.9% of patients receiving prasugrel (P < 0.001). There was significant reductions in the rates of myocardial infarction (9.7% for clopidogrel *vs* 7.4% for prasugrel; P < 0.001), urgent target-vessel revascularization (3.7% *vs* 2.5%; P < 0.001), and stent thrombosis (2.4% *vs* 1.1%; P < 0.001). Major bleeding was increased with prasugrel 2.4% in comparison with 1.8% of patients with clopidogrel (P = 0.03). The prasugrel group had higher rates of life-threatening bleeding (1.4% *vs* 0.9%; P = 0.01), including nonfatal bleeding (P = 0.23) and fatal bleeding (0.4% *vs* 0.1%; P = 0.002). While prasugrel significantly reduced the rates of ischemic events and stent thrombosis, it increased the risk of major bleeding and did not reduce mortality. The benefits of prasugrel must be carefully weighed against the increased risk of bleeding. Prasugrel may be a preferred agent in younger, high risk acute coronary syndrome patients with large area of myocardium at risk and low bleeding risk. Prasugrel should not be used in patients with history of prior stroke, transient ischemic attacks, age greater than or equal 75, or body weight less than 60 kg. A lower dose of prasugrel 5 mg once daily has been suggested in patients who are at higher risk for bleeding. However, prasugrel 5 mg/d has not been prospectively studied.

Ticagrelor

Ticagrelor is a cyclopentyl triazolo pyrimidine that acts on the platelet P_2Y_{12} receptor as an antagonist. It does not require conversion to active metabolite and is a reversible agent. In The Study of Platelet Inhibition and Patient Outcomes trail[170] 18624 patients with acute coronary syndromes, were randomized to ticagrelor (180-mg loading dose, followed by 90 mg twice daily) or clopidogrel (300-to-600-mg loading dose, followed by 75 mg daily). Thirty-five percent of the patients had STEMI. Overall, at 12 mo, the composite end-point of death from vascular causes, myocardial infarction, or stroke occurred in 9.8% of patients receiving ticagrelor *vs* 11.7% of patients receiving clopidogrel (P < 0.001). The rate of death from any cause was also reduced with ticagrelor (4.5% *vs* 5.9%, P < 0.001). In addition, there were reductions in the rates of myocardial infarction (5.8% in the ticagrelor group *vs* 6.9% in the clopidogrel group, P = 0.005) and death from vascular causes (4.0% *vs* 5.1%, P = 0.001). There was no difference in the frequency of stroke alone (1.5% *vs* 1.3%, P = 0.22) or the rates of major bleeding (11.6% and 11.2%, P = 0.43). However, ticagrelor was associated with a higher rate of major non CABG related bleeding (4.5% *vs* 3.8%, P = 0.03), including more instances of fatal intracranial bleeding.

In a pre-specified subgroup analysis of the Study of Platelet Inhibition and Patient Outcomes trail, the net benefit of ticagrelor was smaller in the North American cohort. This was attributed to chance alone or alternatively to the frequent use of higher dose of aspirin

for maintenance therapy. Based on this observation, the dose of aspirin when used in combination with ticagrelor for maintenance therapy should not exceed 100 mg a day.

When considering adding a second drug to aspirin for dual antiplatelet therapy (DAPT), the decision should be individualized. The anti-ischemic benefits should be carefully weighed against patient comorbidities, risk of bleeding, need for long term treatment with an oral anticoagulant, cost, compliance, and the possibility of surgical procedures during the following year.

DURATION OF ANTIPLATELET THERAPY

Current guidelines[171] support uninterrupted use of dual antiplatelet therapy for at least one year in post ACS patients regardless of invasive or conservative treatment or the type of stent (BMS *vs* DES). Recently, there has been significant data supporting the discontinuation of dual antiplatelet therapy 3 to 6 mo after a PCI in the setting of acute coronary syndrome.

In the Efficacy of Xience/Promus *vs* Cypher in Reducing Late Loss after Stenting trail[172] 1443 patients undergoing implantation of drug-eluting stents were randomized to receive 6- or 12-mo DAPT. The primary end point of target vessel failure at 12 mo was 4.8% in the 6-mo DAPT group and 4.3% in the 12-mo DAPT group (P = 0.001 for non-inferiority). This study was underpowered for evaluation of death and MI.

In the Prolonging dual antiplatelet treatment after grading stent-induced intimal hyperplasia trial[173] 2013 patients were randomly assigned to receive bare-metal, zotarolimus-eluting, paclitaxel-eluting, or everolimus-eluting stent implantation. At 30 d, patients in each stent group were randomly allocated to receive up to 6 or 24 mo of clopidogrel therapy in addition to aspirin. The primary composite endpoint of death from any cause, myocardial infarction, or cerebrovascular accident at 24 mo was similar.

In the Real Safety and Efficacy of a 3-mo dual antiplatelet Therapy following E-ZES Implantation trail[174], 2117 patients with coronary artery stenosis were randomized to 2 groups according to DAPT duration and stent type: 3-mo DAPT following zotarolimus-eluting stent (E-ZES) implantation *vs* 12-mo DAPT following the other (sirolimus, everolimus DES implantation). The primary composite endpoint of cardiovascular death, myocardial infarction, stent thrombosis, target vessel revascularization, or bleeding at 1 year occurred in 4.7% patients assigned to E-ZES + 3-mo DAPT compared with 4.7% patients assigned to the standard therapy (P = 0.001 for noninferiority).

In the recently published Optimized Duration of Clopidogrel Therapy Following Treatment With the Endeavor Zotarolimus - Eluting Stent in the Real World Clinical Practice trail[175], 3119 patients undergoing PCI using zotarolimus DES were randomly assigned to 3 mo *vs* 12 mo of dual antiplatelet therapy. The primary composite end point of all-cause death, myocardial infarction, stroke, or major bleeding occurred in 6.0% *vs* 5.8%, respectively P = 0.002 for noninferiority.

Although the data from these trials is reassuring and supports the discontinuation of dual anti-platelet therapy at the end of 6 mo, it is important to note that these trial included patients with stable coronary disease and low risk acute coronary syndrome. Caution should be used in extrapolating this data to patients with STEMI. At this time, dual anti-platelet therapy should be continued for at least one year without interruption when tolerated in patients with ACS.

Role of glycoprotein IIb/IIIa receptor antagonists

Role of glycoprotein Ⅱb/Ⅲa (GP Ⅱb/Ⅲa) receptor antagonists in the setting of STEMI was extensively studied prior to routine use of dual antiplatelet therapy. Addition of a GP Ⅱb/Ⅲa receptor antagonist to combination of DAPT plus unfractionated heparin or bivalirudin failed to show benefit[176-178]. However, in a meta-analysis by De Luca[179] where 722 patients with STEMI from seven randomized trials were included, early administration of abciximab compared to late/peri-procedural administration was associated with reduction in mortality (20% *vs* 24.6% P = 0.02), improvement in pre-procedural (TIMI) 3 flow (21.6% *vs* 10.1%, P < 0.0001), post-procedural TIMI 3 flow (90% *vs* 84.8%, P = 0.04), post-procedural myocardial blush grade (52.0% *vs* 43.2%, P = 0.03), ST-segment resolution (58.4% *vs* 43.5%, P < 0.0001) and distal embolization (10.1% *vs* 16.2%, P = 0.02). There was no difference in the rates of major bleeding complications between early and late abciximab administration (3.3% *vs* 2.3%, P = 0.4). Adjunctive use of GP Ⅱb/Ⅲa inhibitors can be considered at the time P-PCI if there is evidence of large thrombus or inadequate response to a P_2Y_{12} antagonist[179,180]. Based on the data from the HORIZONS-AMI[158] and CICERO[181] trials, a GP Ⅱb/Ⅲa receptor antagonist can be used as adjunct to bivalirudin in the presence of large thrombus or for "bail-out use" for procedure related dissection. Similar findings were also confirmed in a recent meta-analysis by Shimada *et al*[182]. In a recent MI trail[183], intra-coronary infusion of abciximab reduced infarct size at 30 d. This approach should be considered on an individual patient basis[182,183].

ROLE OF CARDIOPROTECTION IN STEMI

Despite significant improvements in every area of STEMI management, adverse event rates continue to be high. Although, reperfusion therapy and the adjunctive pharmacotherapy help reestablish coronary flow, restoration of coronary blood flow can cause further injury to cardiac myocytes. This type of injury is called lethal reperfusion injury. In animal models, close to 50% of the final infarct size is due to lethal reperfusion injury[184]. This injury results from oxidative stress[185,186], calcium overload[187,188], inflammation[189] and rapid restoration of pH[189]. Understanding these

mechanisms at a cellular level has led to renewed interest in designing treatment strategies that target pathways that mediate lethal reperfusion injury. These strategies mediate their cardioprotective effect by multiple signaling pathways such as reperfusion injury salvage kinase (RISK) group of protective kinases. The cardioprotective signaling pathways inhibit the mitochondrial permeability transition pore and multiple other molecules[190].

PRECONDITIONING

Repeated, brief episodes of coronary occlusion with myocardial ischemia alternating with coronary reperfusion before a prolonged episode of ischemia, is a powerful way to limit infarct size. This is known as ischemic pre-conditioning[191]. However due to the fact that the brief episodes of ischemia need to be applied prior to an ischemic event, this approach has limited value in the setting of STEMI.

POST CONDITIONING

Applying the principles of preconditioning after the ischemic event has been shown to be beneficial in animal models[192,193]. In a small randomized control trial, Staat *et al*[194] showed that post-conditioning by 4 cycles of 1-min coronary angioplasty balloon inflations followed by 1 min of balloon deflation within 1 min of coronary reflow after deployment of a coronary stent reduced infarct size and improved myocardial blush grades. Similar findings have also been noted in other small studies that used different balloon inflation and deflation protocols[195]. A significant limitation of catheter/balloon based post-conditioning is that it is limited to cardiac catheterization laboratories at the time of P-PCI.

Post conditioning by cyclosporine

Cyclosporine has been shown to be cardioprotective by inhibiting the mitochondrial permeability transition pore[196]. In a small randomized study of 58 patients, single bolus of 2.5 milligrams of intravenous cyclosporine, compared to placebo reduced infarct size by 40% as quantified by the degree of plasma creatine-kinase elevation[197]. The cardioprotective effect of cyclosporine appears to be promising.

REMOTE ISCHEMIC CONDITIONING

Transient, repeated episodes of ischemia when applied to an organ distant from the heart have been shown to reduce infarct size[198]. This is called remote ischemic conditioning. One proposed mechanism is the release of a chemical by the distant organ that promotes cardiac conditioning. Another possibility is afferent neural pathway stimulation. In a study by Bøtker *et al*[199], 333 patients with a suspected first STEMI were randomly assigned in a 1:1 ratio to receive P-PCI with or without remote conditioning that consisted of intermittent arm ischemia through four cycles of 5-min inflation and 5-min deflation of a blood-pressure cuff. The patients received remote conditioning during transport to hospital, and P-PCI in hospital. The primary endpoint of myocardial salvage index at 30 d, measured by myocardial perfusion imaging, was significantly improved in the preconditioning group (0.75 in the remote conditioning group *vs* 0.55 in the control group, $P = 0.0333$). Given the ease of use and potential universal applicability of this approach, large-scale trials are underway to study this treatment.

ROLE OF ADENOSINE

Adenosine, by mediating its effects *via* A_1 and A_3 receptors appears to play a key role in promoting a cardioprotective a state. Although the mechanisms are complex, inhibition of the formation of mitochondrial permeability transition pores appears to be a primary mechanism[200]. Intravenous infusion of adenosine in patients with STEMI was tested in the Acute Myocardial Infarction Study of Adenosine Ⅰ trail[201]. Although there was 33% relative reduction in the infarct size, this was mostly limited to individuals with large anterior wall MI. Based on this study, the Acute Myocardial Infarction Study of Adenosine Ⅱ trail[202] randomized 2118 patients to 3-h intravenous infusion of low-dose adenosine (50 μg/kg per minute), high-dose adenosine (70 μg/kg per minute), or placebo before PCI or within 15 min of the initiation of fibrinolysis. There was no difference in the composite endpoint of death, new-onset congestive heart failure, or rehospitalization for congestive heart failure within 6 mo. However, subsequent post-hoc and subgroup analyses showed that there was significant reduction in the infarct size in those who received the high dose and those who received adenosine within 3 h of onset of symptoms[203]. Although the routine use of adenosine is currently not supported, early administration of high dose adenosine may reduce infarct size in patients with anterior wall STEMI with large areas of myocardium at risk.

ROLE OF BETA BLOCKERS

Most of the data on the role of routine use of beta blockers in STEMI either predates or involves thrombolytic therapy. There is very limited data on the cardioprotective benefits of beta blockers in the setting of PPCI. In the recently published Effect of Metoprolol in Cardioprotection during an Acute Myocardial Infarction trail[204], 270 patients with STEMI (Killip Class 2 or less) presenting within 6 h of onset of symptoms were randomized to receive intravenous metoprolol or no metoprolol. The primary endpoint of infarct size by magnetic resonance imaging was smaller after intravenous metoprolol compared with control (25.6

± 15.3 gm *vs* 32.0 ± 22.2 gm, *P* = 0.012). This trail illustrates that a commonly used inexpensive medication may play a significant role in cardioprotection in the setting of reperfusion by P-PCI for STEMI. Larger clinical trials that are powered to analyze hard clinical endpoints are needed to definitively understand the role of intravenous beta blockers in the setting of P-PCI.

The ideal duration of treatment with beta blockers after a STEMI is not well established. At the present time most patients are treated indefinitely with beta blockers after a STEMI. This is mostly based on evidence from a large meta-analysis that included 50000 patients and showed a 23% reduction in mortality at a mean follow up of 1.4 years[205]. Lower rates of reperfusion, suboptimal utilization of medical therapy, and short duration of follow up limit this data.

In a recent meta-analysis by Bangalore *et al*[206], that included 21000 patients with mean follow up of 44 mo, the primary composite outcome of cardiovascular death, nonfatal MI, or nonfatal stroke in post myocardial infarction patients were not significantly different the group that was treated with β-blockers compared with those who were not. (16.93% *vs* 18.60% respectively, *P* = 0.14). At the present time, the data on duration of therapy with beta blockers after an MI in inconclusive. In patients with preserved left ventricular ejection fraction and without any evidence of arrhythmias and ischemia, beta blockers can most likely be stopped after one year.

ROLE OF ATRIAL NATRIURETIC PEPTIDES

The cardioptotective effects of atrial natriuretic peptides (ANP) was tested in the Human Atrial Natriuretic Peptide and Nicorandil as Adjunts to Reperfusion Treatment trail[207]. Following reperfusion by either PCI or fibrinolytic therapy, 569 patients with STEMI were randomized to receive a continuous infusion of ANP or placebo for 3 d. Compared to placebo, there was 14.7% reduction in infarct size by area under the curve for total creatine kinase. ANP infusion was also associated improved ejection fraction at 6 to 12 mo compared with controls (44.7% *vs* 42.5%). Over a median follow-up time of 2.7 years, cardiac death and rates of hospitalization were also reduced in the ANP group. Further large-scale studies are needed to fully understand the cardioprotective role of ANP.

ROLE OF HYPOTHERMIA

In animal models, moderate hypothermia (28 ℃-32 ℃) offers cardioprotection by altering signaling pathways[208]. The Cooling as an Adjunctive Therapy to Percutaneous Intervention in Patients With Acute Myocardial Infarction trail[209] failed to show overall statistically significant reduction in infarct size. However, in patients with large anterior STEMI who were cooled to temperatures of less than 35 ℃ prior to reperfusion there was a reduction in the infarct size (9.3% in treated patients *vs* 18.2% in controls; *P* = 0.05).

The Rapid Cooling by Cold Saline and Endovascular Cooling before Reperfusion in patients with ST-elevation Myocardial Infarction trail[210] randomized 20 patients with STEMI undergoing P-PCI to rapid hypothermia by an endovascular catheter or standard therapy. Temperatures < 35 ℃ were obtained in all hypothermia patients prior to reperfusion. The primary end point of infarct size by cardiac MRI was reduced by 38% in the hypothermia group.

However, in the CHILL-MI[211] trail, hypothermia resulted in only a trend towards reduction in infarct size in patients who presented within 4 h of symptoms onset and were cooled to 33 ℃ large scale randomized trials are needed to determine if hypothermia during or immediately prior to P-PCI will result in significant reductions in infarct size and clinical endpoints.

STEM CELLS IN THE TREATMENT OF ACUTE MYOCARDIAL INFARCTION

Because atherosclerotic coronary vascular disease is the major cause of death in the United States and has recently become a major cause of death throughout the world, stem cell therapy is being investigated in patients with acute myocardial infarction (AMI) to limit myocardial damage and possibly regenerate myocardium[212-214]. Two populations of stem cells have been examined in patients with myocardial infarctions and/or ischemic cardiomyopathies: adult bone marrow mononuclear cells and cardiac stem cells. Adult bone marrow mononuclear cells from bone marrow aspirates contain approximately 0.5%-3% hematopoietic and mesenchymal "progenitor" cells that secrete growth factors and cytokines that can limit myocardial inflammation and infarct size. These cells have limited ability to replicate, do not trans-differentiate into myocytes, but can chemoattract endogenous patient stem cells for repair of cardiac injury. Cardiac stem cells are specific undifferentiated progenitor cells found in the right atrial appendage and the ventricular apices of the heart that have paracrine effects, can chemoattract patient stem cells, and may transdifferentiate into myocytes for cardiac repair.

The initial ten year experience with autologous human bone marrow mononuclear cells (BMCs) in the treatment of patients with AMIs showed significant approximately 2%-3% (range 1.9%-5.4%) increases in left ventricular ejection fraction (LVEF), decreases in left ventricular end-systolic volume of -4.8 mL (range -1.4 to -8.2 mL) and reductions in myocardial infarction size of approximately 5.5% (-1.9% to -9.1%)[213,214]. These studies established that the direct intramyocardial or intracoronary administration of bone marrow mononuclear cells was safe and that significant side effects did not occur with administration of these

cells[213-214], see Tables 5 and 6[215-237]. However many of the initial clinical bone marrow cell studies consisted of small numbers of patients and not all the studies randomized patients to treatment with either bone marrow mononuclear cells or placebo (Tables 5 and 6)[215-237].

Since the initial treatment of patients with AMIs with BMCs, major questions have persisted about the use of these cells: what is the optimal cell for AMI treatment; when is the optimal time to inject cells for treatment; what is the viability of the stem cells prior to injection into patients; what is the best parameter to monitor cardiac patients after stem cell treatment. The LateTIME, the TIME, and the Swiss Myocardial Infarction Trials were multi-center trials that addressed the questions whether adult BMCs limit myocardial damage in comparison with patients treated with placebo and what is the optimal time of cell administration after AMIs.

LATETIME TRIAL

The LateTIME trial was a randomized double blind, placebo-controlled trial designed to determine if delivery of adult BMCs two to 3 wk after primarily anterior wall myocardial infarction would be safe and effective in limiting infarct size and improving left ventricular (LV) function[238]. All patients in this study were successfully treated initially with primary percutaneous coronary intervention (PCI) within a median time of 4 h after the onset of chest pain. Bone marrow mononuclear cells were isolated from bone marrow aspirates in each center with a closed, automated system (Sepax, Biosafe). The Infarct volume and LV global and regional LV function were measured by magnetic resonance imaging (MRI) with gadolinium prior to intracoronary injection and 6 mo after injection. The LVEF prior to intracoronary infusion of cells or placebo in 87 AMI patients averaged 48.7% in the bone marrow cell (BMC) group and 45.3% in the placebo group. 150 × 10^6 autologous BMCs or placebo were infused in 87 patients. The changes between baseline and 6 mo in the BMC group for infarct volume, LVEF, wall motion in the infarct zone, and wall motion in the border zone of the infarction were not statistically different from the placebo group[238].

TIME TRIAL

The TIME Trial was a double-blind, placebo controlled trial that investigated the intracoronary administration of autologous BMCs or placebo in 120 patients three or seven days after primarily anterior AMI[239]. The TIME Trial was based on the REPAIR AMI trial that reported that delivery of BMCs to patients 5 to 7 d after AMI resulted in a 5.1% absolute increase in LVEF[239,240].

All patients had successful coronary reperfusion with coronary angioplasty within a median time of 3-4 h of the onset of ischemic symptoms. The mean LVEF in these patients was $\leqslant$ 45% by echocardiography. The mean time from PCI to bone marrow aspiration and cell processing was 3.3 d in the day 3 group and 7.4 d in the day 7 group. Bone marrow mononuclear cells were isolated in each center with a closed, automated system (Sepax, Biosafe) and the cells or placebo were infused into the coronary arteries within 12 h of aspiration and cell processing. The BMC contained 2.3% CD34 and 1.1% CD34 plus CD131 hematopoietic cells. All patients had baseline cardiac MRIs with gadolinium at day 3 or at day 7 after AMI and the MRIs were repeated at 6 mo after the AMI.

Forty-three patients received BMCs on day 3 and 36 patients received bone marrow cells on day 7 after AMI. Each patient received approximately 147 × 10^6 BMCs within 12 h of aspiration and cell processing. Forty-one patients received a placebo. The median time from bone marrow aspiration to infusion directly into the infarct related coronary artery was 8.3 h. In addition, all patients received heparin during the procedure as well as aspirin and clopidogrel.

The differences between the BMC treatment and the placebo treatment in the 3 d group and in the 7 d group were not significant. When both BMC groups were combined (n = 75) to include patients with MRI measurements at baseline and 6 mo and compared with the combined placebo group (n = 37), LVEF in the BMC group increased from 45.2% at baseline to 48.3% at 6 mo while in the combined placebo group the LVEF increased from 44.5% to 47.8% (P = NS). Moreover, there was no significant difference between the changes in regional wall motion in the infarct zone and the border zone between BMC and placebo groups. Infarct volumes uniformly decreased in both groups but the differences between groups were not statistically significant. No difference was observed in global or regional function in patients stratified by myocardial ischemic time.

SWISS MULTICENTER INTRACORONARY STEM CELL STUDY IN ACUTE MYOCARDIAL INFARCTION TRIAL

The Swiss Study[241] randomized patients with AMIs with LVEF < 45% as measured by ventriculography or echocardiography, who had been successfully treated with PCI of the infarct related artery within a median of 5 h of onset of chest pain, to either the intracoronary administration of 140-160 million autologous BMCs at a median of 6 d after AMI (early group, n = 58) or at a median of 24 d after AMI (late group, n = 49) or to a placebo group (n = 60). Ninety-two percent of the patients had anterior wall infarctions. Bone marrow aspirates were performed only in patients assigned to the BMC treatment. Each 10 mL aspirate was treated with 1000 IU Heparin to prevent clot formation. The

Table 5 Bone marrow and circulating progenitor cells in coronary artery disease patients

Ref.	*n*	Randomize	Time post PCI and/or MI	Cell dose	Injection route	Baseline LVEF	LVEF change	Duration	Other findings
Assmus *et al*[215]	92	Yes	2348-2470 d	22 ± 10^6 CPC $205 \pm 110 \times 10^6$ BMC	IC	CPC 39% ± 10% BMC: 41% ± 11%	CPC -0.4% BMC 2.90%	3 mo	Pts with previous MI; ↑ LVEF in BMC but not CPC
Bartunek *et al*[216]	35	Cohort	10 d	$12.6 \pm 2.2 \times 10^6$	IC	45% ± 2.5%	7%	4 mo	↑ LV regional function, perfusion; restenosis ↑
Chen *et al*[217]	69	Yes	18.4 ± 0.5 d	$8\text{-}10 \times 10^9$	IC	49% ± 9%	18%	6 mo	↑ LVEF by ventriculogram ↑ perfusion; ↓ ESV
Erbs *et al*[218]	26	Yes	225 ± 87 d	$69 \pm 14 \times 10^6$	IC	51.7% ± 3.7%	7.20%	3 mo	Pts with chronic CAD occlusion Rxed with CPC; ↓ EF by MRI; infarct size 16%
Ge *et al*[219]	20	Yes	1 d	$39 \pm 22 \times 10^6$	IC	53.8% ± 9.2%	4.80%	6 mo	↑ Perfusion by SPECT
Hendrikx *et al*[220]	20	Yes	217 ± 162 d	$60 \pm 31 \times 10^6$ IM	IM	42.9% ± 10.3%	5%	4 mo	CABG in Pts with previous CAD ↑ Regional but not global LV function; 6/9 with induced ventricular tachycardia
Janssens *et al*[221]	67	Yes	1 d	172×10^6	IC	48.5 ± 7.2	3.30%	4 mo	↓ Infarct size
Kang *et al*[222]	96	Yes	< 14 d AMI; > 14 d OMI	$1\text{-}2 \times 10^9$	IC	52.0 ± 9.9	5.10% AMI	6 mo	G-CSF for 3 d; ↓ ESV and infarct size in AMI; = EF, ESV and infarct size in OMI
Katritsis *et al*[223]	22	Cohort	224 ± 464 d	$2\text{-}4 \times 10^6$	IC	39.7% ± 9.3%	1.60%	4 mo	↑ Regional but not global LV function
Lunde *et al*[224,225]	100	Yes	6 ± 1.3 d	68×10^6 (median) $54\text{-}130 \times 10^6$	IC	41.3 ± 11.0	=	6-12 mo	↑ LVEF in treated and controls; = EDV and infarct size
Meyer *et al*[226]	60	Yes	4.8 ± 1.3	$24.6 \pm 9.4 \times 10^8$	IC	50 ± 10	5.90%	18 ± 6 mo	↑ LVEF by MRI significant at 6 but not 18 mo
Mocini *et al*[227]	36	Cohort	AMI < 6 mo	$292 \pm 232 \times 10^6$ IM	IM	46% ± 6%	5%	3-12 mo	CABG in all; troponin increased
Perin *et al*[228]	20	Cohort	ICM	$25.5 \pm 6.3 \times 10^6$	IM Trans-Endo-cardial	30% ± 6%	5.10%	12 mo	LVEF = Controls; ↑ LV perfusion ↑ Exercise
Ruan *et al*[229]	20	Yes	Approximately 1 d	NR	IC	53.5% ± 5.8%	5.80%	6 mo	↑ LV segmental contraction
Schächinger *et al*[230,231]	204	Yes	3-8 d	2.4×10^8	IC	48.3% ± 9.2%	6%-7%	4-12 mo	↑ EF when Rx > 4 d post MI and when EF ↑ ≤ 48.9; LV perfusion
Strauer *et al*[232]	20	Cohort	5-9 d	$2.8 \pm 2.2 \times 10^7$ IM	IC	57% ± 8%	5%	3 mo	↑ Regional but not global LVEF; ↓ ESV and ↓ Infarct size
Li *et al*[234]	70	Yes	7 ± 5 d	$7.3 \pm 7.3 \times 10^7$	IC	50% ± 8.2%	7%	6 mo	G-CSF for 5 d; ↓ LV ESV, ↓ LV wall motion score

NR: Not recorded or equals no change; CPC: Circulating progenitor cells; BMC: Bone marrow cells; ICM: Ischemic cardiomyopathy; IC: Intracoronary injection; IM: Intramyocardial injection; AMI: Acute myocardial infarction; OMI: Old myocardial infarction; G-CSF: Granulocyte colony stimulating factor; ESV: LV end-systolic volume; SPECT: Single photon emission computer tomography. Adapted from Henning[213].

BMCs were isolated by density gradient centrifugation at a centralized processing facility and contained 1% to 1.3% $CD34^+$ hematopoietic cells. However, the median percentage of mononuclear cells that exhibit migration capacity was only 29%[241].

Cardiac magnetic resonance imaging with gadolinium was performed on patients at baseline prior to cell infusion and at 4 mo after the injection of BMCs into the infarct-related coronary artery and were compared with MRIs of control patients treated with best medical care. At 4 mo after coronary infusion, there were no significant differences in infarct scar size or LV myocardial wall thickening in patients treated with BMCs at either 5-7 d or 3-4 wk after AMI in comparison with control patients. Moreover, LV function did not significantly improve at 4 mo after the intracoronary infusion of autologous BMCs in either the early or late treated groups in comparison with the placebo group.

Table 6 Stem cells in the treatment of patients with acute myocardial infarction

Ref.	*n*	Random-ized	Time post MI	Cell dose	Baseline	LVEF	Duration	Other findings
Strauer *et al*[232]	20	Cohort	8 d	$2.8 \pm 2.2 \times 10^7$	57% ± 8%	5%	3 mo	↑ Regional but not global LVEF ↓ LV ESV and infarct size
Bartunek *et al*[216]	35	Cohort	10 d	$12.6 \pm 2.2 \times 10^6$	45% ± 2.5%	7%	4 mo	↑ LV regional function, ↑ perfusion; ↑↑ restenosis
Li *et al*[234]	70	Yes	6 d	$7.3 \pm 7.3 \times 10^7$	50 ± 8.2	7%	6 mo	↓ LV ESV, LV wall motion score
Janssens *et al*[221]	67	Yes	1 d	172×10^6	48.5 ± 7.2	3.30%	4 mo	↓ Infarct size
Meyer *et al*[226] and Wollert *et al*[237]	60	Yes	4.8 d	24.6×10^8	50.0 ± 10.0	=	6-18 mo	↑ LVEF at 6 but not at 18 mo
Kang *et al*[222]	96	Yes	4 d	$1\text{-}2 \times 10^9$	52.0 ± 9.9	5.1% AMI	6 mo	↓ LV ESV and infarction in acute MI; = ESV and = old MI
Lunde *et al*[224,225]	100	Yes	6 d	68×10^6	41.3 ± 11.0	=	6-12 mo	LVEF ↑ in treated and controls; = EDV and infarct size
Ge *et al*[219]	20	Yes	1 d	4×10^7	53.8 ± 9.2	4.80%	6 mo	↑ LV regional wall perfusion by SPECT
Meluzín *et al*[235,236]	66	Yes	5-9 d	$10^7\text{-}10^8$	42 ± 0.0	3-5	3-12 mo	↑ LVEF 3% @10^7 ↑ LVEF 5%-7% @ 10^8 3-12 mo
Schächinger *et al*[230,231]	204	Yes	3-8 d	2.4×10^8	48.3 ± 9.2	6-7	4-12 mo	↑ EF when Rx > 4 d post MI and when EF < 48.9%; ↑ LV perfusion

Adapted from Henning[213]. MI: Myocardial infarctions; LVEF: Left ventricular ejection fraction.

However, patients with NT-proBNP levels at baseline above 1437 ng/L experienced a greater increase in LVEF of 7.1% in the early group and 9% for the late BMC group. In all cell and placebo treatment groups, LV scar as determined by late gadolinium enhancement on MRI decreased by more than 10 g with a 4%-5% decrease in the ratio of myocardial scar to myocardial mass.

ASSESSMENT OF LATETIME, TIME, AND SWISS TRIALS

Several variables in these studies contributed to the lack of significant improvement of AMI patients treated with BMCs in comparison with placebo treated patients.

Early percutaneous coronary intervention

Patients with AMIs in the LateTIME, TIME, and Swiss Multicenter Trials were treated with PCI within a median of 4 to 5 h of the onset of chest pain. Thereafter, the patients were treated with American and European Heart Association guided best medical therapy. Consequently, AMI sizes and the extent of LV remodeling in the different trial patients were significantly limited and the differences between BMC treated patients and placebo treated patients were small. Although the initial qualifying LV ejection fractions by echocardiography after PCI in the LateTIME and TIME trial patients were ⩽ 45%, the LVEFs by MRI at the time of BMC injection were greater than 45%. Bone marrow mononuclear cells are much less effective in patients with small myocardial infarctions with near normal LVEFs. In addition, placebo treated patients continue to improve with best medical therapy after AMIs as demonstrated by the control patients in the Bone Marrow Transfer to Enhance ST-elevation Infarct Regeneration (BOOST) trial in which the LVEFs equaled or exceeded the increases in the LVEFs in the BMC treated patients at 18 mo after AMI[242]. In addition, the Valsartan in Acute Myocardial infarction Trial and trials of neuro-hormonal blockade of patients with AMIs have demonstrated that optimal medical therapy can increase LVEF by a mean of 2.7% at 20 mo[243]. Consequently much larger numbers of patients will be required in clinical trials to demonstrate statistically significant differences between BMC treated patients and placebo treated patients who receive PCI early after the onset of AMI and guideline directed optimal medical therapy.

Heterogeneous bone marrow cell populations

Unfractionated adult BMCs contain less than 3% $CD34^+$ and 1% $CD34^+/CD133^+$ hematopoietic progenitor cells and ⩽ 1% $CD105^+$ mesenchymal stem cells in healthy subjects when marrow cells are separated by Ficoll density gradient-based separation. However, both $CD34^+$ endothelial colony number and the mesenchymal cell colony number were significantly decreased amongst subjects that participated in the LateTime and Time Trials[244]. In addition, the marrow aspirates in the LateTIME and TIME Trials were separated by an automated cell process system (Sepax, Biosafe), which recovered only 23.6% of the total nucleated cells[245]. Consequently, the BMCs delivered in the LateTIME and TIME trials contained smaller numbers of $CD34^+$ and $CD105^+$ cells than earlier BMC studies. Moreover, stem cell motility can decline by as much as 68% 72 h after harvest from the bone marrow[246]. In addition, 140-150 × 10^6 unfractionated BMCs may not be the most optimal dose of BMCs for stem cell treatment of patients with AMI. In this regard, BMCs from patients with advanced age and patients with chronic diseases, such as ischemic heart disease or diabetes mellitus, are often functionally impaired, propagate poorly,

and have a shortened life span[246-248]. Consequently, BMC colony forming units and cell migration capability must be determined in addition to bone marrow cell number and viability prior to use in the treatment of AMI. Furthermore, BMCs produced only a modest increase in the LVEF of approximately 2%-3% in earlier analyses of stem cell trials of patients with AMIs or ischemic cardiomyopathies[213,214]. Despite well conducted clinical trials, unfractionated BMCs selectively infused into an infarct related coronary artery have a small therapeutic effect and may not be the most optimal cells for the treatment of patients with AMIs.

Red blood cell contamination of stem cells

Red blood cell contamination of bone marrow mononuclear cells can significantly decrease the migration ability and the efficacy of BMCs. Large numbers of red blood cells in the cell preparations cause reduced BMC viability, decreased colony forming capacity, and are associated with reduced recovery of LVEF in patients with AMIs[249]. In patients in the REPAIR-AMI Trial, contamination of the bone marrow cells with red blood cells prior to infusion into patients with AMI independently predicted reduced recovery of LVEF[249]. Moreover, the addition of red blood cells to BMCs dose-dependently decreased neovascularization in ischemic hind-limbs compared to treatment with BMCs without red blood cells[249]. The mechanism by which red blood cells interfere with bone marrow cell propagation, migration and neovascularization involves a dose-dependent reduction of BMC mitochondrial membrane potential and a decrease in BMC mitochondrial adenosine triphosphate (ATP) production[249]. As a consequence of decreased mitochondrial metabolism and function, stem cell self-renewal and differentiation are decreased.

Heparin decreases stem cell migration

Heparin can bind to the chemoattractant stromal derived factor-1 (SDF-1), which is released from ischemic myocardium, and also bind to its receptor CXCR4 on stem cells and thereby block CXCR4 signaling and stem cell migration to injured myocardium[250]. Heparin, in a dose-dependent manner, can inhibit SDF-1 induced BMC migration and homing of BMCs to areas of myocardial ischemia[250-253]. Incubation of BMCs with 20 U/mL of heparin for 30 min abrogates SDF-1 BMC migration by 84% *in-vitro* and significantly reduces the homing of injected BMCs to injured and infarcted myocardium by 50% in research animals[250]. Decreased migratory capacity of BMCs also correlates with reduced neovascularization and decreased functional capacity in subjects with limb ischemia[251]. In addition, heparin decreases the concentration of vascular endothelial growth factor (VEGF) in ischemic tissue and thereby decreases neovascularization[252]. Heparin also interferes with activation of the cell survival factor Akt (Protein Kinase B) by SDF-1-CXCR4 signaling and in this manner interferes with cell survival and growth. In contrast, the thrombin inhibitor bivalirudin does not interfere with BMC homing or SDF-1/CXCR4 signaling and does not decrease VEGF[252].

Stem cell expulsion from myocardium

Ninety to 97% of unfractionated BMCs leave the myocardium in less than 2 h after injection directly into the myocardium or into the coronary arteries[254,255]. Most of the cells are ejected out of the myocardium through the myocardial injection sites or through the coronary veins and lymphatics into the right heart due to the massaging action of the contracting myocardium. The cells are ultimately lodged in the lungs, liver, spleen and kidneys. In addition, approximately 12% of BMCs are retained in the catheter delivery system after injection[255]. With the intravenous injection of BMCs or other cells for cardiac repair the majority of the cells become entrapped in the lungs. Consequently, fourfold greater numbers of cells are required above that required for intramyocardial or intracoronary injection for repair of myocardial infarctions[256].

Future bone marrow cell studies

The BAMI Trial (The effect of intracoronary reinfusion of bone marrow derived mononuclear cells on all-cause mortality in acute myocardial infarction) is recruiting 3000 patients with LVEFs $\leqslant$ 45% within 7 d of AMIs, who have undergone successful coronary reperfusion therapy, for randomization into treatment with either intracoronary autologous unfractionated bone marrow mononuclear cells or placebo[257]. Hopefully the BAMI Trial will avoid the important variables that have been described in this paper and will provide definitive answers to the questions whether BMCs can significantly decrease patient mortality due to myocardial infarction, substantially reduce infarct size and increase LVEF over three years in comparison with patients treated with best medical therapy.

CARDIAC STEM CELLS

Cardiovascular investigators have sought alternatives to BMCs for cardiac repair in patients with ischemic heart disease. Cardiac stem cells, which are multi-potent progenitor cells, are present in niches in the heart and contribute to the physiological turnover of myocytes and vascular endothelial cells in the heart. The number of cardiac stem cells in the heart is estimated at one cardiac stem cell per 10000 cardiac myocytes[258]. Consequently, endogenous cardiac stem cells are not normally able to reverse heart damage due to myocardial infarctions. The turnover of cardiac myocytes occurs at rates estimated to be 1% to as much as 22% per year and is dependent on the age, sex, and the health of the individual[259,260]. Two major types of autologous cardiac stem cells have been investigated in patients with injured and infarcted myocardium in the SCIPIO and CADUCEUS clinical trials: C-kit + lineage negative cardiac stem cells

isolated from right atrial appendages and cardiosphere derived cells (CDCs) grown from right ventricular cardiac muscle biopsies.

C-KIT + STEM CELLS

C-kit is a receptor for stem cell factor, which is released from the ischemic myocardium, and is important in the chemoattraction of stem cells to apoptotic, injured and necrotic myocardium. C-kit + stem cells have the capacity for self-renewal, clonogenicity and multi-potency[261,262]. These stem cells can express the cardiac transcription factors GATA-4, Nkx2.5 and MEF2 and can differentiate into myogenic, vascular endothelial and smooth muscles cells *in-vitro*[261,262]. In research animals with AMIs, cardiac stem cells can form new myocardium[262].

Autologous C-kit cardiac stem cells from right atrial appendages have recently been used for the treatment of patients with myocardial infarctions and ischemic cardiomyopathies in the open labeled Cardiac Stem Cell Infusion in Patients with Ischemic Cardiomyopathy (SCIPIO) Trial[263-265]. In this trial C-kit positive stem cells were isolated during coronary artery bypass surgery from the right atrial appendages of patients with LVEFs < 40%. The cells were then propagated in the laboratory. Four mo later, a maximum of one million cardiac stem cells were injected directly into the patient's saphenous vein grafts and coronary arteries supplying infarcted myocardium. The two year results of this trial have been presented at the American Heart Association Scientific Sessions in November of 2012 and 2013. In the SCIPIO trial the LVEF, measured by three-dimensional echocardiography and by MRI with gadolinium in patients who received cardiac stem cells, increased in 12 patients in absolute units by 11.9% at 2 years[265]. Left ventricular scar, determined by MRI, decreased by as much as 20.4 g at 2 years (n = 6) and was associated with an increase in viable myocardium of 17.9 g (n = 6) at 2 years[265]. New York Heart Association Functional Class score decreased in these patients by 0.77 at 2 years (n = 13). A left internal mammary graft dissection occurred in one treated patient, which was treated with a graft stent, and a peri-procedural myocardial infarction occurred in a second treated patient[264]. In this study, C-kit cardiac stem cells were proposed to chemoattract patients' native stem cells to areas of myocardial injury and also to transdifferentiate to myocytes for cardiac repair. A Phase 2 trial of safety and efficacy of C-kit cardiac stem cells in a larger group of patients is currently being planned.

CARDIOSPHERE DERIVED CELLS

Percutaneous endomyocardial biopsy specimens of the right ventricular septal wall and apex in patients, when grown in culture, can yield spherical multicellular clusters termed "cardiospheres". Cardiospheres are a mixture of stromal, mesenchymal and hematopoietic progenitor cells that contain cells that express CD 105 (commonly associated with mesenchymal stem cells) and partially express C-kit[266,267]. Cardiosphere derived cells (CDCs), when injected into the border of myocardial infarctions in mice, engraft and increase viable myocardium[268]. The functional benefit of CDCs is predominantly due to the secretion of growth factors and the recruitment of endogenous stem cells to injured and infarcted myocardium for myocyte generation. In this regard, cardiospheres and CDCs secrete the growth factors angiopoietin-2, basic fibroblastic growth factor, hepatocyte growth factor, insulin-like growth factor 1, stromal derived factor-1 and vascular endothelial growth factor which are beneficial in repair of injured myocardium[267,268].

Autologous CDCs have been investigated in the open labeled Cardiosphere-derived Autologous Stem Cells to Reverse Ventricular Dysfunction (CADUCEUS) Trial[269,270]. In this trial, 17 patients, post myocardial infarction with LVEFs of 25%-45%, underwent endo-myocardial biopsies of the right ventricular septum. Cardiosphere derived cells were obtain from cultures of the endomyocardial biopsies and the cells were propagated. In this trial, between 12.5 and 25 million CDCs were then given directly into the infarct related coronary artery of each of the 17 patients 1.5 to 3 mo after their myocardial infarctions. The one year followup of 12 of the 17 patients treated with autologous CDCs and 8 control patients have been reported[270]. Left ventricular scar by MRI significantly decreased by a mean of 11.9 g in CDC-treated patients and by 1.7 g in control patients. Left ventricular viable mass increased by a mean of 22.6 g in treated patients in comparison with 1.8 g in control patients. Left ventricular ejection fractions did not significantly increase but the regional wall function of infarcted segments did increase and correlated with the decrease in LV myocardial scar size[269,270]. Covariate statistical analysis demonstrated that the lower percentage of infused $CD90^+$ cells caused the greatest reductions in scar size in patients with large infarctions. A Phase 2 study of CDCs (ALLSTAR Trial) is currently in progress that involves allogeneic CDCs for the treatment of patients after myocardial infarction.

ASSESSMENT OF THE SCIPIO AND CADUCEUS TRIALS

In the SCIPIO trial 1545 patients were evaluated. Two hundred thirteen patients had LVEFs < 40% and 20 patients were treated with C-kit + stem cells. Twelve of 20 patients had MRI determinations of left ventricular function whereas the control patients did not have MRI determinations of left ventricular function. In the CADUCEUS Trial 436 patients were evaluated and 17 patients received CDCs. Consequently, these

trials report a highly selected patient population and the results of these trials cannot be applied to all patients with myocardial infarctions and ischemic cardiomyopathies. Much larger trials are necessary of each of these cell types in patients with myocardial infarctions.

In each of these studies, LV infarction was defined by MRI of delayed enhancement of myocardium in the region of coronary artery occlusion/reperfusion due to gadolinium that leaked from myocardial capillaries and pooled in the myocardial interstitial spaces and intracellular spaces. In these patients the gadolinium volume of distribution was increased and washout from the myocardium was reduced. However, cardiac stem cells can incorporate into damaged blood vessels, chemoattract endogenous stem cells that can form entirely new blood vessels, and can also secrete angiogenic growth factors that stimulate new blood vessels from preexisting vessels. Consequently, the blood vessels in the damaged myocardium of patients treated with these stem cells were less permeable to gadolinium[271]. Infarct scars can potentially appear smaller on MRI due to less gadolinium leak as well as contracture of the myocardial infarction. Moreover inter-scan variability and intra- and inter-observer variability in infarct measurements and interpreting MRI scans can account for some myocardial changes between pre- and post-stem cell infusion[272]. Rebuttals to these arguments against the use of contrast enhanced MRI in estimating infarct size and myocardial regeneration after stem cell treatment have been published[273]. The rebuttal is based on a porcine myocardial infarction study in which allogeneic CDCs decreased infarct scar size and led to cardiomyocyte hyperplasia on MRI and also on histological examination[273]. Nevertheless, anatomical and histological examinations of myocardial biopsies of infarcted hearts of patients or myocardial autopsy examinations of patients treated with these stem cells are necessary to determine if infarct fibrosis is significantly decreased and if substantial generation of new myocytes occurs. Trials of larger numbers of patients treated with C-kit + cardiac stem cells and cardiosphere derived cells for longer times are warranted to determine the precise mechanisms of action of these stem cells and their clinical benefit.

FUTURE DIRECTIONS

The LateTIME, TIME, Swiss, SCIPO and CAUDUCEUS Trials demonstrate that stem cells can be safely administered to patients with acute myocardial infarctions and ischemic cardiomyopathies and do not have significant adverse effects. Specific bone marrow cell subsets, such as unconditioned or conditioned mesenchymal cells or $CD34^+$ hematopoietic cells, may prove to be more efficacious in myocardial infarction repair than unfractionated BMCs[274,275]. In this regard, bone marrow mesenchymal stem cells or mesenchymal stem cells conditioned with cardiogenic growth factors have been reported to be beneficial in increasing LV function and functional capacity in patients with ischemic cardiomyopathies[275]. In addition, mesenchymal stem cells may enhance the beneficial effects of C-kit cardiac stem cells when these cells are administered together[276]. The large size of mesenchymal stem cells, however, requires that these cells be most safely delivered into the heart by direct myocardial injection rather than intracoronary injection in order to avoid problems of cell clumping and coronary occlusion. Mesenchymal stem cells and also umbilical cord stem cells are reported to be "immunoprivileged" and lack Class II human leukocyte antigens[277,278]. If allogeneic stem cells prove to be safe and effective in limiting myocardial damage and LV remodeling after myocardial infarction in patients, then these cells might become an "off the shelf" product that surpasses the significant limitations of inter-patient variability of unfractionated bone marrow mononuclear cells. Since the functional benefit of stem cells appears to be predominantly due to the secretion of biologically active factors, the ultimate rejection of allogeneic stem cells may not be of major concern if the rejection is delayed long enough to allow these cells to exert their paracrine effects. Nevertheless, stem cell trials must be performed in patients with large myocardial infarctions and LVEFs by MRI less than 40% at the time of stem cell administration because stem cells may not be efficacious in patients with small infarctions and near normal or normal LVEFs. In these studies, substantial stem cell viability, colony forming and migration capabilities must be established prior to infusion in patients.

A major problem with all stem cell trials is the short term engraftment and survival of stem cells in injured and infarcted myocardium. The cells that remain in the myocardium do not survive due to ischemia, inflammation, or anoikis or migrate from the myocardium in one to two weeks[254,255]. Consequently, stem engraftment in the heart must be increased in order to significantly enhance their beneficial effects. Possible treatment options include "conditioning" of the myocardium prior to stem cell delivery or co-delivery of stem cells directly into the myocardium with extracellular matrix molecules, nanofibers, hydrogels, or fibrin glues[213,214,279]. Co-delivery of stem cells with other molecules will require direct intramyocardial cell injection at the time of cardiac surgery or cardiac catheterization which appears to produce the greatest functional benefit[280]. Alternatively, stem cells can be administered in patches that are applied directly to the epicardial surface of the damaged myocardium at the time of cardiac surgery[213,214]. Direct stem cell to myocyte contact and interactions may be crucial in eliciting beneficial myocyte functional effects. In addition, genetic engineering of stem cells must be developed that facilitate the homing of stem cells to ischemic myocardium and the retention of the stem cells within the myocardium after intracoronary or intravenous injection.

A major mechanism of action of stem cells studied

to date in myocardial repair is the secretion of growth factors, chemokines, anti-inflammatory cytokines and exosomes or microparticles, which contain proteins, messenger ribonucleic acids and micro-ribonucleic acids. Hypoxic stress appears to increase the paracrine effects of stem cells[281,282]. Biologically active factors from stem cells can suppress inflammatory cytokines and inflammatory cells in the injured myocardium, improve myocardial metabolism, promote angiogenesis, inhibit myocyte and endothelial cell apoptosis, recruit endogenous progenitor cells to injured myocardium, and possibly stimulate surviving myocytes to re-enter the cell cycle and proliferate. The most efficacious stem cell biologically active factors must be identified, purified, and the pharmacologic effects established in research animals and ultimately in patients with injured myocardium.

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REVIEW

Mitochondrial function and regulation of macrophage sterol metabolism and inflammatory responses

Annette Graham, Anne-Marie Allen

Annette Graham, Anne-Marie Allen, Department of Life Sciences, School of Health and Life Sciences and the Institute for Applied Health Research (Diabetes Research Group), Glasgow Caledonian University, G4 0BA Glasgow, United Kingdom
Author contributions: Graham A and Allen AM conceived, drafted and approved the manuscript.
Conflict-of-interest: An international patent application (PCT/GB2014/052585) has been filed on behalf of Glasgow Caledonian University, relating to cholesterol modulation. No other conflicting interests, commercial, personal, political, religious, intellectual or otherwise, exist relating to this manuscript.

Correspondence to: Annette Graham, PhD, Department of Life Sciences, School of Health and Life Sciences and the Institute for Applied Health Research (Diabetes Research Group), Glasgow Caledonian University, Cowcaddens Road, G4 0BA Glasgow, United Kingdom. ann.graham@gcu.ac.uk
Telephone: +44-141-3313722
Fax: +44-141-3313208
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Abstract

The aim of this review is to explore the role of mitochondria in regulating macrophage sterol homeostasis and inflammatory responses within the aetiology of atherosclerosis. Macrophage generation of oxysterol activators of liver X receptors (LXRs), *via* sterol 27-hydroxylase, is regulated by the rate of flux of cholesterol to the inner mitochondrial membrane, *via* a complex of cholesterol trafficking proteins. Oxysterols are key signalling molecules, regulating the transcriptional activity of LXRs which coordinate macrophage sterol metabolism and cytokine production, key features influencing the impact of these cells within atherosclerotic lesions. The precise identity of the complex of proteins mediating mitochondrial cholesterol trafficking in macrophages remains a matter of debate, but may include steroidogenic acute regulatory protein and translocator protein. There is clear evidence that targeting either of these proteins enhances removal of cholesterol *via* LXRα-dependent induction of ATP binding cassette transporters (ABCA1, ABCG1) and limits the production of inflammatory cytokines; interventions which influence mitochondrial structure and bioenergetics also impact on removal of cholesterol from macrophages. Thus, molecules which can sustain or improve mitochondrial structure, the function of the electron transport chain, or increase the activity of components of the protein complex involved in cholesterol transfer, may therefore have utility in limiting or regressing atheroma development, reducing the incidence of coronary heart disease and myocardial infarction.

Key words: Atherosclerosis; Macrophage; Cholesterol; High density lipoproteins; Apolipoproteins; ATP binding cassette transporters; Scavenger receptor B1; Mitochondria (dys)function; Sterol 27-hydroxylase; Liver X receptors

Core tip: Mitochondrial cholesterol trafficking to CYP27A1 located on the inner mitochondrial membrane regulates the formation of oxysterol ligands for liver X receptors (LXRs) in sterol-laden macrophage "foam" cells. In turn, ligation of LXRα has profound implications for sterol removal and inflammatory responses in macrophage "foam" cells, both factors which may contribute to the effective resolution of atherosclerotic lesions and reductions in the incidence of coronary heart disease and its sequelae.

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INTRODUCTION

Coronary heart disease (CHD) is the major cause of morbidity and mortality worldwide, and the single largest cause of disease burden, determined according to disability-adjusted life years, the sum of life lost and years lived with disability[1,2]. Genetic factors contribute to coronary heart disease, fuelled by behavioural (smoking, physical inactivity, unhealthy diet, excess alcohol intake), metabolic (hypertension, diabetes, elevated serum cholesterol, overweight and obesity) and environmental (poverty, stress, educational status) factors[1-3].

Atherosclerosis is the primary cause of coronary heart disease characterised by chronic and unresolved inflammatory responses at sites of perturbed laminar blood flow in large and medium-sized arteries[4-6]. Activation of the arterial endothelial layer allows the accumulation of low density lipoprotein (LDL) within the intima of the vessel, where it can become modified *via* oxidation or crosslinking, triggering the recruitment of monocytes, neutrophils, lymphocytes and circulating stem cells to sites of inflammation[4-6]. Within this complex microenvironment, monocytes differentiate into macrophages which lie within a broad phenotypic spectrum, ranging from pro- (M1) to anti-inflammatory (M2)[6].

Arterial macrophages become laden with excess cholesterol and cholesteryl esters, part *via* the unregulated uptake of modified LDL by scavenger receptors (*e.g.*, CD36, CD68, LOX-1 and SR-AI/AII), and by phagocytosis of apoptotic cells, resulting in formation of "foam cells", a hallmark of early "fatty streak", developing, and unstable atherosclerotic lesions[7-10]. During the early phase of lesion development, this process may represent a protective mechanism; however, in more advanced lesions, cholesterol-laden macrophages, by releasing inflammatory cytokines and matrix metalloproteinases, contribute to chronic unresolved inflammation[10], accelerating the disease process and acute thrombotic events such as cerebrovascular stroke or myocardial infarction.

Thus, removal of cholesterol from macrophage "foam cells" may achieve successful regression and stabilisation of atheroma, and the importance of this pathway in protecting against CHD is supported by epidemiological studies in humans, and in genetically modified mice in which components of this pathway have been overexpressed or deleted. For example, HDL-cholesterol (HDL-C) emerged as an independent risk factor for cardiovascular disease in the Framingham Heart Study, offering a risk reduction of 2%-3% for each 1 mg/dL increase in HDL-C concentration[11,12]. HDL particles also possess antioxidant, anti-thrombotic and pro-fibrinolytic properties, and can counteract the chronic inflammation[13-16], proliferation of haemopoietic stem cells[17] and leucocytosis[10,18] which promote atherosclerosis. However, increasing the level of HDL-C, with niacin[19,20], fibrates[20] or dolcetrapib (dal-OUTCOMES Ⅲ trial)[20,21], does not necessarily confer protection against CHD[19-21] and in patients with systemic inflammation, coronary heart disease, chronic renal disease or diabetes, the protective properties of HDL are lost, and the particles transformed into those with pro-atherogenic potential[22-24]. Thus, it is not just the level, but the quality, composition (including levels of cargo molecules such as sphingosine-1-phosphate)[25] and function of HDL particles that are important.

Some, but not all, of the beneficial effects associated with HDL are mediated *via* the interaction of ATP binding cassette (ABC) transporters, such as ABCA1, ABCG1 and ABCG4, with apolipoproteins and HDL (Figure 1). While ABCA1 promotes efflux of cholesterol and phospholipids to lipid-poor apolipoproteins, such as apoA-I and apoE[13], ABCG1 and ABCG4 promote efflux of cholesterol, oxysterols and desmosterol to HDL[26]. Thus, these transporters together in a sequential manner to generate nascent HDL, which can then mature to HDL_3 and HDL_2 within the reverse cholesterol transport pathway in the bloodstream[25].

Both rare and common genetic variations in ABCA1 influence the levels of HDL-C[26] and risk of ischaemic heart disease (IHD). However, the association between ABCA1 variants and coronary disease seem to be independent of the plasma level of HDL-C[27]. Instead, cholesterol efflux from macrophages is strongly linked to atherosclerosis and provides a novel way of assessing cardiovascular risk that provides a greater level of prediction than HDL-C[28]. Thus the expression and activity of the ABCA1 protein, and the quality and functionality of the nascent HDL generated, may prove valuable discriminants of the risk of cardiovascular disease[29].

Importantly, macrophage ABCA1 expression and cholesterol accumulation are intrinsically linked to the inflammatory status of these cells. Excess cholesterol proves cytotoxic and pro-inflammatory if recycling *via* ABCA1 is disrupted in macrophages[30-33]. Enhanced Toll-like receptor signalling is noted in ABCA1/ABCG1 null macrophages, resulting in increased expression of pro-inflammatory genes, and free cholesterol accumulation[34], while activation of Toll-like receptors 3 and 4 represses induction of ABCA1 and reduces macrophage cholesterol efflux[35]. Conversely, interleukin-6 (IL-6) attenuates pro-inflammatory responses and stimulates efflux of cholesterol *via* ABCA1 in human macrophages[36]. In good agreement with this integrated paradigm, macrophage ABCA1 limits inflammatory responses *via* ApoA-I dependent activation of the Jak2/Stat3 pathway[37,38], while macrophage sterol

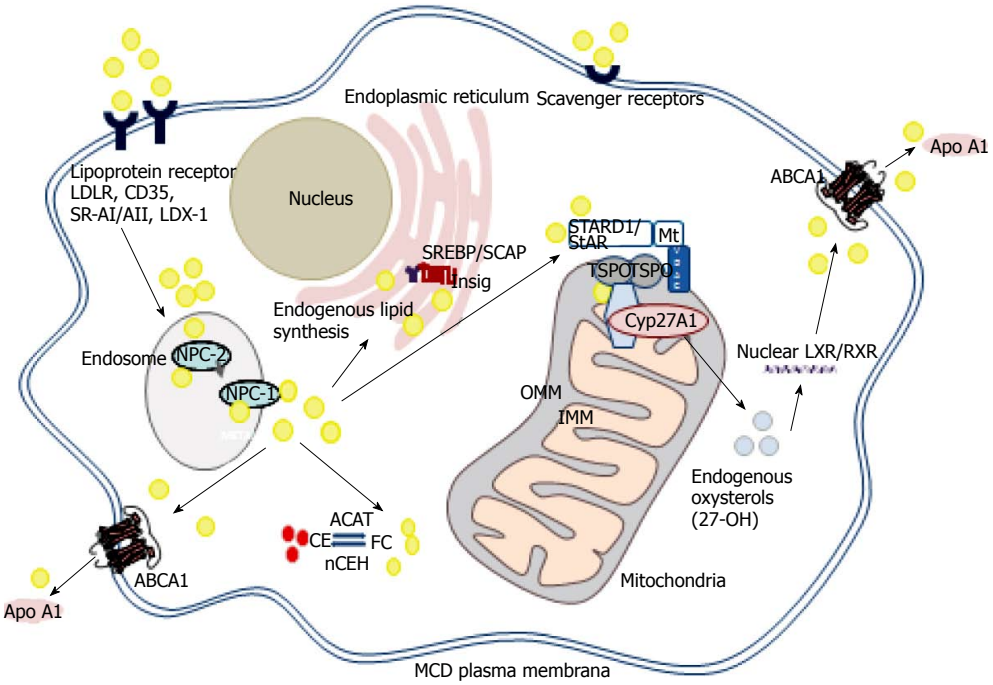

Figure 1 The role of mitochondrial cholesterol trafficking in regulation of macrophage sterol metabolism. Increased expression of steroidogenic acute regulatory protein (StAR, STARD1) or 18 kDa translocator protein (TSPO) drive cholesterol trafficking to mitochondrial sterol 27-hydroxylase (CYP27A1), enhancing endogenous production of oxysterols (24-, 25- and 27-hydroxycholesterol), in turn activating liver X receptors (LXR) and enhancing cholesterol efflux to apolipoprotein A-I (Apo AI) *via* ATP binding cassette transporter A1 (ABCA1). One current model for cholesterol transfer from the outer (OMM) to inner (IMM) mitochondrial membrane, derived from studies in steroidogenic cells, involves a complex of proteins, including StAR, TSPO, voltage-dependent anion channel (VDAC), regulatory subunits of protein kinase A (PKA-R1α), acyl CoA binding domains-1 and -3, ATPase family AAA domain-containing protein 3A (ASTAD3A) and optic atrophy type 1 proteins. Exogenous cholesterol delivered to the endocytic pathway *via* lipoprotein or scavenger receptors is transported either to the plasma membrane, enhancing cholesterol efflux *via* ABCA1, to lipid poor acceptors such as Apo AI or Apo E, or delivered to the endoplasmic reticulum (ER), retaining the Sterol Regulatory Element Binding Protein (SREBP)/SREBP-cleavage activating protein (SCAP) complex, in turn reducing cholesterol biosynthesis. Oxysterols enhance this process by binding to Insig-1/2 (insulin-induced gene-1 or -2). Excess cholesterol is esterified *via* Acyl CoA: Cholesterol Acyltransferase-1 (ACAT-1), and stored in lipid droplets within the cytoplasm as "foamy" droplets. nCEH: Neutral cholesteryl ester hydrolase; FC: Free cholesterol; CE: Cholesteryl ester: NPC-1/NPC-2: Niemann-Pick C1/C2 protein; StAR: Steroidogenic acute regulatory protein: RXR: Retinoic acid receptor.

accumulation activates Liver X Receptor nuclear (LXR) transcription factors, achieving induction of ABCA1 and ABCG1 and repression of inflammation (below)[39,40].

MACROPHAGE LIPID METABOLISM AND INFLAMMATION ARE REGULATED BY LIVER X RECEPTORS

Activation of nuclear LXRs (LXRα/β) is marshals cellular responses to increasing levels of sterol, promoting cholesterol efflux (above)[39-43]. Liver X receptors form heterodimeric complexes with retinoic acid receptors (RXRs), and bind to imperfect direct repeats of the nuclear receptor half-site TGACCT[39-43]. Ligand binding dissociates co-repressor proteins, destined for ubiquitination and proteasomal degradation, and engages co-activator proteins such as histone demethylases and G-protein pathway suppressor-2 (GPS2), stimulating target gene transcription[44].

Activation of LXRα also represses cholesterol biosynthesis *via* novel negative LXR DNA-response elements within the promoter region of squalene synthase and lanosterol 14α-demethylase and suppresses uptake of LDL[45,46]. Oxysterols also bind to Insig-1/2, facilitating sequestration of sterol-regulatory element binding proteins (SREBPs) at the endoplasmic reticulum, ensuring repression of cholesterol biosynthesis and uptake[45]. Deletions of LXRα and LXRβ in murine models of atheroma cause lipid accumulation within the aortic root, even in the absence of an atherogenic diet[47,48].

It is also evident that LXRs modulate innate and adaptive immune responses mediated by macrophages, neutrophils, lymphocytes, neutrophils and dendritic cells[45], decreasing cytokine-mediated expression of a range of pro-inflammatory genes. This is achieved *via* a mechanism involving nuclear receptor co-repressor (NCoR), silencing mediator of retinoid and thyroid receptors (SMRT) and inhibition of nuclear factor kappa B (NFκB) signalling[45,48,49]. Activation of LXRs is also achieved by phagocytosis of apoptotic cells by macrophages increasing expression of receptor tyrosine kinase (*Mertk*), amplifying phagocytosis and cell clearance, and reducing production of inflammatory mediators[50]. Absence of LXR signalling enhances the apoptosis of macrophages challenged with *Listeria monocytogenes*, *Escherichia coli* or *Salmonella typhinurium*, *via* loss of the anti-apoptotic factor AIM/

Spα[51,52].

MACROPHAGE GENERATION OF OXYSTEROL LIGANDS FOR LIVER X RECEPTORS

High levels of mitochondrial sterol 27-hydroxylase (CYP27A1) are found in human macrophages, and this enzyme can produce modified sterols, proven to act as LXR ligands *in vitro* and *in vivo*[53-56]. Loss of CYP27A1 leads to the lipid storage disease, cerebrotendinous xanthomatosis (CTX), which triggers accumulation of cholesterol and cholestanol in brain and tendons, progressive neurological deterioration, xanthomas and, as a secondary complication, accelerated atherosclerosis[57,58].

The rate-limiting step controlling CYP27A1 activity is the flux of cholesterol from the outer to the inner mitochondrial membrane, *via* a mitochondrial cholesterol trafficking complex (discussed below)[59]. Mitochondrial oxysterols therefore act as key cell signalling molecules, the levels of which can be moderated by sulfation (SULT2B1b), esterification (ACAT-1) or metabolism to soluble bile acid derivatives[53]. Conceivably, this process could be "uncoupled" by accumulation of free cholesterol at the interface between endoplasmic reticulum (ER) and mitochondrial membranes, triggering ER stress and proteasomal degradation of ABCA1, and opening of the permeability transition pore in mitochondria[53]. Esterification of excess oxysterols may then result: over 85% of the 27-hydroxycholesterol in human atherosclerotic lesions is esterified and incapable of activating LXRα and its downstream pathways[60,61]. Loss of this protective pathway predicates mitochondrial damage, apoptosis and cytotoxicity, features associated with addition of exogenous atheroma-relevant oxysterols ($\geqslant$ 20 μmol/L) to cultured cells[62].

Thus, it is clear that the biological impact of oxysterols are not solely restricted to LXR activation[63-67]. For example, oxysterols also serve as endogenous ligands for G-protein coupled receptor 183 (Epstein-Barr virus-induced gene 2, *EBI2*)[63], function as selective estrogen receptor modulators[64], bind to the Smoothened molecule to modulate Hedgehog signalling[65], while CYP27A1-derived 7α and 7β, 27-hydroxycholesterol modify innate and adaptive immune responses by acting as agonists of retinoic acid-related (RAR) orphan receptor gamma t (ROPRγτ)[66].

Acute exposure of macrophages to exogenous oxysterols induce rapid (< second) oscillations in cytoplasmic [Ca^{2+}] triggered by influx from the extracellular medium, followed by sustained increases in [Ca^{2+}] mediated by translocation of TRPC1 (transient receptor potential, canonical) channels into lipid rafts in the plasma membrane[68]. Calcium transfer between ER and mitochondria is facilitated by mitochondria-associated membranes, which act as a hub for lipid transfer, regulation of mitochondrial morphology (fission, fusion and trafficking), apoptosis, autophagy and ER stress[69], although the role of endogenously generated oxysterols in these processes remains unknown at present. Certainly, chronic exposure to exogenous oxysterol congeners can activate calcium release from the ER, increasing dephosphorylation of Bcl-2 antagonist of cell death by the calcium-dependent phosphatase calcineurin, and promoting apoptosis[68].

TARGETING PROTEIN CONSTITUENTS OF THE MITOCHONDRIAL CHOLESTEROL TRAFFICKING COMPLEX: IMPACT ON MACROPHAGE STEROL METABOLISM AND INFLAMMATION

Despite intensive investigations in steroidogenic cells and tissues, the nature of the mitochondrial cholesterol trafficking complex remains a matter of debate. One recent model suggests a basal complex, forming contact sites between the outer and inner mitochondrial membranes, composed of the 18 kDa translocator protein (TSPO), adenine nucleotide transporter (ANT) and voltage-dependent anion channel (VDAC)[70-72]. In hormone-stimulated steroidogenic tissues, a "transduceosome" complex is formed, involving recruitment of the regulatory subunits of protein kinase A (PKA-R1α) and acyl CoA binding domain proteins-1 and -3. Elevated levels of cyclic adenosine monophosphate (cAMP) release PKA catalytic subunits to phosphorylate 37 kDa steroidogenic acute regulatory protein at the outer mitochondrial membrane; import of both StAR and cholesterol into the inner mitochondrial membrane and matrix facilitate both proteolytic processing of StAR to its 30 kDa form, and conversion of cholesterol into pregnenolone by CYP11A1[70-72]. However, a dynamic 800 kDa bioactive protein complex in steroidogenic cells has also been described, which does not involve ANT, but is composed of TSPO, VDAC, CYP11A1, ATPase family AAA domain-containing protein 3A (ASTAD3A) and optic atrophy type 1 proteins[73]; in this model, StAR facilitated binding of cholesterol to the 800 kDa complex, enhancing steroidogenesis.

Importantly, there is a growing realisation that key mitochondrial cholesterol trafficking proteins, such as StAR, play an important role in non-steroidogenic tissues[74]. This, combined with conflicting results regarding the impact of genetic deletion of TSPO on steroidogenesis and viability in mice[75-78], may lead to increased consideration of alternate functions for these proteins[74]. For example, StAR is expressed in endothelial cells, monocytes and macrophages[79-82], albeit at levels far lower than those found in adrenal or gonadal tissues[74]. By contrast, other components of the mitochondrial trafficking complex, such as TSPO, are widely expressed in a variety of tissues, including macrophages[78,81].

Importantly, both StAR and TSPO appear to impact on macrophage lipid and inflammatory phenotype, in

part *via* the pathway involving sterol 27-hydroxylase, activation of LXRα and upregulation of ABCA1/ABCG1 mRNA and protein[81-83], arguing a functional role for these proteins in mediating cholesterol supply to CYP27A1. Overexpression of StAR decreased macrophage lipid content[82,83], repressed inflammation[82] and apoptosis[84] and increased macrophage cholesterol efflux[82,83], while a viral vector expressing StAR reduced aortic lipids and atheroma in apoE$^{-/-}$ mice[85]. However, exploiting any putative anti-atherogenic properties of StAR could prove problematic, due to the associated induction of lipogenesis in macrophages[83,86], presumably *via* LXRα dependent induction of *Srebp1c*[87].

This led to focus on other components of the mitochondrial cholesterol trafficking complex and, in particular, TSPO[81]. Transient overexpression of TSPO in human (THP-1) macrophages increased the levels of ABCA1 mRNA and protein, and enhanced efflux of cholesterol to apoA-I, HDL and human serum, a finding reversed by gene knockdown of TSPO. Small molecule TSPO ligands also increased cholesterol efflux, an effect that was amplified in macrophages genetically engineered to overexpress TSPO[81]. Notably, TSPO overexpression caused a decline in macrophage total neutral lipid mass, without induction of lipogenesis, and effectively prevented "foam cell" formation following exposure to modified LDL[81]. These effects were associated with induction of both LXRα and PPARα the latter providing a plausible mechanism for the observed reductions in macrophage lipid mass[81]. Notably, overexpression of some of the other proposed components of the mitochondrial cholesterol trafficking complex, such as VDAC, ANT and ACBD1, discussed above, exerted minimal effects on the macrophage cholesterol efflux pathway[81].

Expression of TSPO is upregulated by exposure to modified LDL in human macrophages[81], and TSPO ligands have been used to image vascular inflammation in CD68 positive macrophages at sites of disturbed flow in murine carotid arteries[88], and macrophage burden[89] and intraplaque inflammation[90] within human carotid atherosclerotic lesions. Despite this evident association with inflammation, it appears that upregulation of TSPO, or signalling *via* this protein, may represent an adaptive mechanism designed to limit tissue damage. Overexpression of TSPO in microglia decreased production of pro-inflammatory cytokines, reflected in increased expression of alternately activated M2 stage-related genes and mediated *via* repression of NF-κB activation[91]. Similarly, TSPO ligands inhibited the proliferation of retinal microglial cells, and repressed the output of reactive oxygen species and TNFα[92]. In good agreement, levels of TSPO are higher in dystrophic murine retina, and in microglia treated with LPS, while TSPO ligand XBD173 repressed the expression of chemokine (C-C motif) ligand 2 (CCL2), IL-6 and iNOS[93]. The TSPO ligand, PK11195 has proved effective in ameliorating the severity of disease in an experimental murine model of multiple sclerosis, by reducing inflammatory responses and promoting oligodendroglial regeneration[94]. TSPO has also been posited as a novel target for Alzheimer's disease[95], anxiety, psychiatric and neurologic disorders[96-99], pain[100], cancer[101] and vascular dysfunction[88-90,102]. At present, it is not known how many of these effects are related to the cholesterol trafficking function of TSPO, although LXRs influence expression of an array of genes involved in cholesterol homeostasis, glucose metabolism, inflammation and Alzheimer's disease[103]. It is also clear that some of the reported effects of TSPO and its ligands may require re-evaluation, given the lack of phenotype recently reported in healthy TSPO$^{-/-}$ mice[75,76].

MITOCHONDRIAL STRUCTURE AND BIOENERGETICS: IMPACT ON CHOLESTEROL HOMEOSTASIS

Mitochondria exhibit constant movement, fusion and fission[104]. The mitochondrial membrane protein mitofusin (Mfn2) is involved in maintaining mitochondrial morphology, energy provision, and cellular growth and apoptosis[105-107]. Recently, Mfn2 has emerged as a regulator of macrophage cholesterol efflux, *via* upregulation of peroxisome proliferator activated receptor-γ (PPARγ) ABCA1, ABCG1 and scavenger receptor-B1 (SR-B1), reflected in marked reductions in cholesterol mass[107]. Overexpression of Mfn2 attenuates the formation of atherosclerotic lesions in rabbit carotid arteries, and levels of Mfn2 are progressively reduced during lesion formation in apoE$^{-/-}$ mice during atherogenesis; levels of Mfn2 are also reduced in atherosclerotic, compared with non-atherosclerotic, human arteries[107].

Remodelling of the inner mitochondrial membrane by optic atrophy 1 (OPA1) also alters the efficiency of mitochondrial cholesterol trafficking, at least in steroidogenic cells[108,109]. Increased steroidogenesis is reported in trophoblasts undergoing syncytilisation, which express increased levels of the pro-fission mitochondrial shaping protein Drp1 increased, and decreased levels of Opa1 and mitofusin. An inverse relationship between levels of Opa1 and steroidogenesis were also evidenced in cells genetically manipulated to express higher levels of Opa1, while accumulation of cholesterol at the inner mitochondrial membrane was observed in mitochondria lacking Opa1[108,109].

Finally, it is self-evident that ATP is needed to mount an effective non-adaptive immune response, and to fuel cholesterol biosynthesis and the activity of ABC transporters that determine the rate of macrophage cholesterol efflux. However, more subtle changes in mitochondrial function or loss of bioenergetic capacity, the emerging concept of the Bioenergetic Health Index (BHI)[110], have been shown to reduce the efficiency of mitochondrial cholesterol trafficking and hormone biosynthesis in steroidogenic tissues[111,112]. Dissipation of the mitochondrial membrane potential ($\Delta\psi_m$ using carbonyl cyanide *m*-chlorophenylhydrazone), inhibition of electron transport at complex Ⅲ (using antimycin), reduction of pH (nigericin) and inhibition

of ATP synthase (oligomycin) blocked the formation of progesterone and synthesis or import of StAR protein in Leydig cells[111,112].

A parallel study in macrophages supports the notion that acute loss of mitochondrial function is also associated with dysregulated cholesterol homeostasis[113]. Cholesterol efflux was inhibited by nigericin and oligomycin in RAW 264.7 macrophages; levels of ABCA1 protein decreased in response to oligomycin treatment, despite paradoxical increases in *Abca1* mRNA[113,114], reflecting findings in carotid atherosclerotic lesions[114] Further, while oligomycin treatment did not alter cholesterol biosynthesis, cholesterol esterification was significantly inhibited, promoting apoptosis. Oligomycin induced expression of genes involved in cholesterol efflux (*Abca1, Abcg4, Stard1*) and cholesterol biosynthesis *(Hmgr, Mvk, Scap, Srepb2*) arguing that loss of coordinated regulation of sterol homeostasis is caused by loss of mitochondrial ATP generation[113]. In turn, accumulation of free cholesterol or fatty acids can trigger mitochondrial dysfunction, which could promote inflammation *via* loss of LXRα-dependent repression of NF-κB (above) and upregulation of cytokine expression, but also by NLRP3 inflammasome-dependent and –independent pathways[115].

QUESTIONS FOR THE FUTURE

This review summarizes the current evidence that, in part, macrophage sterol homeostasis, and inflammatory responses, can be linked to mitochondrial cholesterol trafficking, and mitochondrial structure and bioenergetics. Whether proteins involved in mitochondrial structure, fission, fusion or organelle dynamics can also impact on these processes is currently uninvestigated and an area of keen interest. More particularly, mitochondria-mediated hormetic effects in aging[116,117] suggest a retrograde signalling pathway by which mitochondrial dysfunction in a single distinct tissue elicits the mitochondrial stress response in some (but not all) distal tissues. In turn, this suggests that loss of effective mitochondrial function, such as that caused by hepatic insulin resistance for example, may be transmitted *via* "mitokines" to peripheral tissues, promoting vascular dysfunction and cardiovascular disease. These exciting findings offer some intriguing possibilities for therapeutic strategies aimed at sustaining or improving mitochondrial function.

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ORIGINAL ARTICLE

Case Control Study

Quantification of epicardial fat: Which method can predict significant coronary artery disease?

Zizi Saad, Mohamed El-Rawy, Ragab H Donkol, Sami Boghattas

Zizi Saad, Mohamed El-Rawy, Cardiology Department, Aseer Central Hospital, Abha, Saudi Arabia and Faculty of Medicine, Zagazig University, Ash Sharqia Governorate 44516, Egypt
Ragab H Donkol, Radiology Department, Aseer Central Hospital, Abha, Saudi Arabia and Faculty of Medicine, Cairo University, Giza 12411, Egypt
Sami Boghattas, Radiology Department, Aseer Central Hospital, Abha 61321, Saudi Arabia
Author contributions: Saad Z and Donkol RH designed the study, performed CCTA studies, and wrote the manuscript; El-Rawy M and Donkol RH shared selection of cases, clinical and echocardiographic assessment as well as collection of data and interpreted CCTA scans; Boghattas S analyzed the data.
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Informed consent: All participants provided written informed consent prior to study enrollment.
Conflict-of-interest: Corresponding author with no conflict of interest regarding the study.
Data sharing: Technical appendix, statistical code, and dataset are available. Participants gave informed consent for data sharing. No additional data are available.

Correspondence to: Ragab H Donkol, MD, Radiology Department, Aseer Central Hospital, Abha, Saudi Arabia and Faculty of Medicine, Cairo University, PO Box 34, Giza 12411, Egypt. ragabhani@hotmail.com
Telephone: +966-72291169
Fax: +966-38552244
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Abstract

AIM: To compare the predictive value of three methods of epicardial fat (EF) assessment for presence of significant coronary artery disease (CAD) [*i.e.*, epicardial fat volume (EFV), EFV indexed with body surface area (EFV/BSA) and EFV indexed with body mass index (EFV/BMI)].

METHODS: The study was performed on 170 patients (85 women and 85 men) with clinical suspicion of CAD. They aged 26-89 years with a median age of 54 years. The patients were classified into three groups: Group 1: 58 patients with normal coronary arteries; group 2: 48 patients with non-significant CAD and group 3: 64 patients with significant CAD. The three methods for assessment of epicardial fat were retrospectively studied to determine the best method to predict the presence of significant CAD.

RESULTS: The three methods for epicardial fat quantification and measurements, *i.e.*, EFV, EFV/BSA and EFV/BMI with post- hoc analysis showed a significant difference between patients with significant coronary artery disease compared to the normal group. Receiver operating characteristic curve analysis showed no significant difference between the three methods of epicardial fat measurements, the area under curve ranging between 0.6 and 0.62. The optimal cut-off was 80.3 cm^3 for EFV, 2.4 cm^3/m^2 for EFV indexed with BMI and 41.7 $cm^3/(kg/m^2)$ for EFV indexed with BSA. For this cut-off the sensitivity ranged between 0.92 and 0.94, while specificity varied from 0.31 to 0.35.

CONCLUSION: Any one of the three methods for assessment of epicardial fat can be used to predict significant CAD since all have the same equivalent predictive value.

Key words: Quantification of epicardial fat; Coronary heart disease; Epicardial fat volume

Core tip: There is a great correlation between the volume of epicardial fat and presence of significant coronary artery disease. There are different methods for quantification of epicardial fat volume (EFV). The aim of the study is to compare the predictive value of the three methods used for quantification of epicardial fat (EFV, EFV indexed with body surface area and EFV indexed with body mass index) for presence of significant coronary artery disease. The study concluded the three methods for assessment of epicardial fat have the same equivalent predictive value for significant coronary artery disease and any one of them can be used as a sensitive predictor for significant coronary artery disease.

Saad Z, El-Rawy M, Donkol RH, Boghattas S. Quantification of epicardial fat: Which method can predict significant coronary artery disease? *World J Cardiol* 2015; 7(5): 287-292 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i5/287.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i5.287

INTRODUCTION

Epicardial fat (EF) is the visceral fat of the heart deposited under the visceral layer of the pericardium. Under normal physiological conditions, EF tissue displays biochemical, mechanical and thermogenic cardioprotective properties. Under pathological circumstances, EF can be strongly related to the development of coronary artery disease (CAD). The accumulation of EF is known to be a rich source of free fatty acids and a number of proinflammatory cytokines[1-3].

It has been hypothesized that EF may act as a paracrine (immunological) organ that influences the coronary arteries by promoting chronic inflammation and endothelial dysfunction[4-6].

Several imaging modalities can be used to quantify EF volume (EFV) such as echocardiography, computed tomography, and magnetic resonance imaging[7]. Due to distinct low attenuation values of fat on computed tomography (CT). ECG-gated cardiac CT with its high spatial resolution and true volume coverage of the heart, allows accurate measurement of epicardial and thoracic fat distances and volumes[6,7].

Besides EFV, some authors derived a second parameter, the body surface area indexed EFV (EFV/BSA)[8,9]; however, comparison between these two parameters has not been considered. Moreover, despite the predictive value of higher body mass index for cardiovascular event[10,11], the added value of body mass index (BMI)-adjusted EFV (EFV/BMI) also has not been previously assessed as well.

The aim of the current study is to compare the predictive value of the three methods for EF assessment (EFV, EFV/BSA and EFV/BMI) for presence of significant CAD.

MATERIALS AND METHODS

Patients

A total of 170 consecutive patients with clinical suspicion of coronary artery disease (CAD) aged 26-89 years with a median age of 54 years (85 women and 85 men) were included in our study From November 2012 through February 2014. Patients underwent 128-MDCT according to appropriate use criteria for cardiac computed tomography[12]. All participants provided written informed consent and the study was approved by institutional ethics committee.

The patients were divided into 3 groups according to the severity of coronary artery stenosis assessed by quantitative coronary angiography. Patients in group 1 have normal coronary arteries. Patients in group 2 were have non-significant coronary artery stenosis with percent-diameter stenosis less than 50%. Patients in group 3 have significant CAD with percent-diameter stenosis more than 50% or occlusion[13].

Multidetector computed tomography

Our patients were scanned with a 128-multidetector computed tomography (MDCT) dual source scanner (SOMATOM Flash; Siemens Medical Solutions, Erlangen, Germany). Patients with uncontrolled heart rate (> 65 beats per minute) received oral beta blocker (metoprolol 50 mg) before the CT scan. Sublingual nitroglycerin 0.5 mg was administrated before the scan to achieve coronary vasodilation. A non-contrast CT scan was performed to determine the total calcium burden of the coronary tree (sequential scan with 32 Å~ 0.6-mm collimation, tube current 60 mA at 120 kV). Contrast-enhanced CT angiography data were acquired with the use of a spiral scan with 32 Å~ 0.6-mm collimation, 330-ms gantry rotation, pitch of 0.2, and tube voltage at 120 kV.

Sequential ECG-triggering scans were performed in 142 patients with controlled heart rate and retrospective ECG-gating scans with tube current modulation were performed in 28 patients due to heart rate variability. Intravenous contrast agent (60-90 mL; 350 mg iodine/mL) was injected with flow rate of 5.0 mL/s followed by a 30-mL saline chaser[1].

Image interpretation: The total calcium score was calculated and interpretation of CCTA was analysed for all patients using commercially available software packages "Syngo Via", Siemens Healthcare, Forchheim, Germany.

Measurement of EFV

EFV was measured blindly by two observers for all patients using an offline workstation (Aquarius NetStation; TeraRecon Inc., San Mateo, CA). Using the 3.0-mm-thick axial slices used for calcium scoring, the parietal

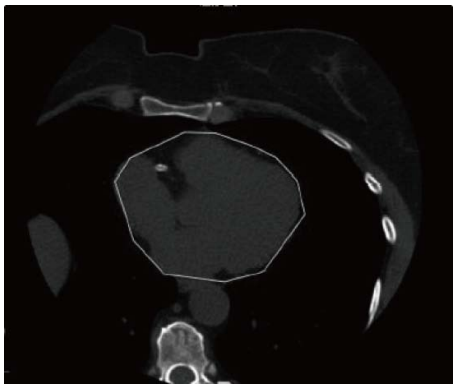

Figure 1 Measurement of epicardial fat volume; the electronic line delineates the contour of epicardiumin in noncontrast computed tomography.

pericardium was traced manually in every 4th slice starting from the aortic root to the cardiac apex[1] (Figure 1). The computer software then automatically interpolated and traced the parietal pericardium in all slices interposed between the manually traced slices. The automatically traced slices were examined and verified for accuracy. Fat voxels were identified using threshold attenuation values of -30 to -190 HU (Figure 2).

Division the estimated EFV of every patient by patient's BSA calculated the indexed EFV to BSA (EFV/BSA). Also, division of EFV by patient's BMI calculated the indexed EFV to BMI (EFVB/MI).

Statistical analysis

The statistical analysis was performed by StatDirect 2.8.0 and Mecalc 9.2.1.0 (for ROC curve analysis and comparison). Because the analyzed quantitative variables (EFV, EFV/BMI, EFV/BSA, BMI, BSA and age) were not normally distributed (Shapiro and Wilk test), the values are expressed as medians with interquartile range. Dichotomous data are presented as frequency (percent).

Differences in characteristics of patients were compared using Pearson's χ^2 test for dichotomous variables (or Fisher's exact test when appropriate) and Kruskall-Wallis test for continuous test.

To compare the predictive performance for risk of significant CAD between the three EF parameters, we plotted receiver operating characteristic (ROC) curve from which the optimal cutoff was derived and we calculated the area under curve (AUC). AUC, optimal cutoff, sensitivity and specificity for each EFP and pairwise comparison of AUC have been determined. A two-tailed *P*-value less than 0.05 were considered as statistically significant; significant differences are presented with asterisk in the tables. The statistical methods of this study were reviewed by a biostatistician.

RESULTS

General characteristics

Table 1 illustrated the important clinical findings and measurements of the patients among the three groups of patients.

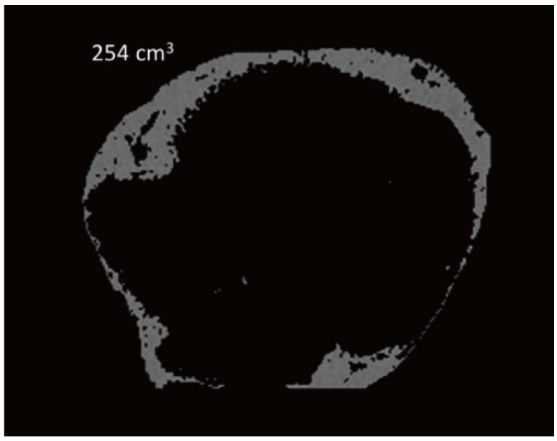

Figure 2 Measurement of epicardial fat volume; the fat voxels were identified using threshold attenuation values (-30 to -190 HU) and the volume of epicardial fat is measures.

Correlation of EFP with CT coronary angiography results

Comparison of EF parameters with CT coronary angiography results is presented in Table 2. Comparison of EFP medians revealed a progressive increase from group 1 to group 3. The medians where 81, 104 and 113 for EFV (cm^3), 2.7, 3.7 and 3.8 for EVV/BMI (cm^3/m^2) and 45.6, 53.9 and 61.8 for EFV/BSA [$cm^3/(kg/m^2)$]. Nevertheless, the post- hoc analysis showed significant difference only between groups 1 and group 3 for all EFP: $P = 0.002$ for EFV, $P = 0.012$ for EFVBMI, and $P = 0.007$ for EFVBSA.

ROC curves analysis

ROC curve for the three the EFP is presented in Figure 3. AUC was 0.62 for EFV, 0.6 for EFVBMI and 0.61 for EFVBSA and pairwise comparison failed to show significant difference. The optimal cut-off was 80.3 cm^3 for EFV, 2.4 cm^3/m^2 for EFVBMI and 41.7 $cm^3/(kg/m^2)$ for EFVBSA. Sensitivity and specificity were respectively 0.92 and 0.35 for EFV, 0.93 and 0.31 for EFVBMI, and 0.94 and 0.32 for EFVBSA.

DISCUSSION

Noninvasive quantitative measurement of epicardial fat volume from CT is feasible, and may play a clinical role in cardiovascular risk assessment[6]. It have shown its reproducibility and correlation to CAD presence, severity, and prognosis[14-16].

Recently, multiple studies have shown a deleterious relationship between epicardial fat burden and coronary atherosclerosis, arrhythmogenesis and major adverse cardiovascular events (MACE)[17,18]. In this study, the epicardial fat volume is correlated with the presence of coronary artery stenosis. In agreement with this findings, Alexopoulos *et al*[19] observed on coronary CT angiography a significant increase in epicardial fat

Table 1 General characteristics of the patients among the three groups (total number of patients 170) *n* (%)

	Group 1 *n* = 58	Group 2 *n* = 48	Group 3 *n* = 64	*P*
Age	48.5 (40.3-50.0)	56 (48.5-64.5)	55 (50-62)	0.004
Male	21 (36.2)	32 (57.1)	32 (50)	0.008
Hypertension	24 (41.4)	29 (60.4)	37 (57.8)	0.090
Diabetes mellitus	23 (39.7)	34 (70.8)	43 (67.2)	0.001
Smoking	5 (8.6)	4 (8.3)	14 (21.8)	0.065
Familial history	7 (12.1)	2 (4.2)	6 (9.4)	0.351
BMI	28.4 (25.9-33.9)	29.5 (25.9-33.1)	31.6 (29.3-33.1)	0.007
BSA	1.80 (1.74-1.88)	1.85 (1.78-1.94)	1.88 (1.81-1.95)	0.093

BMI: Body mass index; BSA: Body surface area.

Table 2 Comparison of three parameters for measuring the epicardial fat with computed tomography coronary angiography results

	Group 1	Group 2	Group 3	*P*
EFV (cm^3)	81 (59.4-124)	104 (83.5-126)	113 (92-138)	0.004
EFVBMI (cm^3/m^2)	2.7 (1.9-3.9)	3.7 (2.5-4.3)	3.8 (3.0-4.6)	0.014
EFVBSA [$cm^3/(kg/m^2)$]	45.6 (35.3-66.9)	53.9 (44.4-68.6)	61.8 (48.1-75.4)	0.011

EFV: Epicardial fat volume; EFVBMI: EFV indexed with body mass index; EFVBSA: EFV indexed with body surface area.

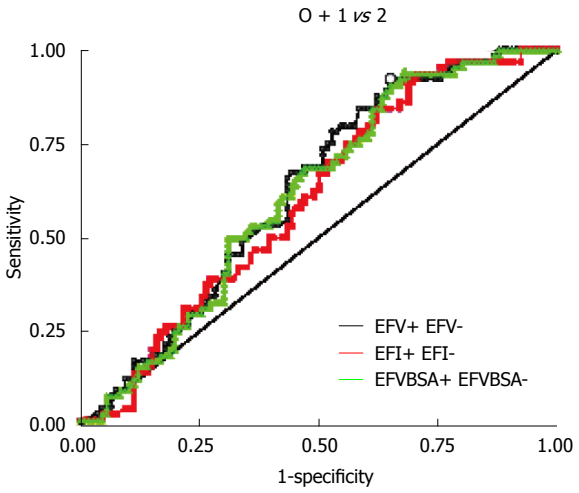

Figure 3 Receiver operating characteristic curves compare the three parameters for measuring the epicardial fat. AUC was 0.62 for EFV, 0.6 for EFVBMI, and 0.61 for EFVBSA (non-significant pairwise comparison). EFV: Epicardial fat volume; EFVBMI: EFV indexed with body mass index; EFVBSA: EFV indexed with body surface area.

volume with increasing coronary luminal stenosis; epicardial fat volume was also larger in patients with mixed or noncalcified plaques.

A recent study by Nakazato *et al*[20] assessed the relationship of epicardial fat volume to weight, BMI and waist circumference, and evaluated whether changes in these parameters over a 4-year period influenced epicardial fat volume measured by noncontrast CT in a relatively healthy asymptomatic population. They found that weight, BMI and waist circumference demonstrated moderate cross-sectional relationships to epicardial fat volume, and that changes in these parameters were related to epicardial fat volume change.

In another study, Shmilovich *et al*[9] assessed the body surface area indexed EVF in a healthy population and validated it as a predictor of major adverse cardiovascular events.

By reviewing the literature, the two parameters EFV and EFV/BSA, have no clear evidence of differences in their accuracies and predictive values[9,18-20]. In the current study we compared the predictive value of three EFP for the presence of significant CAD. To our knowledge, no similar studies have been performed before and EFV/BMI has not previously assessed also, the predictive value of EF/VBSA for CAD has not been compared with EFV.

The major outcome of the present study, derived from the ROC curve analysis, is that the three methods for assessing the EF have an equivalent predictive value for significant CAD and any one of them can be used as a predictor for significant CAD. Therefore, the clinical impact of the indexed EFV seems to be limited. To note that in the series using the indexed EFV to BSA, the benefit of such indexing has not been evaluated and ROC curve analysis has not been applied for assessment of possible increase in the predictive value for total occlusion of coronary arteries[16] or major adverse cardiovascular events[9].

According to the traditional academic point system, the accuracies of the three methods for measuring EF are classified as poor considering that the AUC are in the range 0.6-0.7. This limited accuracy is related mainly to the low specificity, ranging from 0.31 to 0.35, while the sensitivity is high, varying from 0.92 to 0.94, therefore the three methods can be considered as sensitive but poorly specific predictors for significant CAD. Consequently, indexing EFV by BSA or BMI doesn't improve significantly the sensitivity and, more

importantly, the specificity of EF for significant CAD.

In the current study, the optimal cut-off was 80.3 cm^3 for EFV, 2.4 cm^3/m^2 for EFVBMI and 41.7 $cm^3/(kg/m^2)$ for EFVBSA. The optimal cut-off for EFVBSA was lower than the value 50 cm^3/m^2 reported by Ueno *et al*[16] (labeled VEAT in their series), despite a higher median value in our series: 53.7 cm^3/m^2 *vs* 47.1 cm^3/m^2. This difference is likely related to the definition of the end-point itself: significant CAD in the current study *vs* total occlusion of the coronary arteries in the series of Ueno *et al*[16]. More recently, Shmilovich *et al*[9] derived the threshold for the upper normal limit of indexed EF to BSA in a healthy population. The indexed EFV was also non-normally distributed, and the 75th-percentile was 47.1 cm^3/m^2, while in our series the 75th-percentile for the group 1 (composed of patients without CAD) 1 was 66.9 cm^3/m^2. Clearly, these values cannot be compared considering the major differences in the design of the two studies, the series of Shmilovich *et al*[9] including only asymptomatic patients with low risk and without clinical or biological risk factors for CAD.

Analysis of the correlation between the EFP and CT coronary angiography results revealed a trend of increase form group 1 to group 3. This result is similar to the conclusion of Taguchi *et al*[21] that pericardial fat is the strongest independent variable for the severity of CAD. Nevertheless, only groups 1 and 3 differed significantly. The overlap of values, for all EFP, between groups 2 and 3 may explain in great part the low specificity observed for the optimal cut-off. This point merits further analysis to achieve a higher predictive value of EF for significant CAD.

Relatively small number of studied patients with no available follow up data to observe the outcome or prognostic value of EFP considered a study limitation. Additionally our study may be affected by selection bias as we evaluate patients with clinical suspicion of CAD.

In conclusion, the major outcome of the present study that the three methods for assessment of EF have the same equivalent predictive value for significant CAD and any one of them can be used as a sensitive predictor for significant CAD. Additionally, we provided a threshold for each one of the three EFP. For further validation of this threshold additional larger study is recommended.

COMMENTS

Background

Epicardial fat (EF) under normal physiological conditions, EF tissue displays biochemical, mechanical and thermogenic cardio protective properties. Under pathological circumstances, EF can be strongly related to the development of coronary artery disease (CAD). Several imaging modalities can be used to quantify EF volume (EFV) such as echocardiography, computed tomography, and magnetic resonance imaging.

Research frontiers

ECG-gated cardiac computed tomography with its high spatial resolution and true volume coverage of the heart, allows accurate measurement of epicardial fat volumes. Besides EFV, some authors derived a second parameter, the body surface area indexed EFV (EFV/BSA); however, comparison between these two parameters has not been considered. Moreover, despite the predictive value of higher body mass index for cardiovascular event, the added value of body mass index (BMI)-adjusted EFV (EFV/BMI) also has not been previously assessed as well. The current study compares the predictive value of the three methods for EF assessment (EFV, EFV/BSA and EFV/BMI) for presence of significant CAD.

Innovations and breakthroughs

In the current study the authors compared the predictive value of three EFP for the presence of significant CAD. To our knowledge, no similar studies have been performed before and EFV/BMI has not previously assessed also, the predictive value of EF/VBSA for CAD has not been compared with EFV. The major outcome of the present study is that the three methods for assessing the EF have an equivalent predictive value for significant CAD. Furthermore the authors provided a threshold for each one of the three EF parameters.

Applications

The study results suggested that; any one of the three methods for assessment of epicardial fat can be used to predict significant CAD since all have the same equivalent predictive value.

Terminology

EF is the visceral fat of the heart deposited under the visceral layer of the pericardium and has the same origin as abdominal visceral fat.

Peer-review

This is a good study in which the authors compare the predictive value of three methods used for quantification of epicardial fat [*i.e.*, EFV, EFV indexed with body surface area (EFV/BSA) and EFV indexed with body mass index (EFV/BMI)] for presence of significant CAD. The results are interesting and suggest that any one of the three methods for assessment of epicardial fat can be used to predict significant CAD since all have the same equivalent predictive value.

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CASE REPORT

Myocarditis in athlete and myocardial bridge: An innocent bystander?

Federico Quaranta, Emanuele Guerra, Fabio Sperandii, Francesco De Santis, Fabio Pigozzi, Leonardo Calò, Paolo Borrione

Federico Quaranta, Emanuele Guerra, Fabio Sperandii, Francesco De Santis, Fabio Pigozzi, Paolo Borrione, Department of Movement, Human and Health Science, University of Rome "Foro Italico", 00194 Rome, Italy
Leonardo Calò, Department of Cardiology, Policlinico Casilino, ASL Rome B, 00169 Rome, Italy
Author contributions: Quaranta F, Guerra E, Sperandii F and De Santis F contributed in the clinical follow up of the patient and in the review of the literature; Quaranta F, Guerra E and Borrione P contributed in the conception and writing of the article; Pigozzi F, Calò L and Borrione P contributed in writing the article as well as in the final approval of the manuscript.
Informed consent: The patients provided informed written consent prior to study enrollment.
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Correspondence to: Paolo Borrione, MD, Department of Movement, Human and Health Science, University of Rome "Foro Italico", Piazza de Bosis, 00194 Rome, Italy. paolo.borrione@uniroma4.it
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Abstract

Myocarditis is a bacterial or viral inflammatory disease, often unnoticed or misdiagnosed. Athletes with myocarditis must stop practicing their activity since International medical Literature described some cases of sudden death. In the present report, we describe a case of an asymptomatic, apparently healthy, competitive athletes, who was diagnosed a myocarditis and as incidental finding a myocardial bridging. We focused the attention on the importance of anamnesis, electrocardiogram and athletes' entourage for the diagnosis of such insidious pathologies and we evaluated the follow up, focusing the attention on electrocardiogram changes as well as on restitution ad integrum and prognosis, especially for the athletes.

Key words: Myocarditis; Sudden cardiac death; Pre-participation screening

Core tip: Pre participation screening allows sports physicians to diagnose potential causes of sudden death in athletes. We describe a case of an athlete with a previous myocarditis, and an incidental myocardial bridging, suspended from competition and followed in the later time. We also pointed out the question of the possible scarring of myocarditis in relation to the restart of physical activity and training.

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INTRODUCTION

Myocarditis is an inflammatory disease of the myocardium,

possibly determined by both bacterial and viral infections. Coxsackievirus B, enteroviruses adenovirus and parvovirus B19 are often involved in acute processes[1]. Myocardial involvement caused by granulomatous or (auto) immune processes with unknown pathogenetic mechanisms, but this is less common than virus-induced myocarditis or post-infectious inflammatory cardiomyopathy.

Myocarditis is frequently anticipated by flu-like signs and symptoms like chills, fever, headache, muscle aches, general malaise. Moreover, gastrointestinal symptoms such as decreased appetite, nausea, vomiting, and diarrhoea are commonly reported. Cardiac signs and symptoms may appear a few hours to a few days since the beginning of myocardial inflammation and consist in chest pain, palpitations, dyspnea, hypotension, gallop rhythm, rales, jugular venous dilatation, and cardiac tamponade.

In rare cases, the inflammatory condition causes acute hemodynamic compromise, possibly leading to heart failure, complex arrhythmias and fatal events[2]. In reported cases, Myocarditis scenario can overlap Ischemic disease[3], and acute coronary syndrome, even an ST-segment elevation myocardial infarction.

Nevertheless, myocarditis can be undiagnosed and completely asymptomatic with not perceived short lasting fever (5-7 d).

In the absence of structural heart diseases myocarditis accounts for approximately 10% of recent-onset cardiomyopathy in adults. Early fulminant disease is still associated with a high mortality rate. Patients who survive the critical phase have a fairly good prognosis and survival from myocarditis is approximately 60%-70%. In the remaining patients progressive chronic heart failure and unpredictable sudden cardiac death remain a serious concern, often occurring years after the initial clinical event and sometimes despite complete recovery of the myocardial function.

Diagnosis is commonly carried out by integrating different methods[4].

Electrocardiogram (ECG): a sensitive cheap tool of diagnosis. Abnormal ST-T waves and conduction block are frequently observed in myocarditis as well as isolated or complex/polymorphic ventricular arrhythmias present at rest or during stress test[5]. A gradual increase in the width of the QRS complex can be a sign of exacerbation of myocarditis. Continuous ECG monitoring is crucial to detect arrhythmic burden and presence of threatening rhythm disorder.

Echocardiography: in case of echo alterations, myocarditis can be confirmed by transient wall thickening, reduced wall motion and reduced cardiac chamber size in addition to pericardial effusion on echocardiography and increase of echo reflection[6].

Cardiac magnetic resonance imaging (MRI): in addition to the cinematic mode on MRI, T1-weighted early signal enhancement and gadolinium-delayed imaging of the heart are useful to make a diagnosis of myocarditis[7]. T2-weighted images reveal the regions of the heart affected by inflammation. Cardiac magnetic resonance (CMR) has emerged as a leading modality in the noninvasive diagnosis of myocarditis due to its ability to detect myocardial edema, hyperaemia, necrosis and fibrosis in a safe and reproducible fashion. The presence of late enhancement gadolinium (LGE) has been reported in 24%-95% of patients with myocarditis, typically being multifocal and located in the epicardium of the lateral wall and mid wall of the septum. Moreover, the long term prognosis can be determined by the magnitude of damage at MRI, since the left ventricle end diastolic volume, the presence of LGE in the septum and the total amount of LGE are the strongest independent predictors of impaired ventricular function and ventricular dilatation at follow up[8].

Blood Biochemistry: during the acute phase of myocarditis, it is possible to observe a plasma increased elevation of C-reactive protein, aspartate aminotransferase, lactate dehydrogenase, MB form of creatine kinase, and cardiac troponin T[9].

Other techniques may include computed tomography (CT) of the heart to assess possible artery disease[2,10], chest X-ray (qualitative quick assessment of cardiac enlargement and pulmonary congestion); cardiac catheterization, including endomyocardial biopsy (the gold standard for diagnosis but an invasive method not risk free and usually performed only after the acute phase and in clinical stability of the patient)[11].

Myocarditis is reported as a cause of Sudden death in athletes[2].

Italian guidelines for the pre-participation screening sustain that "subjects with certain diagnosis of myocarditis cannot be involved in any kind of sport activity, until the illness process is completely resolved, and however not before of at least 6 mo since the diagnosis was made. The competitive sport activity can be restarted only if clinical evaluation and instrumental evaluations demonstrate the lack of alterations in myocardial contractile function as well as the absence of relevant arrhythmias"[12].

CASE REPORT

A 41-year-old male, regularly involved in competitive sport (American football and weightlifting) came, on march 2013, to our Sport's Medicine laboratory, for the annual mandatory pre-participation screening. Football screening protocol include Cardiological examination, basal ECG, Stress Test. Family and personal history was negative. Clinical examination was unremarkable.

Rest-ECG showed deep T-wave inversion in $\geqslant$ 2 contiguous anterior, inferior or lateral leads, particularly in leads Ⅰ, Ⅱ, Ⅲ, aVF and in V4-V5-V6. This pattern was absent in all the previous ECGs the patient made for previous screening, and in particular it was absent recently, when he underwent job medical exam (ECG January 2013) (Figure 1).

The patient underwent echocardiography which

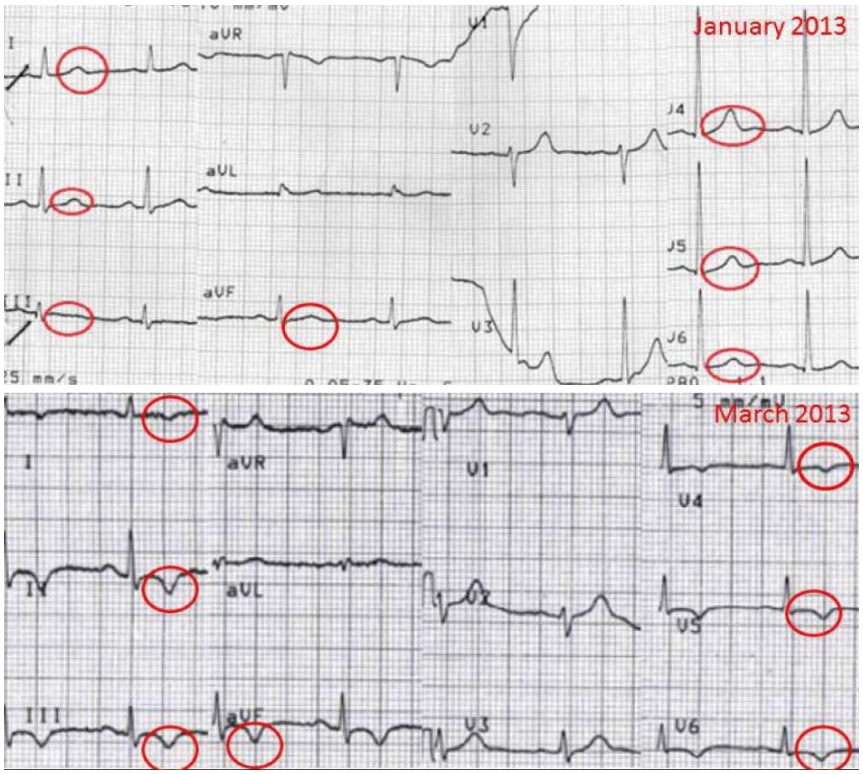

Figure 1 Electrocardiogram changes: Ventricular.

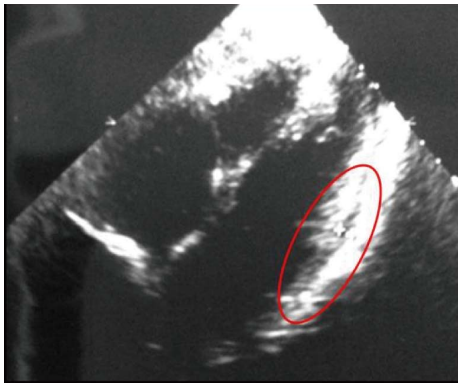

Figure 2 Echocardiogram (March 2013): Echo-free space in the lateral wall and hyper echogenicity of the lateral portion of the left ventricle.

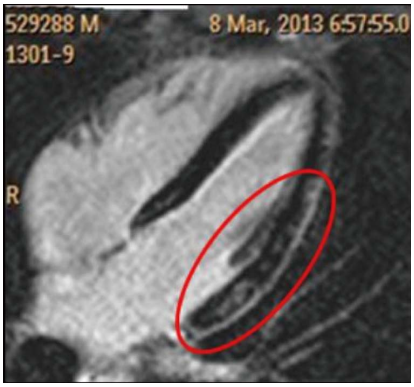

Figure 3 Cardiac magnetic resonance: Areas of intramural and subepicardial delayed enhancement in the lateral wall, suggestive of fibrosis.

showed a mild echo-free space with pericardial effusion in the lateral wall and a slight increase in the hyper echogenicity level of the lateral portion of the left ventricle (Figure 2).

As the patient was completely asymptomatic and actually training and competing a cardiac exercise stress test was performed. T-waves inversion was stable all along the duration of the test. Patient reached 168 beats per minute at peak exercise heart rate and 200 watt of peak mechanical Power. Blood pressure kinetics was normal. No presence of Arrhythmias. No symptoms were reported. Holter ECG monitoring showed absence of ventricular arrhythmias.

The patients underwent through blood examination and biochemistry, which didn't show any alterations.

Through anamnesis the patient remembered having experienced a "persistent flu" on January 2013, characterized by elevated fever (40 ℃) and atypical chest pain which he considered aspecific and not to be referred to his General practitioner, for several days. He was administered a broad-spectrum antibiotic therapy (Cephalexin) and symptoms completely disappeared within 4 d.

As a third level examination we performed a cardiac MRI which showed the presence of some areas of intramural and subepicardial delayed enhancement in the lateral wall, suggestive of fibrosis, in a contest of normal dimensions and function (Figure 3). The characteristic pattern of LGE in myocarditis is patchy or multifocal in a subepicardial or intramyocardial distribution, often involving the lateral wall. This feature is not pathognomonic but is clearly distinct from ischemic heart disease, which typically presents with subendocardial or transmural LGE within a

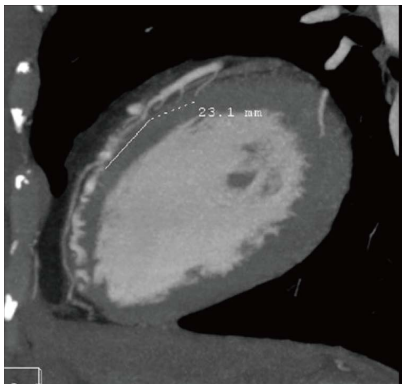

Figure 4 Tomographic computed scan: Myocardial bridge (23 mm).

coronary artery territory.

Despite it, considering the age of the athlete and possible overlapping with ischemic disease even if in absence of wall abnormalities at echo scan of the heart, we excluded coronary involvement through a CT scan of coronary arteries. As a collateral finding a coronary abnormal 23 mm superficial intramiocardial course in the medium tract of Left descending artery was found.

The intramuscular course of coronary arteries can be detected and characterized by Computed Coronary Tomographic Angiography (CCTA). CCTA is an easy and reliable tool for comprehensive *in vivo* diagnosis of the intramuscular course of coronary arteries. It is generally estimated that the myocardial bridging can be detected in about one-third of the adults in autopsy series, whereas the reported incidence in angiographic series is much lower < 5%. The incidence of myocardial bridging in CCTA studies (30.5%) is in concordance with the reported incidence in autopsy series[13].

We concluded for Myocarditis in association with incidental finding of Myocardial bridge (Figure 4).

According to cardiologic Italian guidelines for competitive sports, we recommended to avoid physical overload and sport practice. A therapy with low dose Bisoprolol was started and a follow up was based on monthly ECG and a repetition of echocardiography and stress-test at three months' time.

T wave inversion pattern showed a gradual reduction in amplitude up to complete reversal after 4 mo (Figure 5).

Echocardiogram performed in October 2013 was normal and no sign of effusion was present. Stress test was within normal limits and no repolarization abnormalities could be found.

Clinical evaluation, normalization of basal ECG and echocardiogram demonstrated the complete electrical recovery of the athlete from myocarditis. He continued to be excluded from competitive sports, according to cardiologic Italian guidelines for competitive sports for the presence of intramiocardial Coronary bridge.

After one year of follow up the patient did not experience any cardiological symptoms, clinical examination was unremarkable and electrocardiographic and echocardiographic findings as well as stress test were within normal limits.

DISCUSSION

Myocarditis is a rare recognised cause of sudden death in athletes, its signs and symptoms may overlap ischemic disease as well as cardiomyopathy and it can be minimally symptomatic or able to determine rare fatal events[14]. Even in asymptomatic subjects, recognition of this disease can modify prognosis since inflammatory cardiomyopathy can determine adverse event also many years after apparently total restitution ad integrum, this is the reason why indication for clinical follow up and for adequate exercise prescription can change the patient "scenario".

Intramiocardial bridge is a recognised cause of sudden death in athletes too[15]. The prevalence of this abnormality shows a wide range of frequency, up to 44% with 64-slice coronary CT scan[16]. In the last years Computed Scan has allowed diagnosis even in asymptomatic subjects. This finding requires, accordingly to cardiological Italian guidelines for competitive sports, to avoid competition and high intensity training.

With this report, we firstly intend to remark the relevance of anamnesis and combined techniques such as basal ECG[17], echocardiography, MRI and Computed Scan of the heart, in the diagnosis or exclusion of myocarditis when clearing athletes for competition. ECG is reported to be normal in over 32% of subjects with acute myocarditis. This can also be due to the time at which the ECG is performed, for the transitory nature of abnormalities[18]. MRI has a sensitivity of 81%, specificity of 71% and accuracy of 81% for diagnosing acute myocarditis[5].

Moreover, T wave inversion were in the same location of increased echo reflex and in proximity of mild echo free area interpreted as mild pericardial effusion, in lateral portion of left ventricle. Such data was also confirmed at MRI and at Computed Scan of the heart, with no sign of oedema, but with presence of fibrosis, indicating a past inflammatory based necrosis. This information is crucial to possibly diagnose Myocarditis as well as for the prognosis.

Secondly, we would like to underline the importance of a proper dialogue between sport physicians and the athletes, the medical team and even, in some cases, the family doctor. A focal point is that even if mandatory pre-participation screening in our country is annually based, it seems essential that sports physicians recommend to re-evaluate athletes experiencing persistent flues or not common signs and symptoms such as atypical thoracic pain, asthenia and palpitations. This information is to be extended to athletes and general practitioner.

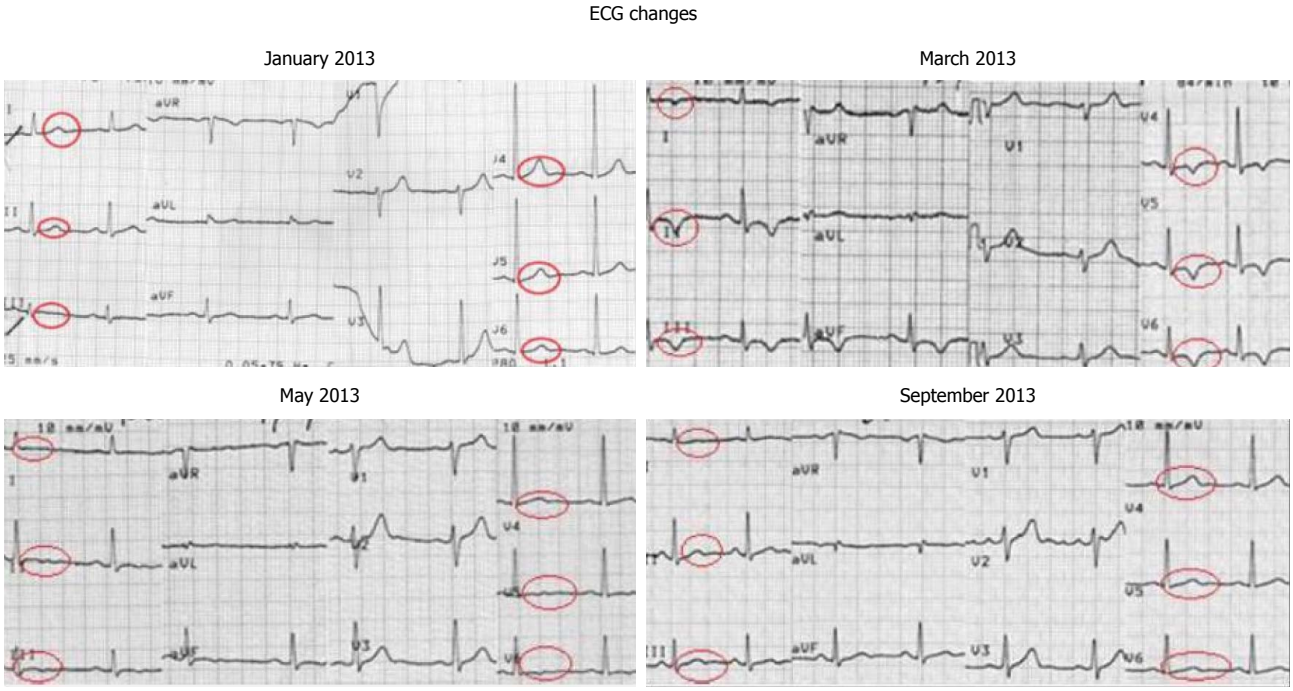

Figure 5 Electrocardiogram follow up.

According to actual Italian guidelines, a period of 6 mo following restitution ad integrum is sufficient to be again eligible for sport competition. With MRI, myocardial damage, previously not detected can be well appreciated as fibrosis or sub epicardial or intramural damage.

It seems important to note that the presence of delayed enhancement and fibrosis is not considered as a risk predictor in previous Italian Cardiological Guidelines for pre participation screening, in fact a complete reversal of T waves abnormalities, with preserved function and absence of symptoms and arrhythmias is considered sufficient for a restitution and integrum. Are now this concept still valid? Which is the role of Myocardial scar in determining a good prognosis? Is an ECG reversal to normality also a sign of better prognosis?

COMMENTS

Case characteristics

The athlete during the pre-participation screening was asymptomatic, he reported elevated fever (40 °C), atypical chest pain with asthenia and persistent flu, 2 mo before.

Clinical diagnosis

The athlete, with a previously normal electrocardiogram (ECG), showed T wave inversion in most leads, after a "persistent flu" with elevated fever 2 mo later.

Differential diagnosis

Differential diagnosis with myocardial infarction was performed through computed tomography (CT) scan which didn't show coronary artery disease as well as through echocardiography which showed normal systolic function.

Laboratory diagnosis

The patient underwent blood examination (C-reactive protein, aspartate aminotransferase, lactate dehydrogenase, MB form of creatine kinase, and cardiac troponin T) which didn't show any alterations.

Imaging diagnosis

The authors performed the following instrumental examinations: (1) ECG: T wave inversion in leads I, II, III, aVF and in V4-V5-V6; (2) Echocardiogram: mild echo-free space with pericardial effusion in the lateral wall and a slight increase in the hyper echogenicity level of the lateral portion of the left ventricle; (3) magnetic resonance imaging (MRI): areas of intramural and subepicardial delayed enhancement in the lateral wall, suggestive of fibrosis; and (4) CT scan: coronary abnormal 23 mm superficial intramiocardial course in the medium tract of left descending artery.

Pathological diagnosis

The authors didn't perform any histological examination.

Treatment

After the detection of the intramyocardial bridge the authors started a therapy with low dose bisoprolol and the authors suspended the athlete from competition and training. A monthly follow up was started.

Term explanation

Pre participation screening is the annual medical evaluation mandatory for competitive athlete in Italy.

Experiences and lessons

It would be desirable that sports physicians recommend to re-evaluate athletes experiencing persistent flues or not common signs and symptoms such as atypical thoracic pain, asthenia and palpitations, during the sports season; this information is to be extended to athletes and general practitioner.

Peer-review

This case report highlights the importance of anamnesis and 12 leads ECG examination for the athletes in diagnosis of subtle transitory myocardial alterations due to infections. Moreover, despite a normalization of ECG during follow up, after a myocarditis, the authors emphasized the eventuality to focus on the possibility of myocardial scars, which can be easily detected with MRI.

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Xiu-Xia Song, Vice Director
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
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EDITORIAL

Left atrial physiology and pathophysiology: Role of deformation imaging

Johannes Tammo Kowallick, Joachim Lotz, Gerd Hasenfuß, Andreas Schuster

Johannes Tammo Kowallick, Joachim Lotz, Gerd Hasenfuß, Andreas Schuster, DZHK (German Centre for Cardiovascular Research), partner site Göttingen, D-37075 Göttingen, Germany
Johannes Tammo Kowallick, Joachim Lotz, Institute for Diagnostic and Interventional Radiology, Georg-August University, D-37075 Göttingen, Germany
Johannes Tammo Kowallick, Andreas Schuster, Department of Biomedical Engineering, Division of Imaging Sciences, The Rayne Institute, St Thomas Hospital, King's College School of Medicine London, London SE1 7EH, United Kingdom
Gerd Hasenfuß, Andreas Schuster, Department of Cardiology and Pneumology, Georg-August University, D-37075 Göttingen, Germany

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Correspondence to: Dr. Andreas Schuster, MD, PhD, FESC, Lecturer, Department of Cardiology and Pneumology, Georg-August University, Robert-Koch-Strasse 40, D-37075 Göttingen, Germany. andreas_schuster@gmx.net
Telephone: +49-551-3920400
Fax: +49-551-396389

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Abstract

The left atrium (LA) acts as a modulator of left ventricular (LV) filling. Although there is considerable evidence to support the use of LA maximum and minimum volumes for disease prediction, theoretical considerations and a growing body of literature suggest to focus on the quantification of the three basic LA functions: (1) Reservoir function: collection of pulmonary venous return during LV systole; (2) Conduit function: passage of blood to the left ventricle during early LV diastole; and (3) Contractile booster pump function (augmentation of ventricular filling during late LV diastole. Tremendous advances in our ability to non-invasively characterize all three elements of atrial function include speckle tracking echocardiography (STE), and more recently cardiovascular magnetic resonance myocardial feature tracking (CMR-FT). Corresponding imaging biomarkers are increasingly recognized to have incremental roles in determining prognosis and risk stratification in cardiac dysfunction of different origins. The current editorial introduces the role of STE and CMR-FT for the functional assessment of LA deformation as determined by strain and strain rate imaging and provides an outlook of how this exciting field may develop in the future.

Key words: Left atrium; Strain; Strain rate; Physiology; Pathophysiology; Cardiovascular magnetic resonance; Echocardiography; Feature tracking; Speckle tracking; Diastolic dysfunction

Core tip: Recent advances in speckle tracking echocardiography (STE) and cardiovascular magnetic resonance myocardial feature tracking (CMR-FT) allow a detailed quantification of left atrium (LA) dynamics in terms of strain and strain rate imaging. Corresponding imaging biomarkers are progressively found to have the potential to predict the outcome in a variety of cardiovascular disease states. The current editorial introduces the role of STE and CMR-FT for the functional assessment of LA deformation and provides an outlook of how this exciting field may evolve in the future.

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INTRODUCTION

Heart failure of different origins including ischemic aetiology remains a major determinant of mortality[1]. Left atrial (LA) enlargement has been shown to be a sensitive parameter for the prediction of adverse cardiac events[2,3]. The interplay between LA enlargement and atrial remodelling in the development of atrial fibrillation (AF) has been demonstrated[4,5]. However, the pure relation of LA pathology to its enlargement within different diseases may oversimplify cardiovascular physiology. It is important to note that the LA does not merely represent a stiff chamber, which passively transports blood from the pulmonary veins to the left ventricle (LV), but a more complex and active chamber. Its role should rather be described as a dynamic modulation of LV filling by functioning as a reservoir, conduit and contractile booster pump[6,7]. There have been tremendous advances in terms of our ability to characterize all three elements of atrial function using non-invasive imaging techniques[8]. Recent advances include LA deformation analysis using speckle tracking echocardiography (STE)[9,10] as well as cardiovascular magnetic resonance myocardial feature tracking (CMR-FT)[7,11]. Corresponding imaging biomarkers are progressively found to have the potential to predict the outcome in a variety of cardiovascular disease states[6]. The current editorial introduces the role of STE and CMR-FT for the quantification of LA dynamics as expressed by strain and strain rate (SR) imaging and provides an outlook of how this exciting field may evolve in the future.

LA DEFORMATION ANALYSIS

Besides conventional techniques to analyse LA functional parameters (*e.g.*, pulmonary venous velocity, LA phasic volumes, mitral valve inflow velocity or mitral annular velocity; recent advances in deformation analysis allow to quantify LA longitudinal strain and SR using STE or - more recently - CMR-FT[7,11] (Figure 1). Strain and SR represent the magnitude and rate of myocardial deformation (please see the review by Gorcsan and Tanaka for in depth explanation[12]). LA strain profiles result in three aspects of LA physiology: passive strain (ε_E, representing LA conduit function), active strain (ε_A, representing LA contractile booster pump function) and total strain (ε_S, representing atrial reservoir function)[7] (Figure 1 and Table 1). Correspondingly, three SR parameters can be quantified: peak positive strain rate (SR_S, representing LA reservoir function), peak early negative strain rate (SR_E, representing LA conduit function) and peak late negative strain rate (SR_A, representing LA contractile booster pump function)[7] (Figure 1 and Table 1). It is interesting to speculate on the physiological relevance of the three LA functional elements: LA reservoir function as a measure of LA compliance and LA active relaxation may represent a compensatory mechanism at early stages of congestive LV failure. Conversely, LA conduit function as a measure of LA compliance is already affected by early diastolic LV relaxation abnormalities with changes in LV stiffness and compliance. Lastly LA booster pump function representing LA contractility has impact on ventricular filling and cardiac output[13].

LA deformation quantification comprises challenges that are not present when dealing with ventricular strain and SR imaging. These include the insertion of pulmonary veins and the presence of the LA appendage, the thin LA wall and the variable LA geometry[7]. Notwithstanding these facts, 2D STE and CMR-FT have both shown good performance and reproducibility of LA deformation analysis[7,14]. However, using two-dimensional representations of 3D structures may oversimplify the complex LA anatomy. Through-plane motion or reduced STE imaging quality with poor imaging windows can affect LA deformation analysis and may be difficult to correct. Recent advances in STE provide three-dimensional imaging that eliminates the effects of through-plane motion in patients with sufficient imaging windows and may allow the comprehensive analysis of global and regional LA strain[15,16]. At the present time, similar developments for CMR-FT based on three-dimensional imaging have not yet been introduced.

STE

Two-dimensional STE makes use of offline software analysis using conventional gray scale B-mode images, which are typically acquired during a breath-hold. The frame rate is set between 50 and 70 frames/sec. Speckles can be described as acoustic markers distributed within the myocardium, which can be

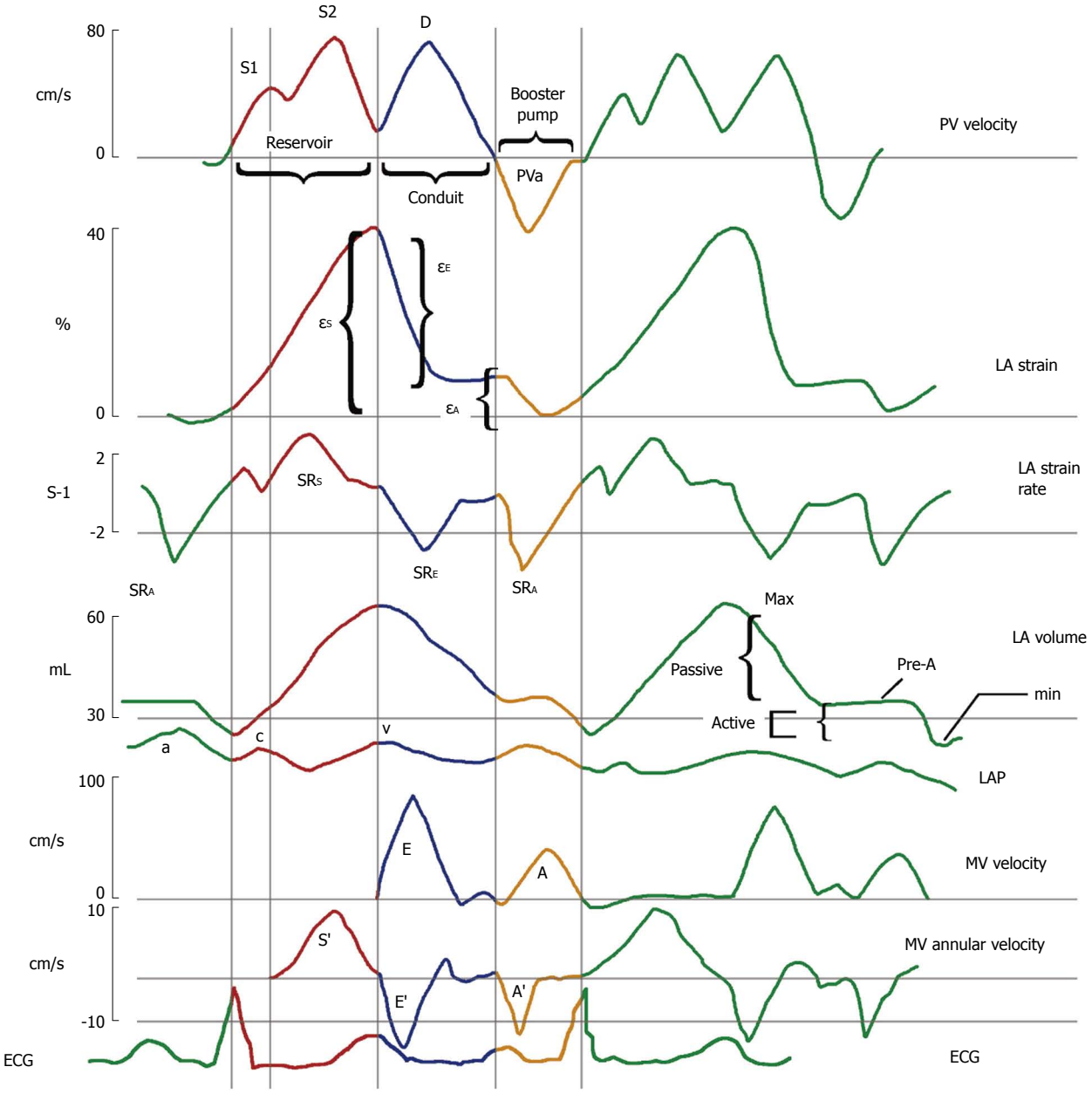

Figure 1 Left atrial physiology imaging using different methods. The figure displays pulmonary venous (PV) velocity, left atrial (LA) strain (ε), LA strain rate (SR), LA volume, left atrial pressure (LAP), and mitral spectral and tissue Doppler. Displayed are two cardiac cycles and the color-coded imaging of reservoir, conduit, and booster pump functions in red, blue, and yellow lines are shown within the first cardiac cycle, respectively. Reprinted from Journal of the American College of Cardiology, Vol 63, Brian D. Hoit, Left atrial size and function: role in prognosis, 493-505, 2014 with permission from Elsevier[6].

tracked from frame to frame[17]. This provides local myocardial displacement information, which can be utilized for the calculation of velocity, strain or SR. It is important to note that there is currently a lack of standardization for LA STE resulting in different approaches to calculate LA deformation indexes: LA strain and SR have been calculated by averaging values from a 15-segment model[18] (six equidistant segments in the apical 4-chamber view, six in the 2-chamber view and three in the 3-chamber view) or from a 12-segment model[14] (six equidistant segments in the 4-chamber view and six segments in the 2-chamber view). Usually, strain and SR indexes are averaged from a total of three consecutive cardiac cycles that provide stable electrocardiographic recording. Furthermore, it is important to understand that there are two different approaches to quantify LA strain with STE. Based on the reference point set at the onset of the P wave (corresponding to the beginning of the atrial cycle)[10,19] or set at the QRS-complex (corresponding to the beginning of the ventricular cycle)[14,20], different LA strain profiles are generated. The description above and the explanation in Figure 1 represent strain profiles acquired with the reference point set at the onset of the QRS-complex resulting in the acquisition of reservoir, conduit and booster pump

Table 1 Left atrial strain and strain rate indexes as determined by speckle tracking echocardiography and cardiovascular magnetic resonance myocardial feature tracking

LA function	Strain	Strain rate
Reservoir	ε_S	SR_S
Conduit	ε_E	SR_E
Booster pump	ε_A	SR_A

Nomenclature refers to the QRS complex set as the reference point and is therefore applicable to both peckle tracking echocardiography and cardiovascular magnetic resonance myocardial feature tracking. LA: Left atrial; ε: Strain; SR: Strain rate.

function[6]. In contrast, if the reference point is set at the onset of the P wave, LA strain profiles display early negative strain (representing LA booster pump function) followed by peak positive strain (representing LA conduit function) while their sum corresponds to LA reservoir function[6].

The wide availability and high temporal resolution of echocardiographic real time images are advantages of STE. However, due to the far-field location of the LA, the main drawback of STE is its dependency on high quality images that frequently cannot be guaranteed in patients with limited acoustic windows[14].

CMR-FT

CMR-FT allows tracking of tissue voxel motion directly from standard steady-state free precession (SSFP) cine CMR images and derivation of myocardial deformation (Figure 2) without the need for additional sequence acquisition as compared to myocardial MR Tagging[7]. Therefore, this technique appears particularly applicable from a clinical perspective and can be easily implemented into a running CMR laboratory. Although CMR-FT was initially validated for ventricular function analysis[21-27], its applicability has recently been extended to quantitative longitudinal LA strain and SR analysis[7]. Typically, LA endocardial borders are initially traced in the 2- and 4-chamber views at the minimum atrial volume after atrial contraction[7]. Subsequently, an automatic tracking algorithm is applied. According to STE, LA contours are divided into six segments[20] and subsequently averaged to global strain and SR indexes using a 12-segment model (six equidistant segments in the 4 and 2-chamber views). CMR-FT benefits from high quality CMR images allowing robust contouring of the thin LA myocardium. Furthermore, CMR includes the acquisition of standardised and highly reproducible imaging planes, which is particularly important in longitudinal studies with repeated measurements[28]. Future studies will need to address whether or not CMR-FT has better inter-study reproducibility than STE. On the other hand, low temporal resolution of CMR images might affect deformation analysis, *e.g.*, the ability to accurately quantify peak strain rates[7]. Future evaluations will have to compare STE and CMR-FT regarding the analysis of LA dynamics to determine whether or not results are interchangeable between modalities or one approach should be preferred over the other.

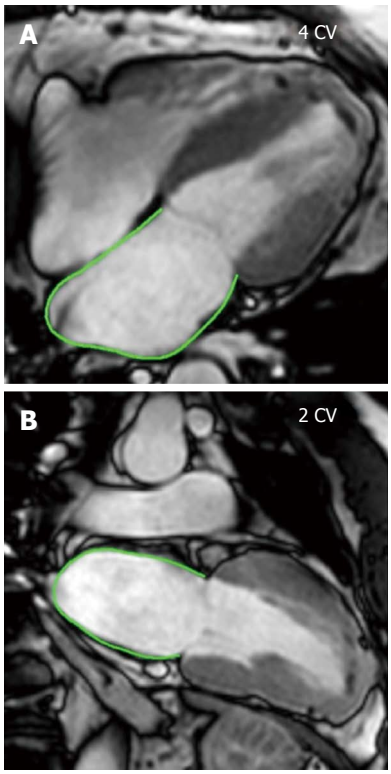

Figure 2 Cardiovascular magnetic resonance myocardial feature tracking of the left atrium in a patient with hypertrophic cardiomyopathy. Cardiovascular magnetic resonance myocardial feature tracking is performed in 4-chamber (A) and 2-chamber (B) views. Contours are based on 48 features, which are tracked throughout the cardiac cycle to generate longitudinal strain and strain rate profiles as displayed in Figure 1.

FUTURE POTENTIAL OF LA DEFORMATION QUANTIFICATION

A growing body of literature suggests to focus on the quantification of the three basic LA functions rather than on the LA volumes only: LA reservoir function has shown to closely correlate with LV filling pressures[29] and has demonstrated to be a sensitive biomarker for the prediction of adverse cardiac events independently of other measures of cardiac dysfunction in patients with heart failure[30]. Strong association between LA conduit function and recurrent atrial fibrillation after pulmonary vein isolation has been described[31]. Accordingly, there has been tremendous effort to study LA dynamics with STE. Mounting evidence suggests that impaired LA strain and SR dynamics have the potential to serve as imaging biomarkers for the prognosis and risk stratification or the decision to intervene in heart failure[32,33], hypertension[34], coronary artery disease[35], atrial fibrillation[36], valvular heart disease[20] and cardiomyopathies[37,38] (please see reviews by Hoit[6] and

Viera *et al*[17] for in depth information).

CMR-FT has been introduced more recently[7]. However, recent studies were able to demonstrate an association of impaired LA reservoir function and the development of heart failure in the general population[39]. Impaired reservoir function as determined by volumetric indexes, strain and SR measurements is also closely related to LV fibrosis[40]. With respect to previous reports on the relevance of LV fibrosis[41], LA reservoir function may also represent a promising target for risk stratification. Furthermore, initial experiences on patients with hypertrophic cardiomyopathy (HCM) and heart failure with preserved ejection fraction (HFpEF) demonstrate impaired LA reservoir and conduit function in HCM and HFpEF[7], when compared to healthy controls. In contrast, patients with HCM exhibit increased LA booster pump function while this is decreased in HFpEF[7]. Future studies will need to investigate whether or not this might refer to a potential compensatory mechanism in HCM, as opposed to a complete impairment of LA dynamics in HFpEF[7]. LA CMR-FT has not been applied to patients with atrial fibrillation yet. Deteriorated image quality, which is frequently present in patients with atrial fibrillation, might negatively impact on CMR-FT quality. It remains to be investigated whether or not CMR-FT is feasible in patients with atrial fibrillation using both, conventional ECG-gated SSFP sequences or real-time CMR techniques[42,43], which have demonstrated improved image quality in arrhythmic patients as compared to conventional ECG-gated techniques[44].

CONCLUSION

LA physiology and pathophysiology as quantified by STE and CMR-FT carry promising clinical and prognostic implications. Future studies will need to apply LA deformation imaging to support our understanding of heart failure development and risk stratification in valvular heart disease, atrial fibrillation, hypertension, coronary artery disease and different types of cardiomyopathy.

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EDITORIAL

Perspective of future drugs targeting sterile 20/SPS1-related proline/alanine-rich kinase for blood pressure control

Gen-Min Lin, Pang-Yen Liu, Ching-Fen Wu, Wen-Been Wang, Chih-Lu Han

Gen-Min Lin, Department of Medicine, Hualien Armed Forces General Hospital, Hualien 971, Taiwan
Pang-Yen Liu, Division of Cardiology, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei 104, Taiwan
Ching-Fen Wu, Department of Internal Medicine, Mennorite Christian Hospital, Hualien 970, Taiwan
Wen-Been Wang, Department of Medicine, Tri-Service General Hospital-Song Shan Branch 105, Taiwan
Chih-Lu Han, Department of Medicine, Taipei Veterans General Hospital, Taipei 112, Taiwan

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Correspondence to: Gen-Min Lin, MD, Department of Medicine, Hualien Armed Forces General Hospital, No.163, Jiali Rd., Xincheng Township, Hualien County 97144, Hualien 971, Taiwan. farmer507@yahoo.com.tw
Telephone: +886-3-8260601
Fax: +886-3-8261370

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Abstract

According to a genome-wide association study, intronic SNPs within the human sterile 20/SPS1-related proline/alanine-rich kinase (*SPAK*) gene was linked to 20% of the general population and may be associated with elevated blood pressure. As cell volume changes, mammalian SPAK kinases respond to phosphorylate and regulate cation-coupled chloride co-transporter activity. To our knowledge, phosphorylation of upstream with-no-lysine (K) (WNK) kinases would activate SPAK kinases. The activation of WNK-OSR1/SPAK cascade on the kidneys and aortic tissue is related to the development of hypertension. Several regulators of the WNK pathway such as the Kelch kinase protein 3 - Cullin 3 E3 ligase, hyperinsulinemia, and low potassium intake to mediate hypertension have been identified. In addition, the SPAK kinases may affect the action of renin-angiotensin-aldosterone system on blood pressure as well. In 2010, two SPAK knock-in and knock-out mouse models have clarified the pathogenesis of lowering blood pressure by influencing the receptors on the kidneys and aortic smooth muscle. More recently, two novel SPAK inhibitors for mice, Stock 1S-14279 and Closantel were discovered in 2014. Targeting of SPAK seems to be promising for future antihypertensive therapy. Therefore we raised some viewpoints for the issue for the antihypertensive therapy on the SPAK (gene or kinase).

Key words: With-no-lysine (K) kinase; Oxidative stress-responsive kinase 1/SPS1-related proline/alanine-rich kinase kinase; Na-Cl co-transporter; Na^+-K^+-$2Cl^-$ cotransporter; Hypertension

Core tip: According to a genome-wide association study, intronic SNPs within the human sterile 20/SPS1-related proline/alanine-rich kinase (*SPAK*) gene was linked to 20% of the general population and may be associated with elevated blood pressure. Based on current studies, targeting of SPAK seems to be promising for future antihypertensive therapy. Therefore, we raised some viewpoints regarding the issue for antihypertensive therapy on the SPAK (gene or kinase).

Lin GM, Liu PY, Wu CF, Wang WB, Han CL. Perspective of future drugs targeting sterile 20/SPS1-related proline/alanine-rich kinase for blood pressure control. *World J Cardiol* 2015; 7(6): 306-310 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i6/306.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i6.306

THE WITH-NO-LYSINE (K) KINASES AND HYPERTENSION AND HYPERKALEMIA

Pseudohypoaldosteronism type Ⅱ, a disease characterizing hypertension with hyperkalemia has been caused by mutations in WNK [with-no-lysine (K)] 1 and WNK4[1]. WNK4 is predominantly produced in the kidneys and epithelial tissues and hence the expression of WNK4 is more restricted than that of WNK1. WNK4 has been shown as a potent inhibitor of diverse epithelial transporters including the renal outer medullary potassium ion channel and the thiazide-sensitive sodium chloride co-transporter (NCC)[2]. In addition, paracellular chloride ion flux is enhanced by WNK4 activity[2]. Importantly, mutations in WNK4 have divergent effects on these transport pathways. WNK4 mutations could increase the inhibition of the renal outer medullary potassium ion channel, relieve the inhibition of NCC, and further promote paracellular chloride ion flux[2,3]. These findings can support a model in which WNK4, as a molecular switch, can alter the balance between potassium ion secretion and chloride ion reabsorption and explain the physiological abnormalities in patients with pseudohypoaldosteronism type Ⅱ. Other WNK kinases also distribute in diverse epithelia throughout the body and are involved in chloride ion flux, suggesting that these kinases may generally participate in the regulation of chloride ion flux.

MOLECULAR PATHWAYS FOR WNK-SPAK/OSR1-NCC/NKCC TO CONTROL BLOOD PRESSURE

As cell volume changes, mammalian SPAK (SPS1-related proline/alanine-rich kinase) and OSR1 (oxidative stress-responsive kinase 1) kinases respond to phosphorylate and regulate cation-coupled chloride cotransporter activity[4]. Phosphorylation of upstream WNK kinases would activate SPAK and OSR1. There are four mammalian WNK kinases: WNK1-WNK4. In humans, WNK1 and WNK4 mutations result in hyperkalemia and hypertension partly by altering renal sodium and potassium transport. WNK1 and WNK4 recruit an endocytic scaffold protein, intersectin, and thereby stimulate endocytosis of ROMK1. This recruitment occurs between the PXXP motif of the WNKs and the SH3 domain of intersectin which is independent of WNK kinase activity. WNKs regulate cation-chloride-coupled cotransporters, Na^+-K^+-2Cl^- cotransporter (NKCC) 1 and NKCC2 (and NCC, under some conditions) dependent on kinase activity[5]. OSR1 and SPAK, two Ste20-related protein kinases, which are bound with and phosphorylated by WNK1 and WNK4, in turn bind with and phosphorylate cation-chloride-coupled cotransporters to increase their activity. Binding of OSR1/SPAK to upstream WNKs and downstream cation-chloride-coupled cotransporters are both mediated by a docking site in the C-terminus of OSR1/SPAK and RFX[V/I] motifs present in WNKs or in NKCCs and NCC[5].

Several regulators of the activation of WNK kinase have been identified in recent animal studies as the Kelch kinase protein 3-Cullin 3 E3 ligase, low potassium intake, hyperinsulinemia, and some hormones (angiotensin Ⅱ, aldosterone and vasopressin), which may act on the kidneys or aortic tissues to affect blood pressure[6-10]. Chávez-Canales *et al*[11] showed that WNK4 could decrease the WNK1 and WNK3-mediated activation of NCC in the kidneys. This finding suggests that WNK kinases form a network in which WNK4 associates with WNK1 and WNK3 to regulate NCC. In addition, the activity of OSR1/SPAK in the kidneys could be enhanced by AMP-activated protein kinase resulting in sodium retention *via* phosphorylation of NKCC2 in obesity[12]. The effect of vasopressin on sodium reabsorption is mediated by SPAK along the distal nephron to control blood pressure as well[13]. Figure 1 shows the potential mechanisms of hypertension related to the WNK-SPAK/OSR1-NCC/NKCC cascade.

SPAK KNOCK-IN AND KNOCK-OUT MOUSE MODELS AND THE EXPRESSION AND FUNCTION OF NCC/NKCC IN THE KIDNEY AND AORTIC TISSUE

Since intronic SNPs within the human *SPAK* gene (also known as *Stk*39) was linked to 20% of the general population and may be associated with hypertension in a genome-wide association study, targeting of SPAK seems to be promising for future

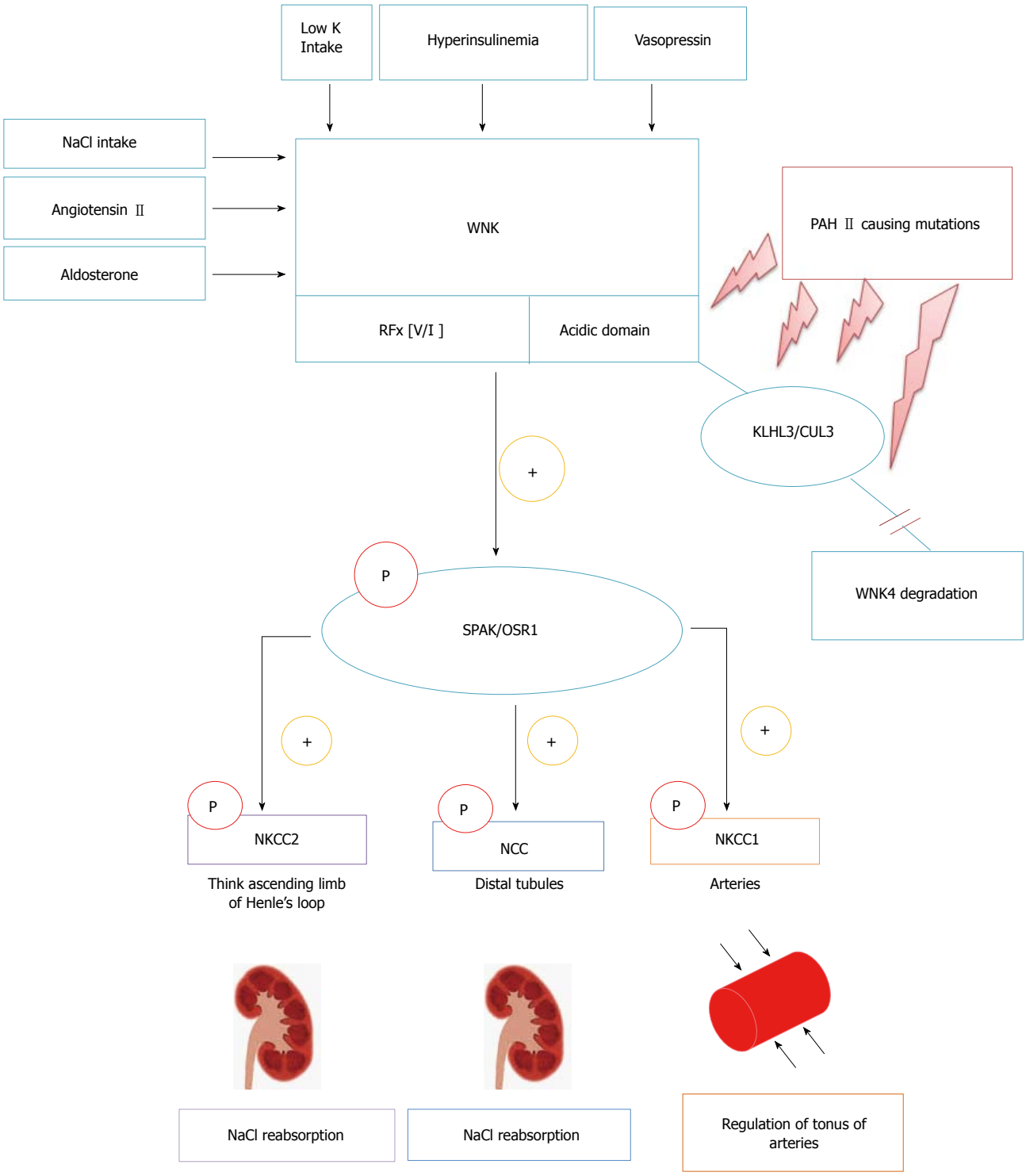

Figure 1 Potential mechanisms of With-no-lysine (K) - SPS1-related proline/alanine-rich kinase/oxidative stress-responsive kinase 1 - Na-Cl co-transporter/ Na^+-K^+-2Cl ($^-$) cotransporter to contribute to hypertension. Several regulators of the activation of WNK cascade, such as KLHL3/CUL3, low potassium intake, hyperinsulinemia and some hormones (angiotensin Ⅱ, aldosterone and vasopressin) may act on the kidneys or aortic tissues. SPAK and OSR1 are activated *via* phosphorylation by upstream WNK kinases using docking site in the RFX (V/I). As a result, SPAK/OSR1 may regulate cation-chloride-coupled cotransporters in kidneys, tonus of aortic tissues, and blood pressure. PAH Ⅱ causing mutations in acidic domain of WNK4, KLHL3 and Cullin 3 activate SPAK/OSR1-NCC signaling by decreasing WNK4 degradation and accumulation of WNK4. KLHL3: Kelch kinase protein-3; CUL3: Cullin3; PAH Ⅱ: Pseudohypoaldosteronism type Ⅱ; WNK: With-no-lysine (K); SPAK: SPS1-related proline/alanine-rich kinase; NCC: Na-Cl co-transporter; NKCC: Na^+-K^+-2Cl ($^-$) cotransporter; OSR1: Oxidative stress-responsive kinase 1.

antihypertensive therapy[14]. In 2010, Yang *et al*[15] generated SPAK null mice in which exons 9 and 10 of the *Stk*39 gene were deleted to investigate its role in the kidneys and aortic blood vessels[15]. Earlier, Rafiqi *et al*[16] had generated knock-in mice in which SPAK could not respond to the WNK kinases. Both

the homozygous SPAK knock-in ($SPAK^{243A/243A}$) and knock-out mice ($SPAK^{-/-}$) demonstrated the same phenotype of hypotension. Rafiqi *et al*[16] accounted for the mechanisms of hypotension in knock-out mice as possibly by lowering expression and phosphorylation of NKCC2 and NCC in the kidneys. Yang *et al*[15] further pointed out that the impaired vasoconstriction may be caused by both reduced function in aortic tissues and NKCC1 phosphorylation in addition to defects of NCC in the kidneys leading to hypotension in their SPAK null mice. However, some different characteristics are present between the SPAK knock-in and knock-out mice that need to be explained. For example, Yang *et al*[15] reasoned the increased NKCC2 phosphorylation in the SPAK null mice due to compensatory up-regulation of OSR1 in the kidneys, which is contrary to the decreased NKCC2 phosphorylation and normal activity of OSR1 in the SPAK inactivated mice.

A PERSPECTIVE FOR DRUG DEVELOPMENT TARGETING OF SPAK TO LOWER BLOOD PRESSURE

To our best knowledge, the SPAK knock-in mice ($SPAK^{+/243A}$/$SPAK^{243A/243A}$) have partial or complete inactivated SPAK function together with WNK1/4 when binding to a cluster of conserved Thr residues which are located at the N-terminal cytosolic domain of the electroneutral cation-coupled chloride cotransporters (SCL2). Because OSR1 binds to a similar cytosolic site on SCL2 with SPAK, to design a drug blocking the binding site between SPAK/OSR1 and SCL2 may affect OSR1 function and result in a hazardous effect. Therefore, the SPAK knock-in mice are more like a model for developing a new drug to target the SPAK protein instead of the binding site of SCL2. As drugs within the cells would inactivate SPAK, they would be competitive antagonist for the site of the N-terminal cytosolic domain of SCL2 with OSR1. As a result, the activity of OSR1 would not be enhanced in SPAK knock-in mice, would subsequently lead to reduced activation of NKCC2 in the kidneys when all the SPAK is inactivated. From this point of view, could we be convinced whether targeting the protein component of SPAK is a promising route? The answer may be derived partly from the blood pressure of $SPAK^{+/243A}$ knock-in mice, which was not reported by Rafiqi *et al*[16]. Although $SPAK^{+/-}$ knock-out mice were observed to have the phenotype of hypotension, this result could not be translated to the knock-in mice directly. Since the SPAK knock-out mice had secondary hyperaldosteronism implying an aldosterone-resistant status which the SPAK knock-in mice did not have, hypotension in SPAK null mice may be associated with this condition rather than the reduction of NKCC1 activity that Yang *et al*[15] proposed. A more definite proof of this would require tissue-specific SPAK knock-out in the vasculature[17], the distribution of SPAK in reference to OSR1 in the arterial vessels in mice should also be estimated. Given that the $SPAK^{+/243A}$ knock-in mice had either a normal range or only a little lower than normal blood pressure, drugs targeting SPAK would work ineffectively. Apparently, the importance of SPAK for the activation of different SCL2 is variable according to their affinity (Kd, dissociation constant) and distributions in tissues. Pharmacokinetically, a drug should be bound to at least half of the SPAK contents to achieve 20% reduction of the epithelial functional NCC and 20% up-regulation of the functional NKCC2 in the kidneys and 40% down regulation of functional NKCC1 in the kidneys and vasculature similar to $SPAK^{+/243A}$ knock-in model[16]. How to determine the optimal drug concentration to obtain the goal of lowering blood pressure would also be a challenge due to different SPAK contents in the tissues and the competition from OSR1.

Alternatively, targeting the gene of *SPAK* in the kidneys and vasculature to produce the heterogeneous knock-out genotype of $SPAK^{+/-}$ with the phenotype of hypotension is a more difficult task. Secondary hyprerreninemia and hyperaldosteronism standing for an aldosterone-resistant status should be highlighted in which they may be harmful to the heart with predominant OSR1 and less SPAK[16,18]. In addition, whether it is useful in people with primary or secondary hyperaldosteronsim should be tested by a hypertensive mouse model with hyperaldosteronism.

Finally, there are some uncertainties regarding the inhibition of SPAK to control blood pressure including the adverse effects of infertility and reduced gastrointestinal glands secretion ability and the protective benefits from sepsis associated with the reduction of NKCC1[19-21]. Recently, Kikuchi *et al*[22] have discovered one small-molecule compound (Stock 1S-14279) and an antiparasitic agent (Closantel) that could inhibit SPAK-regulated phosphorylation and activation of NCC and NKCC1 *in vitro* and in mice[22].

The safety and efficacy of these novel SPAK inhibitors for mice and SPAK knock-in or knock-out mice could provide future models for the control of blood pressure and drug design for human beings. In summary, targeting of the gene or protein of SPAK should be evaluated systematically and the interactions among WNT, OSR1, SCL2 and Renin-Angiotensin-Aldosterone system would need further investigations.

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Preliminary experience with drug-coated balloon angioplasty in primary percutaneous coronary intervention

Hee Hwa Ho, Julian Tan, Yau Wei Ooi, Kwok Kong Loh, Than Htike Aung, Nwe Tun Yin, Dasdo Antonius Sinaga, Fahim Haider Jafary, Paul Jau Lueng Ong

Hee Hwa Ho, Julian Tan, Yau Wei Ooi, Kwok Kong Loh, Than Htike Aung, Nwe Tun Yin, Dasdo Antonius Sinaga, Fahim Haider Jafary, Paul Jau Lueng Ong, Department of Cardiology, Tan Tock Seng Hospital, 308433 Singapore, Singapore

Author contributions: Ho HH designed this study, data analysis, data intepretation and writing manuscript; Tan J, Ooi YW, Loh KK, Jafary FH and Ong PJL designed this study, data interpretation; Aung TH, Yin NT and Sinaga DA collected data and data analysis.

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Correspondence to: Hee Hwa HO, FACC, Department of Cardiology, Tan Tock Seng Hospital, 11, Jalan Tan Tock Seng, 308433 Singapore, Singapore. hokai_wah@yahoo.com
Telephone: +65-63-577831
Fax: +65-63-577772

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Abstract

We evaluated the clinical feasibility of using drug-coated balloon (DCB) angioplasty in patients undergoing primary percutaneous coronary intervention (PPCI). Between January 2010 to September 2014, 89 ST-elevation myocardial infarction patients (83% male, mean age 59 ± 14 years) with a total of 89 coronary lesions were treated with DCB during PPCI. Clinical outcomes are reported at 30 d follow-up. Left anterior descending artery was the most common target vessel for PCI (37%). Twenty-eight percent of the patients had underlying diabetes mellitus. Mean left ventricular ejection fraction was 44% ± 11%. DCB-only PCI was the predominant approach (96%) with the remaining 4% of patients receiving bail-out stenting. Thrombolysis in Myocardial Infarction (TIMI) 3 flow was successfully restored in 98% of patients. An average of 1.2 ± 0.5 DCB were used per patient, with mean DCB diameter of 2.6 ± 0.5 mm and average length of 23.2 ± 10.2 mm. At 30-d follow-up, there were 4 deaths (4.5%). No patients experienced abrupt closure of the infarct-related artery and there was no reported target-lesion failure. Our preliminary experience showed that DCB angioplasty in PPCI was feasible and associated with a high rate of TIMI 3 flow and low 30-d ischaemic event.

Key words: Acute myocardial infarction; Drug coated balloon; Efficacy; Primary angioplasty; Safety

Core tip: Primary percutaneous coronary intervention (PPCI) is the preferred reperfusion therapy for ST-elevation myocardial infarction (STEMI). Stent implantation is considered as a routine during PPCI as it is associated with reduction of ischaemic end-points. Drug-coated balloon (DCB) has emerged as a new therapeutic option to treat coronary artery disease as stent technology has certain limitations. There is however limited data on the feasibility of using DCB as primary therapy in PPCI. We evaluated the clinical safety and efficacy of using paclitaxel-coated balloon in patients undergoing PPCI

for STEMI and report on our 30 d clinical outcomes.

Ho HH, Tan J, Ooi YW, Loh KK, Aung TH, Yin NT, Sinaga DA, Jafary FH, Ong PJL. Preliminary experience with drug-coated balloon angioplasty in primary percutaneous coronary intervention. *World J Cardiol* 2015; 7(6): 311-314 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i6/311.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i6.311

INTRODUCTION

Primary percutaneous coronary intervention (PPCI) is the preferred reperfusion therapy for ST-elevation myocardial infarction (STEMI) if performed in a timely fashion[1]. Stent implantation[2-5] whether with bare metal stent (BMS) or drug-eluting stent (DES) is considered as a routine during PPCI as it is associated with reduction of early ischaemia, restenosis and re-occlusion of culprit artery in comparison with pure old balloon angioplasty (POBA).

Drug-coated balloon (DCB)[6-8] has emerged as a new therapeutic option to treat coronary artery disease (CAD) as stent technology has certain limitations. There is however limited data on the feasibility of using DCB as primary therapy in PPCI. Previous clinical studies[2-3] had shown no difference in the mortality rates between those who received stents or POBA during PPCI with the main difference driven largely by lower rate of target vessel revascularization (TVR) in the stenting group.

It is possible that DCB could close this gap for the POBA group and we therefore evaluated the clinical feasibility (*i.e.*, safety and efficacy) of using paclitaxel-coated balloon in our cohort of South-East Asian patients undergoing PPCI for STEMI.

RESEARCH

Between January 2010 to September 2014, 89 STEMI patients with a total of 89 coronary lesions were treated with SeQuent Please DCB (B.Braun, Melsungen, Germany) as primary therapy during PPCI. The PPCI strategy was to perform thrombus aspiration followed by predilatation of the lesion site before treatment with DCB. Bail-out stenting was performed only when there was significant vessel recoil/coronary dissection. Clinical outcomes are reported at 30 d follow-up.

RESULTS

Table 1 shows the baseline clinical characteristics, angiographic features, procedural data and clinical outcomes of the study patients. The mean age of the patients at presentation was 59 ± 14 years with male preponderance (83%). Twenty-eight percent of the patients had underlying diabetes mellitus. Mean left ventricular ejection fraction was 44% ± 11%. The majority of patients presented with inferior STEMI (55%) with the left anterior descending artery (LAD) being the most common target vessel for PCI (37%) followed by right coronary artery (33%), left circumflex (13%) and others (17%).

Table 1 Baseline clinical characteristics, angiographic features, procedural data and clinical outcomes of patients *n* (%)

	***n* = 89**
Mean age (yr)	59 ± 14
Male: female	74:15 (83:17)
Ever smokers	50 (56)
Diabetes	25 (28)
Hyperlipidemia	41 (46)
Hypertension	49 (55)
Previous myocardial infarction	9 (10)
LVEF	44% ± 11%
Presentation	
Anterior MI	40 (45)
Inferior MI	49 (55)
Target vessel	
LAD	33 (37)
CIRC	12 (13)
RCA	29 (33)
Others	15 (17)
Reference diameter, mm	2.4 ± 0.4
Thrombus aspiration	50 (56)
Predilatation with non-coated balloon	89 (100)
Glycoprotein 2b/3a inhibitors	71 (80)
TIMI flow	
Post-procedural TIMI 2 flow	2 (2)
Post-procedural TIMI 3 flow	87 (98)
30-d clinical outcomes	
Mortality	4 (4.5)
Target vessel revascularisation	0 (0)
Target vessel MI	0 (0)
Target lesion thrombosis	0 (0)

LVEF: Left ventricular ejection fraction; MI: Myocardial infarction; LAD: Left anterior descending artery; CIRC: Left circumflex artery; RCA: Right coronary artery; TIMI: Thrombolysis in myocardial infarction.

Thrombus aspiration was performed in 50 patients (56%) with glycoprotein 2b/3a inhibitors administered in 71 patients (80%). Pre-procedural Thrombolysis in Myocardial Infarction (TIMI) flow was 0 in 70% of patients. At the end of PPCI, TIMI 3 flow was successfully restored in 98% of patients with residual stenosis of 29%.

DCB-only PCI was the predominant approach (96% of patients) with the remaining 4% of patients receiving bail-out stenting for significant recoil/dissection after treatment with DCB. An average of 1.2 ± 0.5 DCB were used per patient, with mean DCB diameter of 2.6 ± 0.5 mm and average length of 23.2 ± 10.2 mm. The mean inflation pressure for DCB was 10 ± 3 atm and mean inflation time was 54 ± 22 s.

At 30-d follow-up, there were 4 deaths (4.5%). Three patients succumbed due to cardiogenic shock and 1 died of sepsis. No patient experienced abrupt closure of the infarct-related artery (IRA) and there

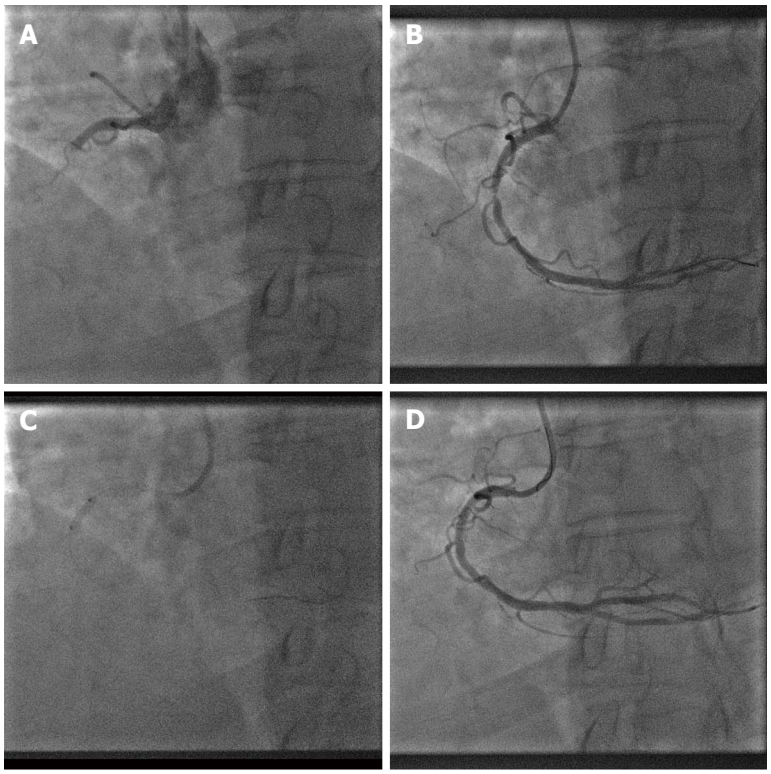

Figure 1 Thrombus aspiration (for visible thrombus) and predilatation with a non-coated balloon prior to drug-coated balloon angioplasty as the final step. A: Baseline coronary angiography showing acute thrombotic occlusion of mid right coronary artery (RCA); B: Mid RCA after thrombus aspiration; C: Predilatation of mid RCA with non-coated balloon; D: Final angiography of mid RCA (after DCB angioplasty).

was no reported TVR, target-vessel-MI or target lesion thrombosis.

DISCUSSION

When compared with fibrinolytic therapy, PPCI in STEMI[1] reduced the rates of death, reinfarction, and stroke. The use of POBA in PPCI has been superseded by routine stenting[2-3] in the contemporary era as the former approach was associated with recurrent ischemia, restenosis, and reocclusion of the IRA. However, prior studies so far had not shown any difference in the mortality rates between those who received stents or POBA during PPCI. The main difference is consistently a lower rate of TVR in the stenting group and it is possible that DCB could close this gap for selected group of patients in the POBA arm. From our preliminary experiences, we demonstrated that the use of DCB as primary therapy for STEMI patients in PPCI was feasible and associated with a high rate of final TIMI 3 flow and low 30-d major adverse cardiac event (MACE).

In recent years, DCB[6-8] has emerged as a viable therapeutic option for treating CAD as the current DES technology has limitations like late stent thrombosis and prolonged dual anti-platelet therapy. Paclitaxel is the drug of choice for all the commercially available DCBs because of its highly lipophilic properties which allows rapid diffusion into the vessel wall and sustained anti-proliferative effect despite its short contact with the vessel wall. The best long term results with DCB is achieved with a DCB-only approach when compared to DCB plus BMS as the former approach is associated with a lower late lumen loss and lower target vessel revascularization.

To gain the utmost benefit from DCB[9], adequate lesion preparation is necessary so that we can maximize balloon contact area to vessel wall for a minimum of 30 s. Similarly in PPCI, we advocate 2 key steps, *i.e.,* thrombus aspiration (for visible thrombus) and predilatation with a non-coated balloon prior to DCB angioplasty as the final step (Figure 1). Removal of thrombus will enable DCB to have better contact with the vessel wall and initial predilatation of the lesion will also allow us to evaluate whether the patient can tolerate prolonged balloon inflation with DCB.

Coronary dissection (iatrogenic) occurs inevitably as result of POBA and abrupt closure of vessel remains one of the most fearful complications. Having good knowledge on the different grades of coronary dissection according to the National Heart, Lung, and Blood Institute (NHLBI) classification[10], one can carefully select patients for DCB angioplasty during PPCI and in our study, no patients experienced abrupt closure of IRA. Only 4% of our patients required bailout stenting for significant recoil/dissection (> Type B dissection). The incidence of abrupt closure of IRA is also significantly reduced in the current era of more

potent anti-platelet agents.

There were several limitations to our study. The number of patients is relatively small and our study was a single-center registry, subject to selection and operator bias. All patients in our study received treatment with SeQuent Please DCB and our results could only be extrapolated to those who had received similar therapy. Whether similar results would be seen with patients receiving other types of DCB is unknown as not all DCBs are equal in terms of clinical efficacy.

In conclusion, the use of DCB as primary therapy in PPCI represents a novel approach in treating STEMI patients. Our preliminary experiences were favourable ie a high rate of final TIMI 3 flow and low 30-d MACE. This approach is possible with appropriate patient selection and by performing 2 key preconditioning steps. Further studies with longer follow-up are necessary to confirm our preliminary findings.

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MINIREVIEWS

Nomenclature, categorization and usage of formulae to adjust QT interval for heart rate

Simon W Rabkin, Xin Bo Cheng

Simon W Rabkin, Xin Bo Cheng, Department of Medicine, Division of Cardiology, University of British Columbia, Vancouver V5Z 1M9, Canada

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Correspondence to: Dr. Simon W Rabkin, MD, FRCPC, FACC, Department of Medicine, Division of Cardiology, University of British Columbia, Level 9 2775 Laurel St, Vancouver V5Z 1M9, Canada. rabkin@mail.ubc.ca
Telephone: +1-604-8755847
Fax: +1-604-8755849

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Abstract

Assessment of the QT interval on a standard 12 lead electrocardiogram is of value in the recognition of a number of conditions. A critical part of its use is the adjustment for the effect of heart rate on QT interval. A systematic search was conducted to identify studies that proposed formulae to standardize the QT interval by heart rate. A nomenclature was developed for current and subsequent equations based on whether they are corrective (QTc) or predictive (QTp). QTc formulae attempt to separate the dependence of the length of the QT interval from the length of the RR interval. QTp formulae utilize heart rate and the output QTp is compared to the uncorrected QT interval. The nomenclature consists of the first letter of the first author's name followed by the next two consonance (whenever possible) in capital letters; with subscripts in lower case alphabetical letter if the first author develops more than one equation. The single exception was the Framingham equation, because this cohort has developed its own "name" amongst cardiovascular studies. Equations were further categorized according to whether they were linear, rational, exponential, logarithmic, or power based. Data show that a person's QT interval adjusted for heart rate can vary dramatically with the different QTc and QTp formulae depending on the person's heart rate and QT interval. The differences in the QT interval adjustment equations encompasses values that are considered normal or significant prolonged. To further compare the equations, we considered that the slope of QTc versus heart rate should be zero if there was no correlation between QT and heart rate. Reviewing a sample of 107 patient ECGs from a hospital setting, the rank order of the slope - from best (closest to zero) to worst was QTcDMT, QTcRTHa, QTcHDG, QTcGOT, QTcFRM, QTcFRD, QTcBZT and QTcMYD. For two recent formulae based on large data sets specifically QTcDMT and QTcRTHa, there was no significant deviation of the slope from zero. In summary a nomenclature permits easy reference to QT formulae that adjust for heart rate. Twenty different formulae can produce discordant calculations of an adjusted QT interval. While the formulae developed by Bazett and Fridericia (QTcBZT and QTcFRD respectively) may continue to be used clinically, recent formulae from large population studies specifically QTcDMT and QTcRTHa appear to be better

to adjust QT for heart rate in clinical practice.

Key words: QT interval; Heart rate adjustment

Core tip: We propose a nomenclature for QT-heart rate adjustment formulae consisting of the first letter of the first author's name followed by the next two consonance with subscripts if the author develops more than one equation. Twenty different QT-heart rate formulae produced discordant calculations of adjusted QT interval. Formulae were categorization into predictive or corrective (QTc) and into linear, rational, exponential, logarithmic, or power based. QTc equations are the most suitable for clinical application. Based on the ability to minimize the slope of a best fit linear relationship between QTc and heart rate, the new formulae QTcDMT and QTcRTHa warrant introduction into clinical practice.

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INTRODUCTION

Assessment of the QT interval on a standard 12 lead electrocardiogram is of value in the recognition of conditions such as electrolyte disturbances, drug-induced cardiac toxicity, genetic abnormalities of cardiac channels (channelopathies) and autonomic nervous system dysregulation[1-6]. Prolonged QT interval has been considered a useful biomarker for electrolyte abnormalities such as hypokalemia or hypomagnesemia[2]. In addition, the duration of the QT interval has been found in epidemiologic studies to identify individuals at high risk of subsequent sudden death[7].

QT interval is highly dependent on heart rate, so that utilization of the QT interval, requires adjustment for the impact of heart rate on the QT interval. Formulae for the adjustment of the QT interval, for heart rate, have been used clinically for almost one hundred years[8,9]. While the original proposals of Bazett[8] and Fridericia[9] remain the most popular methods, there were many other possible choices for heart rate correction proposed in the early years of electrocardiography, as reviewed by Simonson *et al*[10]. There have been considerable concerns about the precision and the validity of the standard QT interval-heart rate adjustments approaches[11-15] that have led to the recent development of QT adjustment formulae from larger numbers of persons[16,17]. Pharmacovigilance data that identified the association of drug-induced sudden cardiac death with prolonged QT interval, generated recommendations by drug approval and monitoring agencies and has led to recommendations to evaluate the effect of drugs on the QT interval - the "Thorough QT Study" (TQT)[18]. Such studies require the careful assessment of the QT interval. The need for evaluation of the QT interval has generated research into how best to isolate the effect of a drug on the QT interval and minimize other factors such as heart rate which changes over time and might influence the QT interval. That research categorized QT-heart rate correction equations and expanded the development of more rate correction approaches that were based on large population studies[16,17,19-21]. This literature has often not been translated to the clinic. The objective of this study is several fold. The first objective was to assemble and review the different QT-heart rate adjustment formulae so as to construct a reference nomenclature which reflects their nature and aids future discussion. The next objective was to compare the QT-heart rate adjustment formulae. The third objective was to assess how well the clinical impact of current widely used methods, which were based on small samples of apparently healthy individuals, compare with the recently proposed formulae that have been based on large sample sizes, often population based.

Our review began with a specific and comprehensive literature search so that all relevant QT interval formulae would be included for our analysis. Second, we applied eligibility criteria to all formulae to limit formulae to those with broad clinical application. Third, we obtained ECGs from a hospital setting to apply the selected formulae to QT and heart rate values. Finally, we compared the most preferred formulae.

LITERATURE SEARCH

A systematic search was conducted to identify studies that proposed equations to standardize the QT interval by heart rate. We searched the Medline and EMbase databases using the PubMed and OvidSP platforms. The full electronic search strategy used was "QT interva" and "heart rate" and reference value. The reference list of publications was searched for other publications so that additional papers from these reference lists were also used for our review.

ELIGIBILITY CRITERIA

Studies that met the following criteria were included: (1) an original study (2) development of the equation in an apparently normal population (3) an adult population (4) clear presentation of the equation, its parameters and conditions (5) equations should be based on ECG measured QT interval. Papers that dealt with cardiac systole rather than QT interval

Table 1 QT correction equations

Ref.	Sample size	Population characteristics	Nomenclature
Linear function			
Sagie *et al* (1992) (Framingham)[26]	5018	Men (2239) and women (2779), aged 28 to 62 yr	QTcFRM
Rational functions			
Hodges *et al* (1983)[31]	607	Men (303) and women (304), aged 20 to 89 yr	QTcHDG
Rautaharju *et al* (2014)[17]	57595	Men and women, aged 5 to 90 yr	QTcRTHa
Power functions			
Bazett (1920)[8]	39	Men (20) and women (19), aged 14 to 53 yr	QTcBTZ
Fridericia (1920)[9]	50	Men and women, aged 30 to 81 yr	QTcFRD
Mayeda (1934)[24]	200	Men (135) and women (65), aged 18 to 64 yr	QTcMYD
Kawataki *et al* (1984)[32]	9	9 male subjects aged 18 to 71 yr, taken at rest, during exercise, and after drug administration	QTcKWT
Dmitrienko *et al* (2005)[16]	13039	Men (6351) and women (6688), aged 4 to 99 yr	QTcDMT
Goto *et al* (2008)[25]	1276	Men aged 20 to 35 yr	QTcGOT
Rautaharju *et al* (2014)[17]	57595	Men and women, aged 5 to 90 yr	QTcRTHb

measurements were excluded except for the early clinical papers. Papers were also excluded if the QT interval was measured mainly in cases with electrolyte abnormalities, only children or in persons with electronic pacemakers.

ECG QT MEASUREMENT

Resting ECGs from a hospital ECG service were evaluated. Only ECGs with sinus rhythm and without bundle branch block, ST elevation myocardial infarction or significant ST-T wave changes were considered. There were 107 ECGs that were anonymously obtained from an acute care hospital. No clinical information is available similar to the usual clinical ECG interpretation setting. ECGs were acquired and digitally analyzed. ECG waveform were sampled at least at 500 samples per second using the Marquette 12SL analysis program (GE Healthcare, Milwaukee, WI, United States). The QT interval is measured "from the earliest detection of depolarization in any lead (QRS onset) to the latest detection of repolarization in any lead (T offset) (The Marquette 12SL analysis program was Marquette™ 12SL™ ECG Analysis Program, GE Healthcare Milwaukee, WI, United States). The QT interval and heart rate measured by the analysis program was used in the heart rate adjustment formulae.

VALIDATION OF EXISTING QT CORRECTION FORMULAE

Recognizing that the goal of the of each formula is to produce QTc values that do not correlated with heart rate or RR interval so that the slope of QTc/RR regression should zero, we calculated the linear slope of eight corrective formulae for the 107 persons with various heart rates. The eight corrective formulae were selected based on clinical usage and relevance.

Statistical analysis

A linear regression model was used to calculate the slope of QTc *vs* heart rate relationship. The goodness of fit of the data to the linear regression (line) is indicated by the standard deviation of residuals.

CATEGORIZATION OF QT FORMULAE

Over 25 different equations were identified. After examination of the formulae, a nomenclature was developed. Formulae were categorized into QT correction or prediction formulae. A correction formula is defined as a formula which attempts to separate the dependence of the length of the QT interval from the length of the RR interval (Table 1). The correction formulae are identified by the subscript with a lower case c. The other category includes predictive formulae, which are defined as formulae that predict an "optimal" QT interval length given the heart rate. The prediction equations are identified by the subscript with a lower case p or QTp (Table 2). Our rationale for this division is based on how each type of formulae is used. For a QTc equation, the patient's heart rate and QT interval are used to calculate a QTc value, which is compared to a standard value. For a QTp equation only the person's heart rate is required, then, the output QTp will be compared to the patient's uncorrected QT interval. QTc limits would be anticipated to be different for each equation, and the same applies to the difference between uncorrected QT and QTp.

Formulae were then divided according to the nature of correction - classified as linear, rational, power, logarithmic, or exponential[20,22] (Table 1). For our naming convention, we identify formulae by the first letter of the first author's name followed by the next two consonants (whenever possible) in capital letters. If the first author develops more than one equation, the equations are labelled by the lower case alphabetical letter as subscript. The only exception to this rule was the Framingham study which has had many authors over the years and is a population based study that has developed its own name and reputation amongst cardiovascular studies.

Table 2 QT prediction equations

Ref.	Sample size	Population characteristics	Equation
Linear functions			
Adams (1936)[33]	104	Men (50) and women (54), mean age 28 yr	QTpADM
Schlamowitz (1946)[34]	650	Men (650) aged 18 to 44 yr	QTpSCH
Karjalainen *et al* (1981)[27]	324 ECGs	Men (military personnel) aged 18 to 28 yr	QTpKRJ
Simonson *et al* (1962)[10]	960	Men (649) and women (311), aged 20 to 59 yr	QTpSMN
Rational functions			
Boudoulas *et al* (1981)[35]	200	Men (100) and women (100), aged 18 to 79 yr	QTpBRL
Hodges *et al* (1983)[31]	607	Men (303) and women (304), aged 20 to 89 yr	QTpHDG
Wohlfart and Pahlm (1994)[36]	37	Men (16) and women (21), aged 38 to 68 yr, taken at rest and during exercise	QTpWHL
Klingfield *et al* (1995)[37]	94	Men, mean age 48 yr, taken at rest and during exercise	QTpKLN
Power functions			
Bazett (1920)[8]	39	Men (20) and women (19), aged 14 to 53 yr	QTpBZT
Fridericia (1920)[9]	50	Men and women, aged 30 to 81 yr	QTpFRD
Mayeda (1934)[24]	200	Men (135) and women (65), aged 18 to 64 yrs	QTpMYD
Schlomka and Raab (1936)[30]	336	Men and women	QTpSCH
Shipley and Hallaran (1936)[23]	200	Men and women, aged 22 to 35 yr	QTpSHP
Hegglin and Holzmann (1937)[38]	700	Men and women	QTpHGG
Kawataki *et al* (1984)[32]	9	Men aged 18 to 71 yr	QTpKWT
Goto *et al* (2008)[25]	1276	Men aged 20 to 35 yr	QTpGOT
Logarithmic functions			
Ashman (1942)[39]	1083	Men (432), women (425), and children (226)	QTpASH
Merri *et al* (1989)[40]	364	Men (191) and women (173) aged 10 to 81 yr	QTpMRR
Exponential functions			
Sarma *et al* (1984)[28]	16	Men (10) aged 18 to 30 yr, taken at rest and during exercise	QTpSRM
Lecocq *et al* (1989)[41]	11	Men (5) and women (6), aged 22 to 26 yr, taken at rest, during exercise, and after drug administration	QTpLCC
Arrowood *et al* (1993)[42]	16	16 subjects, aged 21 to 62 yr	QTpARR

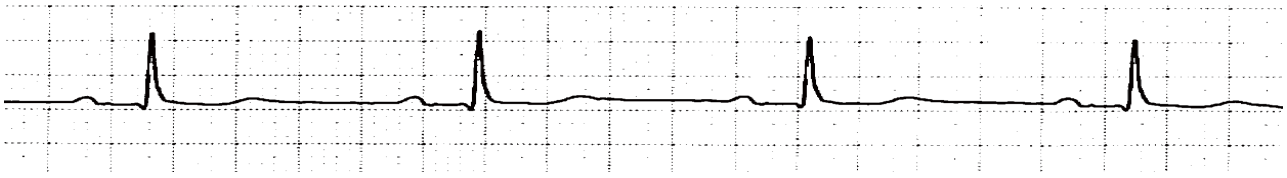
The proportion of men and women is provided when available.

Correction formulae

The majority of corrective formulae utilize a power function to adjust the heart rate (Table 1). The first and still widely used correction equations were: Bazett's proposal, based on a very small sample of normal subjects, that the QT interval varied according to the square root of the heart rate or cycle length (RR interval)[8] and Fridericia's proposal[9] that the cube root of the RR interval was the best adjustment formula. The original Bazett formula which included constants was examined and had the constants eliminated producing the widely used Bazett formula[23]. Dmitrienko *et al*[16] reported on the ECGs from 13039 individuals (men and women) who had ECGs as part of their baseline assessment in clinical drug trials, conducted in 2000 and 2001, sponsored by Eli Lilly and Company. This correction formula was obtained by fitting a linear model to log-transformed QT and RR data. Mayeda[24] examined the ECGs of 200 apparently healthy Japanese individuals. Goto *et al*[25] studied the relationship between RR and QT, using the bootstrap method, in resting ECGs of 1276 healthy young Japanese men. The major linear equation was developed by Sagie *et al*[26] from the Framingham population in the United States. The sample size used to develop or test the equations varied dramatically between studies. The most recent equation, developed by Rautaharju *et al*[17], was based on pooled data from three different sources-two population studies and one large study of baseline ECGs prior to drug testing for a potential effect on QT, and consisted of 57595 individuals. These authors suggested two equations a rational and a power function formula.

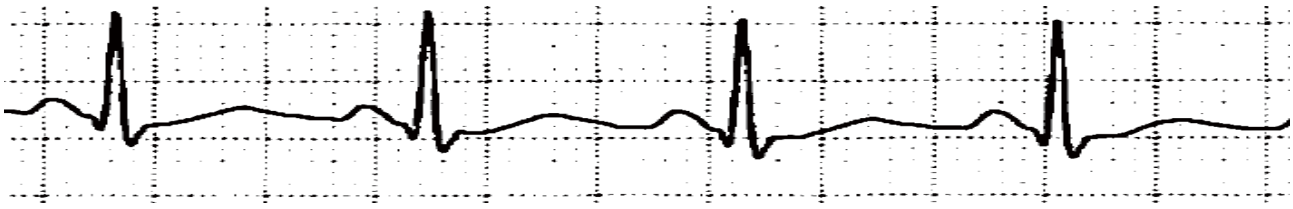
Predictive equations

The largest number of predictive equations also utilizes a power function to adjust for heart rate. The next most frequent adjustment formulae are linear or rational equations (Table 2). Some of the authors have both corrective and predictive equations which differ by the presence of relevant constants in the predictive equation. Simonson *et al*[10] proposed a logarithmic and a linear equation to predict QT interval based on the RR interval. They concluded that "because the logarithmic …and linear ….regression equations gave identical results within the error of measurement, the simpler linear equation … was used for further analysis."[10]. Some of the predictive equations tried to adjust for the nonlinearity of the QT-RR interval relationship by considering different heart rate ranges. Karjalainen *et al*[27] measured the QT intervals in 324 electrocardiograms of healthy young men and weighted the sample for low and high heart rates equally. They concluded that the QT-RR relation does not permit the use of one simple adjustment equation and proposed formulae that provided different



QT	444	QTpADM	405		
QTcFRM	439	QTpSCH	379	QTpSCH	388
QTcHDG	441	QTpKRJ	397	QTpSHP	404
QTcRTH$_a$	439	QTpSMN	408	QTpHGG	397
QTcBZT	437	QTpBRL	405	QTpKWT	454
QTcFRD	439	QTpHDG	395	QTpGOT	440
QTcMYD	435	QTpWHL	388	QTpASH	396
QTcKWT	440	QTpKLN	404	QTpMRR	423
QTcDMT	437	QTpBZT	376	QTpSRM	467
QTcGOT	439	QTpFRD	386	QTpLCC	410
QTcRTH$_b$	439	QTpMYD	424	QTpARR	425

Figure 1 The QTc and QTp heart rate corrections for the uncorrected QT interval measured by computerized assessment of a digitized ECG.



QT	354	QTpADM	332		
QTcFRM	422	QTpSCM	282	QTpSCL	309
QTcHDG	436	QTpKTJ	314	QTpSHP	297
QTcRTH$_a$	446	QTpSMN	337	QTpHGG	292
QTcBZT	473	QTpBRD	307	QTpKWT	389
QTcFRD	429	QTpHDG	309	QTpGOT	357
QTcMYD	502	QTpWHP	327	QTpASH	304
QTcKWT	409	QTpKLN	340	QTpMRR	323
QTcDMT	450	QTpBZT	277	QTpSRM	362
QTcGOT	431	QTpFRD	315	QTpLCC	340
QTcRTH$_b$	429	QTpMYD	293	QTpARR	325

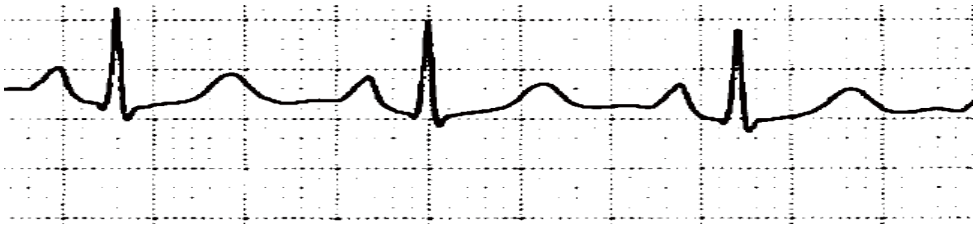
Figure 2 The QTc and QTp heart rate corrections for the uncorrected QT interval measured by computerized assessment of a digitized ECG.

parameters according to the heart rate[27]. Some predictive equations attempted to evaluate the QT-RR interval relationship using interventions to vary heart rate. Sarma *et al*[28] studied 10 healthy, normal men who exercised on a stationary bicycle, and 6 patients with rate-programmable VVI pacemakers whose rates were changed by an external programme, and developed an equation with an exponential function.

The sample size used to develop QTp equations varied between studies but overall the sample sizes were smaller than those used to develop the QTc equations. A number of equations were derived as QTp equations and were subsequently modified to QTc equations with Bazett and Fridericia being the most well-known[8,9].

Application of formulae

To illustrate the application of the various QT adjustment approaches, each of them was applied to three different ECGs (Figures 1-3). The closest correlation between the equations occurred, as expected, in a 71 years old man with a heart rate of 58 bpm where the difference in QTc was 6 milliseconds (ms) (435 to 441 ms) and for QTp the difference was 91 ms with a range from 376 to 467 ms (Figure 1). This is because QTc formulae are largely based on the assumption that the QT interval is accurate at the heart rate of 60 bpm. However, not all QTp equations are based on "normal heart rate" being at 60 bpm. In contrast, a man aged 53 years with a heart rate of 107 bpm, had a QTc ranging from 409 to 502 ms and QTp from 277 to 389 ms (Figure 2). The discrepancy between QTcBZT and QTcFRD was 44 ms. A 53 years old woman had QTc ranging from 424 to 487 ms and QTp from 305 to 408 ms (Figure 3). The discrepancy between QTcBZT and QTcFRD was 30 ms. The differences in the QT interval adjustment between formulae is readily apparent. Importantly the range encompasses values that are considered significant QT prolongation which raise the possibility of the presence of one of the causes for prolonged QT using one equation but a normal QT when considering another equation. The difference between QTcBZT and QTcFRD



QT	384	QTpADM	364		
QTcFRM	434	QTpSCH	305	QTpSCH	329
QTcHDG	435	QTpKRJ	341	QTpSHP	341
$QTcRTH_a$	446	QTpSMN	353	QTpHGG	320
QTcBZT	468	QTpBRL	351	QTpKWT	408
QTcFRD	438	QTpHDG	340	QTpGOT	380
QTcMYD	487	QTpWHL	350	QTpASH	340
QTcKWT	424	QTpKLN	364	QTpMRR	364
QTcDMT	452	QTpBZT	328	QTpSRM	400
QTcGOT	439	QTpFRD	335	QTpLCC	369
$QTcRTH_b$	442	QTpMYD	327	QTpARR	357

Figure 3 The QTc and QTp heart rate corrections for the uncorrected QT interval measured by computerized assessment of a digitized ECG.

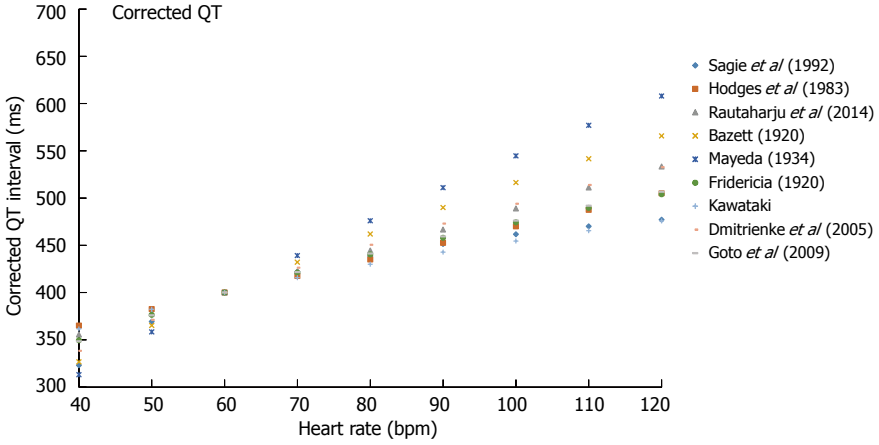


Figure 4 The corrected QT interval for the different correction formulae for a 50 years old man with a QT of 400 ms.

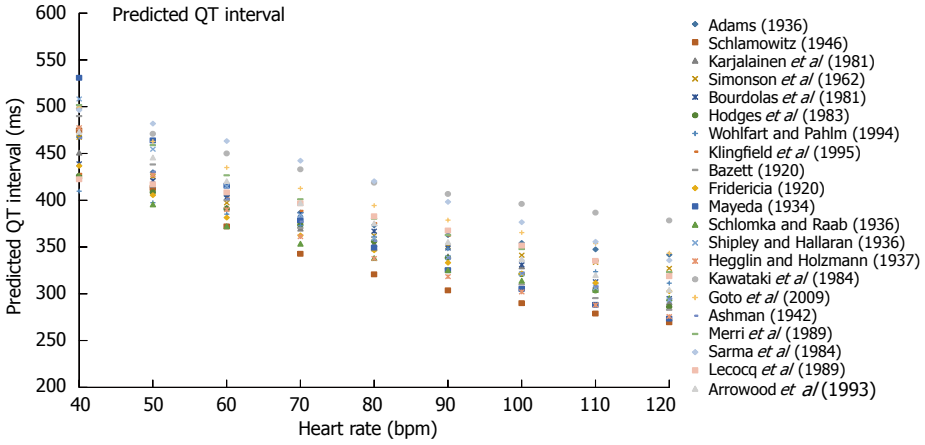


Figure 5 The predicted QT interval for the different correction formulae for a 50 years old man with a QT of 400 ms.

is apparent yet the value used to diagnose prolonged QT syndrome maybe considered to be the same by some clinicians.

To further illustrate the effect of using each of the correction equations an example is used of of an uncorrected QT interval 400 ms in a 50 years old man (Figure 4). By definition, all QTc equations show equipoise at a heart rate of 60 bpm. The discrepancy

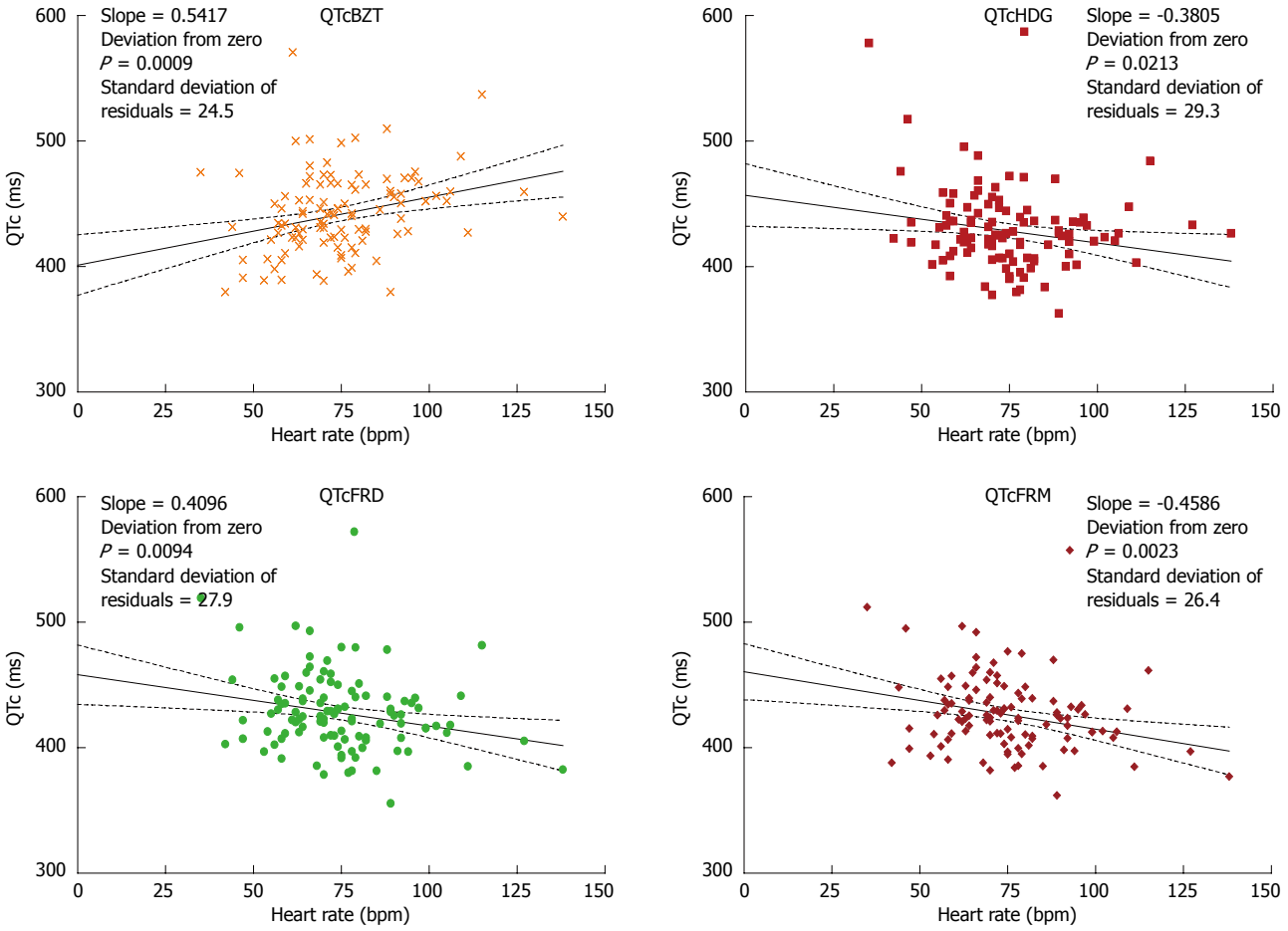


Figure 6 The relationship between QTc and heart rate for QTcBZT, QTcFRD, QTcHDG, and QTcFRM. The slope of the line and how significantly it deviates from zero is shown in the insert. The goodness of fit of the data to the linear regression (line) is shown by the standard deviation of residuals.

in QTc between the different formulae is apparent at slower and faster heart rates with the magnitude of the dispersion increasing at slower and faster heart rates. At a heart rate of 100 bpm, QTc ranges from 462 ms (QTcFRM) to 546 ms (QTcMYD) with QTcBZT at 516 ms and QTcFRD at 474 ms. At a heart rate of 40 bpm, QTc ranges from 313 ms (QTcMYD) to 400 ms (QTcRTHb) with QTcBZT at 327 ms and QTcFRD at 349 ms. The variation among formulae are non-linear, which is again as a result of QTc values being equipoise at 60 bpm.

Prediction equations also show a considerable range of reported QTp values. Considering the same 50 years old man with a QT of 400 ms (Figure 5), at a heart rate of 40 bpm, QTp ranges from 313 (QTpWHL) to 530 ms (QTpMYD) with QTpBZT at 453 and QTpFRD at 436 ms. At a heart rate of 100 bpm, QTp ranges from 290 (QTpSCH) to 396 ms (QTpKWT) with QTpBZT at 287 and QTpFRD at 322 ms. A hypothesis worth considering is that if we combine all QTp equations, which are based on different populations, we may construct an interval where QTp is considered to be normal.

HEART RATE INDEPENDENCE OF QT-HEART RATE FORMULAE

Recognizing that the goal of each formula is to produce QTc values that do not correlated with heart rate, we calculated the linear slope of eight corrective formulae for the 107 persons with various heart rates. The formulae varied in their slope (Figures 6 and 7). Two equations had a slope that was not significantly different from zero namely QTc DMT and QTc RTHa with the former being closest to zero. The other 6 equations had slopes that were significantly different from zero with the largest slope for QTcMYD. Of the equations that showed slopes that deviated from zero QTcBZT was the next largest slope or highest relationship to heart rate. The goodness of fit of the data to the linear regression (line) is shown by the standard deviation of residuals.

QT-heart rate adjustment formulae can generate a range of QT-adjusted values depending on the heart rate. Furthermore a wide range of QT-adjusted values is possible for individuals at any given heart rate. The clinician is confronted with the problem of the correct

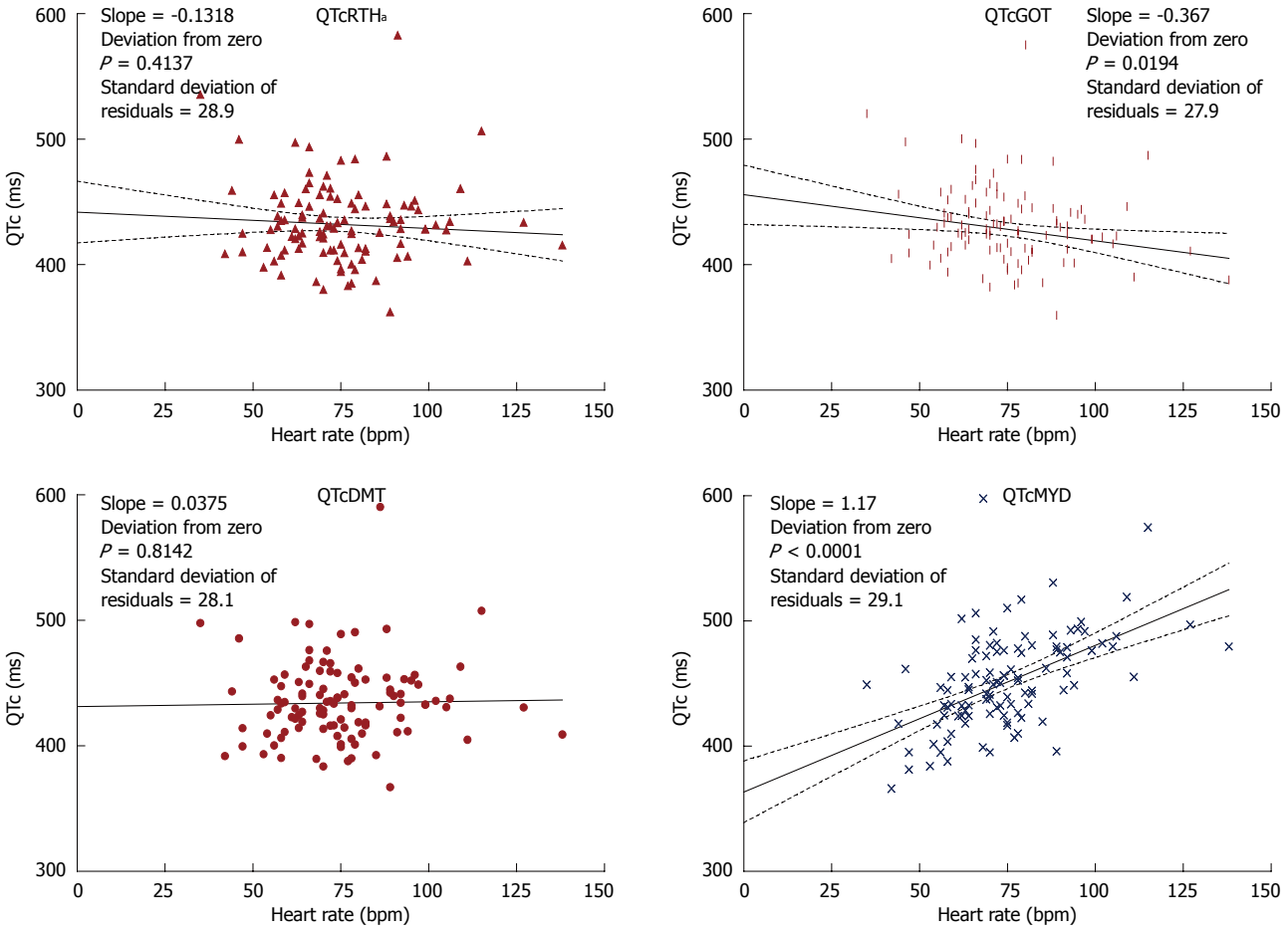


Figure 7 The relationship between QTc and heart rate for QTcRTHa, QTcDMT, QTcGOT, and QTcMYD. The slope of the line and how significantly it deviates from zero is shown in the insert. The goodness of fit of the data to the linear regression (line) is shown by the standard deviation of residuals.

choice for adjusting the QT interval and assessing the implications of the choice.

ABSENCE OF A "GOLD" STANDARD

The central issue is the absence of a true "gold standard" to identify the duration of cardiac repolarization and then to evaluate all the equations against this standard in order to determine the "best" one. The Bazett correction approach (QTcBZT) is a frequently used formula. It has long been known and criticized because it is purportedly "overcorrects" the measured QT interval at fast heart rates and under corrects it at low heart rates"[26]. The absence of a gold standard for heart rate correction makes it difficult to know what the true correction is and what is "over" and "under"-correction. The absence of a "gold" standard has likely played a role in maintaining the use of QTcBZT despite its critics[13,26,29]. While the Fridericia formula (QTcFRD) is believed to be more accurate than QTcBZT, it has already been criticized because it retains the potential for bias at either extreme of heart rate(s)[16] but other formulae also share this feature at clinically relevant faster heart rates.

QTp vs QTc

There are more QTp than QTc equations perhaps because of the manner in which QT adjustment equations are derived. Most QTp equations utilize regressions of population data to a pre-determined form. The procedure is briefly as follows. First, the uncorrected QT interval raw data are presented in a scatter plot. Then, a pre-determined form of equation is selected. The pre-determined form may be linear, rational, exponential, logarithmic, or power based. Next a statistical procedure to minimize the "error" generates parameters for the model. The regressed equation becomes the QTp equation. The error is usually quantified in the form of square of the residuals, but the method to quantify error is ultimately up to the discretion of each author. Predicted QT equations are dependent on the population from which they are derived. Several equations share the same raw form, but only differ in constants. For example, Fridericia[9] and Schlomka and Raab[30] both contain the RR interval raised to the power of one third and Goto *et al*[25] raises RR to the power of 0.3409, which approximates the power of one third. The

reason behind differing constants for the same power may have several explanations. First, there may be variations between studies in selection criteria, genetic factors of the subjects, or environmental factors. Second, there may be systematic differences in data collection such as defining the end of the T wave which is essential for QT measurement. Third, the sample sizes may not be large enough to ensure accuracy for statistical modeling.

The large number of different QTp equations and the multiple different parameters across the different equations is perhaps the reason that clinicians often opt to use QTc formulae rather than QTp formulae. Generally, QTc formulae are stripped of constants and coefficients contained in the initial formula. A presumed advantage of this approach is that some sources of variation due to subject selection, systematic errors, and sample sizes are minimized or eliminated while the important coefficients are determined from the trends of data sets.

A disadvantage of QTc formulae is that while some constants are removed to be less reflective of the sample size, the same constants played a role in the value of regressed parameters. For example, the power parameters without the accompanying constants in QTcBZT and QTcFRD will not minimize the error in the initial sample. This transformation is essentially changing the form of the initial pre-determined equation, without regressing to minimize the error.

A better approach might be fitting the initial data sets without a multiplicative coefficient.

Another problem with QTc formulae is the lack of specific limits for the definition of "prolonged QT" for each QT correction equation. Assuming that each equation is derived from a sample of normal healthy subjects, it is possible to calculate a confidence interval for the predicted duration of the QT interval. There has not, however, been a standard way to determine the confidence interval of a transformed QTc equation from the original data for each formula.

QTc and QTp equations have been categorized according to whether the equations are linear, hyperbolic, parabolic, logarithmic, shifted logarithmic, exponential or general additive models[20,22]. Our classification and nomenclature simplifies the categorization. From usual clinical data, it appears that most recent power correction equations agree with each other, and all equations may use the same limit for prolonged QT. The agreement among power-based QT correction equations is generally good because most resting heart rates are sufficiently close to 60, and that the RR interval is close to 1. For example, at a heart rate of 70 beats per minute, the RR is 1.17 s. The square root of 1.17 is 1.08 and the cube root of 1.17 is 1.05, where the difference is less than 3%. Hence, it is not a coincidence that most power correction equations agree among commonly encountered heart rates. The nature of the equation demands it near the "normal heart rates". The reported phenomenon that some equations fail at higher or lower heart rates is intrinsic to the choice of a power-based model in the regression process. Rautaharju and Zhang[15] concluded that pure power functions generate a rate-dependent bias in the upper and lower ranges of the adjusted QT distribution that can be reduced by incorporating an intercept. We found, however, that approach still led to a rate dependency but agree that the approach minimizes such rate dependency.

From our discussion, it is clear that neither corrective nor predictive formulae have an absolute theoretical benefit over the other. In fact, corrective formulae are often incorrectly derived from predictive equations. However, we advocate for the use of corrective formulae on the basis that they are already readily adopted clinically, and upper limits are already determined by clinicians through decades of experience. Clinically, it is more logical and customary to see if a given measured value (QTc in this case) is within a pre-determined range via a QTc formula rather than a QTp formula. With a QTp formula, an absolute value must be calculated, and such operations can lead to errors in certain instances. To use corrective formulae, more work needs to be done to systemically determine the appropriate upper and lower limits for the duration of QTc for each formula.

QTc HEART RATE INDEPENDENCE

We constructed scatter plots from ECG data obtained from the patient group (Figures 6 and 7). Each QTc formula was applied according to their stated form. This includes any available considerations given to age and gender. Our evaluation begins with Bazett (QTcBZT) and Fridericia (QTcFRD), both commonly used equations in clinical practice. We observe that QTcFRD is associated with a smaller slope with a linear regression line, and this translates into QTcFRD being superior to QTcBZT in attempting to separate the dependence of QT duration on heart rate. However, it is also clear that newer equations with larger sample sizes can achieve much higher accuracy than either QTcFRD or QTcBZT. The rank order of the slope was from best (closest to zero) was QTcDMT, QTcRTHa, QTcHDG, QTcGOT, QTcFRM, QTcFRD, QTcBZT and QTcMYD. As an example, QTcDMT has a slope of 0.04, which is more accurate than other equations studied. Hence, we conclude that QTcDMT should be used in future practice as it best separates the dependence of QTc from heart rate. QTcRTHa was the next best and warrants similar consideration.

There are many steps to take before QTcDMT or QTcRTHa replaces QTcBZT or QTcFRD. First, our results should be corroborated with a larger sized clinical study, with more subjects with well-defined clinical or physiological states. Second, it is essential to determine the upper and lower limits for a normal "QTcDMT" value. We recommend that the upper

and lower bound be set at 95% inclusion of all test subjects, which can be achieved by ranking the results obtained or *via* resampling methods, such as bootstrap or jackknife methods. Lastly, it is important to validate the upper and lower limits in a clinical setting in comparison to a standard by defining the correlation - sensitivity and specificity of newer QTc formulae - QTcDMT or QTcRTH in the detection of electrolyte disturbances, drug-induced cardiac toxicity, genetic abnormalities of cardiac channels (channelopathies) and autonomic nervous system dysregulation.

CONCLUSION

In summary, the clinician has a choice of over 20 different equations to adjust the QT interval to minimize the effect of heart rate on the QT interval. These equations should be referred to by a standard nomenclature such as the one proposed here in. The clinician should recognize that at some heart rates, there will be marked discordances between formulae both for QTc and QTp. We believe that QTc equations are preferred over QTp equations because there are more easily adopted in the clinical setting. Some equations have a slope of their QTc to heart rate close to zero but the fit of the equations may not be ideal. While none of the formulae may completely eliminate the effect of heart rate on the QT interval, some of the recent formulae based on large population samples appear to be better that the older heart rate adjustment formulae. In particular, we have found that QTcDMT and to some extent QTcRTH$_a$ are significantly more accurate than other formulae studied. Larger clinical studies are required to validate their precision. In addition, the lower and upper limits of the newer equations specifically QTcDMT and QTcRTH$_a$ should be tested under a clinical setting to compare them to the current commonly used equations such as QTcBZT and QTcFRD. With these caveats, QTcDMT and QTcRTH$_a$ warrant consideration for implementation in clinical practice.

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MINIREVIEWS

Closing patent foramen ovale in cryptogenic stroke: The underscored importance of other interatrial shunt variants

Gianluca Rigatelli, Alberto Rigatelli

Gianluca Rigatelli, Section of Adult Congenital and Adult Heart Disease, Cardiovascular Diagnosis and Endoluminal Interventions, Rovigo General Hospital, 45100 Rovigo, Italy
Alberto Rigatelli, Urgency Medicine, Verona University Medical School, 37045 Verona, Italy

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Correspondence to: Gianluca Rigatelli, MD, PhD, Section of Adult Congenital and Adult Heart Disease, Cardiovascular Diagnosis and Endoluminal Interventions, Rovigo General Hospital, Viale Tre martiri, 45100 Rovigo, Italy. jackyheart71@yahoo.it
Telephone: +39-347-1912016
Fax: +39-425-394513

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Abstract

Recent trials and metanalysis even not fully conclusive and still debated, at least suggested that mechanical device-based closure of patent foramen ovale (PFO) is more effective than medical therapy in prevent recurrence of stroke. In a proportion ranging from 20% to nearly 40% of patients in literature, PFO is associated to atrial septal aneurysm (ASA): ASA is a well-known entity often associated with additional fenestration. Additionally small atrial septal defects ("Flat ASD") can present with signs of paradoxical embolism and cannot be easily detected by transthoracic echocardiography or even by transesophageal echocardiography and are usually discovered by intracardiac echocardiography at the moment of transcatheter closure. This evidence might change potentially the anatomical diagnosis from PFO to fenestrated ASA or as we called it to "hybrid defect", being a bidirectional flow through a small ASD or/and an additional fenestration, often present. Despite the differences in anatomy, pathophysiology and haemodynamic paradoxical embolism may occur in both entities and also may be the first appearance of fenestrated ASA. Because some overlapping do really exist between PFO and hybrid defects, which are often not clearly differentiable by standard diagnostic tools, it is likely that a proportion of patients evaluated for potential transcatheter closure of PFO had actually a different anatomical substrate. These different anatomical and pathophysiologic entities have not been address in any of the previous trials, potentially having an impact on overall results despite the similar mechanical treatment. Neurologists and general cardiologists in charge of clinical management of PFO-related cryptogenic stroke should be aware of the role of hybrid defects in the pathophysiology of paradoxical embolism - mediated cerebral ischemic events in order to apply the correct decision - making process and avoid downgrading of patients with paradoxical embolism-related interatrial shunt variants different from PFO.

Key words: Atrial septal defect; Patent foramen ovale; Echocardiography; Anatomy

Core tip: Recent trials and met analysis suggested that mechanical device-based closure of patent foramen ovale (PFO) is more effective than medical therapy in prevent recurrence of stroke. Fenestrated atrial septal aneurysms and small atrial septal defects (hybrid defects) can present with signs of paradoxical embolism and because they are often not clearly differentiable by standard diagnostic tools, it is likely that a proportion of patients evaluated for transcatheter closure of PFO, had actually a different anatomical substrate. These different anatomical entities have not been address in any of the previous trials, potentially having an impact on overall results.

Rigatelli G, Rigatelli A. Closing patent foramen ovale in cryptogenic stroke: The underscored importance of other interatrial shunt variants. *World J Cardiol* 2015; 7(6): 326-330 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i6/326.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i6.326

INTRODUCTION

Recent trials and metanalysis and in particular, the RESPECT trial[1], even not fully conclusive and still debated, at least suggested that mechanical device-based closure of patent foramen ovale (PFO) is more effective in prevent recurrence of stroke by near 67%. When looking deeply into the number of different metanalysis of PFO closure for cryptogenic (or better paradoxical embolism mediated) stroke[2-7], it appears clear that in a proportion ranging from 20% to nearly 40% of patients PFO is associated to atrial septal aneurysm (ASA): ASA is a well-known entity often associated with additional fenestration[8]. Additionally small atrial septal defects can present with signs of paradoxical embolism and cannot be easily detected by transthoracic echocardiography or even by transesophageal echocardiography and are usually discovered by intracardiac echocardiography at the moment of transcatheter closure[9], confusing even more the diagnosis of PFO which is at the basis of all the studies about effectiveness of transcatheter closure in cryptogenic stroke. This evidence might change potentially the anatomical diagnosis from PFO to fenestrated ASA (or fenestrated secundum atrial septal defect) or to a so called "hybrid defect", [a small single atrial septal defects (ASD) associated with paradoxical embolism], being a bidirectional flow through a small ASD or and additional fenestration often present. The aim of this review is to analyse conjunction points between PFOs and hybrid defects and outlined the role of these type of interatrial shunt in the pathophysiology of paradoxical embolism.

PRACTICAL EPIDEMIOLOGY

Isolated ASD represent 7% of all cardiac anomalies and can present at any age[10]. Adolescents and adults with isolated atrial septal defects are more likely to reach adult age without being diagnosed. Secundum ASD is by far the most common type, occurring in 1/1500 live births, with 65% to 75% involving females[10]. On the other hand, PFO represents an endemic variant in the normal population with a prevalence of 25%-27%[11]. These two entities appear so different that is difficult to find a conjunction ring: nevertheless we use the same philosophy for the treatment. Indeed, device - based closure has been proved to be effective[12,13] in both settings.

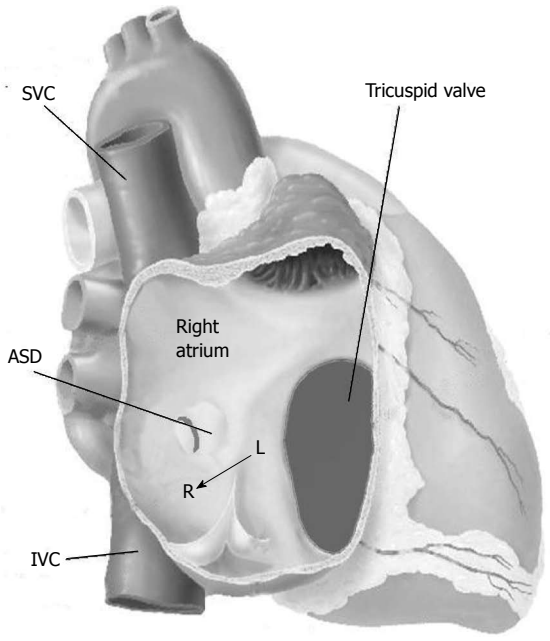


Figure 1 Secundum atrial septal defect is a defect located into the limit of the fossa ovalis. The shunt usually is left-to-right because of the pressure gradient between the left (high pressure) and right (low pressure) atrium. ASD: Atrial septal defect; IVC: Inferior vena cava; L: Left; R: Right; SVC: Superior vena cava.

Anatomy, pathophysiology, and haemodynamic

From an anatomic and pathophysiologic point of view these two entities are absolutely different.

The ostium secundum ASD is a defect of the atrial septum (Figure 1) within the limit of the fossa ovalis and causes usually a left-to-right shunt, being the left atrial pressure higher than the right atrial one. The volume of and direction of flow through an ASD depend on the size of the hole and the relative diastolic filling properties of the left and right chambers. Reduced left ventricle compliance and mitral stenosis increase the left-to-right shunt, whereas reduced right ventricle compliance may decrease the left-to-right shunt or may cause a right-to-left shunt. A Qp/Qs ratio > 1.5:1 or dilation of the right chambers defined a left-to-right shunt as significant[14].

The PFO is defined as the incompetence of the fossa ovale valve determining a right-to-left shunt (Figure 2). The reason because a right-to-left atrial

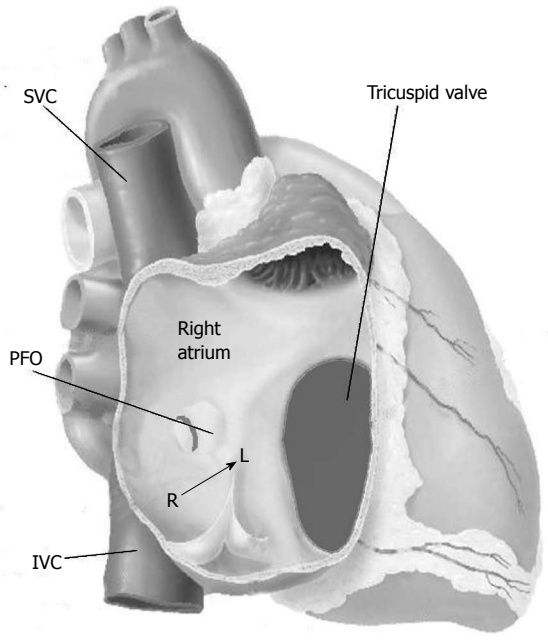


Figure 2 Patent foramen ovale is a communication between the right and left atrium caused by the incompetence of the fossa ovalis valve. The shunt is usually right-to-left despite the gradient pressure between the atria. IVC: Inferior vena cava; L: Left; PFO: Patent foramen ovale; R: Right; SVC: Superior vena cava.

shunting occurs with normal intracardiac pressures and normal or near-normal pulmonary function through a PFO has still not been completely clarified. An explanation may arise from some few considerations. Firstly, a physiologic transient spontaneous reversal of difference between the left and the right pressure is physiologically present during early diastole and during isovolumetric contraction of the right ventricle of each cardiac cycle; this right-to-left gradient may be sustained by physiologic manoeuvres that increase the right atrial pressure such as posture, inspiration, cough or Valsalva manoeuvre, or by situation in which pulmonary vascular resistances results increased, such as acute pulmonary embolism, hypoxemia due to obstructive sleep apnoea, severe chronic obstructive pulmonary disease, right ventricular infarction and positive end-expiratory pressure during neurosurgical procedures in the sitting position. Secondly, another theory explaining the right-to-left shunting through a PFO, is represented by the "so-called" "flow phenomenon". It describes a preferential blood flow from the inferior vena cava towards the atrial septum as a part of the ancient foetal circulation pathway[15].

Thirdly, the increasing stiffness of the right chambers compared to the left chambers caused by aging has been postulated. Finally, conditions such due to mediastinal shift or heart counter-clockwise rotation and/or distortion, following an ascending aorta enlargement, right pneumectomy or pericardial effusion may cause an anatomic disarray of the inferior vena cava relationship with the interatrial septum favouring part of the blood flow to enter the left atrium throughout a PFO[16].

Even from a haemodynamic point of view, ASD obviously differs from PFO. ASD are usually associated with pulmonary hypertension of different degree, an increased Qp/Qs ratio and enlarged right chambers, whereas the usual findings in PFO patients is a normal or slightly elevated pulmonary pressure, normal Qp/Qs ratio, and normal right chambers. Sometimes in presence of a PFO associated with large ASA, a mild impairment of the left atrial function can be observed[17].

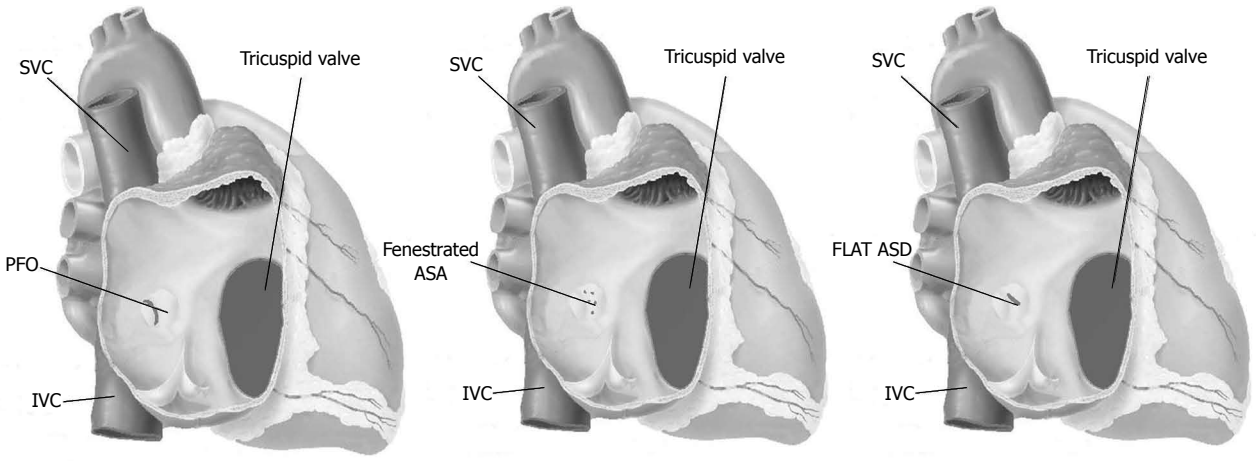
Usually also fenestrated secundum ASD with or without ASA tends to present less right chambers enlargement and only slightly increase in mean pulmonary pressure compared to secundum ASD.

CONJUNCTION POINTS

Despite the gross differences in anatomy and haemodynamic, when we look to the clinical presentation and patho-physiology, we can find some contact points. Excepted for supraventricular arrhythmias and dyspnoea, usually present only in secundum single and fenestrated ASD, paradoxical embolism may occur in both entities and also may be the first appearance of fenestrated ASD with or without ASA.

Usually paradoxical embolism is associated with PFO but occasionally secundum ASD, pulmonary arterio-venous fistula, and other intracardiac septal defects may act as alternative pathophysiological mechanism. Microemboli from a vein thrombotic location, or as recently postulated[17] microthrombotic stratification on the surface of a huge ASA or in the left atrium itself as a result of a left atrial dysfunction induced by the PFO and ASA itself, may navigate to the left side of the circulation through the PFO causing different ischemic syndromes. Differently, the pathophysiology of paradoxical embolism through a secundum ASD is usually caused by a temporaneous right heart pressure increasing which induce a right-to-left shunting which allows a venous thromboembolus to enter the arterial circulation. As an alternative mechanism, Valsalva manoeuvre, coughing, or straining might increase right-to-left component of a bidirectional shunt inducing a paradoxical embolism in ASD patients, in particular in elderly patients, more prone to rapid change of right chambers pressure because of the increasing stiffness of the chambers.

Recently in an analysis of our institutional database we found 24 (6.2%) with a secundum ASD out of 386 patients evaluated for paradoxical embolism. Defects were multifenestrated in 41.6% (10/24). Single ASD (58.3%) had a "flat" elliptical shape with a major axis of 7.6 ± 2.4 and minimal axis of 2.5 ± 1.6 mm when assessed with intracardiac echocardiography. Patients with ASD-related paradoxical embolism had more frequently a deep venous thrombosis, bigger stroke areas compared to PFO patients, and massive curtain shunt on Valsalva maneuver on transcranial



PFO	Hybrid ASD	
	Fenestrated ASA	Flat ASD
R-to-L shunt on TEE	Bidirectional shunt on TEE	
Normal PAP	Normal or slightly increased PAP	
Normal right chambers	Normal or slightly increased right chambers	
Permanent/valsalva shunt on TSD	Valsalva shunt on TSD	

Figure 3 Spectrum of interatrial shunt associated with possible paradoxical embolism and potential related haemodynamic and functional characteristics. ASA: Atrial septal aneurysm; ASD: Atrial septal defect; FO: Fossa ovalis; IVC: Inferior vena cava; PAP: Pulmonary artery pressure; PFO: Patent foramen ovale; R-to-L: Right to left shunt; SVC: Superior vena cava; TCD: Transcranial doppler; TEE: Transesophageal echocardiography.

Doppler. When compared to non-emboligenous ASD, they had lower mean pulmonary pressure, lower mean Qp/Qs, and had bidirectional shunt at rest[18]. As matter of fact, flat elliptical shape ASD and fenestrated ASD with or without ASA appear to represent the conjunction ring between ASD and PFO, being a hybrid hemodynamic and clinical profile compared to each of the others (Figure 3).

FINAL CONSIDERATIONS: ARE THE PAST TRIALS REALLY FOCUSED ON THE PROPER ANATOMICAL ENTITY?

Because some overlapping do really exist between PFO, fenestrated ASD and hybrid defects (Figure 1) which are not always clearly differentiable by standard diagnostic tools, it is likely that a proportion of patients evaluated for potential transcatheter closure of PFO had actually a different anatomical substrate. Past trials and case series used Transeophageal guidance in the majority of patients and the severity of the shunt and presence of permanent shunt has not been evaluated systematically by Transcranial Doppler or transesophageal echocardiography in the enrolment process.

Current modern judgement about medical or mechanical closure is suggested to be based, following the only published multidisciplinary consensus[19], on recurrent stroke or ischemic event with positive neuroimaging studies, severe shunt graded by transcranial Doppler and transesophageal echocardiography, presence of permanent shunt, and of additional anatomical features, such as ASA, tunnel-like opening, and Eustachian valve. The large and permanent shunt in particular, as previously suggested[18,20] is one of the most influent parameters, and it appears clear that in presence of an hybrid defect, it doesn't play the same role as in true PFO, while ASA and Eustachian valve may be more influent facilitating paradoxical shunt through an hybrid defect or a fenestrated ASD when a Valsalva manoeuvre is provoked. These different anatomical and pathophysiologic pictures have not been address in any of the previous trials, potentially having an impact on overall results despite the similar mechanical treatment.

From a practical point of view, patients with deep vein thrombosis and more clinically relevant ischemic syndrome are more likely to have a hybrid defect, whereas patients with no deep vein thrombosis and mild symptomatology are more likely to have a PFO. An ideal screening of a patient with suspected paradoxical embolism should include not only the transthoracic echo, but also the transesophageal echo in order to differentiate between PFO and hybrid defects.

At the light of what we discussed above, neurologists and general cardiologists in charge of clinical management of PFO-related cryptogenic stroke should be aware of the role of hybrid defects and multi-fenestrated ASA in the pathophisiology of paradoxical embolism - mediated cerebral ischemic events in order to apply the correct decision -making process and avoid downgrading of patients with paradoxical embolism-related interatrial shunt variants different from PFO.

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ORIGINAL ARTICLE

Basic Study

Bone morphogenetic protein-4 and transforming growth factor-beta1 mechanisms in acute valvular response to supra-physiologic hemodynamic stresses

Ling Sun, Philippe Sucosky

Ling Sun, 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

AIM: To explore *ex vivo* the role of bone morphogenetic protein-4 (BMP-4) and transforming growth factor-beta1 (TGF-β1) in acute valvular response to fluid shear stress (FSS) abnormalities.

METHODS: Porcine valve leaflets were subjected *ex vivo* to physiologic FSS, supra-physiologic FSS magnitude at normal frequency and supra-physiologic FSS frequency at normal magnitude for 48 h in a double-sided cone-and-plate bioreactor filled with standard culture medium. The role of BMP-4 and TGF-β1 in the valvular response was investigated by promoting or inhibiting the downstream action of those cytokines *via* culture medium supplementation with BMP-4 or the BMP antagonist noggin, and TGF-β1 or the TGF-β1 inhibitor SB-431542, respectively. Fresh porcine leaflets were used as controls. Each experimental group consisted of six leaflet samples. Immunostaining and immunoblotting were performed to assess endothelial activation in terms of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 expressions, paracrine signaling in terms of BMP-4 and TGF-β1 expressions and extracellular matrix (ECM) remodeling in terms of cathepsin L, cathepsin S, metalloproteinases (MMP)-2 and MMP-9 expressions. Immunostained images were quantified by normalizing the intensities of positively stained regions by the number of cells in each image while immunoblots were quantified by densitometry.

RESULTS: Regardless of the culture medium, physiologic FSS maintained valvular homeostasis. Tissue exposure to supra-physiologic FSS magnitude in standard medium stimulated paracrine signaling (TGF-β1: 467% ± 22% *vs* 100% ± 6% in fresh

controls, BMP-4: 258% ± 22% *vs* 100% ± 4% in fresh controls; $P < 0.05$) and ECM degradation (MMP-2: 941% ± 90% *vs* 100% ± 19% in fresh controls, MMP-9: 1219% ± 190% *vs* 100% ± 16% in fresh controls, cathepsin L: 1187% ± 175% *vs* 100% ± 12% in fresh controls, cathepsin S: 603% ± 88% *vs* 100% ± 13% in fresh controls; $P < 0.05$), while BMP-4 supplementation also promoted fibrosa activation and TGF-β1 inhibition reduced MMP-9 expression to the native tissue level (MMP-9: 308% ± 153% with TGF-β1 inhibition *vs* 100% ± 16% in fresh control; $P > 0.05$). Supra-physiologic FSS frequency had no effect on endothelial activation and paracrine signaling regardless of the culture medium but TGF-β1 silencing attenuated FSS-induced ECM degradation *via* MMP-9 downregulation (MMP-9: 302% ± 182% *vs* 100% ± 42% in fresh controls; $P > 0.05$).

CONCLUSION: Valvular tissue is sensitive to FSS abnormalities. The TGF-β1 inhibitor SB-431542 is a potential candidate molecule for attenuating the effects of FSS abnormalities on valvular remodeling.

Key words: Aortic valve; Fluid shear stress; Calcification; Bone morphogenetic protein; Transforming growth factor beta

Core tip: Although flow abnormalities have been shown to promote valvular pathogenesis in a bone morphogenetic protein-4 (BMP-4)- and transforming growth factor-beta1 (TGF-β1)-dependent manner, the mode of action of those molecules in response to fluid shear stress (FSS) abnormalities remains unknown. This *ex vivo* study aimed at isolating the role played by those cytokines in the acute response of porcine leaflets to supra-physiologic FSS magnitude/frequency. The study reveals that: (1) valvular endothelial activation is weakly regulated by BMP-4 in response to FSS abnormalities; (2) TGF-β1 silencing attenuates FSS-induced extracellular matrix degradation *via* MMP-9 downregulation; and (3) BMP-4 and TGF-β1 do not synergistically interact in response to FSS abnormalities.

Sun L, Sucosky P. Bone morphogenetic protein-4 and transforming growth factor-beta1 mechanisms in acute valvular response to supra-physiologic hemodynamic stresses. *World J Cardiol* 2015; 7(6): 331-343 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i6/331.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i6.331

INTRODUCTION

Calcific aortic valve disease (CAVD) affects 3% of the general population above 75 years of age and is the first indication for valvular replacement worldwide[1,2]. The formation of calcific lesions on the valve leaflets involves active processes including inflammation[3,4], extracellular matrix (ECM) remodeling[5-7] and osteogenesis[8-10]. Calcified valves typically exhibit increased expression of cytokines such as transforming growth factor-beta1 (TGF-β1)[11] and bone morphogenic protein-4 (BMP-4)[12]. While genetic defects[13] and conventional cardiovascular risk factors[2] have been identified as potential triggers of CAVD, blood flow abnormalities have emerged as a potential concomitant contributor[14-16]. Aging, hypertension and anatomical valve defects such as the bicuspid aortic valve, which are risk factors for CAVD[17-21], generate hemodynamic alterations that result in an abnormal friction force or "fluid shear stress" (FSS) on both sides of the leaflets[22-24].

To date, the evidence of causality between hemodynamic abnormalities and valvular pathogenesis has been provided *ex vivo*, using sophisticated bioreactors aimed at replicating the characteristics of the leaflet FSS environment[25,26]. Due to the challenge to replicate the native side-specific leaflet FSS (*i.e.*, unidirectional on the ventricularis, oscillatory on the fibrosa), early studies investigated the role played by FSS abnormalities in valvular pathogenesis by subjecting only one leaflet surface at a time to flow. Studies conducted using this simplified model demonstrated the capability of combined alterations in FSS magnitude and pulsatility to stimulate inflammation on the leaflet fibrosa in a TGF-β1- and BMP-4-dependent manner[27], and the capability of elevated FSS to activate the fibrosa endothelium *via* synergies between BMP-4 and TGF-β1 pathways[28]. Recent advances in bioreactor design have enabled the replication of the native side-specific leaflet FSS in the laboratory setting. Simultaneous exposure of both leaflet surfaces to abnormalities in FSS magnitude and/or frequency has revealed the high sensitivity of the leaflet tissue to elevated FSS magnitude or frequency, and the ability of FSS abnormalities to promote paracrine signaling and ECM degradation[29].

The clear involvement of BMP-4 and TGF-β1 in hemodynamically induced CAVD provides a rationale for considering those molecules for targeted cell-based therapies aimed at attenuating or blocking the downstream pathological cascade. However, the upstream role of and potential synergies between TGF-β1 and BMP-4 in the transduction of FSS abnormalities have not been evidenced in the native (*i.e.*, side-specific) leaflet FSS environment. Supported by our previous results, the present study addresses the hypothesis that TGF-β1 and BMP-4 synergistically interact to regulate valvular pathogenesis in response to side-specific alterations in FSS magnitude or frequency. This hypothesis was tested *ex vivo* by exploring the effects of each cytokine on the downstream FSS-induced pathological response. This dependence was characterized by measuring endothelial activation, paracrine signaling and ECM

remodeling events secondary to FSS alterations after silencing or promoting pharmacologically the expression of each molecule.

MATERIALS AND METHODS

Experimental conditions

Porcine aortic valve leaflets were subjected to physiologic and supra-physiologic FSS environments *ex vivo* using our double-sided cone-and-plate bioreactor (Figure 1A)[26]. This device has been previously validated mechanically and biologically and implemented in different *ex vivo* studies to subject simultaneously but independently both leaflet surfaces to desired side-specific and time-varying FSS[29,30]. Fresh porcine valves (6-12 mo) were obtained from a local abattoir (Martin's Custom Butchering, Wakarusa, IN), immediately transported to the laboratory in ice-cold phosphate buffered saline. A circular section of 7 mm in diameter was excised from the base of each leaflet. Two samples from each valve were used as experimental samples while the third sample served as fresh control. Six experimental samples were mounted in the bioreactor, exposing both their aortic and ventricular sides to FSS. All experiments were conducted for 48 h, a duration sufficient for valve leaflets to transduce FSS abnormalities into a pathological response[29].

Consistent with our previous studies[27-29], the physiologic FSS environment consisted of a unidirectional FSS varying between 0 and 80 dyn/cm^2 on the ventricularis (leaflet surface facing the ventricle) and a reciprocal FSS varying between -8 and +10 dyn/cm^2 on the fibrosa (leaflet surface facing the aorta; Figure 1B). The two supra-physiologic FSS environments consisted of supra-physiologic FSS magnitude (*i.e.*, twice the physiologic level) at physiologic frequency (Figure 1C) and supra-physiologic FSS frequency (*i.e.*, twice the physiologic frequency) at physiologic magnitude (Figure 1D). Those abnormal FSS environments were selected based on their demonstrated ability to stimulate acute CAVD mechanisms[29].

In order to isolate the possible synergies between BMP-4 and TGF-β1, the experiments were conducted using standard culture medium (Dulbecco's Modified Eagle Medium, Sigma) as well as four additional culture medium variations. The downstream action of BMP-4 was blocked by supplementing the standard culture medium with noggin, a well-known BMP antagonist[31-33], while TGF-β1 signaling was blocked by supplementing the medium with SB-431542, a small molecule inhibitor specifically targeting the TGF-β type-I receptor[34,35]. The inhibitor concentrations used in this study (noggin: 100 ng/mL; SB-431542: 1 μmol/L) have been shown to effectively inhibit BMP- and TGF-β1 signaling in response to stretch and FSS abnormalities *ex vivo*[27,28,36]. Conversely, BMP-4 and TGF-β1 signaling were promoted by supplementing the standard culture medium with recombinant BMP-4 and TGF-β1, respectively, using concentrations (BMP-4: 10 ng/mL; TGF-β1: 10 ng/mL) previously established to effectively enhance paracrine signaling processes in valvular tissue[27,28]. Fresh porcine leaflets were used as controls.

Biological characterization

Endothelial activation was assessed in terms of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Paracrine signaling events were characterized in terms of the cytokines BMP-4 and TGF-β1. ECM remodeling and degradation were quantified in terms of matrix metalloproteinases (MMP-2 and -9) and cathepsins (cathepsin L and S). Detailed immunostaining and immunoblotting protocols are described in Supplementary Material.

Statistical analysis

Each experimental group consisted of six leaflet samples. Data from each group were quantified as mean ± SD error and then normalized to the values measured in the fresh control. Following this procedure, all biomarker expressions were expressed in terms of a normalized mean value ± normalized standard error. Data from all experiments were tested for normality by the Anderson-Darling method, then analyzed using ANOVA followed by the Bonferroni post-hoc test. A *P*-value of less than 0.05 was used as a measure of statistical significance. The statistical review of the study was performed by a biomedical statistician (Dr. Jun Li, Department of Applied and Computational Mathematics and Statistics, University of Notre Dame, Notre Dame, IN, United States).

RESULTS

BMP-4 supplementation promotes endothelial activation in response to supra-physiologic FSS magnitude

Immunostaining was performed to examine endothelial activation in response to all three FSS environments using standard, pro- and anti-osteogenic culture media. Tissue conditioned under physiologic FSS did not exhibit any positive staining for ICAM-1 or VCAM-1, regardless of the culture medium (Figure 2A). Exposure of leaflet tissue to supra-physiologic FSS magnitude exhibited a similar trend except when BMP-4 was added to the culture medium, which resulted in ICAM-1 expression on the endothelial lining of the fibrosa (Figure 2B). Similarly to the results obtained under physiologic FSS, supra-physiologic FSS frequency did not promote cell adhesion molecule expression with any culture medium (Figure 2C).

Synergistic effects of BMP-4 and TGF-β1

Potential synergies between BMP-4 and TGF-β1 signaling in response to FSS abnormalities were investigated by quantifying the expression of one cytokine following the pharmacological inhibition or supplementation

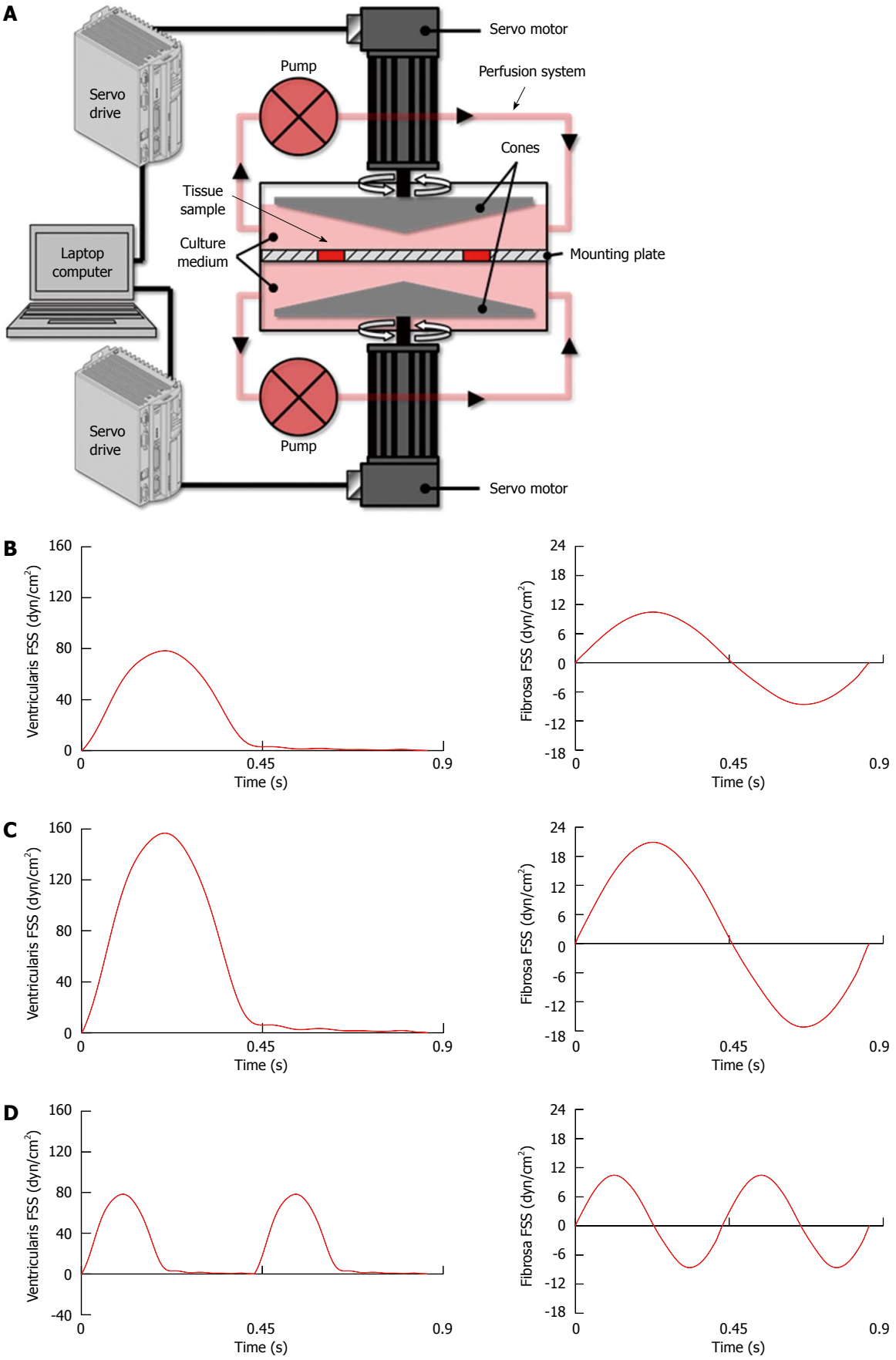


Figure 1 Tissue conditioning methodology and fluid shear stress environments. A: Schematic of the double-sided cone-and-plate bioreactor; B: Physiologic fluid shear stress (FSS); C: Supra-physiologic FSS magnitude at physiologic frequency; D: Supra-physiologic FSS frequency at physiologic magnitude.

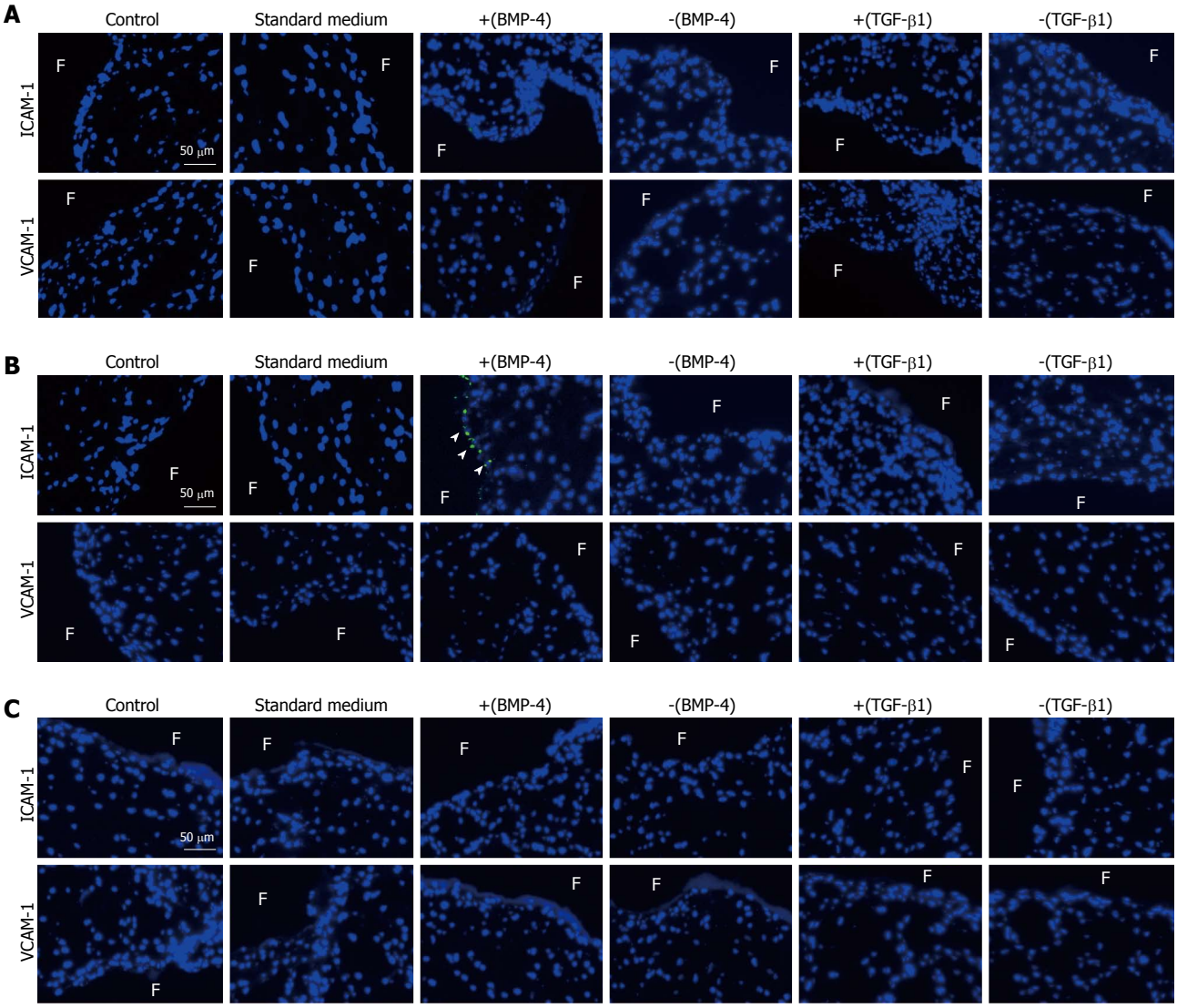


Figure 2 Intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 immunostaining. Tissue exposed for 48 h to: (A) physiologic fluid shear stress (FSS), (B) supra-physiologic FSS magnitude, and (C) supra-physiologic FSS frequency in standard medium and medium supplemented with BMP-4 [+(BMP-4)], noggin [-(BMP-4)], TGF-β1 [+(TGF-β1)] or SB-431542 [-(TGF-β1)] (F: Fibrosa; green: Positively stained cells; blue: Cell nucleus). ICAM-1: Intercellular adhesion molecule-1; VCAM-1: Vascular cell adhesion molecule-1; TGF-β1: Transforming growth factor-beta1; BMP-4: Bone morphogenic protein-4.

of the other. Western blot results indicate that under physiologic FSS, medium supplementation with BMP-4 or noggin had no significant effect on TGF-β1 expression, which remained statistically similar to the levels measured in fresh controls and in tissue conditioned using the standard medium (Figure 3A). Similarly, no significant difference in BMP-4 expression was detected between any culture medium treatment groups. In contrast, analysis of tissue conditioned to supra-physiologic FSS magnitude (Figure 3B) revealed a significant 4.7-fold, 6.2-fold and 4.1-fold increase in TGF-β1 expression using standard medium, medium supplemented with BMP-4 and medium supplemented with noggin, respectively, relative to the fresh controls (467% ± 22%, 624% ± 100%, 405% ± 38%, respectively, *vs* 100% ± 6%; $P < 0.05$). However, no statistical difference in TGF-β1 expression was detected between the standard medium, BMP-4 treatment and noggin treatment groups. While supra-physiologic FSS also resulted in a significant 2.6-fold, 2.2-fold and 2.1-fold increase in BMP-4 expression using standard medium, medium supplemented with TGF-β1 and medium supplemented with SB-431542, respectively, relative to the fresh controls (258% ± 22%, 219% ± 33%, 207% ± 13%, respectively, *vs* 100% ± 4%; $P < 0.05$), no statistical difference in BMP-4 expression was detected between those three culture medium groups. Lastly, exposure of leaflet tissue to supra-physiologic FSS frequency using the five culture media (Figure 3C) produced results similar to those obtained under physiologic FSS, in which no significant difference in TGF-β1 or BMP-4 expression was detected between the fresh controls, the standard medium group, the pro-osteogenic medium groups (BMP-4 or TGF-β1 treatment) and the anti-osteogenic medium groups (noggin or SB-431542 treatment).

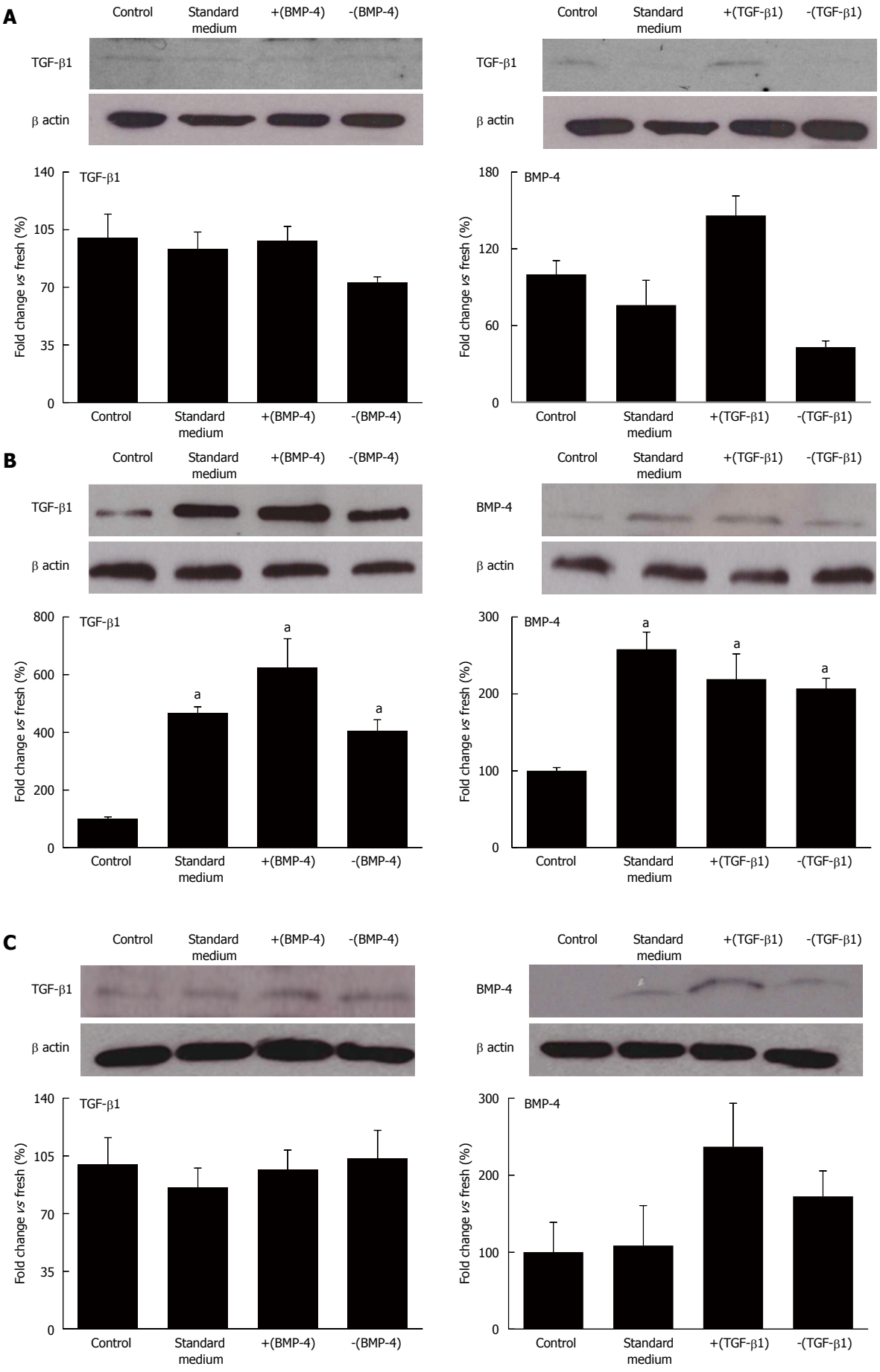


Figure 3 Transforming growth factor-beta1 and bone morphogenic protein-4 immunoblotting. Tissue exposed for 48 h to: (A) physiologic fluid shear stress (FSS), (B) supra-physiologic FSS magnitude, and (C) supra-physiologic FSS frequency standard medium and medium supplemented with BMP-4 [+(BMP-4)], noggin [-(BMP-4)], TGF-β1 [+(TGF-β1)] or SB-431542 [-(TGF-β1)] ($^aP < 0.05$ *vs* fresh control). TGF-β1: Transforming growth factor-beta1; BMP-4: Bone morphogenic protein-4.

TGF-β1 silencing reduces MMP-9 expression in response to FSS abnormalities

MMP-2 and MMP-9 immunostaining was performed in leaflet tissue exposed to physiologic and supra-physiologic FSS using the five culture media to characterize the downstream action of BMP-4 and TGF-β1 on ECM degradation. No significant difference in MMP-2 and MMP-9 expression was detected between any culture medium treatment group and the fresh controls in leaflets subjected to physiologic FSS (Figure 4A). Leaflet exposure to supra-physiologic FSS magnitude resulted in a significant 9.4-fold, 8.4-fold and 11.1-fold increase in MMP-2 expression using standard medium, medium supplemented with BMP-4 and medium supplemented with TGF-β1, respectively, relative to the fresh controls (941% ± 90%, 842% ± 126%, 1108% ± 170%, respectively, *vs* 100% ± 19%; $P < 0.05$; Figure 4B) but no difference in expression was detected between the five culture media. A significant 12.2-fold, 9.3-fold and 16.2-fold increase in MMP-9 expression was also observed with the standard medium, noggin and TGF-β1 treatment groups, respectively, relative to the fresh controls (1219% ± 190%, 931% ± 104%, 1621% ± 261%, respectively, *vs* 100% ± 16%; $P < 0.05$), with no significant difference in MMP-9 expression between the five culture media. In contrast, TGF-β1 silencing resulted in a significant 75% and 81% reduction in MMP-9 expression relative to the standard culture medium and TGF-β1 treatment group, respectively, and resulted in a MMP-9 expression level statistically similar to that measured in fresh controls. Lastly, supra-physiologic FSS frequency resulted in a significant 12.6-fold, 13.2-fold, 14.5-fold and 13.4-fold increase in MMP-2 expression using standard medium, medium supplemented with BMP-4, medium supplemented with TGF-β1 and medium supplemented with SB-431542, respectively, relative to the fresh controls (1264% ± 145%, 1318% ± 239%, 1447% ± 278%, 1339% ± 314%, respectively, *vs* 100% ± 21%; $P < 0.05$) without any significant difference between any culture medium groups (Figure 4C). This FSS environment also resulted in a significant 15.7-fold, 14.9-fold and 17.3-fold increase in MMP-9 expression in the standard culture medium, noggin and TGF-β1 treatment groups, respectively, relative to the fresh controls (1571% ± 191%, 1488% ± 316%, 1728% ± 268%, respectively, *vs* 100% ± 42%; $P < 0.05$). TGF-β1 silencing resulted in a significant 81% and 83% reduction in MMP-9 expression relative to the standard culture medium and TGF-β1 treatment group, respectively (302% ± 182% *vs* 1571% ± 191% and 1728% ± 268%, respectively; $P < 0.05$), and resulted in a MMP-9 expression level statistically similar to that measured in fresh controls.

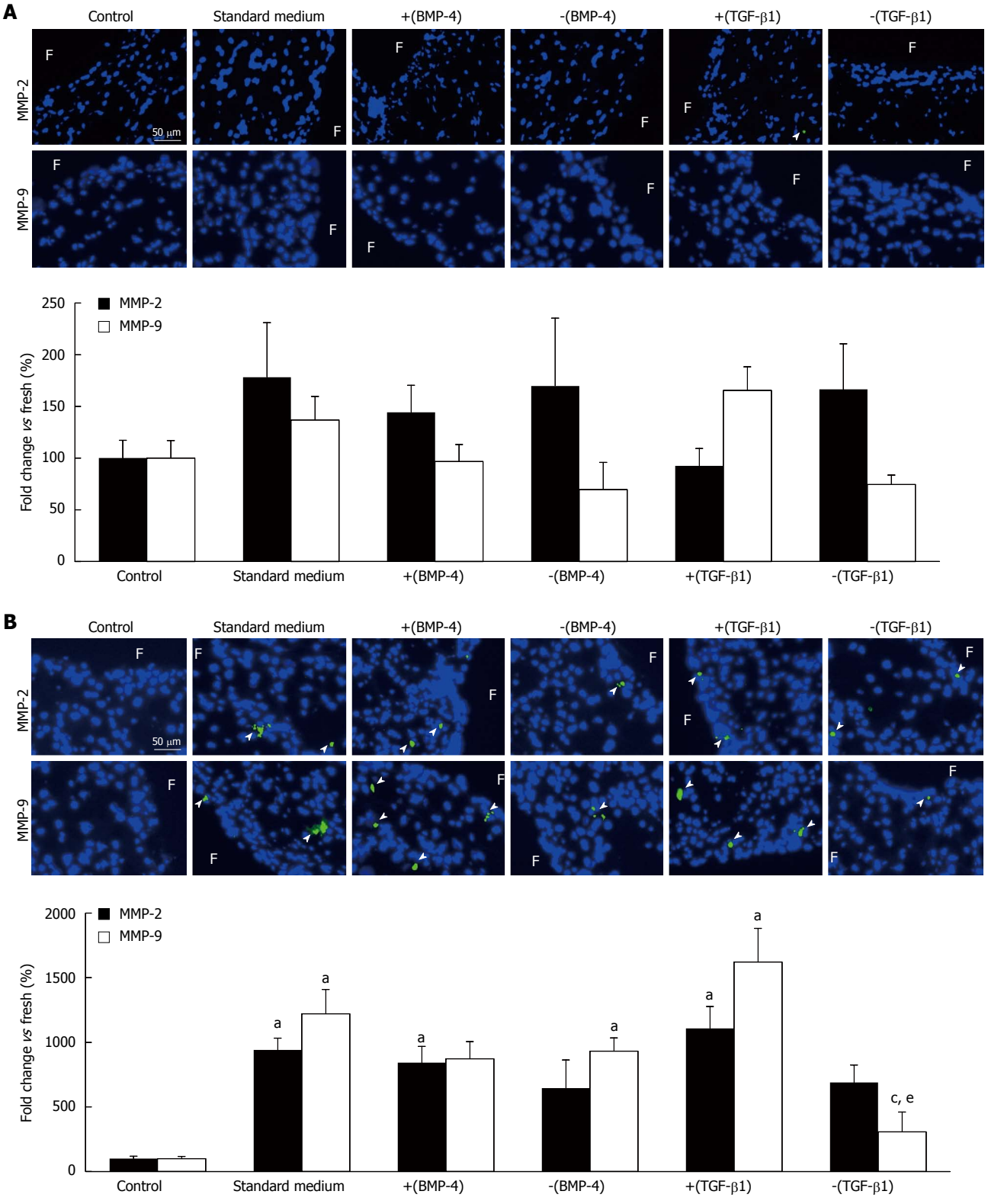
BMP-4 and TGF-β1 do not synergistically regulate cathepsin expression in response to FSS

The synergistic effects of BMP-4 and TGF-β1 on FSS-mediated protease expression were characterized *via* cathepsin L and cathepsin S immunostaining. Under physiologic FSS, no significant difference in cathepsin L and cathepsin S expression was detected between any culture medium treatment group and the fresh controls (Figure 5A). Tissue exposure to supra-physiologic FSS magnitude resulted in a significant 11.9-fold and 15.5-fold increase in cathepsin L expression using the standard medium and medium supplemented with TGF-β1, respectively, relative to the fresh controls (1187% ± 175%, 1546% ± 171%, respectively, *vs* 100% ± 12%; $P < 0.05$; Figure 5B). The same FSS environment resulted in a significant 6.0-fold, 6.0-fold, 5.5-fold, 5.4-fold and 3.3-fold increase in cathepsin S expression using the standard medium, BMP-4, noggin, TGF-β1 and SB-431542 treatment groups, respectively, relative to the fresh controls (603% ± 88%, 598% ± 96%, 554% ± 94%, 541% ± 92%, 325% ± 57%, respectively, *vs* 100% ± 13%; $P < 0.05$). However, the effects of BMP-4 and TGF-β1 on protease expression remained limited as indicated by the absence of significant difference in cathepsin L and S expression between the different medium treatment groups. Lastly, supra-physiologic FSS frequency did not promote cathepsin L expression, regardless of the culture medium treatment group (Figure 5C). In contrast, the same mechanical treatment resulted in a significant 7.4-fold, 6.4-fold and 7.4-fold increase in cathepsin S expression in the BMP-4, noggin and TGF-β1 treatment groups, respectively, relative to the fresh controls (744% ± 129%, 635% ± 76%, 744% ± 144%, respectively, *vs* 100% ± 7%; $P < 0.05$).

DISCUSSION

In this *ex vivo* study, we investigated the role of the cytokines BMP-4 and TGF-β1 in the acute pathological response of porcine valve leaflets exposed to FSS abnormalities. We demonstrated that: (1) valvular endothelial activation is weakly regulated by BMP-4 in response to FSS abnormalities; (2) TGF-β1 silencing attenuates FSS-induced ECM degradation *via* MMP-9 downregulation; and (3) BMP-4 and TGF-β1 do not synergistically interact in response to FSS abnormalities.

This study first confirms the key role played by FSS in the maintenance of valvular homeostasis. In fact, exposure of leaflet tissue to its native FSS environment did not stimulate any pathological event and resulted in biomarker expressions similar to those measured in fresh tissue, regardless of the culture medium. Valvular tissue has been shown to be sensitive to the forces present in its hemodynamic environment[37,38]. As compared to stretch and pressure which propagate throughout the leaflet and stimulate both valvular endothelial cells (VECs) and interstitial cells (VICs), FSS is an interfacial stress sensed primarily by VECs. Therefore, the ability of FSS alone to maintain leaflet homeostasis in the absence of any other mechanical signal demonstrates the key role played by the


A
Control
Standard medium
+(BMP-4)
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MMP-2
MMP-9
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MMP-9
Fold change vs fresh (%)
250
200
150
100
50
0
Control
Standard medium
+(BMP-4)
-(BMP-4)
+(TGF-β1)
-(TGF-β1)
B
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Standard medium
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2000
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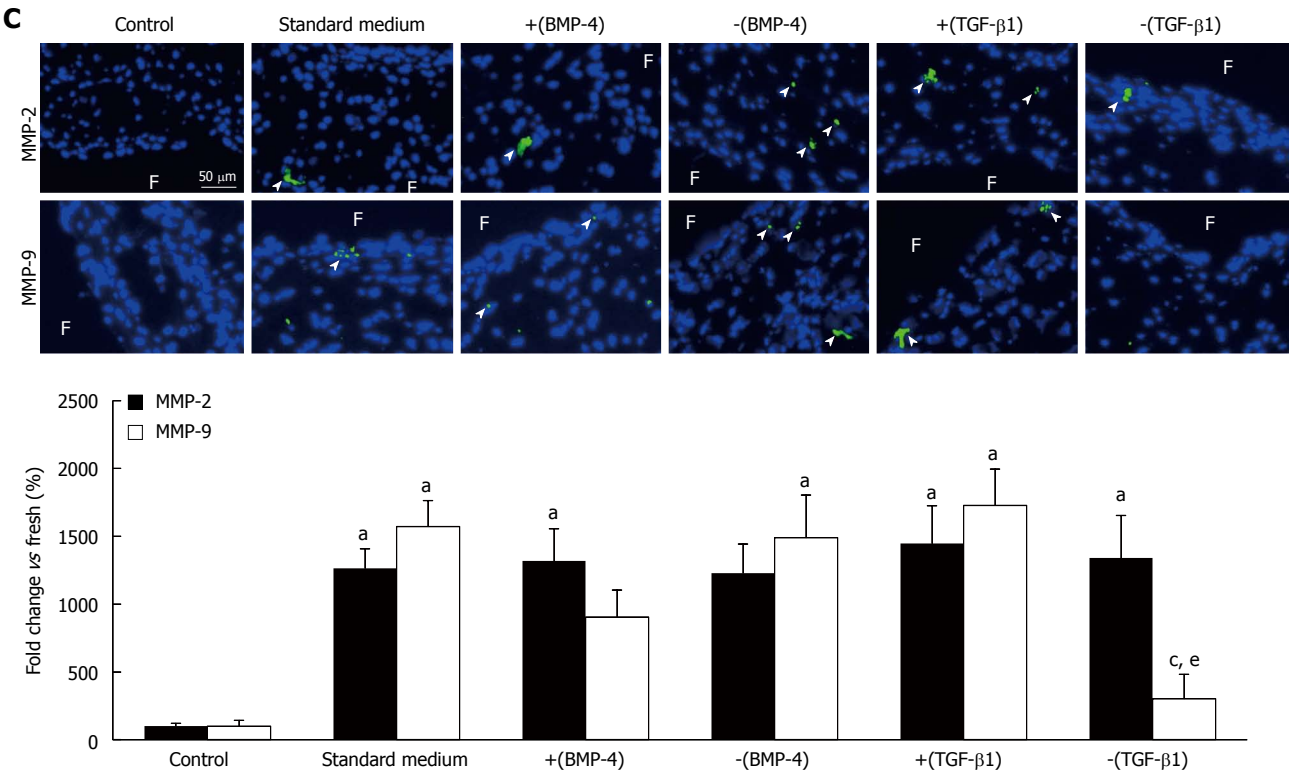
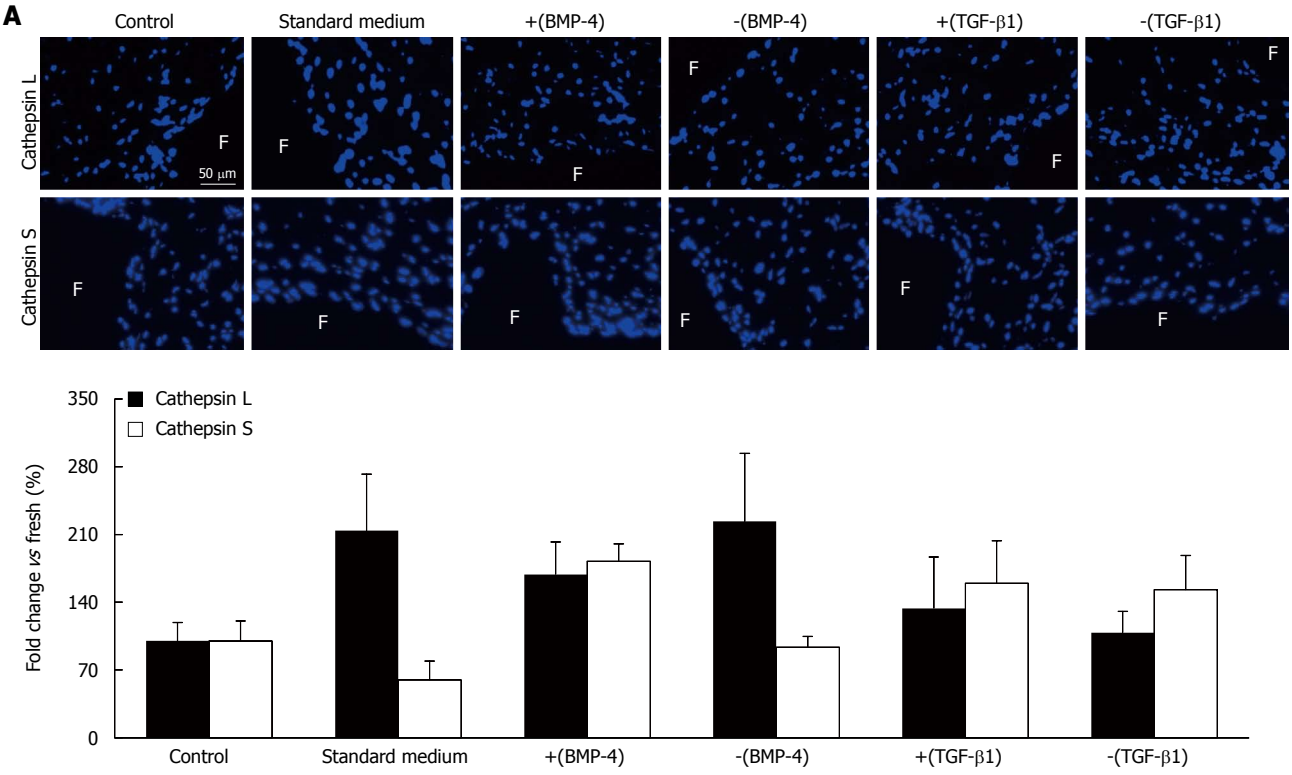


Figure 4 Metalloproteinases-2 and metalloproteinases-9 immunostaining. Tissue exposed for 48 h to: (A) physiologic fluid shear stress (FSS), (B) supra-physiologic FSS magnitude, and (C) supra-physiologic FSS frequency standard medium and medium supplemented with BMP-4 [+(BMP-4)], noggin [-(BMP-4)], TGF-β1 [+(TGF-β1)] or SB-431542 [-(TGF-β1)] (F: Fibrosa; green: Positively stained cells; blue: Cell nucleus; $^{a}P < 0.05$ *vs* fresh control; $^{c}P < 0.05$ *vs* standard culture medium; $^{e}P < 0.05$ *vs* standard medium supplemented with TGF-β1). MMP: Metalloproteinases; TGF-β1: Transforming growth factor-beta1; BMP-4: Bone morphogenic protein-4.



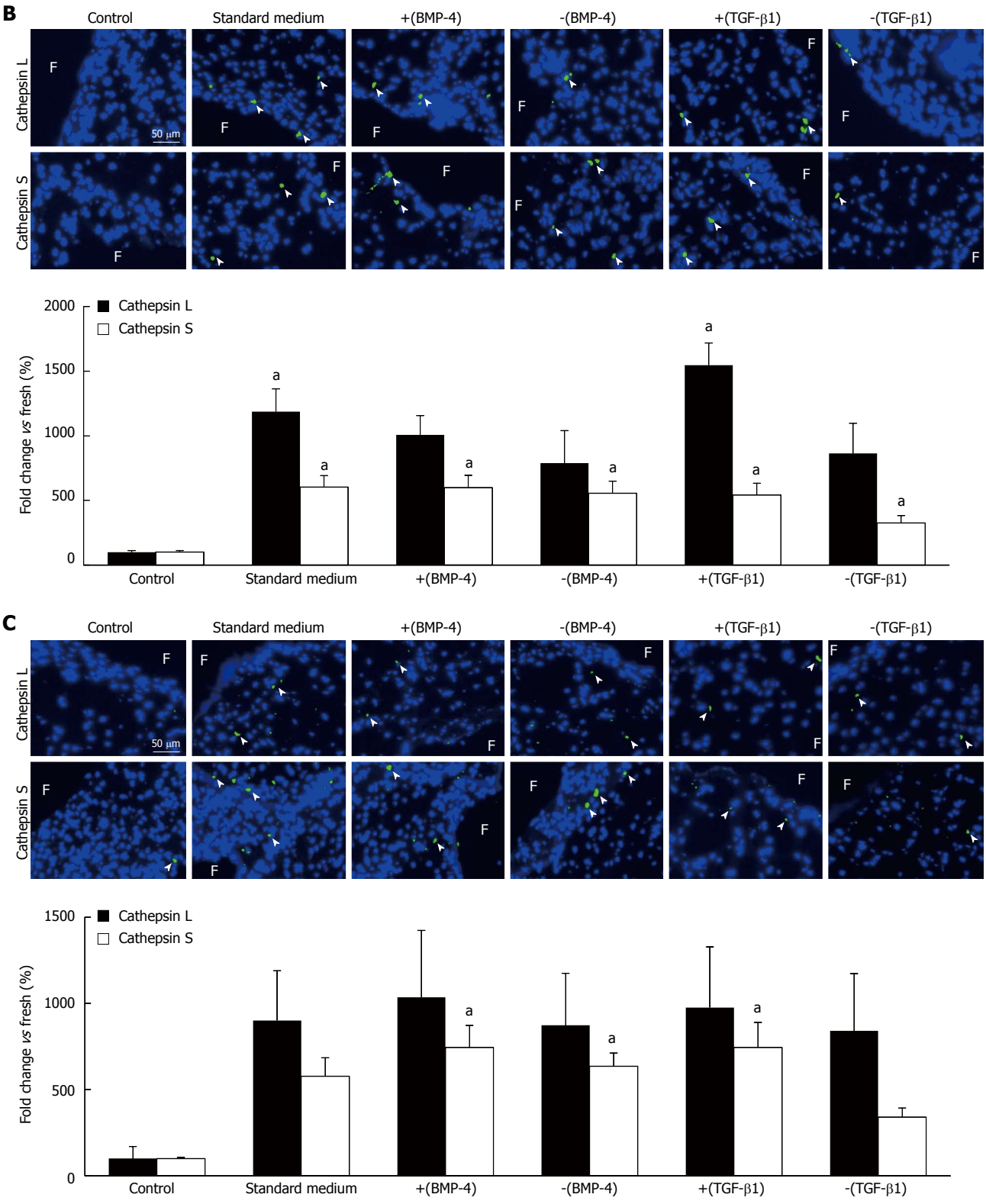


Figure 5 Cathepsin L and cathepsin S immunostaining. Tissue exposed for 48 h to: (A) physiologic fluid shear stress (FSS), (B) supra-physiologic FSS magnitude, and (C) supra-physiologic FSS frequency standard medium and medium supplemented with BMP-4 [+(BMP-4)], noggin [-(BMP-4)], TGF-β1 [+(TGF-β1)] or SB-431542 [-(TGF-β1)] (F: Fibrosa; green: Positively stained cells; blue: Cell nucleus; $^{a}P < 0.05$ *vs* fresh control). TGF-β1: Transforming growth factor-beta1; BMP-4: Bone morphogenic protein-4.

leaflet endothelium in valvular function. In contrast, leaflet exposure to supra-physiologic FSS resulted in increased paracrine signaling and ECM degradation. Those results confirm those of previous *ex vivo* studies on the effects of normal and abnormal FSS on valvular biology[27-29] and on the role played by FSS in bicuspid aortic valve calcification[30].

This study is the first to report the dependence of FSS-mediated valvular ECM degradation on TGF-β1 signaling, as suggested by the dramatic decrease in MMP-9 expression in response to FSS abnormalities following TGF-β1 inhibition. The potential involvement of TGF-β1 in valvular ECM degradation is consistent with previous reports suggesting the modulation of MMP-9 expression by several growth factors and inflammatory cytokines in sheep VICs[39,40]. The ability of the small molecule inhibitor SB-431542 to reduce MMP-9 expression to the level measured in fresh leaflets also suggests the possible use of this molecule in drug-based therapies aimed at attenuating ECM degradation.

Interestingly, this study did not reveal the existence of clear synergies between BMP-4 and TGF-β1 signaling in response to FSS abnormalities, as shown by the inability of the pro- and anti-osteogenic media to alter the expression of those cytokines. This result differs from our previous *ex vivo* results on the effects of single-sided FSS magnitude and/or pulsatility abnormalities on the leaflet fibrosa[27,28], which suggested a downregulation of FSS-induced BMP-4 expression following TGF-β1 inhibition. One possible explanation for this discrepancy is the difference in mechanical environment considered in those studies. While those earlier *ex vivo* studies subjected only one leaflet surface to FSS abnormalities, the present experiments were performed using a more realistic side-specific FSS environment, which potentially attenuated the severity of the pathological response.

Lastly, the interpretation of the results should be put in the perspective of two important considerations. First, noggin is a BMP antagonist that not only binds BMP-4 but also BMP-2, -5, -6 and -7[31,32]. Therefore, the observed biological response following noggin supplementation may be the result of the inhibition of other BMP members. Second, while the study only focuses on the acute biological response, the results may still be relevant to the long-term processes leading to CAVD as valve leaflets respond to FSS alterations in periods as short as 48 h and continued mechanical conditioning for up to 72 h has been shown not to alter that initial response[29].

In summary, this study provides further evidence of the key role played by BMP-4 and TGF-β1 in valvular FSS mechanotransduction and the specific involvement of TGF-β1 in FSS-induced ECM degradation. While inhibition of those cytokines is not sufficient to completely block the FSS-induced pathological response, the TGF-β1 inhibitor SB-431542 emerges as a potential candidate molecule for attenuating the adverse effects of FSS abnormalities on ECM degradation.

COMMENTS

Background

Calcific aortic valve disease (CAVD) is driven by inflammatory, remodeling and osteogenic processes triggered by cardiovascular risk factors and hemodynamic cues. In particular, supra-physiologic fluid shear stress (FSS) environments, which may result from hypertension, aging and valvular defects, have been shown to stimulate early CAVD signaling in a bone morphogenetic protein-4 (BMP-4) and transforming growth factor-beta1 (TGF-β1)-dependent manner. While the demonstrated involvement of BMP-4 and TGF-β1 in early CAVD provides a rationale for considering those molecules in targeted cell-based therapies aimed at attenuating or blocking the downstream pathological cascade, the synergies and modes of action of those molecules in response to FSS abnormalities have not been defined yet.

Research frontiers

The current modality to treat CAVD consists of the complete replacement of the valve by an artificial implant, which only partially restores valvular function and does not address the root cause of the disease. The development of non-invasive pharmacological approaches requires more insights into the basic biology of CAVD. Therefore, the characterization of the signaling pathways involved in the disease and the interacting mechanisms of cardiovascular calcification, micro-scale mechano-transduction and macro-scale hemodynamics are current hotspots in valvular research.

Innovations and breakthroughs

Their previous characterization of the contribution of side-specific FSS magnitude and/or frequency abnormalities to early valvular pathogenesis revealed the sensitivity of the leaflet tissue to elevated FSS magnitude or frequency and the ability of FSS abnormalities to promote paracrine signaling *via* BMP-4- and TGF-β1-dependent pathways. The present study is a logical extension of our previous work as it investigates the upstream roles of BMP-4 and TGF-β1 in the FSS-mediated valvular response. Here, the authors programmed their FSS bioreactor to generate the most unfavorable FSS environment for valvular tissue (*i.e.*, side-specific supra-physiologic FSS magnitude or side-specific supra-physiologic FSS frequency) and the authors aimed at isolating the mechanistic role played by BMP-4 and TGF-β1 in the FSS-mediated pathological response by either promoting or inhibiting pharmacologically the downstream action of those molecules. The results demonstrate for the first time the mechano-sensitivity of BMP-4 and TGF-β1 to alterations in the native valvular FSS environment and their respective role in FSS-mediated pathogenesis. While BMP-4 promotes valvular endothelial activation in response to supra-physiologic FSS, TGF-β1 mediates extracellular matrix (ECM) degradation.

Applications

The ability of the TGF-β1 inhibitor SB-431542 to reduce flow-mediated ECM degradation suggests the possible use of this molecule in non-invasive drug-based therapies aimed at attenuating flow-induced aortic valve pathogenesis.

Terminology

CAVD is the most common valvular disease and is characterized by the formation of calcific lesions on the valve leaflets. FSS is the frictional fluid force resulting from the relative motion between the valve leaflets and the surrounding blood flow.

Peer-review

This is a very well conducted study.

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ORIGINAL ARTICLE

Prospective Study

Utility of electrophysiological studies to predict arrhythmic events

Gabriela Hilfiker, Andreas W Schoenenberger, Paul Erne, Richard Kobza

Gabriela Hilfiker, Paul Erne, Richard Kobza, Division of Cardiology, Luzerner Kantonsspital, 6000 Luzern, Switzerland
Andreas W Schoenenberger, Division of Geriatrics, Department of General Internal Medicine, Inselspital, Bern University Hospital, University of Bern, 3010 Bern, Switzerland
Paul Erne, Hirslanden Klinik St Anna, 6006 Luzern, Switzerland

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Correspondence to: Richard Kobza, MD, Division of Cardiology, Luzerner Kantonsspital, Spitalstrasse, Luzern 16, 6000 Luzern, Switzerland. richard.kobza@luks.ch
Telephone: +41-41-2055208
Fax: +41-41-2055109

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Abstract

AIM: To evaluate the prognostic value of electrophysiological stimulation (EPS) in the risk stratification for tachyarrhythmic events and sudden cardiac death (SCD).

METHODS: We conducted a prospective cohort study and analyzed the long-term follow-up of 265 consecutive patients who underwent programmed ventricular stimulation at the Luzerner Kantonsspital (Lucerne, Switzerland) between October 2003 and April 2012. Patients underwent EPS for SCD risk evaluation because of structural or functional heart disease and/or electrical conduction abnormality and/or after syncope/cardiac arrest. EPS was considered abnormal, if a sustained ventricular tachycardia (VT) was inducible. The primary endpoint of the study was SCD or, in implanted patients, adequate ICD-activation.

RESULTS: During EPS, sustained VT was induced in 125 patients (47.2%) and non-sustained VT in 60 patients (22.6%); in 80 patients (30.2%) no arrhythmia could be induced. In our cohort, 153 patients (57.7%) underwent ICD implantation after the EPS. During follow-up (mean duration 4.8 ± 2.3 years), a primary endpoint event occurred in 49 patients (18.5%). The area under the receiver operating characteristic curve (AUROC) was 0.593 (95%CI: 0.515-0.670) for a left ventricular ejection fraction (LVEF) < 35% and 0.636 (95%CI: 0.563-0.709) for inducible sustained VT during EPS. The AUROC of EPS was higher in the subgroup of patients with LVEF ⩾ 35% (0.681, 95%CI: 0.578-0.785). Cox regression analysis showed that both, sustained VT during EPS (HR: 2.26, 95%CI: 1.22-4.19, P = 0.009) and LVEF < 35% (HR: 2.00, 95%CI: 1.13-3.54, P = 0.018) were independent predictors of primary endpoint events.

CONCLUSION: EPS provides a benefit in risk stratification

for future tachyarrhythmic events and SCD and should especially be considered in patients with LVEF ⩾ 35%.

Key words: Electrophysiologic techniques; Cardiac; Arrhythmia; Cardiac; Sudden cardiac death

Core tip: In our long-term prospective cohort study we could reveal several important findings about the prognostic value of programmed ventricular stimulation for risk stratification of sudden cardiac death (SCD). First, in a mixed population with different cardiac pathologies inducible sustained ventricular tachyarrhythmia during electrophysiological stimulation (EPS) identified those at higher risk for SCD or appropriate implantable cardioverter defibrillators (ICD) activation. Second, left ventricular ejection fraction (LVEF) < 35% was another independent predictor of SCD surrogate. Third, in patients with LVEF > 35% negative EPS had a high negative predictive value for SCD and ICD activation.

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INTRODUCTION

Implantable cardioverter defibrillators (ICD) are an established therapy for primary and secondary sudden cardiac death (SCD) prevention. Randomized trials have shown a significant mortality reduction in implanted patients at high risk for SCD[1-4]. For ICD therapy guidance, evaluation of SCD risk is crucial. Guidelines recommend various non-invasive techniques to recognize patients at higher risk for life-threatening arrhythmias[5]. Factors associated with a significantly increased risk of ventricular tachyarrhythmia include increased resting heart rate, wide QRS, presence of late potentials, presence of heart failure, or lower left ventricular ejection fraction[6-10]. However, currently available methods for SCD risk estimation are still imprecise. Therefore, many patients, who received an ICD, are not going to suffer from a future arrhythmic event and do not benefit from ICD. On the other hand, many patients, who are not recognized at high risk, die from SCD and numbers of SCD victims are still highest in these patients with normal left ventricular ejection fraction (LVEF)[11,12]. It remains an ongoing challenge to predict an individual patient's risk.

Currently, the electrophysiological study (EPS) is widely used for risk stratification and several randomized trials suggest a significant predictive value of this examination. However, many previous studies focused on the predictive value in one subgroup of patients with a specific cardiac pathology. Most data are available from post myocardial infarction patients in whom inducible ventricular tachycardias (VT) or ventricular fibrillation (VF) during EPS indicate higher risk for future arrhythmic events[13]. An improved risk stratification with EPS has especially been shown in patients who were preselected as a high risk population based on previous non-invasive tests[14,15]. Other studies provided a prognostic value of EPS in patients with Brugada syndrome, hypertrophic or dilated cardiomyopathy[16-18]. The discussion about the general prognostic value of electrophysiological testing is ongoing. We therefore performed an induction study of VT or VF by programmed electrical stimulation either for primary prevention of SCD, after documented VT or in SCD survivors. The usefulness of EPS for the prediction of future arrhythmic events was evaluated in a prospective long-term follow-up in patients with different cardiac pathologies.

MATERIALS AND METHODS

Study population

This prospective cohort study evaluated all patients who were examined by EPS at the Luzerner Kantonsspital (Lucerne, Switzerland) between October 2003 and April 2012. Patients underwent EPS for SCD risk evaluation because of structural or functional heart disease and/or electrical conduction abnormality and/or after syncope/cardiac arrest. The study population therefore embraced patients with coronary artery disease (CAD), dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy, impaired LVEF, Brugada or Long QT syndrome, and other rare cardiac diseases (*e.g.*, valvular heart disease, tetralogy of Fallot, variant angina). Some patients who underwent EPS did not have cardiac disease but were assessed because of unclear syncope or family history of cardiomyopathy. Patients who did not provide written informed consent were excluded. The study complies with the Declaration of Helsinki and was approved by the local ethics committee.

Baseline evaluation

All participating patients were evaluated at baseline. Patient history was recorded including cardiovascular risk factors, underlying heart disease, and medication. Electrocardiogram (ECG) was recorded in all patients. LVEF was measured with transthoracic echocardiography in all patients and was determined on two- and four-chamber views using the modified biplane Simpson method.

EPS was part of the baseline evaluation and was performed according to the ACC/AHA/ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death[19]. The

ventricular arrhythmia induction protocol during EPS included programmed stimulation at three basic cycle lengths (600, 450, and 350 ms) and up to three extrastimuli with a minimum coupling interval of 180 ms. A third extrastimulus was introduced during a basic drive cycle length of minimal 500 ms after completion of programmed ventricular stimulation with 1 and 2 extrastimuli during paced cycle lengths of 600, 450, and 350 ms[20]. EPS was considered abnormal, if a sustained VT was inducible.

Follow-up

Patients with ICD were regularly followed-up at Luzerner Kantonsspital in six-month or yearly intervals. In addition, they were followed-up immediately, if shocks occurred. The clinical course and the numbers of appropriate and inappropriate ICD therapies were protocoled at each follow-up visit. In patients who had no ICD, follow-up was obtained from several sources: first, medical records at Luzerner Kantonsspital were studied, if available (*i.e.*, in patients who were re-admitted after the EPS); second, the patients and/or their general practitioner were contacted by phone and interviewed using a structured protocol. In all patients who died, additional information on the circumstances of death was collected. Death was classified as non-cardiac or cardiac. Among cardiac deaths, SCD was defined according to the Hinkle-Thaler method[21].

Endpoints

The primary endpoint of this study was SCD or, in implanted patients, adequate ICD activation [shock or antitachycardia pacing (ATP)]. The secondary endpoint was SCD or adequate ICD shock. For the secondary endpoint, events with ATP were not counted. If a patient experienced more than one endpoint event (*e.g.*, ICD shock in a patient who later died from SCD), only the first endpoint event was counted.

Statistical analysis

We descriptively analyzed baseline characteristics. We then calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the area under the receiver operating characteristic curve (AUROC) with its 95%CI of the EPS for the prediction of both endpoints[22]. The diagnostic accuracy of the EPS was compared to that of the LVEF. For this purpose, LVEF was dichotomized at 35% (< 35% indicating higher risk *vs* ⩾ 35% indicating lower risk). We also performed a Cox regression analysis with age, sex, EPS and LVEF as independent variables. Kaplan-Meier survivor functions were generated to illustrate the ability to stratify the risk of both dichotomized predictors separately[23]. Calculations were done for all study participants together and, in a sensitivity analysis, repeated separate for participants with CAD and DCM. Data were analyzed using Stata 11.2 (StataCorp LP, College Station, TX, United States).

RESULTS

Study population

Overall, 289 patients underwent EPS during the study period. Twenty-four patients (8.3%) were lost to follow-up, resulting in 265 patients who were analyzed. Table 1 shows the baseline characteristics of study participants. Mean age was 57.4 ± 10.7 years with a maximum range from 21.8 to 76.7 years. Most participants were male. CAD was present in a majority of patients.

EPS and therapeutic consequences

The EPS was performed for primary prevention in 209 patients (78.9%). In 56 patients (21.1%) the indication was secondary prevention: twenty-nine cardiac arrest survivors (10.9%), and 27 patients (10.2%) who had documented VT/VF on previous ECGs. During EPS, sustained VT was induced in 125 patients (47.2%) and non-sustained VT in 60 patients (22.6%). In 80 patients (30.2%) no arrhythmia could be induced.

In our cohort, 153 patients (57.7%) underwent ICD implantation after the EPS, and 112 patients (42.3%) received no ICD. Patients were selected for device implantation according to the specific ACC/AHA/ESC guidelines for the underlying heart disease. The decision to implant an ICD was influenced by the result of the EPS if recommended in the guidelines. Antiarrhythmic medication consisted of beta blockers in 214 patients (80.8%), amiodarone in 38 patients (14.3%), and digoxin in 37 patients (14.0%).

Follow-up and endpoint events

The mean duration of the follow-up was 4.8 ± 2.3 years (interquartile range 3.1-6.2 years, maximum range 0.2-9.2 years). During follow-up, 28 patients (10.6%) died, 12 of them due to non-cardiac causes. Among the 16 patients with a cardiac cause of death, SCD occurred in 8 patients. A primary endpoint event occurred in 49 patients (18.5%) with a mean time interval since the EPS of 947 ± 778 d (maximum range 16-3050 d). Table 1 shows baseline characteristics separate for patients with and without primary endpoint event. There were no significant differences between the two groups, except for LVEF which was lower in patients with primary endpoint event, and the findings during EPS that found more sustained VTs in patients with primary endpoint event. A secondary endpoint event occurred in 33 patients (12.5%) with a mean time interval since the EPS of 997 ± 761 d (maximum range 63-2709 d).

Diagnostic accuracy

Table 2 shows the diagnostic accuracy of the EPS and the LVEF for primary and secondary endpoint. The AUROCs of EPS and of LVEF did not significantly differ (P = 0.427 for primary endpoint, and P = 0.676 for

Table 1 Baseline characteristics

Characteristic	All study participants n = 265	Participants without primary endpoint event[1] n = 216	Participants with primary endpoint event[1] n = 49	P value[2]
Age, mean ± SD, yr	57.4 ± 10.7	57.5 ± 10.6	57.2 ± 11.3	0.848
Male sex	230 (86.8%)	185 (85.6%)	45 (91.8%)	0.350
Cardiovascular risk factors				
Hypertension	152 (57.4%)	124 (57.4%)	28 (57.1%)	0.973
Dyslipidemia	161 (60.8%)	131 (60.6%)	30 (61.2%)	0.941
Diabetes mellitus	60 (22.6%)	49 (22.7%)	11 (22.4%)	0.972
Smoking[3]	154 (58.1%)	121 (56.0%)	33 (67.3%)	0.147
Family history of CAD	81 (30.6%)	65 (30.1%)	16 (32.7%)	0.725
Cardiac disease				
CAD	152 (57.4%)	120 (55.6%)	32 (65.3%)	0.213
DCM	58 (21.9%)	45 (20.8%)	13 (26.5%)	0.384
HCM				
Obstructive	1 (0.4%)	1 (0.5%)	0 (0.0%)	1.000
Non-obstructive	3 (1.1%)	2 (0.9%)	1 (2.0%)	0.460
Brugada syndrome	2 (0.8%)	2 (0.9%)	0 (0.0%)	1.000
Long QT	3 (1.1%)	3 (1.4%)	0 (0.0%)	1.000
Other cardiac disease	30 (11.3%)	26 (12.0%)	4 (8.2%)	0.618
Echocardiography				
LVEF, mean ± SD	41.1% ± 15.9%	42.8% ± 16.2%	33.6% ± 11.7%	< 0.001
LVEF < 35%	106 (40.0%)	79 (36.6%)	27 (55.1%)	0.017
EPS				
Induction of sustained VT	125 (47.2%)	91 (42.1%)	34 (69.4%)	0.001
Induction of non-sustained VT	60 (22.6%)	53 (24.5%)	7 (14.3%)	0.122
No VT induction	80 (30.2%)	72 (33.3%)	8 (16.3%)	0.019

[1]Event of primary endpoint (sudden cardiac death, ICD shock or ATP); [2]P value for the comparison of participants without *vs* with endpoint event; [3]Current or former smoker. CAD: Coronary artery disease; DCM: Dilated cardiomyopathy; EPS: Electrophysiological study; HCM: Hypertrophic cardiomyopathy; LVEF: Left ventricular ejection fraction; SD: Standard deviation; VT: Ventricular tachycardia.

Table 2 Diagnostic accuracy of the electrophysiological study and the left ventricular ejection fraction for the primary and secondary endpoint

Predictor variable	Sensitivity	Specificity	PPV	NPV	AUROC (95%CI)
Primary endpoint					
Sustained VT during EPS					
All study participants (n = 265)	69.4%	57.9%	27.2%	89.3%	0.636 (0.563-0.709)
Subgroup of study participants with LVEF < 35% (n = 106)	66.7%	48.1%	30.5%	80.9%	0.574 (0.468-0.680)
Subgroup of study participants with LVEF ⩾ 35% (n = 159)	72.7%	63.5%	24.2%	93.5%	0.681 (0.578-0.785)
LVEF < 35%	55.1%	63.4%	25.5%	86.1%	0.593 (0.515-0.670)
Secondary endpoint					
Sustained VT during EPS					
All study participants (n = 265)	66.7%	55.6%	17.6%	92.1%	0.611 (0.524-0.699)
Subgroup of study participants with LVEF < 35% (n = 106)	61.1%	45.5%	18.6%	85.1%	0.533 (0.406-0.660)
Subgroup of study participants with LVEF ⩾ 35% (n = 159)	73.3%	61.8%	16.7%	95.7%	0.676 (0.553-0.798)
LVEF < 35%	54.5%	62.1%	17.0%	90.6%	0.583 (0.491-0.675)

AUROC: Area under the receiver operating characteristic curve; EPS: Electrophysiological study; NPV: Negative predictive value; PPV: Positive predictive value; VT: Ventricular tachycardia; LVEF: Left ventricular ejection fraction.

secondary endpoint). There was a non-significant trend for a higher AUROC of the EPS in the subgroup of patients with an LVEF ⩾ 35% as compared to the subgroup of patients with LVEF < 35% (P = 0.156 for primary endpoint, and P = 0.113 for secondary endpoint). Cox regression analysis showed that both, sustained VT during EPS (HR: 2.26, 95%CI: 1.22-4.19, P = 0.009) and LVEF < 35% (HR: 2.00, 95%CI: 1.13-3.54, P = 0.018) were independent predictors of primary endpoint events. Kaplan-Meier survivor functions of EPS and LVEF for the primary endpoint are shown in Figure 1.

Sensitivity analysis

Main analysis were repeated separate for participants with CAD and DCM. Among the 152 CAD patients, a primary endpoint event occurred in 32 patients (21.1%). The AUROCs of the EPS (0.604, 95%CI: 0.516-0.693) and of LVEF (0.606, 95%CI: 0.509-0.704) were similar to the overall study population and did not significantly differ (P = 0.975). Among the 58 patients with DCM, a primary endpoint event occurred in 13 patients (22.4%). Due to the low numbers of patients, the AUROC of the EPS (0.625, 95%CI: 0.469-0.781) had a broad 95%CI. The AUROC of LVEF (0.425, 95%CI:

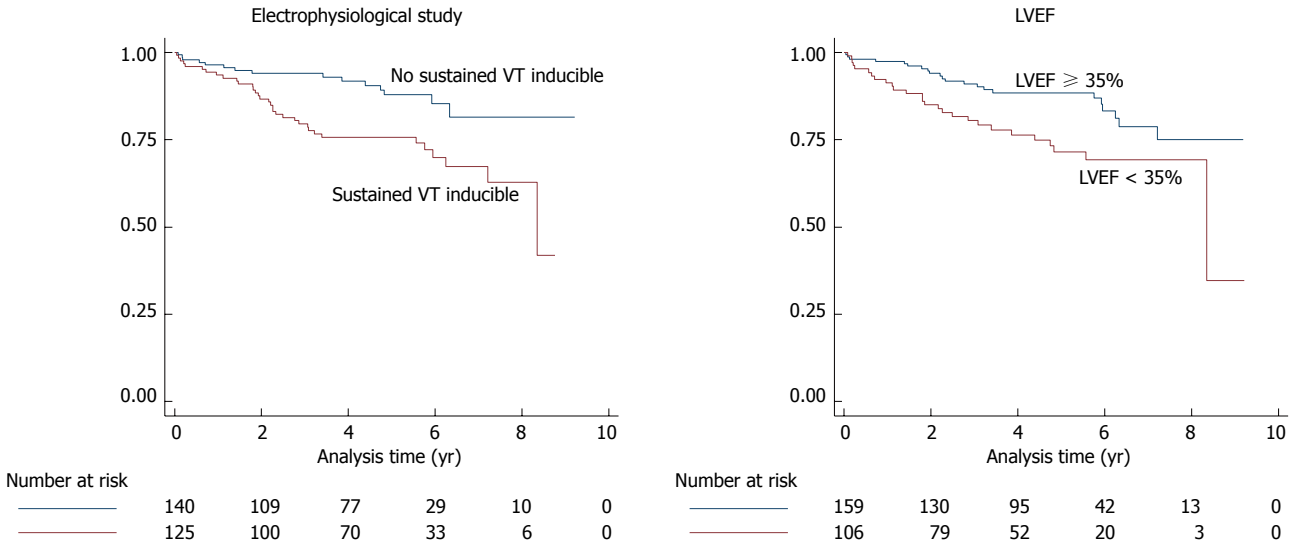

Figure 1 Kaplan-Meier function of primary endpoint event-free survival for patients with or without sustained ventricular tachycardia during the electrophysiological study and for patients with left ventricular ejection fraction < 35% *vs* ⩾ 35%. VT: Ventricular tachycardia; LVEF: Left ventricular ejection fraction.

0.268-0.582) was low (P = 0.105 as compared to the AUROC of the EPS).

DISCUSSION

This long-term prospective cohort study revealed several important findings. First, in a mixed population with different cardiac pathologies inducible sustained ventricular tachyarrhythmia during EPS identified those at higher risk for a SCD surrogate, defined either as appropriate ICD activations and/or as documented SCD. Second, LVEF < 35% was another independent predictor of primary endpoint events. Third, in patients with LVEF > 35% negative EPS had a high negative predictive value for the primary and for the secondary endpoint.

Electrophysiologic testing of ventricular tachycardia was introduced 1972[24]. Amongst others, programmed ventricular stimulation was used to assess the efficacy of antiarrhythmic drugs for suppression of inducible ventricular arrhythmias or the efficacy of antitachycardia surgery[25]. With the availability of ICDs EPS has become an important test for risk stratification to predict SCD[19]. The prognostic value of EPS is based on the assumption that patients with inducible ventricular tachyarrhythmias should have a high likelihood of spontaneous arrhythmic events and that non-inducible patients should be at low risk[26]. In the current guidelines electrophysiologic testing has a class Ⅰ recommendation for diagnostic evaluation of patient with remote myocardial infaction with symptoms suggestive of ventricular arrhythmias, including palpitations, presyncope and syncope. Another class Ⅰ indication is syncope of unkown cause with impaired LV function or structural heart disease[19]. In nonischemic DCM electrophysiologic testing is not recommended for risk stratificiation. However, in a recent study of Gatzoulis *et al*[18] inducibility of VT/VF in patients with idiopathic dilated cardiomyopathy was associated with an increased likelihood of subsequent ICD activation and SCD surrogate. In the present study we have shown, that EPS is useful for the prediction of future arrhythmic events in a collective of patients with different cardiac pathologies. It is well established that the predictive accuracy of LVEF for lifethreatening arrhythmic events is limited[26]. However ICD-Implantation guided by LVEF alone lacks of specificity because in progressive heart failure unexpected SCD accounted for only 30% of deaths while many were due to progressive pump failure not preventable by an ICD.

According to our findings, the additional use of electrophysiologic testing in patients with LVEF < 35% is disputable as the PPV is only improved from 25.5% to 30.5% in cases with a positive EPS. This might not influence the further clinical management of the patient because a 25.5% risk of a future tachyarrhythmic event or SCD already seems to justify ICD implantation. However in patients with LVEF > 35% and a negative EPS the NPV was improved from 86.1% to 93.5%. This means that the risk estimation for future arrhythmic events during the follow-up period of 4.8 ± 2.3 years is reduced from 13.9% to 6.5%. In addition a positive EPS still exhibits a PPV of 24.2% in patients with LVEF > 35%.

Similar results were found in the MUSTT trial[27]. Both, low ejection fraction and inducible sustained ventricular tachycardia during EPS, identified patients at increased mortality risk. Inducible tachyarrhythmias identified patients for whom death was significantly more likely to be arrhythmic and this was observed especially if ejection fraction was higher than or equal to 30%. Due to our findings, invasive testing should especially be considered in this group of higher to

normal LVEF, as it might prevent implantation of ICD in patient who won't benefit.

This study has some limitations. First, the findings of this study are from a single center. Therefore, generalizability is limited and confirmation in an independent sample is of importance. Second, we included patients who underwent EPS for primary prevention as well as for secondary prevention. NPV and PPV of sustained ventricular tachycardia during EPS might differ in these subgroups, however the study population is too small to perform an independent statistical analysis.

EPS provides a benefit in risk stratification for future tachyarrhythmic events and SCD and should especially be considered in patients with LVEF > 35%.

COMMENTS

Background

Patients with preexisting cardiac disease are at higher risk for future cardiac arrhythmias, potentially leading to sudden cardiac death (SCD). In the study the authors evaluated the prognostic value of electrophysiological stimulation (EPS) in the risk stratification for future cardiac arrhythmias.

Research frontiers

Guidelines recommend various non-invasive techniques to recognize patients at higher risk for life-threatening arrhythmias. Currently, the electrophysiological study (EPS) is widely used for risk stratification and several randomized trials suggest a significant predictive value of this examination.

Innovations and breakthroughs

The authors found that in a mixed population with different cardiac pathologies inducible sustained ventricular tachyarrhythmia during EPS identified those at higher risk for SCD or appropriate activation of implantable cardioverter defibrillator (ICD). Furthermore left ventricular ejection fraction (LVEF) < 35% was another independent predictor of SCD surrogate. In patients with LVEF > 35% negative EPS had a high negative predictive value for SCD and ICD activation.

Applications

EPS provides a benefit in risk stratification for future tachyarrhythmic events and SCD and should especially be considered in patients with LVEF > 35%.

Peer-review

The authors have prospectively evaluated the role of programmed ventricular stimulation in the risk stratification of tachyarrhythmic events and sudden cardiac death in a large patient population; the possibility to optimize selection of patients undergoing ICD implantation is very relevant and evidence-based conclusions would be of great clinical importance.

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CASE REPORT

Giant saphenous vein graft pseudoaneurysm to right posterior descending artery presenting with superior vena cava syndrome

Andres Vargas-Estrada, Dianna Edwards, Mohammad Bashir, James Rossen, Firas Zahr

Andres Vargas-Estrada, James Rossen, Firas Zahr, Division of Cardiovascular Medicine, University of Iowa Hospitals and Clinics, Iowa City, IA 52242, United States
Dianna Edwards, Department of Internal Medicine, University of Iowa hospitals and Clinics, Iowa City, IA 52242, United States
Mohammad Bashir, Department of Cardiothoracic Surgery, University of Iowa Hospitals and Clinics, Iowa City, IA 52242, United States

Author contributions: Vargas-Estrada A and Edwards D conceived, designed and wrote the report; Rossen J and Zahr F performed the diagnostic coronary angiogram and made critical revisions to the discussion content; Bashir M provided the intraoperative echocardiographic images, performed the surgical procedure and guided the discussion regarding treatment options; the manuscript has been read and approved by all the authors.

Ethics approval: This is a clinical case report. All patients related identification information has been avoided according to the policy of University of Iowa Hospitals and Clinics and the Health Insurance Portability and Accountability Act (HIPPA) by the United States.
Informed consent: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.
Conflict-of-interest: All authors have no conflict-of interest to disclose.

Correspondence to: Firas Zahr, MD, Division of Cardiovascular Medicine, University of Iowa Hospitals and Clinics, 200 Hawkins Dr., Iowa City, IA 52242,
United States. andres-vargasestrada@uiowa.edu
Telephone: +1-319-3848001
Fax: +1-319-3536343

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Abstract

Saphenous vein grafts (SVG) pseudoaneurysms, especially giant ones, are rare and occur as a late complication of coronary artery bypass grafting. This condition affects both genders and typically occurs within the sixth decade of life. The clinical presentation ranges from an asymptomatic incidental finding on imaging studies to new onset angina, dyspnea, myocardial infarction or symptoms related to compression of neighboring structures. An 82-year-old woman presented with acute onset back pain, dyspnea and was noted to have significantly engorged neck veins. In the emergency department, a chest computed tomographic angiogram with intravenous contrast revealed a ruptured giant bilobed SVG pseudoaneurysm to the right posterior descending artery (RPDA). This imaging modality also demonstrated compression of the superior vena cava (SVC) by the SVG pseudoaneurysm. Coronary angiogram with bypass study was performed to establish the patency of this graft. Endovascular coiling and embolization of the SVG to RPDA was initially considered but disfavored after the coronary angiogram revealed preserved flow from the graft to this arterial branch. After reviewing the angiogram films, a surgical strategy was favored over a percutaneous intervention with a Nitinol self-expanding stent since the latter would have not addressed the superior vena cava compression caused by the giant pseudoaneurysm. Intraoperative transesophageal echocardiogram demonstrated SVC

compression by the giant pseudoaneurysm cranial lobe. Our patient underwent surgical ligation and excision of the giant pseudoaneurysm and the RPDA was regrafted successfully. In summary, saphenous vein grafts pseudoaneurysms can be life-threatening and its therapy should be guided based on the presence of mechanical complications, the patency of the affected vein graft and the involved myocardial territory viability.

Key words: Giant saphenous graft pseudoaneurysm; Late complication of coronary artery bypass grafting; Superior vena cava syndrome; Endovascular coiling and embolization; Nitinol self-expanding stent

Core tip: Saphenous vein grafts (SVG) pseudoaneurysms, especially giant ones, are rare and occur as a late complication of coronary artery bypass grafting. Although unusual, superior vena cava (SVC) syndrome has been reported as a complication of saphenous vein graft pseudoaneurysms causing compression of the SVC. Here we report a case of such condition illustrated with state-of-the-art multi-modality images which were critical for the planning of the most appropriate treatment strategy. SVG pseudoaneurysms can be life-threatening and their therapy should be guided based on the presence of mechanical complications, the patency of the affected vein graft and the involved myocardial territory viability.

Vargas-Estrada A, Edwards D, Bashir M, Rossen J, Zahr F. Giant saphenous vein graft pseudoaneurysm to right posterior descending artery presenting with superior vena cava syndrome. *World J Cardiol* 2015; 7(6): 351-356 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i6/351.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i6.351

INTRODUCTION

Coronary saphenous vein graft pseudoaneurysms are rare complications of coronary artery bypass grafting, occurring several years after the initial procedure. Although very unusual, superior vena cava obstruction has been reported as a complication of a rupture of a coronary artery bypass vein graft[1]. It is uncommon to encounter such patients and the optimal treatment remains uncertain. The improvement of percutaneous interventions and the development of covered and Nitinol self-expanding stents have become an attractive option to spare these subjects from a repeat thoracotomy, however this is not always feasible. To our knowledge, there have been very few similar cases reported, we believe this to be the first reported case of such condition illustrated with state-of-the-art multi-modality imaging.

CASE REPORT

An 82-year-old woman presented to the hospital with complaints of acute onset back pain, dyspnea for twelve hours and significantly engorged neck veins. She had undergone 3-vessel coronary artery bypass graft surgery in 1993. At the time of her coronary artery bypass operation, the left internal mammary artery (LIMA) was used to graft the first diagonal branch. Separate saphenous vein grafts were placed to the left anterior descending artery (LAD) and right posterior descending artery (RPDA). It is unknown to us the reasons for LIMA to diagonal branch anastomosis instead of arterial bypass to LAD which in turn received a venous graft. On her arrival to the emergency department, an electrocardiogram showed sinus rhythm and no ischemic changes. Her cardiac enzymes were negative. Computed tomography with IV contrast of the patient's chest ruled out pulmonary embolism and aortic dissection. Tomographic and 3D reconstruction views identified a ruptured giant bilobed SVG pseudoaneurysm to RPDA causing mass effect on the superior vena cava and right-sided cardiac chambers (Figures 1 and 2). The cranial lobe of the pseudoaneurysm measured 4.7 cm × 5.4 cm and the caudal lobe measured 8.0 cm × 7.0 cm in its larger diameter and demonstrated mural thrombosis. Coronary angiography demonstrated a giant pseudoaneurysm of the SVG with patent flow to the right posterior descending artery (Figure 3). Coiling and embolization of the SVG to RPDA was initially considered but disfavored since the coronary angiogram revealed preserved flow from the graft to RPDA branch. Also a percutaneous intervention with a covered *vs* a Nitinol self-expanding stent to the ruptured SVG was contemplated but ultimately surgical intervention was decided since the former strategy would have not addressed the superior vena cava compression caused by the giant pseudoaneurysm. Intraoperative echocardiography (Figure 4) showed SVC compression by the giant pseudoaneurysm cranial lobe. At surgery, the giant SVG pseudoaneurysm was ligated, excised and the RPDA was regrafted. The patient recovered uneventfully and was discharged on postoperative day 20. After completing her post-surgical rehabilitation, she returned to our clinic six weeks later, asymptomatic and in stable condition.

DISCUSSION

Aneurysmal dilatation of aortocoronary saphenous vein grafts (SVG), first described by Riahi *et al*[2] in 1975 remains a rare yet widely reported phenomenon. This condition is secondary to true aneurysm or pseudoaneurysm, with the former being more common. SVC syndrome is caused by obstruction of blood flow through the superior vena cava due to thrombosis or extrinsic compression. It is a medical emergency

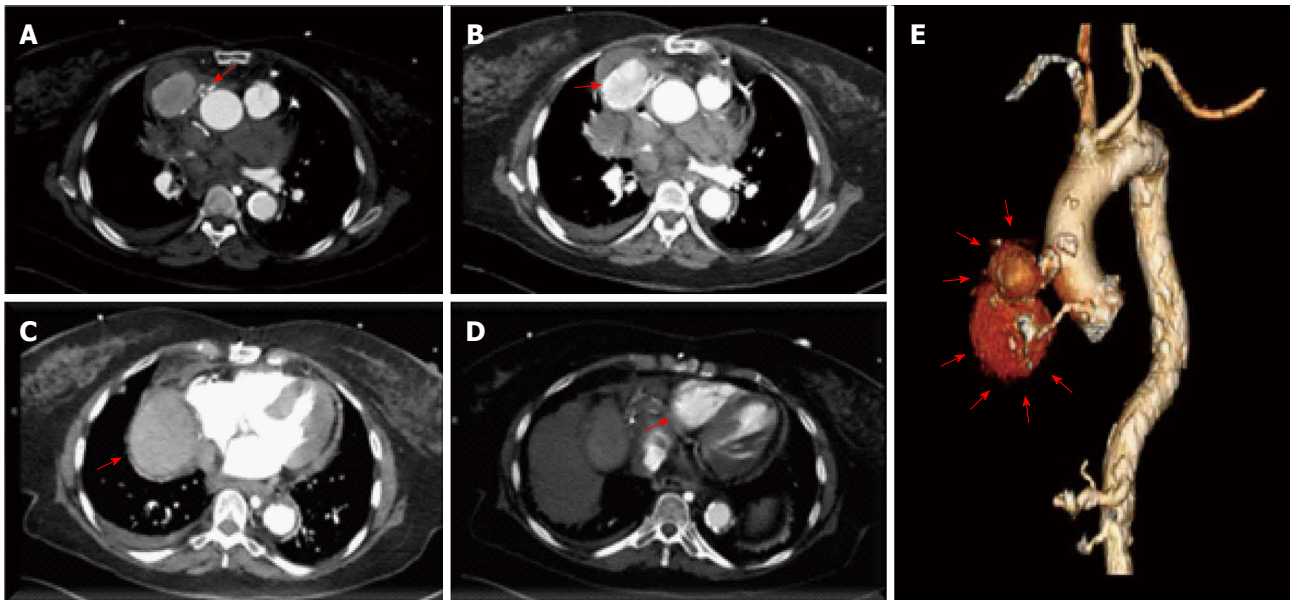

Figure 1 Transaxial tomographic views showing the right coronary artery origin and the saphenous vein grafts pseudoaneurysm lobes. A: The more cranial lobe of the pseudoaneurysm measured 4.7 cm × 5.4 cm in diameter and demonstrated mural thrombosis; B: The caudal lobe of the pseudoaneurysm also demonstrated mural thrombosis and measured 8 cm × 7 cm in its larger diameter; C, D: The caudal lobe of the giant pseudoaneurysm was patent and demonstrated flow into the distal right coronary and right posterior descending artery; E: Computed tomographic 3-D reconstruction of the saphenous vein grafts giant bilobed pseudoaneurysm to the right posterior descending artery.

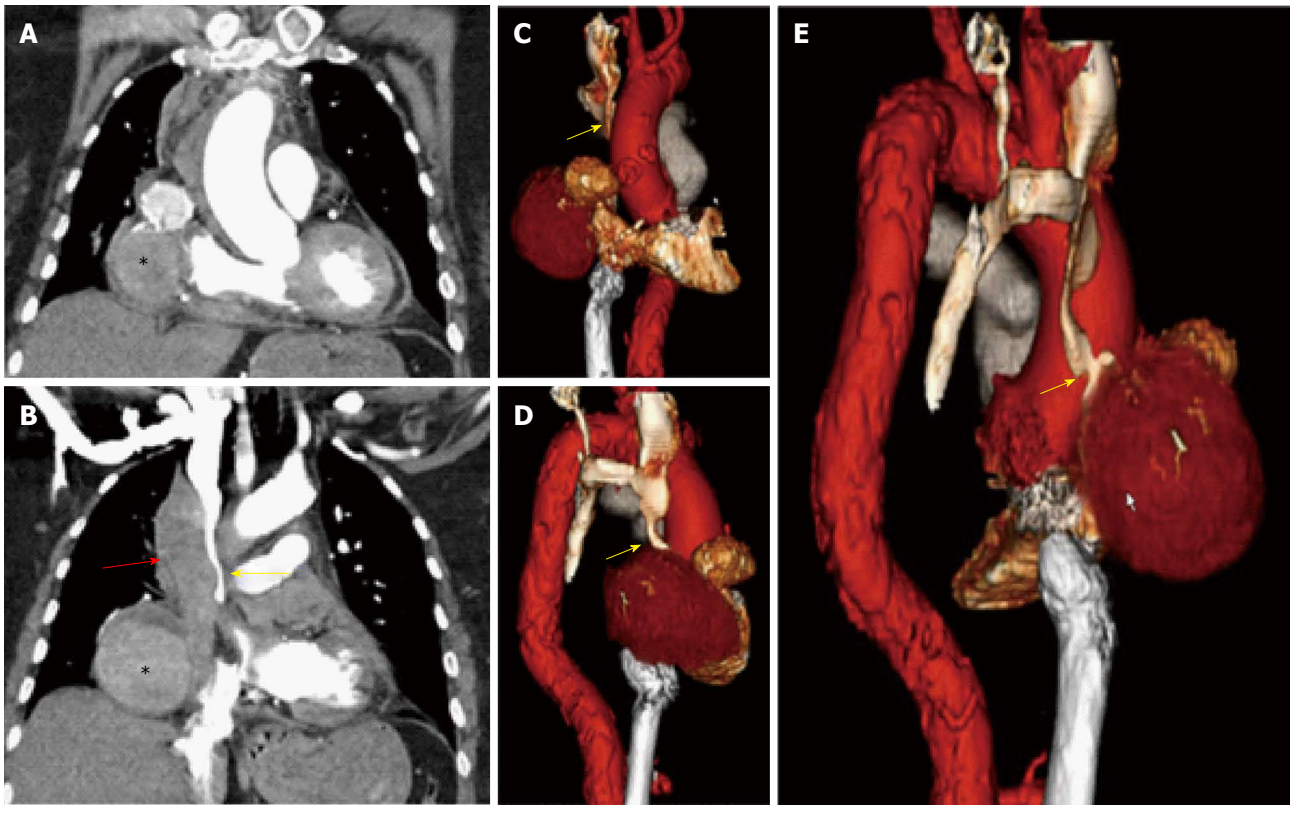

Figure 2 Significantly distended neck veins secondary to compression of the superior vena cava by the giant saphenous vein grafts pseudoaneurysm to right posterior descending artery. A: Coronal tomographic views showing upper and lower lobes of the SVG pseudoaneurysm (asterisks); B: Large hematoma (red arrow) is shown compressing the SVC (yellow arrow); C, D, E: Computed tomographic 3-D reconstruction of the SVG giant bilobed pseudoaneurysm and SVC compression (yellow arrow) in anterior, lateral and posterior views. SVG: Saphenous vein grafts; SVC: Superior vena cava.

and most often manifests in patients with a malignant process within the thorax (particularly lung adenocarcinoma), however as many as 40% of cases are attributable to nonmalignant causes. The first case of

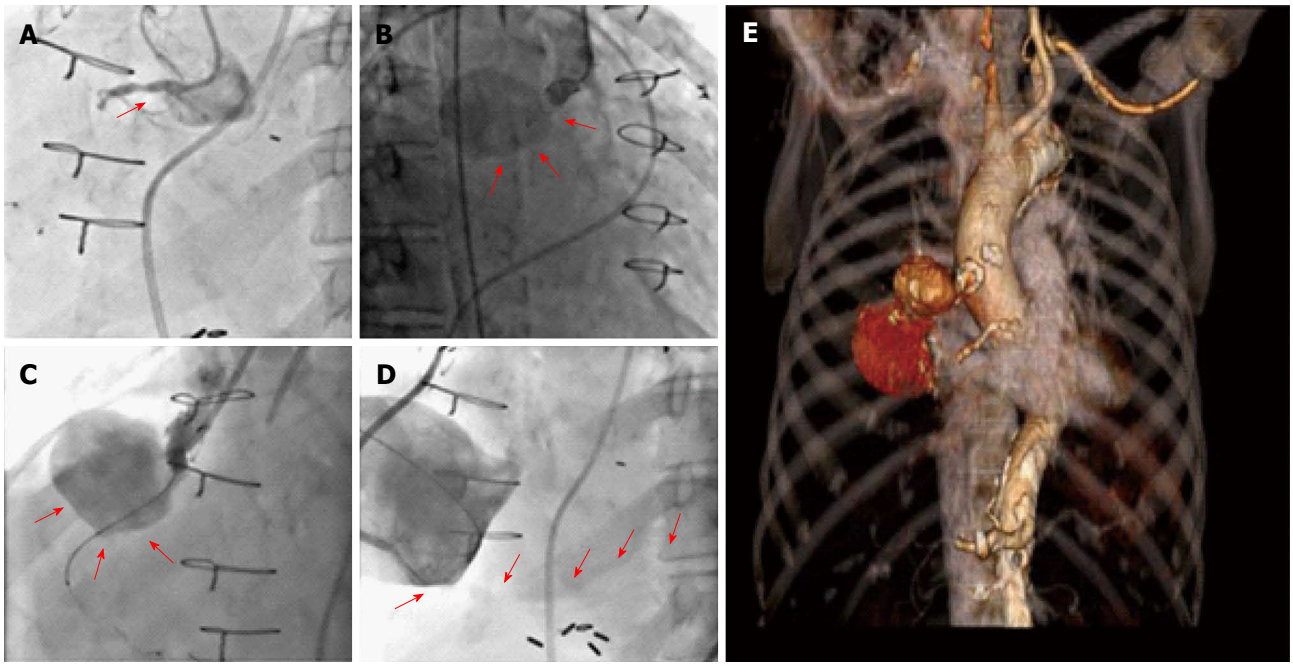

Figure 3 Coronary angiography demonstrated a giant pseudoaneurysm of the saphenous vein grafts with patent flow to the right posterior descending art. A: Coronary angiography showing the ostial and proximal right coronary artery (RCA) occlusion; B: Angiographic image depicting the upper lobe of the SVG to RPDA giant pseudoaneurysm; C: Giant pseudoaneurysm of vein graft with wide neck beginning about 2 cm from the graft origin; D: Injection within the aneurysm sac revealing a patent bypass graft that continues into the distal right coronary artery with a sequential connection to the posterior descending artery; E: Computed tomographic 3-D reconstruction of the SVG giant bilobed pseudoaneurysm to the distal right coronary artery seen in relation to the thoracic structures. SVG: Saphenous vein grafts; RPDA: Right posterior descending artery.

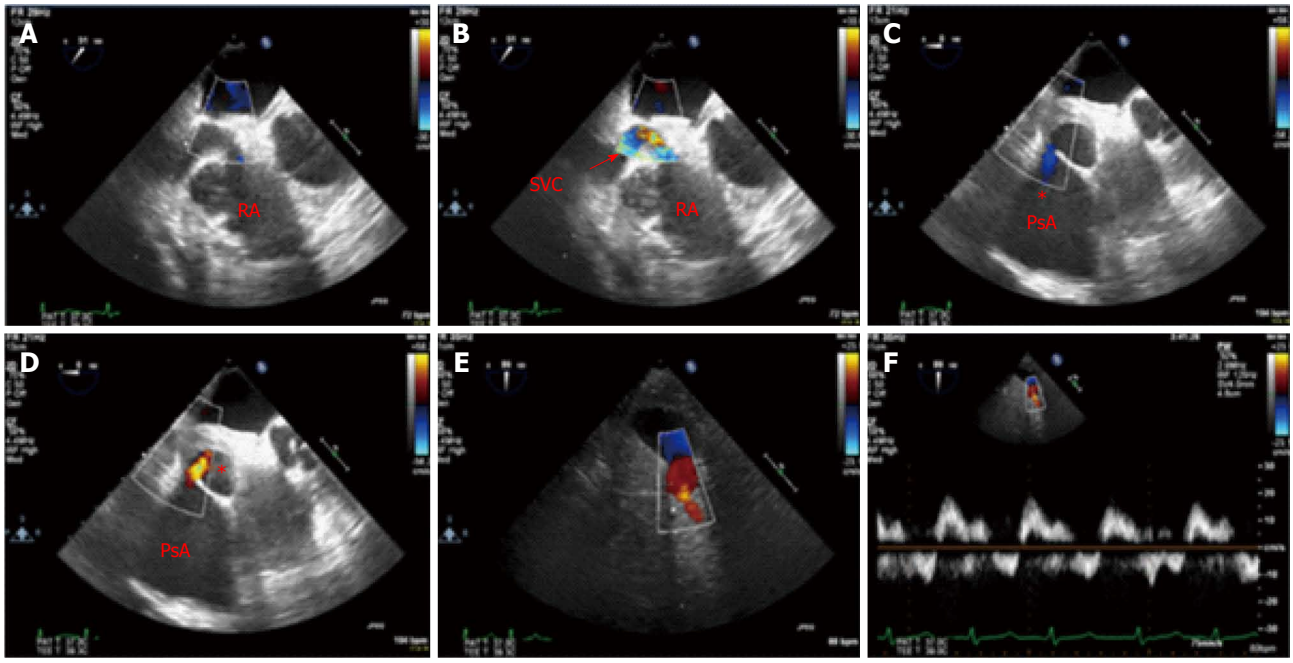

Figure 4 Intraoperative echocardiography showed superior vena cava compression by the giant pseudoaneurysm cranial lobe. A: Intraoperative transesophageal echocardiogram demonstrated normal left ventricular systolic function with left ventricular ejection fraction 60% and the giant pseudoaneurysm of SVG to RPDA in relation to the cardiac structures; B: Echocardiographic view of the pseudoaneurysm upper lobe compressing the superior vena cava (SVC); C, D: Blood flow by color Doppler of the SVC into the right atrium; E, F: Bidirectional flow is seen between the upper and lower pseudoaneurysm (PsA) lobes during diastole and systole respectively. Color Doppler and flow velocities of the giant pseudoaneurysm caudal lobe. RPDA: Right posterior descending artery.

ruptured SVG pseudoaneurysm presenting with SVC syndrome was reported by Rosin and colleagues[3] in 1989. Kavanagh *et al*[4] in 2004 reported the first case in which CT findings of this condition were described. We presented a case of a giant saphenous vein graft pseudoaneurysm to right posterior descending artery in a patient presenting with superior vena cava (SVC) syndrome. The precise incidence of SVG aneurysms

remains difficult to ascertain and more so for SVG pseudoaneurysms. In one case series, an incidence of 0.07% was estimated from a review of 5500 grafts at a single institution[5]. This condition occurs in both genders but predominates in men (87% of reported cases on average within the sixth decade of life). Postulated mechanisms for SVGs aneurysmal dilatation include atherosclerotic degeneration of the graft, vessel wall ischemia secondary to disruption of the vasa vasorum during the harvesting and grafting process, graft endothelial dysfunction and changes in medial smooth muscle cell orientation in the vicinity of valve sites[6]. The clinical presentation is somewhat variable and ranges from an asymptomatic incidental finding on chest X-ray (12%-47%) to new onset angina (46.4%), shortness of breath (12.9%), myocardial infarction (7.7%), hemoptysis (4.8%), hemothorax, or symptoms related to compression of neighboring structures, as well as shock (3.8%) or death[7]. SVGs aneurysms predominate in the right coronary distribution thus compression of right-sided cardiac structures is more common. Our patient presented with significantly distended neck veins secondary to compression of the superior vena cava by the giant SVG pseudoaneurysm to RPDA (Figure 2). The mechanical complications include right atrial compression (11.5% of cases), right ventricular compression (7.2%) and fistula formation (7.7%)[8]. The most feared complication is aneurysm rupture which has been reported as a presenting feature in only a minority of cases. Although frequently identifiable on chest X-ray or echocardiography, coronary angiography is required to evaluate the patency of the coronary arteries and bypass grafts. Confirmation of the size of the aneurysm or pseudoaneurysm and the relationship to the surrounding structures is best achieved with CT angiography or MRA. Different treatments options exist and are applied according to the anatomy of the pseudoaneurysm, mechanical complication and the patency of the affected vein graft. Repeat coronary artery bypass grafting with aneurysmal ligation or excision is the classic approach[9]. In cases in which the affected graft remains patent despite the aneurysmal dilation and in non-surgical candidates for repeat sternotomy, percutaneous management with a covered stent should be considered. In patients in whom preservation of myocardial blood supply is not a concern or non-viable myocardium has been demonstrated, the aneurysmal neck can be occluded by Amplatzer vascular plugs[9] or the aneurysm can be thrombosed by endovascular coiling[10]. Although large-bore Nitinol stents are highly effective for superior vena cava syndrome, in our case it was decided to proceed with re-thoracotomy and excision of the graft pseudoaneurysm after rupture was demonstrated by CT imaging and coronary angiography. The SVC syndrome resolved with the surgical excision of the ruptured SVG pseudoaneurysm. The optimal treatment for this condition remains an area of uncertainty with available data based on case reports and small case series and certainly depends upon the clinical scenario and should be made by a multidisciplinary cardiac team.

COMMENTS

Case characteristics

An 82-year-old woman presented with acute onset back pain, dyspnea and engorged neck veins.

Clinical diagnosis

Dyspnea of twelve hours duration and engorged neck veins, later were found to be caused by superior vena cava syndrome from superior vena cava (SVC) compression by a giant saphenous vein grafts (SVG) pseudoaneurysm.

Differential diagnosis

Any cause for acute onset back pain and dyspnea such as acute coronary syndrome, pulmonary embolism, aortic dissection, *etc.*

Laboratory diagnosis

Lab tests result including cardiac enzymes were unremarkable.

Imaging diagnosis

Multi-imaging modalities including computed tomography chest angiogram, coronary angiogram and TEE revealed a giant bilobed SVG pseudoaneurysm to the right posterior descending artery causing compression of the superior vena cava leading to SVC syndrome.

Treatment

Surgical ligation and excision of the SVG pseudoaneurysm with regrafting of right posterior descending artery.

Experiences and lessons

Saphenous vein graft pseudoaneurysms can present as a late complication following coronary artery bypass grafting. Although most frequently asymptomatic and found incidentally by imaging modalities, the authors should consider this condition in the differential diagnoses for acute coronary syndrome and as a possible explanation of SVC syndrome in patients with prior coronary artery grafting. Different treatments options exist and are applied according to the anatomy of the pseudoaneurysm, mechanical complication and the patency of the affected vein graft.

Peer-review

This is an interesting report for clinical practice.

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CASE REPORT

Long term evolution of magnetic resonance imaging characteristics in a case of atypical left lateral wall hypertrophic cardiomyopathy

Tobias Gassenmaier, Bernhard Petritsch, Andreas S Kunz, Spyridon Gkaniatsas, Philipp D Gaudron, Frank Weidemann, Peter Nordbeck, Meinrad Beer

Tobias Gassenmaier, Bernhard Petritsch, Andreas S Kunz, Department of Diagnostic and Interventional Radiology, University Hospital of Würzburg, 97080 Würzburg, Germany
Spyridon Gkaniatsas, Philipp D Gaudron, Frank Weidemann, Peter Nordbeck, Department of Internal Medicine I, University Hospital of Würzburg, 97080 Würzburg, Germany
Frank Weidemann, Peter Nordbeck, Comprehensive Heart Failure Center, University of Würzburg, 97080 Würzburg, Germany
Meinrad Beer, Department of Diagnostic and Interventional Radiology, University of Ulm, 89070 Ulm, Germany

Author contributions: Gassenmaier T, Petritsch B and Kunz AS wrote the paper and researched the literature; Gkaniatsas S, Gaudron PD, Weidemann F and Nordbeck P contributed to patient's treatment and critical review of the paper; Beer M contributed to imaging and critical review of the paper.

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Informed consent: Informed consent of the patient was obtained where applicable. All details that might disclose the identity of the patient were omitted in this case report.
Conflict-of-interest: None.

Correspondence to: Dr. Tobias Gassenmaier, MD, Department of Diagnostic and Interventional Radiology, University Hospital of Würzburg, Oberdürrbacher Str. 6, 97080 Würzburg, Germany. gassenmaie_t@ukw.de
Telephone: +49-931-20134200
Fax: +49-931-20134209

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Abstract

We are reporting a long-time magnetic resonance imaging (MRI) follow-up in a rare case of cardiac left lateral wall hypertrophy. Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiovascular disorder and a significant cause of sudden cardiac death. Cardiac magnetic resonance (CMR) imaging can be a valuable tool for assessment of detailed information on size, localization, and tissue characteristics of hypertrophied myocardium. However, there is still little knowledge of long-term evolution of HCM as visualized by magnetic resonance imaging. Recently, our group reported a case of left lateral wall HCM as a rare variant of the more common forms, such as septal HCM, or apical HCM. As we now retrieved an old cardiac MRI acquired in this patient more than 20 years ago, we are able to provide the thrilling experience of an ultra-long MRI follow-up presentation in this rare case of left lateral wall hypertrophy. Furthermore, this case outlines the tremendous improvements in imaging quality within the last two decades of CMR imaging.

Key words: Hypertrophic cardiomyopathy; Atypical; Follow-up; Cardiac magnetic resonance imaging; Left lateral wall

Core tip: Cardiac magnetic resonance imaging (MRI) can be a valuable tool for assessment of detailed information on size, localization, and tissue characteristics in cases of hypertrophic cardiomyopathy. We report the thrilling experience of an ultra-long magnetic resonance imaging follow-up presentation in a rare case of left lateral wall hypertrophy with an initial cardiac MRI of patient acquired more than 20 years ago. This case outlines the tremendous improvements in imaging quality within the last two decades of cardiac MR imaging.

Gassenmaier T, Petritsch B, Kunz AS, Gkaniatsas S, Gaudron PD, Weidemann F, Nordbeck P, Beer M. Long term evolution of magnetic resonance imaging characteristics in a case of atypical left lateral wall hypertrophic cardiomyopathy. *World J Cardiol* 2015; 7(6): 357-360 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i6/357.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i6.357

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiovascular disorder and a significant cause of sudden cardiac death. Cardiac magnetic resonance (CMR) imaging can be a valuable tool for assessment of detailed information on size, localization, and tissue characteristics of hypertrophied myocardium. However, there is still little knowledge of long-term evolution of HCM as visualized by magnetic resonance imaging (MRI). Recently, our group reported a case of left lateral wall HCM[1] as a rare variant of the more common forms, such as septal HCM, or apical HCM[2]. As this patient underwent initial cardiac MRI more than 20 years ago, we are able to provide the thrilling experience of an ultra-long MRI follow-up presentation in this rare case of left lateral wall hypertrophy, including the tremendous improvements in imaging quality within the last two decades of CMR imaging.

CASE REPORT

A 52-year-old male presented himself to the emergency department of our institution suffering an episode of nocturnal chest pain. As the electrocardiogram showed ventricular tachycardia, synchronized electrical cardioversion was performed, successfully terminating the arrhythmia. Afterwards, the patient was subjected to sequential clinical investigations, excluding ischemia as cause for the serious symptoms, and CMR imaging, revealing an atypical form of HCM.

CMR was performed five days after the initial arrhythmic event on a MAGNETOM® Trio 3.0 Tesla scanner (Siemens AG Sector Healthcare, Erlangen, Germany). In addition to these current investigations, we were now able to retrieve MR images from the year 1989 (1.5 Tesla Philips Gyroscan, Philips Medical System, Best, the Netherlands) which had been acquired in the pre-PACS era more than 23 years in advance of the current event. This enabled us to present a long term MRI follow-up of this rare manifestation of HCM and to point out the massive improvements in imaging quality of cardiac MRI, due to enormous technical development within the last two decades.

Past images from the year 1989 deliver some limited diagnostic information. Nevertheless, left ventricular wall thickening up to 45 mm was clearly seen in this patient even 23-years ago (Figure 1A). In addition, Gadolinum enhancement (GE) of the noticeable area of interest was depicted after *iv* contrast application (Figure 1B). In the late 1980s, late Gadolinium enhancement (LGE) had not yet been described as a non-invasive method for myocardial tissue characterization, including analysis for myocardial fibrosis.

Current high-quality T1 weighted turbo-spin-echo images with dark blood technique confirmed an extensive, confined thickening of the left ventricular lateral wall up to 45 mm in the 4-chamber view (Figure 1C). Cine-SSFP sequences demonstrated a prolonged longitudinal relaxation of the lateral wall (not shown). After injection of 0.2 mmol/kg intravenous contrast agent (Gadovist®, Bayer HealthCare, Leverkusen, Germany) the myocardial mass showed homogenous contrast enhancement. LGE imaging was acquired 12 min after *iv* contrast administration. PSIR-SSFP images in the 4-chamber (Figure 1D) and short axis views (Figure 2A-C) revealed a homogenous enhancement, corresponding to the left ventricular lateral wall thickening, as it can be typically observed in other, more common forms of HCM.

In addition, a small accompanying circular pericardial effusion (indicated with arrows) was depicted in the present CMR images (Figure 1C and 2B).

Comparison of the studies revealed no significant change over time regarding the extent of wall thickening. It is difficult to judge whether significant change in myocardial fibrosis can be detected by CMR, as LGE techniques had not been described prior to 2001 and the baseline CMR was performed prior to that in 1989[3] (Figure 1, A/B *vs* C/D). In 1989, the contrast enhanced images were acquired about 3 min after Gd administration, a "late-enhancement stop" and the so-called "nulling" technique were not available/invented. However, as far as one can compare the images from 1989 to 2012, no significant change in fibrotic myocardium occurred during this time span. The diagnosis of myocardial fibrosis was already proven in 1989 by myocardial biopsy, showing massive hypertrophy but no signs of malignancy.

Therefore, this intensive myocardial thickening described above has to be considered a highly

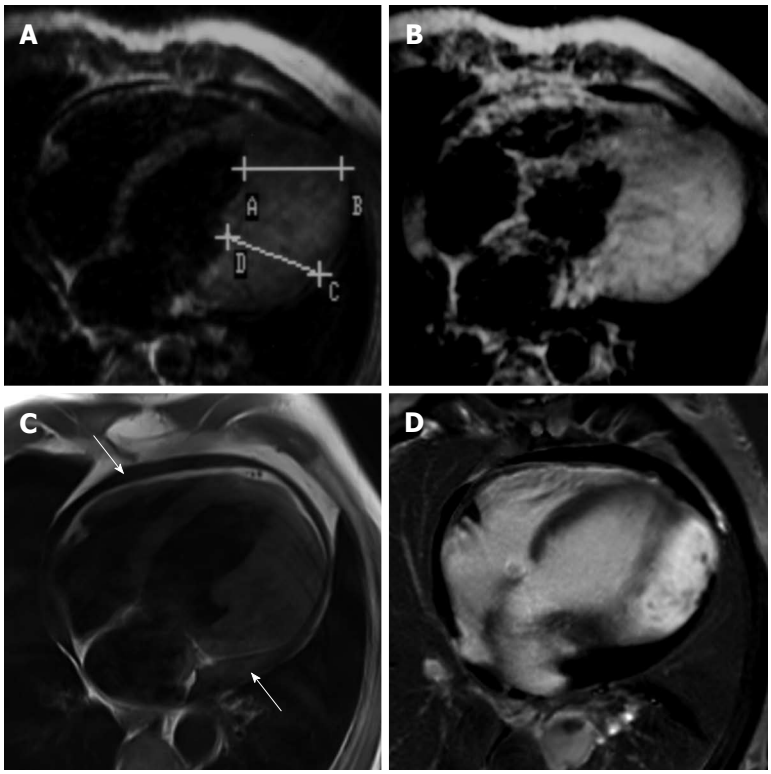

Figure 1 Hypertrophic cardiomyopathy of the left lateral wall in 1989 (A, B) and 2012 (C, D). Note the difference of image quality especially within the GE series from 1989 (B) and the PSIR-SSFP late Gadolinium enhancement (LGE) images in 2012 (D). Within the last 23 years, there was no significant change in respect to morphology (A, C) and LGE (B, D) in this patient. Arrows indicate accompanying circumferential, increasing pericardial effusion.

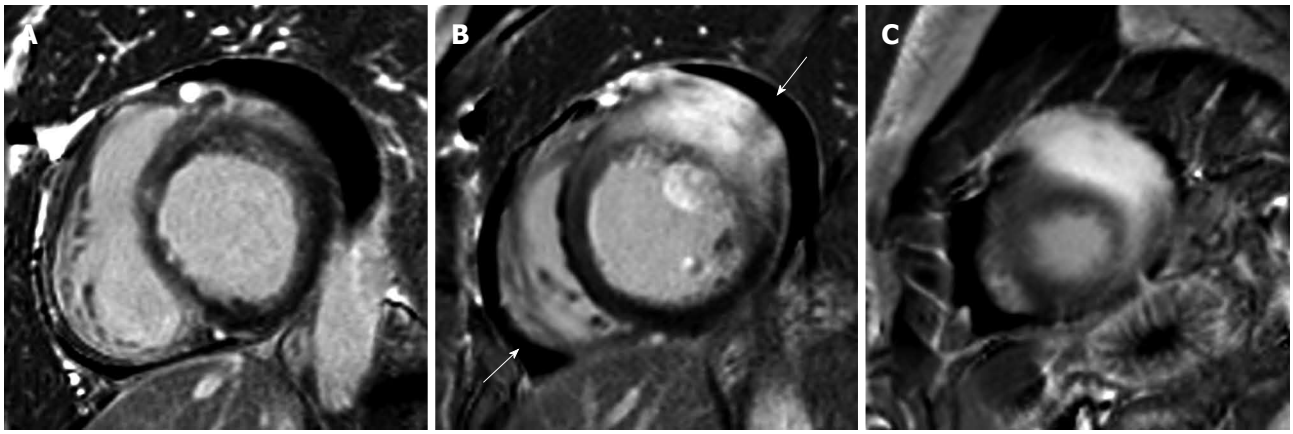

Figure 2 Late Gadolinium enhancement images in basal (A), midventricular (B) and apical (C) slices in the short axis view show significant late Gadolinium enhancement in the area of hypertrophic cardiomyopathy of the left lateral wall. Arrows indicate accompanying pericardial effusion.

uncommon manifestation of HCM limited solely to the left ventricular lateral wall.

Finally, the patient could be discharged in good general condition after the implantation of an ICD for prevention of further episodes of ventricular tachycardia.

DISCUSSION

In a patient with atypical left wall cardiac hypertrophy, CMR was able to provide detailed information on size, localization, and tissue characteristics of the myocardial mass and allowed non-invasive long time assessment of these parameters. Even though limited to the left ventricular lateral wall, our variant of HCM showed some typical characteristics. For instance, LGE is frequently observed in HCM patients and reflects the presence of fibrosis within the myocardium[4,5]. However, the clinical significance of change of LGE over time in HCM is still under debate[6]. Nonetheless, the presence of LGE is an independent risk factor for adverse outcome in HCM and associated with an increased frequency of ventricular tachyarrhythmia, as was the case in our patient[7,8].

However, initial inspection of the LGE images with

the atypical site of LGE and the considerable degree of myocardial hypertrophy raised concerns whether the patient might suffer from a malignant tumor of the myocardium. Pericardial effusion fortified this impression. However, only a short period after the current CMR, reports from the old, previous CMR from 1989 were retrieved. Finally, comparison of the images from 1989 to those of 2012 confirmed that the patient suffered from HCM and excluded a malignant tumor as a potential differential diagnosis.

For future studies, T1 maps might be helpful to differentiate between a cardiac entity (HCM) and non-cardiac entity (tumor). However, this was not performed in the current case, as the utilized MR scanner was not equipped with software applicable for T1 mapping.

COMMENTS

Case characteristics

A 52-year-old male with an episode of nightly chest pain.

Clinical diagnosis

Electrocardiogram showed ventricular tachycardia.

Differential diagnosis

Ischemia, cardiac tumor, atypical hypertrophic cardiomyopathy (HCM).

Laboratory diagnosis

Tests for exclusion of cardiac ischemia were within normal limits.

Imaging diagnosis

Cardiac magnetic resonance imaging showed an extensive, confined thickening of the left ventricular lateral wall up to 45 mm. Comparison with a previous study from 1989 revealed that this left ventricular wall thickening was clearly seen in this patient already 23-years ago.

Pathological diagnosis

Myocardial biopsy had shown massive hypertrophy but no signs of malignancy in 1989.

Treatment

The patient underwent implantation of an ICD for prevention of further episodes of ventricular tachycardia.

Related reports

Isolated left lateral wall hypertrophic cardiomyopathy is a rare variant of the more common forms, such as septal HCM, or apical HCM.

Term explanation

Late gadolinium enhancement is frequently observed in HCM patients and reflects the presence of fibrosis within the myocardium.

Experiences and lessons

This case report outlines the tremendous improvements in imaging quality within the last two decades of cardiac MR imaging.

Peer-review

This article reported a long-time MRI follow-up in a rare case of cardiac left lateral wall hypertrophy. Cardiac magnetic resonance imaging is a valuable tool for assessment characteristics of hypertrophic cardiomyopathy. And this report provided detailed information on location, size and imaging characteristics of hypertrophic cardiomyopathy.

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CASE REPORT

Reverse or inverted apical ballooning in a case of refeeding syndrome

Pablo Robles, Isabel Monedero, Amador Rubio, Javier Botas

Pablo Robles, Isabel Monedero, Amador Rubio, Javier Botas, Departament of Cardiology, Hospital Universitario Fundación Alcorcón, 28922 Alcorcón, Madrid, Spain

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Correspondence to: Pablo Robles, MD, Departament of Cardiology, Hospital Universitario Fundación Alcorcón, Calle Budapest, 1, 28922 Alcorcón, Madrid, Spain. probles@fhalcorcon.es
Telephone: +34-916-817330
Fax: +34-916-219928

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Abstract

Takotsubo cardiomyopathy is characterized by the development of transient left ventricular regional wall motion abnormalities, in the absence of significant coronary artery obstruction. This syndrome usually occurs in women and is frequently associated with an intense emotional or physical stress. It usually involves apical segments, but in the recent years atypical forms have been described. Inverted or reverse Takotsubo is a variant in which the basal and midventricular segments are hypokinetic, sparing contractile function of the apex. In this report we describe the case of a 54-year-old woman, with chronic malnutrition, initially admitted because of hypoglycemia and severe electrolyte disturbance due to a refeeding syndrome. Within the next hours she experienced acute cardiac symptoms and developed heart failure with low cardiac output. Electrocardiogram (ECG), elevation of troponin and echocardiographic findings were consistent with inverted Takotsubo cardiomyopathy. To the best of our knowledge, this is the first incidence reported of inverted Takotsubo triggered by refeeding syndrome.

Key words: Apical ballooning; Refeeding syndrome; Anorexia; Atrial tachycardia; Inverted takotsubo

Core tip: Inverted Takotsubo is a stress-induced cardiomyopathy type that could be encountered in patients suffering from varied physical or emotional triggers. In this report we describe the first case following a refeeding syndrome. There are reported cases of classical apical Takotsubo associated with nutrition disorders, but none of them presenting with the inverted variant.

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INTRODUCTION

Takotsubo cardiomyopathy (also called apical ballooning syndrome or stress-induced cardiomyopathy) is an acute cardiac syndrome characterized by transient and reversible wall-motion abnormalities of the left ventricle.

The clinical features include an onset of acute chest symptoms, electrocardiographic changes, and elevated cardiac enzymes, mimicking myocardial infarction, but in the absence of significant obstructive coronary disease.

It is estimated that this condition probably accounts for 1% to 2% of all cases of suspected acute myocardial infarction. Approximately 90% of all reported cases have been in women and the average age of onset range between 58 and 75 years, with < 3% of the patients being < 50 years[1].

In the most commonly described type of stress cardiomyopathy, the contractile function of the mid and apical segments of the left ventricular is depressed and there is hyperkinesis of the basal walls. Less common (atypical) variants include ventricular hypokinesis restricted to the mid-ventricle (mid-ventricular Takotsubo), hypokinesis of the base and mid-ventricle segments with sparing of the apex (reverse or inverted Takotsubo), and localized hypokinesis[2].

The pathophysiology remains unknown, but this syndrome is frequently triggered by intense emotional or physical stress or by an acute medical illness, so catecholamine mediated myocardial stunning is the most accepted explanation[3]. We present a case of inverted Takotsubo in a woman with chronic malnutrition who experienced a rapid oral nutrition repletion. After that she developed refeeding syndrome, a potentially lethal clinical condition characterized by severe metabolic disturbances in undernourished or starved patients undergoing refeeding. Medical complications of this syndrome include cardiovascular system, but it has not usually been described to trigger Takotsubo's cardiomyopathy. The fact that the patient developed an atypical variant (inverted) instead of the classical type of apical stress cardiomyopathy, also makes this case remarkable.

CASE REPORT

A 54-year-old woman was admitted to our hospital on Christmas day with impaired consciousness and severe hypoglycemia (19 mg/dL). She had a past medical history significant for persistent malnutrition, although main organic causes of weight loss had been excluded. The day before admission the relatives of the patient had urged her to ingest a copious dinner on Christmas Eve.

Physical examination on admission revealed marked emaciation with a body weight of 28 kg. She was 162 cm in height and her body mass index (BMI) was 10.66 kg/m^2 (-45% of her ideal BMI). Her body temperature was normal, but she had bradycardia (55 beats/min) and edema in her lower limbs. Her albumin (1.5 g/dL), phosphate (2.2 mg/dL), magnesium (1.6 mg/dL) and potassium (2.7 mmol/L) levels were low. Liver dysfunctions (AST: 122 IU/L, ALT: 72 IU/L) also were noted, as well as coagulation disorders (PT: 55.9%, APTT: 43 s).

First of all, she was treated with 25 g of 50% glucose administrated intravenously, with recovery of consciousness within a few minutes. Then she started receiving specific treatment for electrolyte replacement. The initial ECG showed sinus bradycardia (Figure 1A). Some hours later, the patient referred heart palpitations and a new ECG (Figure 1B and C) was obtained, showing a supraventricular tachycardia. It was remarkable the ST segment elevation in leads Ⅱ, Ⅲ, aVF, V5-V6. The tachycardia was terminated by the administration of adenosine (Figure 1D). Revising the whole electrocardiographic registry the episode was consistent with paroxysmal atrial tachycardia. In the next hours the clinical state of the patient progressively impaired, with development of acute dyspnea, hypotension and obtundation, suggesting heart failure and low cardiac output. Chest X-ray also demonstrated an impairment respect to the previous one on admission (Figure 2). Echocardiography showed dyskinesia of basal and mid-ventricular segments, with hyperkinesia of left ventricular apex (Figure 3). The ejection fraction was estimated at 25%. Serum troponins were mildly elevated with a peak of 4.2 ng/mL, with non-elevated creatine phosphokinase (CPK) levels (80 UI/L). The patient was treated with noninvasive positive pressure ventilation and inotropic drugs at the Intensive Care Unit. Along the next days her clinical situation progressively improved, and a echocardiogram performed one week later showed recovery of the wall motion abnormalities of the left ventricle, with hyperdynamic ejection fraction (Figure 4). Finally, as a complication she developed respiratory distress due to a Serratia marcescens-induced acute pneumonia, and she died. Subsequent necropsy revealed coronary arteries with non obstructive lesions.

DISCUSSION

Once other causes of weight loss had been excluded, all the evidence (clinical signs and findings, along with information provided by the family) pointed towards our patient in the present case suffered from anorexia nervosa (AN).

In patients with AN, cardiac complications can be present in up to 80% of cases and have been reported as cause of at least one-third of all deaths[4].

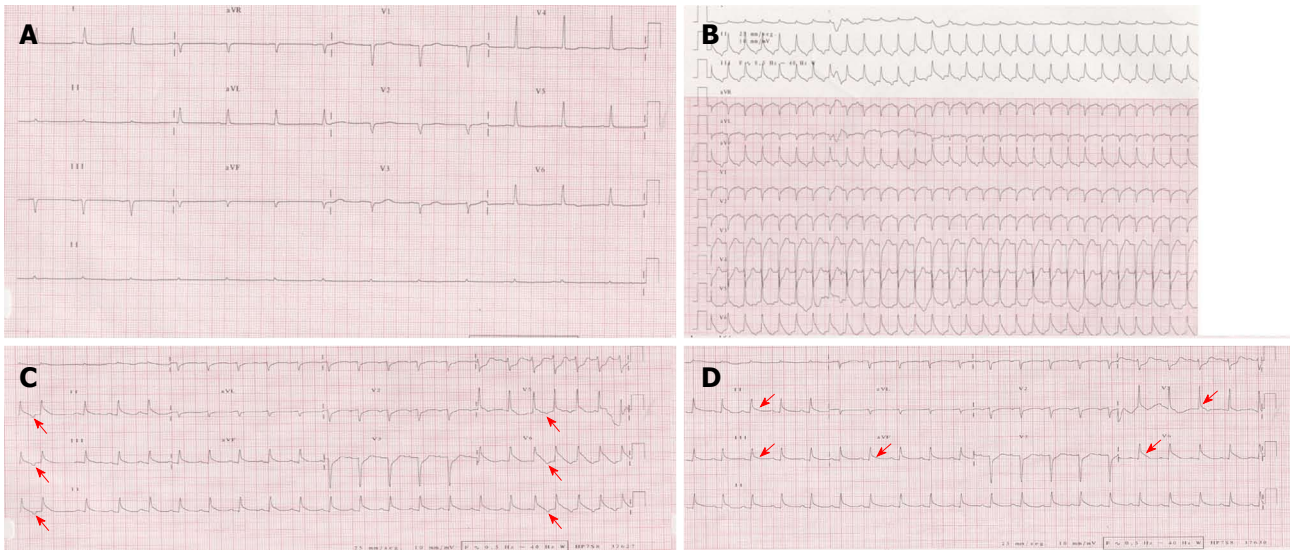

Figure 1 Change process of electrocardiogram. Baseline electrocardiogram (ECG) showed sinus bradycardia and nonspecific repolarization abnormalities (A). Surface ECG of repetitive nonsustained atrial tachycardia (AT). Note that the first P wave of the tachycardia is similar in morphology to the subsequent P waves, consistent with abnormal automaticity as the mechanism of the AT (red arrow). In the setting of posteroseptal AT (originating below and around the coronary sinus ostium), the P wave is positive in lead V1, negative in the inferior leads, and positive in leads aVL and aVR (B and C). ECG after the completion of the tachycardia showed persistent ST elevation in leads Ⅱ, Ⅲ, AVF, V5 and V6 (D) (red arrows).

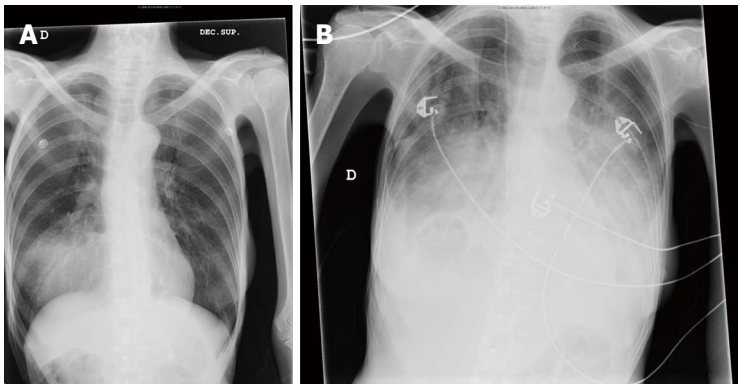

Figure 2 Chest X-ray on admission (A) and after the episode of atrial tachycardia (B) showing signs of severe heart failure.

Main cardiovascular disorders include alterations in hemodynamics (mainly hypotension), in structure (radiographic evidence of decreased cardiac size associated with lower left ventricular mass) and in electrical activity, including sinus bradycardia (present in this patient), reduction in QRS voltage, alterations in ST segment, U waves and prolonged QT interval. QT prolongation may be influenced both by electrolyte abnormalities and psychotropic drugs, with subsequent higher risk of ventricular arrhythmias or torsades de pointes[5,6]. However, left ventricle function generally remains normal, and Takotsubo's cardiomyopathy has only been reported in AN in isolated cases, some of them with hypoglycaemic coma as the precipitating event[7].

Over a chronic severe malnutrition state, the patient had been urged to ingest a copious dinner just before admission. Clinical impairment that she developed within the next hours can be attributed to the appearance of a refeeding syndrome (RF).

RF describes a series of metabolic and biochemical changes that occur as consequence of reintroduction of feeding after a period of starvation or fasting. First reports of the syndrome appeared in the 1950s after observations of malnourished prisoners of war who developed cardiac and neurological symptoms soon after the recommencement of feeding. In 2001 Crook *et al*[8,9] referred to a syndrome of important electrolyte and fluid shifts associated with metabolic abnormalities in malnourished patients undergoing oral, enteral or parenteral refeeding.

This potentially lethal condition encompasses a severe electrolyte disturbance, mainly low serum concentrations of intracellular ions such as phosphate, magnesium, and potassium. Hypophosphataemia is the adopted surrogate marker for diagnosing RF, though low serum phosphate is not pathognomonic. It may produce clinical complications affecting

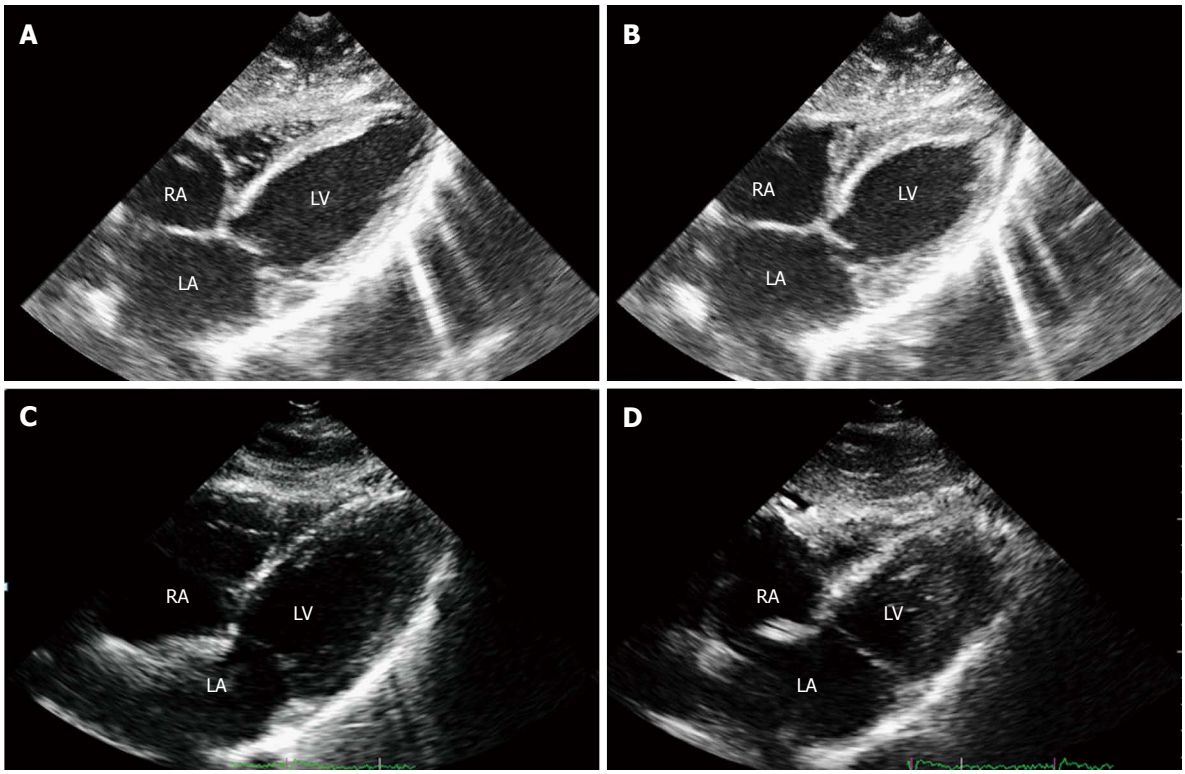

Figure 3 Two-dimensional echocardiogram, subcostal four chambers view, showing the anteroseptal and posterolateral walls of the left ventricle. End-diastolic (A) and mid-systolic (B) frames at the time of acute cardiac symptoms presentation showed dyskinesia of basal and medium segments, with hyperkinesia of the left ventricular apex. One week later, recovery of the wall motion abnormalities was demonstrated, with hyperdynamic ejection fraction (C and D). A previous echocardiogram performed two years before in this patient was similar to this last one. RA: Right atrium; LA: Left atrium; LV: Left ventricle.

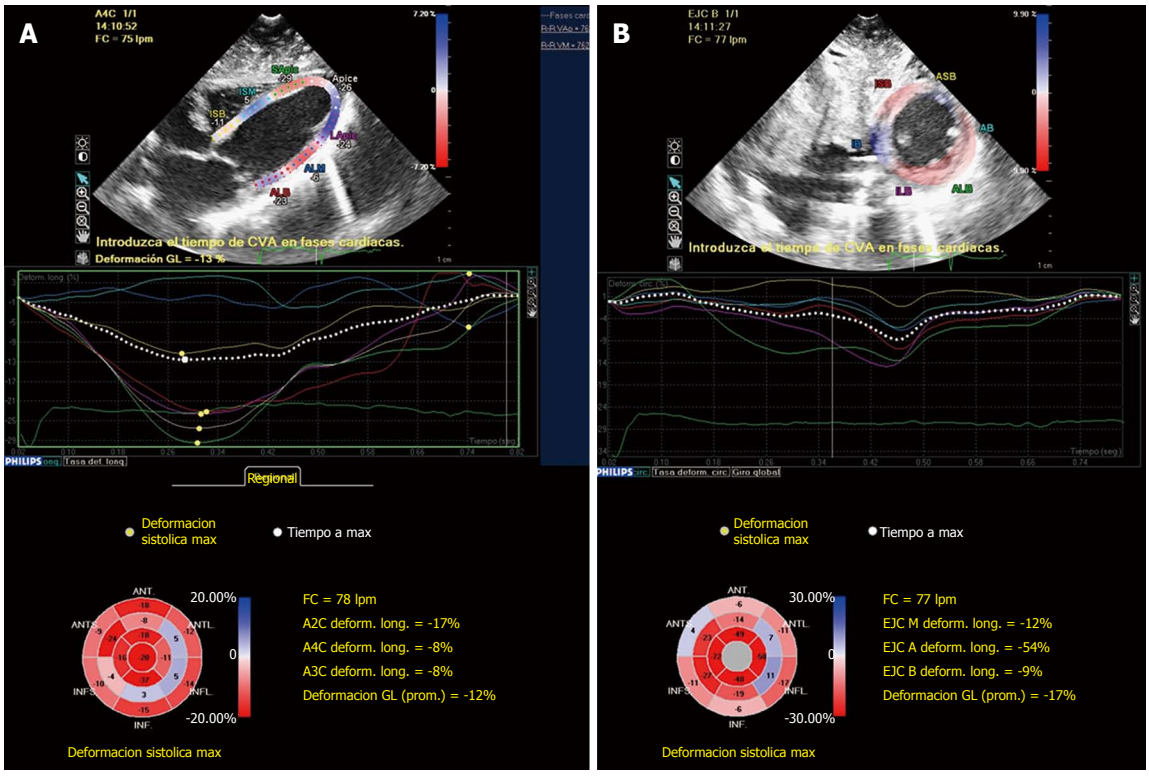

Figure 4 Bull's eye mapping of two-dimensional speckle tracking strain imaging longitudinal (A) and circunferencial (B) showed decreased strain values of the basal and mid-ventricular segments, with normal or increased strain values of the apical segments.

the cardiac, respiratory, haematological, hepatic and neuromuscular systems and leading even to death[10,11].

Atrophy of the heart during starvation renders the patient more vulnerable to fluid overload and heart failure, and electrolyte abnormalities may contribute to ventricular arrhythmias[12]. Nevertheless, our patient developed unusual cardiovascular complications associated with RF, as atrial tachycardia and stress-induced cardiomyopathy.

Automatic atrial tachycardias (caused by abnormal automaticity in cardiac cells) are catecholamine sensitive and the discharge of the abnormal pacemaker involved can be triggered by drugs, various forms of cardiac disease, reduction in extracellular potassium or alterations of autonomic nervous system tone. One or more of them could have influenced in the episode suffered by this patient in the context of RF[13].

Stress-induced (Takotsubo) cardiomyopathy is characterized by the development of transient wall-motion abnormalities in the absence of obstructive coronary artery disease. It was initially described in the Japanese population in 1991 as a syndrome of reversible left ventricular dysfunction with wall-motion abnormalities that involved the apical segments[14]. This condition is typically triggered by severe emotional or physical stress, and it is thought to be caused by a catecholamine-mediated injury. Subarachnoid hemorrhage and pheochromocytoma have been described as common triggers of Takotsubo's cardiomyopathy, which supports this hypothesis, with the exact mechanism of damage caused by catecholamines being less well understood[15].

Various patterns of stress-induced cardiomyopathy have been recently recognized and classified into 4 types based on the involvement of the left ventricle: (1) classic or apical type; (2) reverse or inverted type; (3) mid-ventricular type; and (4) and localized type[16]. We report a case consistent with the inverted type, with dyskinesia of basal and mid-ventricular segments and hyperdynamic contractility of the apex.

Clinical differences affecting inverted type in comparison to common apical and mid-ventricular type have been evaluated by several studies. They conclude that patients with reverse Takotsubo are significantly younger compared with those with other types. It might be due to an asymmetric distribution of adrenergic receptors, which seem to play an important role to determine the area of hypokinesis[17]. The hypothesis is that adrenoceptor density is highest in the apex compared with the base in postmenopausal women, explaining the occurrence of apical variant in older women. The presentation of the inverted type at an early age could be explained by the abundance of adrenoreceptors at the base of the heart, compared with the apex, in younger patients[18].

Release of troponin is higher in inverted Takotsubo compared to other patterns, which might be the consequence of the larger muscle region involved compared to apical type. Nevertheless, in apical and midventricular patterns natriuretic peptides are more elevated and a higher prevalence of significant reversible mitral regurgitation is present, which is clinically translated by more severe heart failure symptoms and higher NYHA functional class[19].

Inverted Takotsubo also seems to be more often associated with either mental or physical stress than other types. Different authors have described cases of inverted type associated with varied physical triggers (pheochromocytoma, pulmonary embolism, cerebellar hemorrhage, pneumomediastinum, *etc.*)[20-22]. Nevertheless to our best knowledge, this is the first report of a case of stress cardiomyopathy presenting with an inverted pattern following a refeeding syndrome.

Regarding to malnourished patients, there are reported cases of classical Takotsubo associated with starvation states of different etiologies, but usually with refractory hypoglycemia as characteristic feature, and none of them presenting with the inverted variant[23]. A particular group would be patients with anorexia nervosa, with some reported cases of development of stress cardiomyopathy maintaining euglycemia; in these cases the syndrome might be triggered by emotional stress or electrolyte disturbances, and neither any of them presenting with the inverted Takotsubo type in the published cases.

In our case, we hypothesize that this particularly unique cardiac manifestations of refeeding syndrome (atrial tachycardia and inverted Takotsubo) might be influenced by hypoglycemia, electrolyte abnormalities, metabolic disturbances, emotional stress....as isolated factors or by a contribution of all of them[24].

COMMENTS

Case characteristics

A 54-year-old woman with chronic malnutrition who experienced a rapid oral nutrition repletion.

Clinical diagnosis

Impaired consciousness, emaciation with a body mass index of 10.66 kg/m^2, bradycardia and edema in her lower limbs.

Differential diagnosis

Hypoglycemia, electrolyte abnormalities, heart failure, renal failure.

Laboratory diagnosis

Severe hypoglycemia (19 mg/dL), low levels of albumin (1.5 g/dL), phosphate (2.2 mg/dL), magnesium (1.6 mg/dL) and potassium (2.7 mmol/L), liver dysfunctions (AST: 122 IU/L, ALT: 72 IU/L) and coagulation disorders (PT: 55.9%; APTT: 43 s).

Imaging diagnosis

Chest X-ray demonstrated marked heart failure signs and echocardiography showed dyskinesia of basal and mid-ventricular segments with hyperkinesia of left ventricular apex, consistent with inverted Takotsubo, with decreased ejection fraction (estimated at 25%).

Pathological diagnosis

Necropsy revealed coronary arteries with non obstructive lesions.

Treatment

The patient was treated with noninvasive positive pressure ventilation and inotropic drugs, but she finally died due to a Serratia marcescens-induced acute pneumonia.

Related reports

Takotsubo cardiomyopathy is a syndrome frequently triggered by intense emotional or physical stress, and although it is thought to be catecholamine mediated, the pathophysiology remains unknown.

Term explanation

Refeeding syndrome describes a series of metabolic and biochemical changes that occur as consequence of reintroduction of feeding after a period of starvation or fasting.

Experiences and lessons

In this report the authors describe the first case of inverted Takotsubo following a refeeding syndrome.

Peer-review

There are reported cases of classical apical Takotsubo associated with nutrition disorders, but none of them presenting with the inverted variant.

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CASE REPORT

Case of angina pectoris at rest and during effort due to coronary spasm and myocardial bridging

Hiroki Teragawa, Yuichi Fujii, Tomohiro Ueda, Daiki Murata, Shuichi Nomura

Hiroki Teragawa, Yuichi Fujii, Tomohiro Ueda, Daiki Murata, Shuichi Nomura, Department of Cardiovascular Medicine, Hiroshima General Hospital of West Japan Railway Company, Higashi-ku, Hiroshima 732-0057, Japan

Author contributions: Teragawa H wrote the manuscript; Fujii Y, Ueda T, Murata D and Nomura S collected data and evaluated the study.

Ethics approval: The study was reviewed and approved by the Hiroshima General Hospital of West Japan Railway Company Institutional Review Board.
Informed consent: Informed consent was obtained from the present patient.
Conflict-of-interest: All the authors have no conflict-of interest.

Correspondence to: Hiroki Teragawa, MD, PhD, Department of Cardiovascular Medicine, Hiroshima General Hospital of West Japan Railway Company, 3-1-36 Futabanosato, Higashi-ku, Hiroshima 732-0057, Japan. hiroteraga71@gmail.com
Telephone: +81-82-2621171
Fax: +81-82-2621499

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Abstract

We present a case of a 71-year-old male who had chest symptoms at rest and during effort. He had felt chest oppression during effort for 1 year, and his chest symptoms had recently worsened. One month before admission he felt chest squeezing at rest in the early morning. He presented at our institution to evaluate his chest symptoms. Electrocardiography and echocardiography failed to show any specific changes. Because of the possibility that his chest symptoms were due to myocardial ischemia, he was admitted to our institution for coronary angiography (CAG). An initial CAG showed mild atherosclerotic changes in the proximal segment of the left anterior descending coronary artery (LAD) and mid-segment of the left circumflex coronary artery. Subsequent spasm provocation testing using acetylcholine revealed a bilateral coronary vasospasm, which was relieved after the intracoronary infusion of nitroglycerin. Finally, a CAG showed myocardial bridging (MB) of the mid-distal segments of the LAD. Fractional flow reserve using the intravenous administration of adenosine triphosphate was positive at 0.77, which jumped up to 0.90 through the myocardial bridging segments when the pressure wire was pulled back. Thus, coronary vasospasm and MB might have contributed to his chest symptoms at rest and during effort. Interventional cardiologists should consider the presence of MB as a potential cause of myocardial ischemia.

Key words: Coronary spasm; Myocardial bridging; Myocardial squeezing; Fractional flow reserve

Core tip: Myocardial bridging (MB), an anomaly in which the myocardium overlies the intramural course of segments of the epicardial coronary arteries, is associated with cardiac events. This may be explained by myocardial ischemia, coronary spasms, and/or mechanical compression of the coronary artery by the MB itself. We encountered a patient with angina pectoris both at rest and during exercise, which was

caused by both coronary spasm and MB-induced direct myocardial ischemia. The latter finding was revealed using a pressure wire. MB sometimes causes two vascular characteristics, coronary spasms and direct myocardial ischemia, whose management is quite different.

Teragawa H, Fujii Y, Ueda T, Murata D, Nomura S. Case of angina pectoris at rest and during effort due to coronary spasm and myocardial bridging. *World J Cardiol* 2015; 7(6): 367-372 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i6/367.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i6.367

INTRODUCTION

Myocardial bridging (MB) is an anomaly in which the myocardium overlies the intramural course of segments of the epicardial coronary arteries[1]. The frequency of MB ranges from 5.4% to 85% in autopsy series[2-4] and from 0.5% to 29.4% on coronary angiography[4-10]. It has been accepted that MB might affect the cardiovascular system[1,8,11-13]. In addition, the presence of MB is associated with myocardial infarction[14-17] and sudden cardiac death[18-20]. Myocardial ischemia due to compression of the coronary artery by MB[21,22] and/or coronary spasm at the MB segments[12,23-25] has been considered a major factor responsible for MB-related cardiac events. In this study, we report a case of angina pectoris at both rest and during exercise due to both coronary spasm and MB-related myocardial ischemia, which was documented using a pressure wire.

CASE REPORT

A 71-year-old male had felt chest oppression on effort, such as when carrying heavy baggage, for 1 year. Recently, his chest symptoms had occurred more frequently. One month before admission he felt chest squeezing at rest in the early morning. He presented at our institution for an evaluation of his chest symptoms in May 2014. Coronary risk factors such as smoking, hypertension, and diabetes mellitus were all absent, although he had a low level of high-density lipoprotein (HDL) cholesterol. His mother had angina pectoris. He had undergone operations for appendicitis and prostate cancer at the ages of 25 and 69 years, respectively. On medical examination, his height was 1.63 m, his weight was 73 kg, and his body mass index was 27.5. His vital signs were stable with a blood pressure of 110/80 mmHg and a pulse of 59 beats/min. No cardiac murmur or abnormal respiratory sounds in the lungs were detected. Blood examinations revealed elevated levels of creatinine (1.06 mg/dL), uric acid (9.4 mg/dL), and triglycerides (227 mg/dL), and a low level of HDL cholesterol (35 mg/dL). Neither a chest X-P, electrocardiogram, nor echocardiography showed any specific changes. He was admitted to our institution for coronary angiography (CAG) because of the possibility that his chest symptoms were due to myocardial ischemia.

An initial CAG showed mild atherosclerotic changes at the proximal segments of the right coronary artery (RCA), the left anterior descending coronary artery (LAD) and the mid-segment of the left circumflex coronary artery (Figure 1). To clarify the cause of his chest symptoms, we performed spasm provocation testing using acetylcholine (ACh). During the spasm provocation test, a pressure wire (PrimeWire Prestige PLUS, Volcano Therapeutics Inc., Rancho Cordova, CA, USA) was inserted into the distal portion of the RCA and distal portion of the LAD. The ratio of the distal pressure, derived from the pressure wire, to the proximal one, derived from the tip of catheter (Pd/Pa), was continuously monitored.

Intracoronary infusion of 50 μg ACh caused a diffuse coronary spasm at the mid-distal portion of the RCA (Figure 2A), which was accompanied by the usual chest symptoms and a reduction in the Pd/Pa from 1.0 to 0.69 at baseline. Because of the prolonged coronary spasm, 600 μg nitroglycerin (NTG) was intracoronarily administered, which relieved the coronary spasm in the RCA (Figure 3A). The subsequent intracoronary infusion of 100 μg ACh in the LCA resulted in no chest symptoms but a diffuse spasm in the mid-distal portion of the LAD (Figure 2B), which was accompanied by a reduction in the Pd/Pa from 0.94 to 0.60 at baseline. The intracoronary infusion of 300 μg NTG relieved the coronary spasm and returned the Pd/Pa to the baseline value of 0.94. A final CAG revealed an MB of the mid-distal segments of the LAD (Figures 3B and C). The length and percent systolic narrowing of the MB segment was 38 mm and 78%, respectively. The fractional flow reserve (FFR) of the LAD, which was assessed using a pressure wire and the intravenous administration of 160 μg/min per kilogram adenosine triphosphate (ATP), was positive at 0.77 from 0.94 at baseline (Figure 4A). It then jumped to 0.90 through the MB segments when the pressure wire was pulled back (Figure 4B). Therefore, multi-vessel coronary spasms and a myocardial bridge may contribute to his chest symptoms at rest and during effort. The following day he was discharged and prescribed diltiazem (300 mg/d). Since then, he has been taking 300 mg/d diltiazem and 15 mg/d nicorandil and his symptoms have been controlled in the outpatient clinic.

DISCUSSION

In this study, we present a case of angina pectoris both during exercise and at rest. These symptoms were due to bilateral coronary spasms and MB-related myocardial ischemia, which was identified using a pressure wire.

Several reports have described MB-related myo-

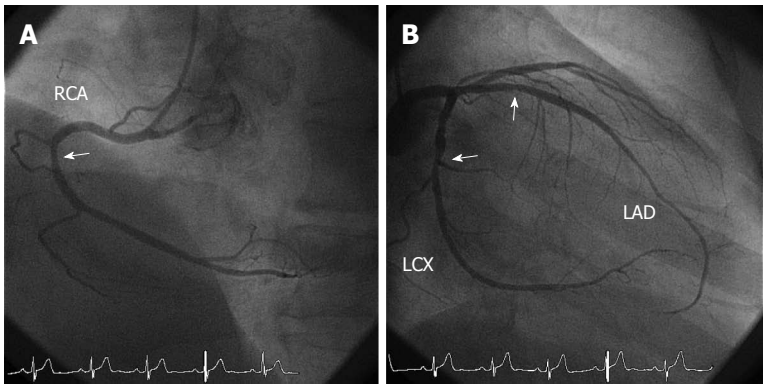

Figure 1 Coronary angiography before spasm provocation tests. A: There were mild atherosclerotic changes at the proximal segment of the right coronary artery (RCA); B: There were mild atherosclerotic changes at the proximal segment of the left anterior descending coronary artery (LAD) and at the mid-segment of the left circumflex coronary artery (LCX). The mild atherosclerotic changes are indicated using arrows.

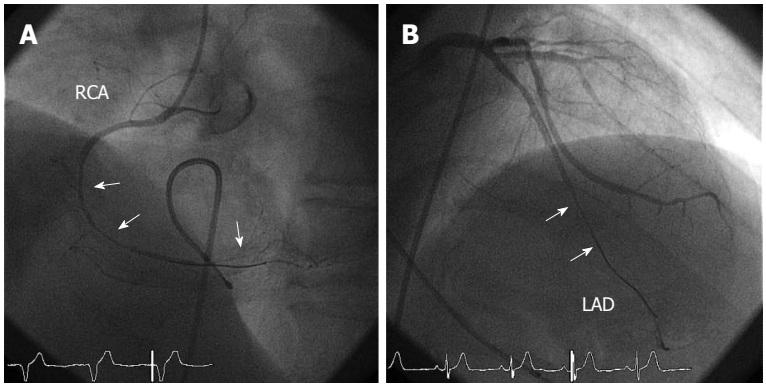

Figure 2 Coronary angiography during the spasm provocation tests. A: In the right coronary artery (RCA), a diffuse coronary spasm occurred at the mid-distal segments after the intracoronary infusion of 50 μg acetylcholine (ACh); B: In the left coronary artery, a diffuse coronary spasm occurred at the mid-distal segments of the left anterior descending coronary artery (LAD) after the intracoronary infusion of 100 μg ACh. The coronary spasm segments are indicated using arrows.

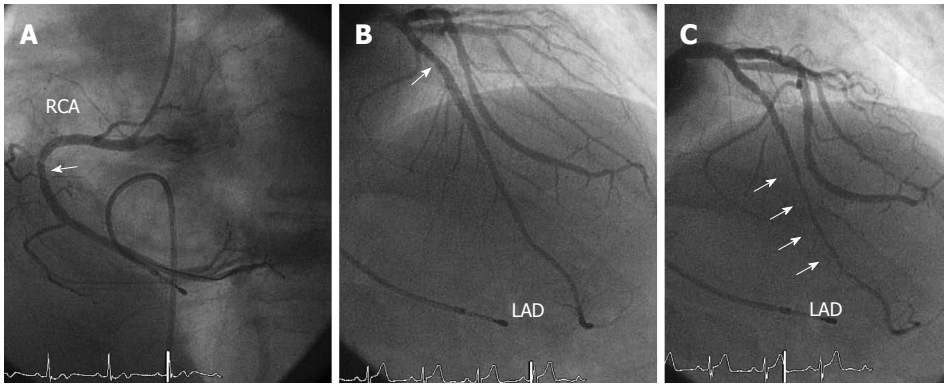

Figure 3 Coronary angiography after the intracoronary infusion of nitroglycerin. A: There was a mild atherosclerotic change at the proximal segment of the right coronary artery (RCA); B: There was a mild atherosclerotic change (indicated with arrows) in the proximal segment of the left anterior descending coronary artery (LAD) at the end-diastolic phase; C: There was myocardial bridging (indicated with arrows) at the mid-distal segments of the LAD at the end-systolic phase.

cardial infarction[14-17] and sudden cardiac death[18-20]. Myocardial ischemia has been suggested to be the main cause of MB-related cardiac events due to mechanical compression of the coronary artery by the MB[21,22] and/or coronary spasm at the MB segments[12,23-25]. It is possible that coronary spasms frequently occur at MB segments because of endothelial dysfunction and/or vascular dysfunction of the coronary artery at MB segments[11,12]. Although the current case had multivessel coronary spasms, the segment of the LAD that underwent coronary spasm was the same as the MB segment, which is consistent with an MB-related coronary spasm. This suggests that cardiologists should consider the possibility of coronary spasm in patients with chest pain and MB on coronary angiograms. Several methods have been used to assess MB-related myocardial ischemia due to mechanical compression of the coronary artery by MB, such as pharmacological stress echocardiography[26], stress myocardial perfusion imaging[27], intracoronary blood flow velocity measurements[21,26], and intracoronary pressure measurements[1,21,26,28]. In the current case, we assessed intracoronary pressure using a pressure wire because this technique has a reliable cutoff value[29] and can be used conveniently in the clinical setting.

Regarding the relationship between intracoronary pressure and MB, reports describing the pressure gradient within the MB segment vary. For example, it has been reported that a pressure gradient within

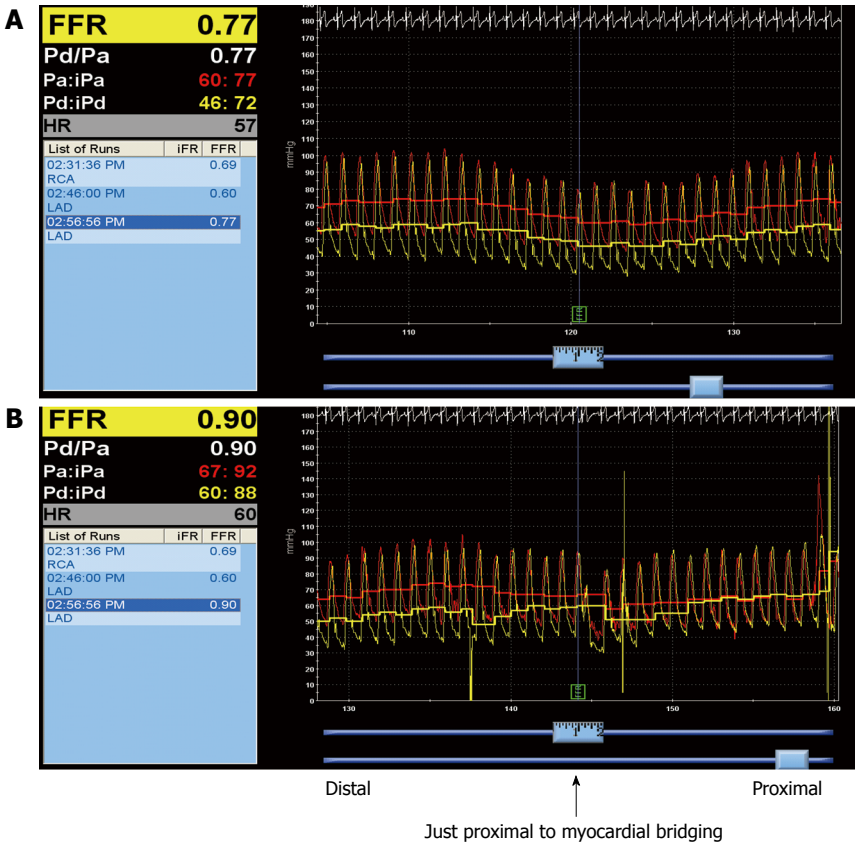

Figure 4 The fractional flow reserve using the intravenous infusion of adenosine triphosphate was 0.77 (A), which jumped up to 0.90 through the myocardial bridging during pullback (B).

the MB segment is present even at baseline[21], only during pharmacological stress[1,26,28], only within the MB segment[26], or both within and beneath MB[1,21,28]. These different results may have been due to differences in the severity and degree of the MB itself as well as differences in the methods used to measure intracoronary pressure. According to the current results, where Pd/Pa was 0.94 and FFR was 0.77 at baseline and the Pd/Pa increased to 0.90 through the MB segments, a pressure gradient was present only during pharmacological stress and within and beneath the MB.

ATP is used frequently to measure FFR in the clinical setting during the assessment of MB. However, it has been reported that dobutamine is more useful as the stress agent during the assessment of MB[1,26,28] because it causes a more severe and longer compression within the MB[28]. Assessing the FFR of the vessel containing the MB can be challenging[30] because atherosclerotic changes often occur proximal to the MB[1], which may reduce FFR. The present case had a minor atherosclerotic lesion proximal to the MB; however, the FFR increased to 0.90 just proximal to the MB when the pressure wire was pulled back. Therefore, in cases with MB and proximal atherosclerotic lesions, assessing FFR using the combination of the pullback method may be more useful.

β-blockers are the mainstay of treatment for symptomatic patients with MB[1]. However, as shown in the present case, coronary spasms sometimes occur in patients with MB, particularly in the MB segments[12]. In general, monotherapy using β-blockers is prohibited in patients with coronary spasms[31]. Furthermore, the use of NTG, which is very effective for relieving coronary spasms, may exacerbate the systolic narrowing of the MB segments[32]. Therefore, it is important to ascertain the presence of coronary spasms in patients with MB. Furthermore, in cases with both MB and coronary spasms, calcium-channel blockers (CCB) or CCB plus β-blockers may be useful. Patients with coronary spasms and MB should be monitored carefully when these drugs are administered. In the present case, CCB with diltiazem plus nicorandil was used to treat the coronary spasm, which was the main pathology in the present case. When chest symptoms are present during exercise the use of β-blockers should be considered. It was reported that percutaneous coronary intervention is useful to relieve chest symptoms in patients with MB[1,22,30,33]; however, it was also reported that the incidence restenosis is relatively high[1,30]. Therefore, pharmacological treatment should be used even in patients with MB and a significantly reduced FFR.

In conclusion, coronary spasms sometimes consolidate in patients with MB, and the presence of coronary spasms should be assessed in such patients.

In addition, intracoronary pressure measurements using a pressure wire may be useful to assess the severity of MB. Interventional cardiologists should keep these concepts in mind.

COMMENTS

Case characteristics

A 71-year-old male presented chest oppression during effort and chest squeezing at rest.

Clinical diagnosis

Angina pectoris due to coronary spasm and myocardial bridging.

Differential diagnosis

Angina pectoris due to significant coronary stenosis, pulmonary thromboembolism.

Laboratory diagnosis

Elevated levels of creatinine (1.06 mg/dL), uric acid (9.4 mg/dL), and triglycerides (227 mg/dL), and a low level of HDL cholesterol (35 mg/dL).

Imaging diagnosis

Coronary angiography showed mild atherosclerotic changes. Spasm provocation testing using acetylcholine showed multi-vessel coronary spasms. Coronary angiography after an intracoronary infusion of nitroglycerin showed myocardial bridging of the left anterior descending coronary artery. The fractional flow reserve using adenosine triphosphate was positive at 0.77.

Treatment

The patient was treated with 300 mg/d diltiazem and 15 mg/d nicorandil.

Related reports

Angina pectoris due to coronary spasms or myocardial bridging is well-known, however, little has been reported regarding angina pectoris at rest and during effort due to both coronary spasms and myocardial bridging.

Term explanation

Myocardial bridging is an anomaly in which the myocardium overlies the intramural course of segments of the epicardial coronary arteries and is associated with cardiac events.

Experiences and lessons

This report presents a case of angina pectoris due to coronary spasm and myocardial bridging. Coronary spasms sometimes consolidate in patients with myocardial bridging, and the presence of coronary spasms should be assessed in such patients. In addition, intracoronary pressure measurements using a pressure wire may be useful to assess the severity of myocardial bridging.

Peer-review

This is an interesting case. The case is well presented and the text well written.

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EDITORIAL

Night time blood pressure dip

Dennis Bloomfield, Alex Park

Dennis Bloomfield, Alex Park, Department of Medicine, Richmond University Medical Center, Staten Island, NY 10310, United States

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Correspondence to: Dennis Bloomfield, MD, Director of Clinical Research, Department of Medicine, Richmond University Medical Center, 366 Bard Avenue, Staten Island, NY 10310, United States. dbloomfieldmd@aol.com
Telephone: +1-718-8182707
Fax: +1-718-8161780

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Abstract

The advent of ambulatory blood pressure monitoring permitted examination of blood pressures during sleep and recognition of the associated circadian fall in pressure during this period. The fall in pressure, called the "dip", is defined as the difference between daytime mean systolic pressure and nighttime mean systolic pressure expressed as a percentage of the day value. Ten percent to 20% is considered normal. Dips less than 10%, referred to as blunted or absent, have been considered as predicting an adverse cardiovascular event. This view and the broader concept that white coat hypertension itself is a forerunner of essential hypertension is disputable. This editorial questions whether mean arterial pressures over many hours accurately represent the systolic load, whether nighttime dipping varies from measure to measure or is a fixed phenomenon, whether the abrupt morning pressure rise is a risk factor or whether none of these issues are as important as the actual night time systolic blood pressure itself. The paper discusses the difference between medicated and nonmedicated white coat hypertensives in regard to the cardiovascular risk and suggests that further work is necessary to consider whether the quality and duration of sleep are important factors.

Key words: Nighttime dip; Ambulatory blood pressure monitor; Blunting; Cardiovascular risk

Core tip: While the blunted or absent nighttime pressure dip in nonmedicated white coat hypertensives is generally believed to be a predictor of adverse cardiovascular events, it does not appear to present the same risk in medicated white coat patients. Of the many measurable pressure issues, including pulse pressure and morning surge, during sleep and with awakening, only the mean systolic pressure appears to be the predictor of risk.

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NIGHT TIME BLOOD PRESSURE DIP

The circadian fall in blood pressure during sleep[1] has been fully examined only since the development of

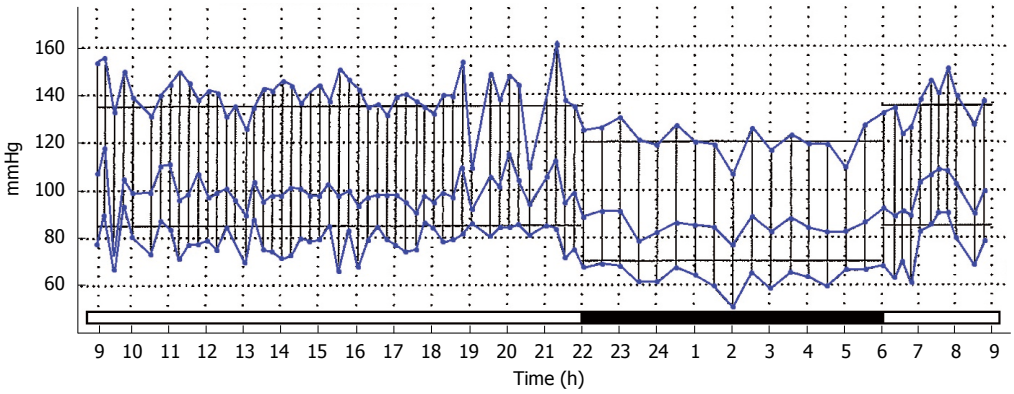

Figure 1 Ambulatory blood pressure recording showing a normal night time dip. In all the ambulatory blood pressure monitoring tracings, the systolic, diastolic, and mean pressures are shown in blue. The pressure scale in mmHg is on the vertical Y-axis on the left and the time scale in hours is on the horizontal X-axis. The duration of sleep corresponding to the night time period is indicated by a heavy black bar.

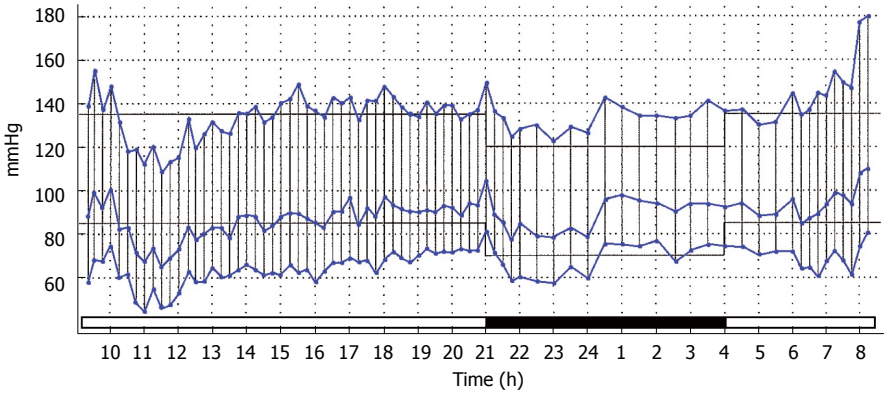

Figure 2 Ambulatory blood pressure recording showing a blunted night time dip.

ambulatory blood pressure monitoring (ABPM) and its occurrence in white coat hypertension (WCH) has not been generally elucidated. First described in 1988, the night time dip has become an accepted measure of cardiovascular risk[1-4]. The dip is defined as the difference between the mean systolic pressure in the day and mean systolic pressure during the night, expressed as a percentage of the day time mean, with the accepted normal between 10% and 20%[5]. A representative ABPM tracing with a normal night time dip is shown in Figure 1.

Dips less than 10% are described as absent or blunted and those in excess of 20% are known as exaggerated or extreme[6]. An example of a blunted dip, such as would be recognized as predicting an adverse cardiovascular event, is shown in Figure 2.

Identification of medical risk factors, particularly cardiovascular ones, carries a couple of requirements. The definition of the conditions must be accepted and unchallengeable and the observations on which this designation is based must be unassailable. These conditions are not met with the night time dip.

The utility of the definition of night time dip is far from practical. It is assumed that the mean systolic pressures are utilized but there is such variation in the actual systolic values during the 24 h that the mean hides much information and bears little relationship to actual daily events. The utilization of average or maximum systolic pressures would be equally inaccurate. In white coat hypertension, mean and average systolic pressures are artificially elevated by the white coat episode.

The ambulatory monitoring data is also soft. It is known that repeated studies do not necessarily provide the same result. Dippers may become non dippers on subsequent testing[7].

Furthermore, the patient-designated "time of sleep" is actually the time of going to bed. The true time of falling asleep clearly cannot be indicated with this methodology. Consequently, the pressures used in the sleep *vs* awake calculations are slightly but inherently inaccurate.

Reported studies in night time dip have almost always been performed in untreated hypertensive patients[8]. In the real world, ABPM is rarely performed in such patients. In our experience, patients are referred for this study to ascertain the effectiveness of the treatment or when progressive medication has failed to control the hypertension. It would be expected that cardiovascular risks would be more evident in the uncontrolled hypertensive patients, however, our studies with medicated patients[8] have

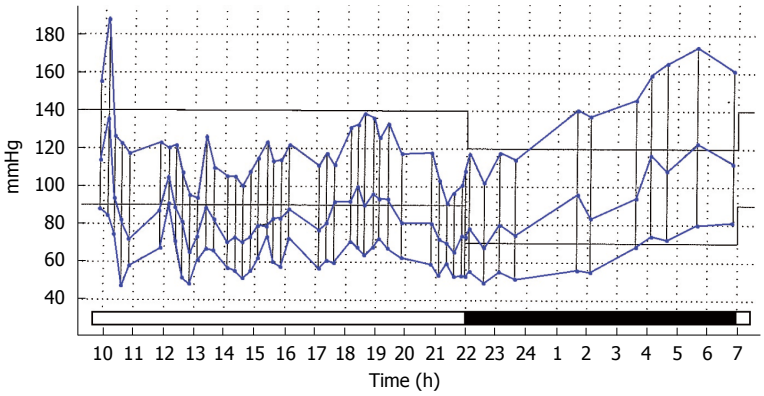

Figure 3 Ambulatory blood pressure recording showing white coat hypertension with a steadily rising pressure of superimposed essential hypertension. The pressure only reaches hypertension levels during sleep when it is likely to be clinically unnoticed.

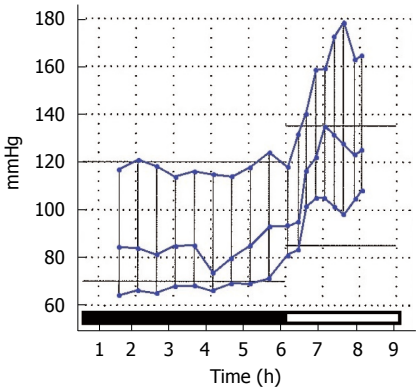

Figure 4 Ambulatory blood pressure recording showing a significantly rapid rise in mean blood pressure upon awakening.

shown no greater incidence of blunted night time dip in uncontrolled hypertensives, controlled hypertensives or white coat hypertensives. Sufficient reduction in the night time pressure may in fact be blunted or negated by protective reflexes if these pressures are already reduced by medication.

Over bridging these considerations is the understanding that the cardiovascular risk associated with the rupture of atheromatous plaques in the coronary and cerebral arteries are essentially systolic issues. They may be related to actual systolic levels or pulse pressure rather than mean pressures or degree of nocturnal dipping.

Masked hypertension, the condition in which the home or the ABPM pressures are significantly higher than the office values must be given considerations here. It is seldom diagnosed in clinical practice as there is little incentive to prescribe ABPM or home pressure devices if the pressure is normal, but occasionally, accidental or incidental incidences of blood pressure measurement may reveal this condition and some explanation is required when masked hypertension is revealed during sleep. This may eliminate the night time dip. Night time narrow peaks of systolic hypertension can occur with dreaming, wider systolic elevations with obstructive sleep apnea and steadily rising pressures are seen with essential hypertension (Figure 3).

A widened pulse pressure has also been recognized as an indicator of cardiovascular risk. White coat hypertensives have a widened pulse pressure during the white-coat episodes but not at night. Analysis of our studies in dippers and non-dippers, hypertensives, white coaters and normal subjects, has found that there is no statistical difference in pulse pressure values between day and night. With the exception of isolated systolic hypertension, a widened pulse pressure occurs in patients who do not have elevated blood pressure as their principle diagnosis and includes those with aortic regurgitation, arteriovenous shunts, thyrotoxicosis and other cardiovascular disorders that, in themselves, increase the risk of death.

Associated with the night time dip, the morning blood pressure surge has been blamed for the increase in cardiovascular events in the morning hours. It is measured as mmHg increase per hour in the mean pressure and it is generally accepted that a rise greater that 10 is significant as a cardiovascular risk factor. Figure 4 shows a typical exaggerated increase in the pressure during the process of awakening.

However, when the rate of rise is continuously calculated during the 24 h ABPM recording, many instances of "significance" are seen to occur at times other than in the morning. Figure 5 adequately depicts this point.

A large study has found that the night time systolic pressure itself, rather than the surge, the dip or the pulse pressure has been shown to correlate more closely with the clinical events[9].

It is clear that the blood pressure at night, in some negative way, impacts the cardiovascular system. What particular element of the pressure, whether it is its depth or its systolic/diastolic width or whether the heart rate or length or quality of sleep is the causative factor remains to be determined.

Unfortunately, the tool to answer these questions, the ambulatory blood pressure monitor, is underutilized in the United States, largely because the study

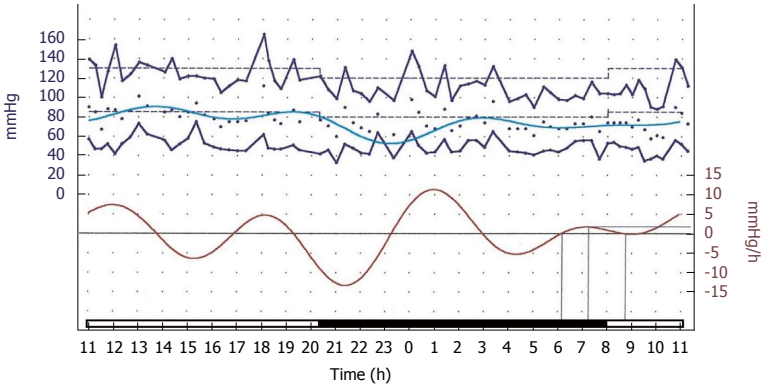

Figure 5 Ambulatory blood pressure recording showing the rate of pressure rise upon awakening at 7 am. At 1 am, the rate of rise reaches cardiac risk significance. The scale in mmHg/h is shown in red on the right.

remains non-reimbursable. As it may hold some basic but unknown secrets of cardiovascular health and disease, the "night time dip" warrants a much more extensive investigation.

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EDITORIAL

Hemoglobin optimization and transfusion strategies in patients undergoing cardiac surgery

Mahdi Najafi, David Faraoni

Mahdi Najafi, Department of Anesthesiology, Tehran Heart Center, Tehran University of Medical Sciences, Tehran 1411713138, Iran

David Faraoni, Department of Anesthesiology, Peri-operative and Pain Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, United States

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Correspondence to: Dr. Mahdi Najafi, Department of Anesthesiology, Tehran Heart Center, Tehran University of Medical Sciences, North Karegar Street, Tehran 1411713138, Iran. najafik@sina.tums.ac.ir
Telephone: +98-21-88029674
Fax: +98-21-88029724

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Abstract

Although red blood cells (RBCs) transfusion is sometimes associated with adverse reactions, anemia could also lead to increased morbidity and mortality in high-risk patients. For these reasons, the definition of perioperative strategies that aims to detect and treat preoperative anemia, prevent excessive blood loss, and define "optimal" transfusion algorithms is crucial. Although the treatment with preoperative iron and erythropoietin has been recommended in some specific conditions, several controversies exist regarding the benefit-to-risk balance associated with these treatments. Further studies are needed to better define the indications, dosage, and route of administration for preoperative iron with or without erythropoietin supplementation. Although restrictive transfusion strategies in patients undergoing cardiac surgery have been shown to effectively reduce the incidence and the amount of RBCs transfusion without increase in side effects, some high-risk patients (*e.g.*, symptomatic acute coronary syndrome) could benefit from higher hemoglobin concentrations. Despite all efforts made last decade, a significant amount of work remains to be done to improve hemoglobin optimization and transfusion strategies in patients undergoing cardiac surgery.

Key words: Cardiac surgery; Blood transfusion; Anemia; Transfusion threshold; Risk factor

Core tip: Anemia and red blood cells transfusion are common during cardiac surgery, and could be associated with adverse reactions. Preoperative hemoglobin optimization through the identification and treatment of anemia and the definition of standardized transfusion algorithm using restrictive transfusion triggers play a central role in the development of Patient Blood Management programs. However, further researches are needed to better define transfusion triggers, based on pathophysiological indices, rather than single hemoglobin thresholds.

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INTRODUCTION

Patients undergoing cardiac surgery are at increased risk of excessive perioperative bleeding, and increased blood product transfusions[1]. Although blood products are safer than ever, transfusion of allogeneic blood products remains associated with a significant incidence of adverse reactions[2]. Last decades the development of Patient Blood Management (PBM) programs improved perioperative management, and decreased the use to blood products through a better identification of both patient-related and procedure-related risk factors[3].

On the one hand, cardiac surgery is among major procedures that significantly influence the distribution of body fluid through the large volumes of fluid administered during cardiopulmonary bypass (CPB), the volume of cardioplegia, and the amount of fluid administered to optimize cardiac output. In addition, the contact between blood and non-endothelial surfaces will lead to the "activation coagulopathy"[4], and all these mechanisms are part of the CPB-induced coagulopathy that significantly influences the requirement for blood products transfusion[5].

On the other hand, with the progress made in medical therapies and interventional cardiology, patients requiring cardiac surgery become older, and arrive to surgery with a huge number of comorbidities, and medications[6]. Patients are usually treated with antiplatelet agents and/or anticoagulants, which will increase the bleeding risk[7]. Although RBCs transfusion could be associated with adverse events; anemia in high-risk patients could also be associated with increased morbidity and mortality[8]. For these reasons, the definition of perioperative strategies that aims to detect and treat preoperative anemia, prevent excessive blood loss, and define "optimal" transfusion algorithms are crucial.

PHYSIOLOGY

Oxygen is major component of cellular homeostasis; the maintenance of an aerobic metabolism through adequate oxygen supply to cells is crucial. Cardiac output and the arterial oxygen concentration (CaO_2) will allow the maintenance of an adequate oxygen delivery (Do_2).

$Do_2 = CO \times CaO_2$, where $CaO_2 = (Hb \times SaO_2 \times 1.39) + (PaO_2 \times 0.0031)$

With SaO_2 corresponding to the arterial oxygen saturation, PaO_2 is the partial arterial pressure in oxygen, 0.0031 is the amount oxygen diluted in plasma, and 1.39 the amount of oxygen linked to 1 g of hemoglobin. Based on this formula, it appears evident that the hemoglobin concentration will play a central role in oxygen transportation throughout the body, and organs.

The adequacy of tissue oxygenation depends on tissue/organ metabolic needs. Tissue oxygenation is adequate when oxygen delivery (Do_2) is at least equal to the rate of oxygen consumed by the tissues (Vo_2: oxygen consumption). The ratio between Do_2 and Vo_2 allow for the determination of the oxygen extraction (O_2 ER), which in physiologic condition correspond to 25%-30% of the oxygen delivery. Interestingly, adaptive mechanisms allow the maintenance of adequate oxygen consumption in the presence of decreased oxygen delivery through an increase in the amount of oxygen extracted by the tissues[9]. Below a certain Do_2 level, the extraction could not be increased and metabolism will be shift to anaerobic metabolism. This change in metabolic characteristics will be associated with increased lactate plasmatic concentration, if prolonged may cause tissue damage[10].

Although a certain degree of anemia could be tolerated, the "critical" hemoglobin level that will not allow the maintenance of an adequate oxygen delivery will depend on different factors that include physiologic status (*e.g.,* sepsis increases oxygen demand) or condition (*e.g.,* anesthesia decreases oxygen demand)[11]. In case of acute and severe anemia, adaptive mechanisms to optimize the balance between oxygen supply and demand are regulated by hypoxia-inducible factors, and included neuronal nitric oxide synthase, erythropoietin, and hypoxia-inducible factors[12]. Although all these mechanisms improve oxygen delivery, organs with higher baseline oxygen extractions, such as the heart, are usually flow-dependent, which is obtained by vasodilatation of the coronary arteries[13]. This will have an important impact in patients suffering from coronary artery disease, and perioperative management of anemia in patients undergoing coronary artery bypass graft (CABG) surgery is crucial[14].

PREOPERATIVE OPTIMIZATION OF HEMOGLOBIN CONCENTRATION

Based on the World Health Organization (WHO), anemia is defined by hemoglobin levels < 12 g/dL in women, and < 13 g/dL in men[3]. If 25% of the general population is anemic, preoperative anemia is reported in 22% to 30% of patients undergoing cardiac surgery[15] Although preoperative anemia was associated with an increased incidence of acute kidney injury and strokes, both short and long-term mortality increased in anemic patients undergoing CABG surgery[16]. In addition, adverse events associated with

hemorrhage, and blood product transfusion was higher in patients with preoperative anemia compared to non-anemic patients[17]. As a consequence, preoperative detection of anemia, and optimization of hemoglobin level is crucial.

Iron deficiency has been shown to be responsible of 29% of the preoperative anemia, followed by the presence of chronic kidney disease in 10.7%[18]. In case of iron deficiency, preoperative iron supplementation should be recommended. In patients undergoing cardiac surgery, Piednoir *et al*[19] reported that on 100 patients undergoing cardiac surgery, 37% had preoperative iron deficiency, from those one third were anemic. The authors also reported that 62% of patients with iron deficiency received RBC transfusion compared to 35% in controls[19]. No study assessed the efficacy of preoperative iron supplementation in patients undergoing cardiac surgery. So far, our experience is based on studies performed in colorectal[20] or orthopedic surgeries[21] that reported a significant reduction in RBCs transfusion in patients that received preoperative oral iron administration. Other authors reported that iv iron administrated within 3-5 wk before orthopedic surgery could significantly increase hemoglobin level[22]. Although a recent meta-analysis reported a significant increase in hemoglobin level, and reduction of RBCs transfusion following the iv administration of iron, this benefit was balanced by an increased incidence of infection. Based on current evidence, oral iron administration (200-300 mg/d) should be recommended in patients with preoperative iron deficiency, while iv supplementation (1000 mg weekly during 3-5 wk) might only be considered in case of contraindication to oral administration or short delay before a surgery that could not be postponed[23].

If the preoperative administration of erythropoietin stimulating agent could be attractive, the benefit-to-risk balance associated with preoperative administration of erythropoietin (EPO) remains weakly studied. Currently, EPO is approved in anemic patients without nutritional deficiency undergoing orthopedic surgery. However, its administration in patients undergoing cardiac surgery is currently prohibited due to an increased incidence of thromboembolic complication reported in pilot studies[24].

RESTRICTIVE *VS* LIBERAL TRANSFUSION TRIGGERS?

Transfusion has been used for years to increase hemoglobin concentration, and oxygen delivery. However, RBCs transfusion is associated with several side effects, and sometimes, increased mortality[25]. On the other hand, anemia has been associated with adverse outcomes ranging from cardiovascular events, heart failure, renal failure, prolonged recovery, and late mortality. Although the safety of blood products transfusion has been extensively enhanced last decades, there are still concerns over the hazards of transfusion, particularly with respect to the high rate of annual consumption of blood products worldwide (around 85 million units)[26]. With regard to complications associated with blood product transfusion, non-infectious risks such as human errors, acute hemolytic and non-hemolytic reactions have overpassed the risk of infection. Based on large studies performed in cardiac patients, blood product transfusion is associated with negative outcomes such as cardiac, pulmonary, renal, and neurologic complications, as well as increased length of hospital stay and death[27]. In addition, these side effects will have a significant impact on health care resource utilization.

As far as the 1990s, Bracey *et al*[28] reported that restrictive transfusion strategies (Hb threshold = 8 g/dL) led to a 20% reduction of RBCs transfusion without increase in the incidence of side effects[28]. However, it took about 10 years before the publication of a second prospective randomized study that compared a restrictive (hematocrit > 24%) *vs* liberal (hematocrit > 30%) transfusion strategy in patients undergoing cardiac surgery[29]. In this study, Hajjar *et al*[29] reported that although the restrictive transfusion strategy was associated with lower intra-operative hemoglobin levels, no difference was observed in term of morbidity and mortality. A Cochrane systematic review of 17 cardiac and non-cardiac trials published in 2010 concluded that restricted transfusion strategy decreases transfusion without increasing adverse outcomes such as cardiac events, thromboembolic complications, and death[30]. However, this review denotes that patients with "serious heart disease" should not be treated in this way. In a recent systematic review with meta-analysis, Curley *et al*[31] reported that only a few studies (n = 7) adequately compared the efficacy and safety of a restrictive transfusion protocol to a liberal approach in patients undergoing cardiac surgery. Although restrictive transfusion strategies significantly reduced the incidence of RBCs transfusion without side effect, the inter-studies variability was important, and further adequately powered studies are needed to assess the appropriate transfusion threshold in the cardiac population.

Based on a pathophysiologic decrease in anemia tolerance in patients with coronary artery disease, perioperative anemia could be associated with poor outcomes, and a specific transfusion strategy could probably be adopted in this high-risk population[14]. In 2013, Carson *et al*[32] published the preliminary results of a prospective randomized multi-center study that aimed to compare a restrictive (Hb > 8 g/dL) *vs* a liberal (Hb > 10 g/dL) transfusion strategy in patients with symptomatic coronary artery disease. The preliminary analysis reported that higher transfusion thresholds were associated with better outcome in this particular population. Recently, Murphy *et al*[33] published the results of a prospective

Table 1 Practice guidelines for red blood cell transfusion

Guidelines	Release date	Hemoglobin threshold definition	Level of evidence
Society of Thoracic Surgeons/Society of Cardiovascular Anesthesiologists[33]	2011	6 g/dL preoperative and on CPB	2C
		7 g/dL postoperative and at risk of ischemia on CPB	2C
British Committee for Standards in Haematology[34]	2012	7 g/dL stable, non-bleeding CAD	C
		8-9 g/dL ACS	
The American Association of Blood Banks[35]	2012	7-8 g/dL in stable patients	1A
		8 g/dL in patients with CVD	2B
		No number for ACS	Uncertain recommendation; very low-quality evidence
European Society of Anesthesiology[36]	2013	7-9 g/dL in bleeding patients	1C
American Society of Anesthesiologists[37]	2015	No number	-

1A: Strong recommendation, high quality evidence; 1B: Strong recommendation, moderate quality evidence; 1C: Strong recommendation, low quality evidence; 2A: Weak recommendation, high quality evidence; 2B: Weak recommendation, moderate quality evidence; 2C: Weak recommendation, low quality evidence; CPB: Cardiopulmonary bypass; CAD: Coronary artery disease; CVD: Cardiovascular disease; ACS: Acute coronary syndrome.

multicenter randomized study (TITRe2 study) that randomized more than 2000 patients undergoing cardiac surgery to a restrictive (Hb threshold < 7.5 g/L) or a liberal transfusion strategy (Hb threshold < 9 d/dL). Interestingly, the authors didn't observe any differences in term of postoperative outcome, mortality, and costs between the two transfusion strategies. The authors concluded that restricted strategy was not superior to a liberal transfusion strategy with respect to morbidity and health care costs. These results confirmed that indication for RBCs transfusion in patients undergoing cardiac surgery might not be guided by a single transfusion threshold and that some patients may benefit from higher hemoglobin level, while other may tolerate lower hemoglobin concentrations.

Recent guidelines emphasized that recommendations on blood transfusion in patients with cardiovascular disease are not supported by strong evidence[26,34-37]. (Table 1) Despite their differences, guidelines generally agreed that RBCs transfusion should not be recommended in case of hemoglobin concentration $\geqslant$ 10 g/L and might be useful in case of hemoglobin concentration < 7 g/L[38]. However, transfusion triggers might be reconsidered in critical scenario, and especially in bleeding situations[26,34-36]. Evidence is particularly limited in clinical contexts such as acute coronary syndrome, where the recommendation for higher hemoglobin threshold is still controversial[26]. Patients with acute coronary syndrome (ACS) or patients who were at risk of end organ ischemia were included in different clinical trials over the past years, but the relationship between ACS, RBCs transfusion, and outcome remains to be determined[26,37]. Further results are waited in this context, but this supports the hypothesis that one single transfusion threshold could not fit to all patients, and that RBCs transfusion should be based on more than a single hemoglobin measurement.

CONCLUSION

Both anemia and transfusion are associated with adverse events and increased morbidity in patients undergoing cardiac surgery. Although the "optimal" RBCs transfusion strategy has not yet been defined, RBCs transfusion should be preferred when its benefits outweigh the risks. Patients with cardiac diseases are more vulnerable to anemia-related hypoxia, and recent data suggested that a restrictive transfusion strategy was not superior to a liberal transfusion, that could be associated with "better" outcome in some high-risk patients with symptomatic coronary artery disease. Each cardiac surgical department might develop standardized transfusion algorithm, based on a multidisciplinary approach. This approach should include preoperative identification of anemic patients, preoperative measures to increase hemoglobin concentration (*e.g.,* iron and/or erythropoietin), intraoperative measure to decrease blood loss, and definition of "optimal" transfusion trigger based on patient's characteristics, rather than a single hemoglobin threshold. Because on current knowledge the "ideal" transfusion thresholds to be recommended in cardiac patients is not yet known, further large prospective studies are urgently needed to determine the efficacy and safety of different transfusion strategies in this high-risk population.

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REVIEW

Impairment of aspirin antiplatelet effects by non-opioid analgesic medication

Amin Polzin, Thomas Hohlfeld, Malte Kelm, Tobias Zeus

Amin Polzin, Malte Kelm, Tobias Zeus, Division of Cardiology, Pulmonology, and Vascular Medicine, Heinrich Heine University Medical Center Dusseldorf, 40225 Dusseldorf, Germany

Thomas Hohlfeld, Institute for Pharmacology and Clinical Pharmacology, Heinrich Heine University, 40225 Dusseldorf, Germany

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Correspondence to: Dr. Med. Amin Polzin, MD, Division of Cardiology, Pulmonology, and Vascular Medicine, Heinrich Heine University Medical Center Dusseldorf, Moorenstrasse 5, 40225 Dusseldorf, Germany. amin.polzin@med.uni-duesseldorf.de
Telephone: +49-211-18800
Fax: +49-211-18812

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Abstract

Aspirin is the mainstay in prophylaxis of cardiovascular diseases. Impaired aspirin antiplatelet effects are associated with enhanced incidence of cardiovascular events. Comedication with non-opioid analgesic drugs has been described to interfere with aspirin, resulting in impaired aspirin antiplatelet effects. Additionally, non-opioid analgesic medication has been shown to enhance the risk of cardiovascular events and death. Pain is very frequent and many patients rely on analgesic drugs to control pain. Therefore effective analgesic options without increased risk of cardiovascular events are desirable. This review focuses on commonly used non-opioid analgesics, interactions with aspirin medication and impact on cardiovascular risk.

Key words: Non-steroidal anti-inflammatory drug; Drug-drug interaction; Pharmacodynamic; Dipyrone; Aspirin; Paracetamol; Metamizole

Core tip: Aspirin is the mainstay in prophylaxis of cardiovascular diseases. Impaired aspirin antiplatelet effects are associated with enhanced incidence of cardiovascular events. Comedication with non-opioid analgesic drugs has been described to interfere with aspirin, resulting in impaired aspirin antiplatelet effects. Additionally, non-opioid analgesic medication has been shown to enhance the risk of cardiovascular events and death. Pain is very frequent and many patients rely on analgesic drugs to control pain. Therefore effective analgesic options without increased risk of cardiovascular events are desirable. This review focuses on commonly used non-opioid analgesics, interactions with aspirin medication and impact on cardiovascular risk.

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INTRODUCTION

Approximately 20% of European adults suffer of acute or chronic pain and rely on analgesic drugs[1,2]. The incidence of pain and usage of non-opioid analgesics is even higher in patients with cardiovascular diseases. Forty percent of patients with coronary artery disease reported intake of non-opioid analgesic drugs[3]. This is not surprising, as the incidence of pain correlates with increasing age[4] and cardiovascular diseases are morbidities of middle to older age patients[5].

Aspirin (acetylsalicylic acid; ASA) is essential secondary prevention of cardio-and cerebrovascular events[6]. It inhibits cyclooxygenase (COX)-1 by irreversible acetylating serine 530 near the active site. This hampers conversion of arachidonic acid to thromboxane (TX) A2 for the life span of the affected platelet[7]. Aspirin has been shown to reduce the incidence of death, myocardial infarction and stroke[8-10]. However, during the last decade substantial inter-individual variation in pharmacodynamic response to aspirin has been described. This is called high on-treatment platelet reactivity (HTPR) (formerly known as "aspirin resistance"). Patients with HTPR have an increased incidence of death, myocardial infarction and stroke[11]. Many potential mechanisms including non-compliance[12,13], impaired absorption[14], genetic polymorphisms[15] increased turnover rate, enteric coating of aspirin[16,17] and COX-1 independent pathways may cause this HTPR[18]. Besides that, non-opioid analgesic medication may impair aspirin antiplatelet effects. In contrast to above mentioned internal factors, this drug-drug interaction is avoidable. Therefore special attention should be paid to this interaction leading to impaired aspirin antiplatelet effects. This review focuses on (1) mechanisms-; (2) laboratory-; and (3) clinical evidence of the aspirin drug-drug interaction with commonly used non-opioid analgesics.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used drugs in the world[19]. They are available on prescription as well as over the counter. In the United States, 70 million NSAID prescriptions- and 30 billion over the counter sales per year were registered[20]. Approximately 83% of United States - American adults use NSAID to relief pain at least once a year, 29% once a week and 15% daily[21]. In Australia, 55% of people consume NSAIDs at least once per month[22].

The term "NSAID" subsumes a variety of drugs with different chemical structures, pharmacokinetics, pharmacodynamics and mechanism of action but similar effects[23]. The main common feature is prevention of prostaglandin formation by inhibition of COX isoforms. This results in desirable anti-inflammatory and analgesic effects due to COX-2 inhibition in inflamed tissues[24]. On the other hand, inhibition of COX-1 in the gastric mucosa impairs maintenance of the mucosal barrier. This results in an increased risk of gastrointestinal events[25]. Additionally NSAIDs may worsen renal function[26] and affect platelets[27]. Based on the above mentioned differences in pharmacokinetics and pharmacodynamics between different NSAIDs, a considerable variability in analgesic as well as anti-inflammatory and antiplatelet effects is not surprising[23,27]. NSAIDs may also impair cardiovascular prognosis[28,29], possibly by inhibition of prostaglandin synthesis in the vasculature resulting in an increase in blood pressure and disturbed endothelial control of thrombogenesis. This aspect must not be confounded with the pharmacodynamic interaction of non-opioid analgesics with aspirin, which is discussed here. However, it is possible that this interaction contributes to the overall cardiovascular risk of NSAIDs. In the following, we will discuss the most commonly used NSAIDs with respect to their potential to interfere with platelet inhibition by low dose aspirin.

Ibuprofen

Ibuprofen was the first propionic acid derivative NSAID. Worldwide more than 100 million patients consumed ibuprofen and it is available in more than 100 countries[30]. Ibuprofen forms hydrogen bonds to arginine 120 and tyrosine 355 near the active site of the COX[27]. *In-vitro* analysis revealed, that ibuprofen inhibits platelet aggregation of human platelets[27]. In healthy individuals, ibuprofen intake led to inhibition of thromboxane formation[31] and platelet aggregation *ex-vivo*. As ibuprofen inhibited COX transiently, platelet function returned to normal within 4 to 6 h[32]. *In-vitro* coincubation with aspirin completely abrogated platelet inhibition and thromboxane formation by aspirin. This has been shown *ex-vivo* in healthy individuals as well with multiple studies demonstrating hampered aspirin antiplatelet effects in ibuprofen co-treated healthy subjects[33-37]. Catella-Lawson *et al*[33] reported that controlled order of intake with single dose ibuprofen (400 mg) two hours after aspirin intake preserves aspirin antiplatelet effects in healthy individuals. However ibuprofen medication three times per day inhibits aspirin antiplatelet effects independently of the above mentioned order of intake. This finding may be consistent even in lower doses of ibuprofen (150 mg)[36]. None of patients on aspirin for secondary prophylaxis of a cerebrovascular event with ibuprofen co-treatment had adequate aspirin induced inhibition of platelet aggregation. Additionally, 72% of patients experienced recurrent ischemic events. After termination of analgesic medication, aspirin antiplatelet effects restored[32]. In patients with cardiovascular diseases, different studies detected increased incidence of death and recurrent myocardial infarction in aspirin and ibuprofen comedicated patients[38-40]. This finding was confirmed in two meta-analyses, investigating the risk of death

and cardiovascular events in patients at increased risk of vascular disease on ibuprofen medication[29,41].

Naproxen

Naproxen is a propionic acid derivative NSAID like ibuprofen. However, its' pharmacokinetics are different. Plasma half-life of ibuprofen is about two hours, whereas the plasma half-life of naproxen is approximately 12 h[42]. Naproxen forms hydrogen bonds to tyrosine 385 and serine 530 in the active site of COX[27]. This leads to dose-dependent, reversible inhibition of platelet activation *in-vitro*. However, increasing concentrations of arachidonic acid can overcome this COX-inhibition. Additionally, ASA administration after pre-incubation with naproxen prevents ASA antiplatelet effects[43]. In healthy individuals, naproxen co-treatment with aspirin impairs aspirin antiplatelet effects as well[32,35,37]. This effect was consistent in over the counter doses as well as prescription doses[44]. However, data of clinical studies are contradictory. Some studies described an increased incidence of cardiovascular events in naproxen treated patients[32,45]. However others described a beneficial effect on the incidence of adverse events[39,40,46,47]. Two meta-analyses described no significant increase of vascular events and death in naproxen medicated patients[29,41]. The reasons for these inconstant results are unclear. Naproxen inhibits aspirin antiplatelet effects in-vitro similar to ibuprofen[27]. However, Capone *et al*[48] described permanent functionally relevant inhibition of *ex-vivo* platelet function in healthy individuals with 500 mg naproxen twice a day. Therefore, most probably the increased plasma half-life and therefore longer lasting reversible inhibition of platelets by naproxen may be responsible for the protective, respectively less harmful effects of naproxen in patients with cardiovascular diseases. The importance of naproxen's potential to interfere with the antiplatelet action of aspirin is presently not clear. Different authors recommended preferring the use of naproxen in patients with increased cardiovascular risk[49,50].

Diclofenac

Diclofenac is a heteroaryl acetic acid NSAID. It is commonly prescribed to alleviate acute and chronic pain[51]. Like other NSAIDs, diclofenac inhibits COX enzymes (COX-2 > COX-1). There may be additional mechanisms of action inducing its anti-inflammatory, antipyretic and analgesic effects, which are not completely understood. Besides affection of arachidonic acid uptake and release, activation of nitric oxide-cGMP antinociceptive pathway, inhibition of thromboxane prostanoid receptor and lipoxygenase enzymes, it may also inhibit peroxisome proliferator activated receptor gamma, block acid-sensing ion channels, alter interleukin-6 production and inhibit substrate P and N-methyl-D-aspartate receptor hyperalgesia[51].

In-vitro incubation of human platelets with diclofenac inhibited thromboxane formation and platelet aggregation[27]. This was reproducible *ex-vivo* after diclofenac treatment of healthy volunteers[31,52]. Additionally, platelet function was inhibited after diclofenac - treatment in patients[53,54]. However, there seems to be no interaction with aspirin treatment. ASA antiplatelet effects including inhibition of thromboxane formation were preserved *in-vitro* after pre-incubation with diclofenac[27]. Additionally, diclofenac treatment in healthy individuals on aspirin revealed sufficient pharmacodynamic response to aspirin as well[33,34]. This may be explained by molecular docking analyses. Diclofenac did not form any hydrogen bond interactions in the hydrophobic active channel of COX. Therefore it appears not to interfere with the ASA induced acetylation of serine 530, preserving aspirin antiplatelet effects despite of diclofenac co-treatment[27].

In aspirin and analgesic co-treated patients with coronary artery disease, MacDonald *et al*[38] described improved outcome of diclofenac co-treated patients in comparison to ibuprofen comedicated patients. In contrast, in a study including 83667 patients after myocardial infarction, diclofenac co-treatment was associated with the highest risk of recurrent myocardial infarction and death during 90 d[39] as well as in one- and five year follow-up[40]. These findings were confirmed in two meta-analysis investigating death and cardiovascular events in patients with analgesic medication. Both reported an increased risk in diclofenac treated patients as well[29,41].

COX-2 INHIBITORS

Anti-inflammatory and analgesic effects of COX-inhibitors are largely mediated by prevention of COX-2 induced prostaglandin formation in inflamed tissues[24]. Impairment of the gastric mucosal barrier resulting in increased incidence of gastrointestinal events, and affection of platelets are mostly caused by COX-1 inhibition[25,27]. Therefore, during the 90's NSAIDs with COX-2 selectivity were developed[55].

Conflicting data has been reported regarding impairment of aspirin antiplatelet effects by COX-2 inhibitors. Despite COX-2 selectivity, celecoxib was shown to form a hydrogen bond in the hydrophobic channel of COX-1 with tyrosine 355[27]. This goes in line with *in-vitro* experiments, demonstrating inhibition of thromboxane formation and platelet aggregation by celecoxib incubation. In ASA and celecoxib co-incubated platelets, ASA antiplatelet effects were inhibited[27]. Celecoxib administration in dogs interfered with the ability of aspirin to inhibit platelet aggregation[56]. In contrast, no impact on platelet function was observed in healthy individuals on celecoxib treatment[35]. Additionally, different groups reported that COX-2 inhibiting co-treatment in aspirin treated healthy individuals did not impair the pharmacodynamic response to aspirin[33,35,37,57]. Regardless, multiple studies reported increased risk of cardiovascular

events and death in patients receiving COX-2 inhibitors independently of concomitant aspirin intake[28,58-60].

PARACETAMOL (ACETAMINOPHEN)

Paracetamol is an aniline derivative. It is one of the most widely used antipyretic and analgesic drugs worldwide, especially as the risk of gastrointestinal bleeding events are lower in comparison to NSAIDs[61]. It is available over the counter in many countries[62]. However, in supra-therapeutic doses depletion of endogenous glutathione occurs, resulting in paracetamol metabolism shunting to toxic pathways causing severe, even fatal, hepatotoxicity[63,64]. To date, the mechanisms of analgesia by paracetamol remain unclear despite of extensive investigations[65]. A plethora of mechanisms have been postulated including activation of the endocannabinoid pathway[66,67], inhibition of the nitric oxide synthase[68,69], and indirect activation of descending serotonergic pathways[70-72]. Additionally, inhibition of cyclooxygenases in a direct-[73-75], or indirect (by converting to their oxidized, inactive form[76]) way has been described. Current opinion suggests that paracetamol performs its analgesic actions by multiple mechanisms predominantly in the central nervous system[65].

In-vitro addition of paracetamol to human platelets was reported to inhibit collagen, epinephrine and arachidonic acid induced platelet aggregation and TX formation[77]. Accordingly, in healthy individuals a reduced arachidonic acid-induced TX formation one hour after single dose of paracetamol was shown. However an effect on platelet aggregation was observed in only one of five investigated individuals[77]. Munsterhjelm *et al*[78] detected an inhibition of platelet aggregation in healthy individuals 10 min after ingestion of paracetamol. Already 90 min after intake of paracetamol, platelet aggregation was restored. Additionally, a combination of paracetamol and diclofenac exhibits an additive effect on platelet inhibition. In comparison to diclofenac treatment in healthy individuals alone, addition of paracetamol preserves inhibition of platelet aggregation and TX formation 90 min after intake. Nevertheless, platelet function normalized after 24 h[52]. In patients, a single dose of paracetamol reduced arachidonic acid induced TX formation, but did not inhibit platelet aggregation in patients[54]. Molecular modelling and docking analyses revealed that paracetamol forms only one single hydrogen-bond to arginine 120 in the hydrophobic channel of COX-1[27]. No aspirin interaction resulting in inhibition of aspirin antiplatelet effects was seen, suggesting that one hydrogen-bond might not be sufficient to induce impairment of aspirin antiplatelet effects[27]. These findings were supported by the results of Catella-Lawson *et al*[33] and Rao *et al*[79], both did not observe altered aspirin antiplatelet effects in presence of paracetamol, either. However an increased incidence of first cardiovascular event in patients with frequent use of paracetamol was observed[80]. Potential reasons for this observation may be a dose dependent risk of renal insufficiency of paracetamol[81] which is a predictor of cardiovascular events[82]. Secondly, an increased blood pressure in patients with paracetamol usage has been described[83-86]. Also, an impairment of endothelial function by depletion of glutathione is thinkable to induce this enhanced risk of cardiovascular events[87].

DIPYRONE (METAMIZOLE)

Dipyrone is a pyrazolinone analgesic with favorable analgesic, spasmolytic and antipyretic effects. Gastrointestinal complications are rare in comparison to NSAIDs like ibuprofen or diclofenac[88]. Due to the risk of agranulocytosis, it has been withdrawn in many countries including the United States. Nevertheless, it is extensively used in Central- and South America and freely available over the counter in Mexico. Therefore despite of the withdrawal by the Food and Drug Administration, there is a wide spread use in the United States as well[89]. Moreover, it is freely available and the most used analgesic in Eastern European countries like Bulgaria[90]. Guidelines of the European Society of Cardiology do not recommend the use of NSAIDs in patients with cardiovascular diseases[91,92]. This may be one of the reasons why dipyrone daily doses tripled during the last decade in European countries like Germany[93]. The exact mechanism of its analgesic effects is complex and not completely understood, yet. Besides COX inhibition, an activation of opioidergic- and cannabinoid system in combination with inhibition of central COX-3 appears to contribute to its analgesic effects. Comedication with opioids causes superadditive analgesic effects. It inhibits both prostaglandin dependent and - independent pathways of fever and exhibits its spasmolytic effects by inhibition of intracellular calcium release[94]. Dipyrone inhibits all COX isoforms. It reversibly binds near the active site of COX, forming hydrogen bonds to tyrosin 385 and serine 530[27]. Dipyrone sterically hinders aspirin access to the active site and serine 530 of COX-1. Plasma half-life of dipyrone is about 2.5 h. Therefore it is 7.5 fold longer available than aspirin with a plasma half-life of only 20 min[95]. *In-vitro* experiments revealed that dipyrone active metabolite impairs ASA induced inhibition of microsomal platelet COX. The active metabolite of dipyrone in therapeutically relevant (low micromolar) concentrations showed little inhibition of platelet aggregation and TX formation. However, it prevented ASA dependent inhibition of platelet aggregation and thromboxane formation caused by arachidonic acid as well as collagen. The effect was reproducible in terms of microsomal platelet COX activity and p-selectin expression as well[96]. Increasing ASA concentrations *in-vitro* overcame this effect[97]. Additionally, previous incubation with ASA before addition of dipyrone preserves ASA antiplatelet effects[98].

In healthy individuals, aspirin intake sufficiently inhibits platelet aggregation, seven days of additional

Table 1 Risk and benefits of non-opioid analgesics

Substance	Platelet inhibition	Aspirin interaction	Benefits	Risks
Aspirin	Irreversible	-	Analgesic, antipyretic, anti-inflammatory Reduction of cardiovascular events and death	Bleeding events
Ibuprofen	Reversible (half-life 1-4 h)	Yes	Analgesic, antipyretic, anti-inflammatory	Death Cardiovascular events Bleeding events
Naproxen	Reversible (half-life 12-24 h)	Yes	Analgesic, antipyretic, anti-inflammatory Reduction of cardiovascular events (?)	Bleeding events
Diclofenac	Reversible (half-life 1-2 h)	No	Analgesic, antipyretic, anti-inflammatory	Death Cardiovascular events Bleeding events
Celecoxib	Reversible (half-life 8-13 h)	Yes	Analgesic, antipyretic, anti-inflammatory Less gastrointestinal events	Death Cardiovascular events
Paracetamol	Reversible (half-life 1-4 h)	No	Analgesic, antipyretic Less gastrointestinal events	Cardiovascular events
Dipyrone	Reversible (half-life 2-4 h)	Yes	Analgesic, antipyretic, anti-inflammatory Less gastrointestinal events	Risk of death and cardiovascular events not investigated

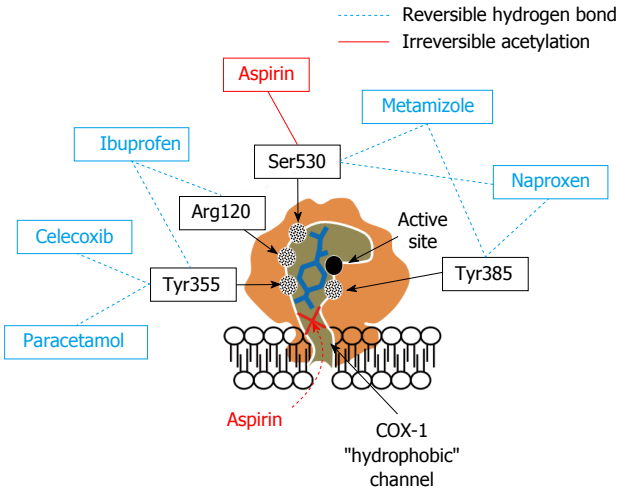

Figure 1 Graphical abstract. Non-opioid analgesics form reversible hydrogen bonds near the active centrum of cyclooxygenase (COX)-1. This prevents (1) aspirin entrance to the hydrophobic channel; (2) irreversible acetylation of Ser530; and (3) platelet inhibition for the remainder of the platelets life-span.

dipyrone intake completely blunts aspirin antiplatelet effects. This effect was reversible within three days of continued aspirin administration after termination of dipyrone intake. However, multiple daily doses of dipyrone were not tested[97]. Interestingly, Börgermann *et al*[99] reported that there was no impaired pharmacodynamic response to aspirin in dipyrone treated healthy individuals. However the duration of aspirin and dipyrone co-treatment was only two days. As aspirin antiplatelet effects are irreversible and persist for the remainder of the affected platelets life-span, it is not surprising, that no relevant differences were observed after two days of additional dipyrone treatment. A partial inhibition of aspirin antiplatelet effects has been described after four days of concomitant intake and complete inhibition after seven days[100]. Furthermore, it has been shown that aspirin intake prior to dipyrone preserves aspirin antiplatelet effects, whereas dipyrone intake prior to aspirin completely blunts aspirin antiplatelet effects measured by platelet aggregation in healthy individuals[97].

In patients with coronary artery disease, residual platelet reactivity despite of aspirin was detected in 50% of dipyrone comedicated patients[101]. Residual platelet TX formation in patients with coronary artery disease correlated with the concentration of dipyrone metabolites. Additionally, in dipyrone treated patients after cardiac surgery, the incidence of HTPR to aspirin nearly tripled postoperatively[99]. The impact of this *in-vitro* and *ex-vivo* effects on clinical outcome has not been investigated yet.

CONCLUSION

The optimal analgesic regimen in patients with pain is challenging. Considering laboratory and clinical data, naproxen and paracetamol seem to display the most favourable benefit/risk ratio. However, increased incidence of adverse events has been described with these analgesics as well. Alternatively, aspirin would be a possible alternative to relieve pain and inhibit platelet function. Yet it is well known, that analgesic doses of aspirin increase the risk of gastrointestinal complications (Figure 1 and Table 1). If medication with non-opioid analgesics is considered indispensable, a strict order of intake, with aspirin medication at least two hours prior to analgesic medication is advisable. However, the optimal analgesic and antiplatelet regimen in patients with increased risk of cardiovascular disease is still unknown and requires further investigation.

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MINIREVIEWS

Peritoneal dialysis for chronic cardiorenal syndrome: Lessons learned from ultrafiltration trials

Amir Kazory

Amir Kazory, Division of Nephrology, Hypertension, and Renal Transplantation, University of Florida, Gainesville, FL 32610-0224, United States

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Correspondence to: Amir Kazory, MD, Division of Nephrology, Hypertension and Renal Transplantation, University of Florida, 1600 SW Archer Road, Gainesville, FL 32610-0224, United States. amir.kazory@medicine.ufl.edu
Telephone: +1-352-2738822
Fax: +1-352-3923581

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Abstract

The current models of cardiorenal syndrome (CRS) are mainly based on a cardiocentric approach; they assume that worsening renal function is an adverse consequence of the decline in cardiac function rather than a separate and independent pathologic phenomenon. If this assumption were true, then mechanical extraction of fluid (*i.e.*, ultrafiltration therapy) would be expected to portend positive impact on renal hemodynamics and function through improvement in cardio-circulatory physiology and reduction in neurohormonal activation. However, currently available ultrafiltration trials, whether in acute heart failure (AHF) or in CRS, have so far failed to show any improvement in renal function; they have reported no impact or even observed adverse renal outcomes in this setting. Moreover, the presence or absence of renal dysfunction seems to affect the overall safety and efficacy of ultrafiltration therapy in AHF. This manuscript briefly reviews cardiorenal physiology in AHF and concludes that therapeutic options for CRS should not only target cardio-circulatory status of the patients, but they need to also have the ability of addressing the adverse homeostatic consequences of the associated decline in renal function. Peritoneal dialysis (PD) can be such an option for the chronic cases of CRS as it has been shown to provide efficient intracorporeal ultrafiltration and sodium extraction in volume overloaded patients while concurrently correcting the metabolic consequences of diminished renal function. Currently available trials on PD in heart failure have shown the safety and efficacy of this therapeutic modality for patients with chronic CRS and suggest that it could represent a pathophysiologically and conceptually relevant option in this setting.

Key words: Cardiorenal syndrome; Peritoneal dialysis; Heart failure; Ultrafiltration

Core tip: This article briefly reviews the clinical significance of renal dysfunction in heart failure and evaluates the results of the ultrafiltration studies in acute heart failure and cardiorenal syndrome (CRS). It concludes that peritoneal dialysis could represent an

efficacious option for chronic CRS due to its ability to simultaneously address renal and cardiac dysfunction in these patients. Recent technical advances such as possibility of initiating peritoneal dialysis (PD) in the acute setting and placement of the PD catheter by interventional radiology could make this home-based therapeutic option even more accessible and intriguing.

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INTRODUCTION

Renal dysfunction is a prevalent feature of heart failure (HF) and portends adverse impact on its potential management options, course, and outcomes. Although several therapeutic strategies have so far been evaluated for patients who present with simultaneous dysfunction of the heart and the kidney [*i.e.,* cardiorenal syndrome (CRS)], the optimal therapy for "chronic" CRS remains largely unknown. This could reflect the paucity of data on the precise mechanisms underlying this syndrome, which is unfortunately unlikely to resolve soon due to its complexity. The contemporary question for clinicians providing care for patients with chronic CRS is whether there exists a safe management strategy that could provide this group of patients with improved outcomes and quality of life compared with conventional therapies. The answer might paradoxically lie in the lessons learned from trials on "acute heart failure" (AHF).

ULTRAFILTRATION FOR HF AND CRS

In the last decade a multitude of attempts have been aimed at finding more efficacious and safer therapies for AHF[1]. While trials on pharmacologic agents such as endothelin receptor antagonists and adenosine receptor antagonists have mostly been disappointing, extracorporeal ultrafiltration has shown promising results that ranged from more efficient fluid and sodium removal to reduction in the rate of re-admission[2,3]. Indeed, ultrafiltration has been recognized as an emerging therapy for patients with AHF which can be used either as an alternative to conventional diuretic-based strategies or as an adjuvant therapy. However, it is noteworthy that patients with significant renal dysfunction have often been excluded from the ultrafiltration trials and as such their favorable results can hardly be extrapolated to common clinical scenarios in which the decline in renal function parallels deterioration in cardiac status[3,4].

In contrast to most previous AHF studies, the recently published Ultrafiltration in Decompensated Heart Failure with Cardiorenal Syndrome (CARRESS-HF) trial examined the role of ultrafiltration in management of patients with AHF who also presented with worsening renal function (WRF)[5]. In this randomized controlled trial that included 188 patients, ultrafiltration was compared to a diuretic-based pharmacologic therapy in patients who were admitted with a primary diagnosis of AHF and experienced acute CRS. Surprisingly, once the renal component was added to the clinical picture, the favorable findings of the previous trials were not observed anymore; ultrafiltration was found to be inferior to pharmacologic therapy with regards to its impact on both renal function and development of serious adverse events, and the recruitment of the patients had to be stopped. Although considered a trial of AHF, the unprecedented design of CARRESS-HF (*e.g.*, inclusion of patients with an increase in serum creatinine level as small as 0.3 mg/dL up to 3 mo prior to admission to the hospital) made it possible for the trial to also recruit and follow patients with cardiorenal physiology of less acuity.

CARDIO-RENAL PHYSIOLOGY AND ULTRAFILTRATION

Renal dysfunction is an established predictor of adverse outcomes in patients with HF. Until recently, the traditional model of therapy for HF mainly focused on low cardiac output (*i.e.*, low forward flow) being the trigger for a cascade of pathologic events ultimately leading to deterioration in renal function. Even though most patients admitted for AHF present with normal blood pressure, a significant subset still experience concomitant WRF and CRS. Therefore, the decrease in renal perfusion pressure secondary to reduced stroke volume, which was once considered the major mechanism, cannot fully explain renal dysfunction of all these patients. Indeed, more recent data suggested that WRF in the setting of AHF would correlate better with the degree of renal venous congestion rather than cardiac output (*i.e.*, high backward pressure)[6]. In this model, alteration in renal function is more related to right atrial and central venous pressure than cardiac index or left ventricular ejection fraction, hence proposing the hypothesis of backward rather than forward failure.

Does ultrafiltration therapy affect cardiac physiology and hemodynamic status? A number of studies including those using invasive methods have reported several beneficial effects on cardio-circulatory parameters such as cardiac index and systemic vascular resistance following ultrafiltration therapy. While the precise mechanisms remain to be determined, these positive results could still be explained to some extent by the aforementioned models of CRS. On the arterial side, efficient fluid removal by ultrafiltration therapy reduces left ventricular end-diastolic volume and pushes the heart towards the left side of the Frank-Starling curve.

Table 1 Proposed benefits of peritoneal dialysis therapy for heart failure

Continuous gentle ultrafiltration with minimal impact on hemodynamic status
Improvement in functional status and symptoms of volume overload
Reduction in number of days of heart failure-related hospitalizations
Restoration of diuretic responsiveness
Reduction in weight and improvement in volume status
Improvement in left ventricular ejection fraction
Sodium sieving effect and possibility of better control of natremia
Removal of pro-inflammatory mediators (medium-sized molecules)
Reduction in intra-abdominal pressure in patients with severe ascites
Improvement in quality of life
Improved atherogenic lipid serum profile
Lack of impact on neurohormonal activity (renin-angiotensin-aldosterone system and sympathetic nervous system)
Improved control of serum potassium level (hence providing the opportunity to use medications such as aldosterone receptor blockers)
Reduction in healthcare cost

Adapted from Courivaud *et al*[9], with permission.

This effect can hinder the intermediary pathways such as activation of renin-angiotensin-aldosterone and sympathetic nervous systems and their downstream adverse effects such as ventricular remodeling and perturbation in renal hemodynamics. On the right side, ultrafiltration extracts fluid directly and exclusively from the venous side of the circulation leading to immediate reduction in preload, ventricular wall stress, and capillary hydrostatic pressure. Decongestion of the venous side of the circulation has also been reported to improve renal vein engorgement without affecting counteracting intermediate pathways such as adenosine receptors and tubuloglomerular feedback that result in a decrease in glomerular filtration rate. We have previously reviewed the currently available data on the interactions between the cardiocirculatory system and the kidney in the setting of AHF, and the potential role of ultrafiltration in modifying these mechanisms[7].

RENAL IMPLICATIONS OF ULTRAFILTRATION TRIALS

The common theme in the above-mentioned models of CRS is the dependence of the renal component on the cardiac status (*i.e.*, a cardiocentric approach). They both assume that WRF is an adverse consequence of the decline in cardiac function rather than a separate and independent phenomenon. If this assumption were true, then ultrafiltration therapy would be expected to portend positive impact on renal hemodynamics and function as it is capable of improving cardio-circulatory physiology and reducing neurohormonal activation. However, available ultrafiltration trials, whether in AHF alone or in CRS, have so far failed to show any improvement in the associated WRF; they have either reported no impact or even observed adverse renal outcomes in this setting[3,5]. This important observation questions the accuracy of the above-mentioned cardiocentric models of WRF in AHF, and suggests that renal component of acute CRS is not merely a consequence of deterioration in cardio-circulatory status or the use of conventional therapies in a subset of patients; it can reflect an independent but related phenomenon that needs to be regarded and managed separately. In this model, a number of maladaptive mechanisms (*e.g.*, inflammation and endothelial cell dysfunction) are shared by the kidney and the heart resulting in a decline in the function of both organs and development of CRS. As such, any therapeutic option for this syndrome should not only target cardio-circulatory status, but it also needs to have the ability of addressing the adverse homeostatic consequences of the decline in renal function. In this respect, ultrafiltration might not be the optimal option for management of all cases of acute CRS, as supported by recent trials such as CARRESS-HF, simply due to the fact that it lacks any clearance property and cannot address the diverse metabolic and homeostatic derangements associated with concomitant renal dysfunction.

PERITONEAL DIALYSIS AND CHRONIC CRS

In the chronic setting, where patients present with various degrees of HF and slowly declining renal function, a therapy with the ability of simultaneously addressing both organs will be conceptually attractive and mechanistically relevant. Peritoneal dialysis (PD) can be such an option. PD has been shown to provide efficient intracorporeal ultrafiltration and sodium extraction in volume overloaded patients (especially through the use of icodextrin solution), while concurrently correcting the metabolic consequences of diminished renal function. It has also been reported to portend less well-characterized benefits such as removal of myocardial depressant factors and improvement in endothelial dysfunction (Table 1). It is noteworthy that not all proposed beneficial mechanisms are exclusive to PD; while many can be the direct consequences of using this specific therapeutic modality (*e.g.*, reduction in intra-abdominal pressure in patients with severe ascites), some can also be achieved through other methods of renal replacement therapy such as hemodialysis (*e.g.*, reduction in weight and improvement in volume status).

Several uncontrolled PD studies have so far reported favorable results for patients with chronic CRS despite the fact that they often used PD as "the last resort" for very sick patient populations who were refractory to alternative options and were not candidates for heart transplant[8-10]. For instance, in a study on 126 patients with refractory heart failure and various degrees of renal dysfunction, Courivaud *et al*[10] reported a 90% reduction in the number of days of hospitalization after initiation of PD (3.3 d/patient per month *vs* 0.3 d/patient per month; $P < 0.0001$).

Table 2 Selected studies on the role of peritoneal dialysis in heart failure

Ref.	Study design	No. of patients	Mean age (yr)	Male gender	NYHA class	EF	Renal function	Main findings	Comment
Koch *et al*[11]	Prospective	118	73.2	60.2%	Ⅲ (49.2%) Ⅳ (50.8%)	43.5%	Creatinine clearance 19.2 mL/min	Significant improvement in body Weight and NYHA class	Negligible incidence of peritonitis and catheter dysfunction
Núñez *et al*[8]	Prospective	25	75.1	72%	Ⅲ or Ⅳ (100%)	40%	eGFR 33 mL/min per 1.73 m^2	Significant improvement in patients' clinical status and NYHA class	Marked reduction in the number of days hospitalized for acute heart failure
Bertoli *et al*[12]	Multicenter retrospective	48	74	81%	Ⅱ (6%) Ⅲ (48%) Ⅳ (46%)	30%	eGFR 21 mL/min per 1.73 m^2	Significant improvement in NYHA class and reduction in the number of days hospitalized	Significant reduction in pulmonary artery pressure and improvement in EF
Courivaud *et al*[10]	Retrospective	126	72	69%	N/A	38%	eGFR 33.5 mL/min per 1.73 m^2	Significant reduction in the number of days hospitalized for acute heart failure	Improvement in cardiac function in patients with a an EF of 30% or less

NYHA: New York Heart Association.

The results of selected studies on the role of PD in HF are summarized in Table 2. Since HF is the single most common reason for hospitalization of patients over 65 and the majority of its cost is related to the in-hospital care, use of this home-based therapy for chronic CRS could potentially lead to significant savings in healthcare expenditure while providing a better quality of life for patients. The advantages, potential mechanisms, safety, and efficacy of this therapeutic modality for patients with HF has been discussed elsewhere[9]. In patients with significant residual renal function who do not require dialytic support, nocturnal automated PD or a single night time exchange with icodextrin solution could be sufficient to maintain euvolemia. Depending on the severity of HF, degree of volume overload, symptoms, and comorbidities, the PD therapy can be customized and some patients could use it only a few nights a week rather than every night. In patients with more severe renal dysfunction who require dialytic support for clearance, continuous ambulatory PD or automated PD with day time icodextrin exchange could have the greatest promise to generate the needed gentle continuous ultrafiltration while providing adequate clearance.

A major concern regarding the use of PD in this patient population has been that its morbidity might replace the morbidity from HF. This issue seems to be less compelling nowadays with reasonably low incidence of PD-related complications such as peritonitis, catheter dysfunction, and hernias as reported by most studies as well as the reports on the reduction in HF-related hospitalization after initiation of PD. Moreover, although the data are not consistent, it appears that PD does not alter the natural history of the disease and as such is unlikely to have a significant effect on survival of these patients. Finally, it should be noted that the current literature on the use of PD in the setting of HF still suffers from significant limitations which could hamper its more widespread use (*e.g.*, lack of an appropriately matched control group and relatively short follow-up periods). This could explain the fact that despite aforementioned advantages of this modality, PD is not yet considered by the professional cardiology societies as a therapeutic option for HF. Future prospective randomized studies with longer follow-up periods could address the knowledge gap and prove helpful in this regard.

In summary, based on the currently available data, PD represents one of the few options for patients with chronic CRS that not only is pathophysiologically and conceptually relevant, but is also reported to be safe and effective in several clinical trials. Recent technical advances such as possibility of initiating PD in the acute setting and placement of the PD catheter by interventional radiology could make this home-based therapeutic option even more accessible and intriguing[13].

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Prognostic impact of atrial fibrillation on clinical outcomes of acute coronary syndromes, heart failure and chronic kidney disease

Nileshkumar J Patel, Aashay Patel, Kanishk Agnihotri, Dhaval Pau, Samir Patel, Badal Thakkar, Nikhil Nalluri, Deepak Asti, Ritesh Kanotra, Sabeeda Kadavath, Shilpkumar Arora, Nilay Patel, Achint Patel, Azfar Sheikh, Neil Patel, Apurva O Badheka, Abhishek Deshmukh, Hakan Paydak, Juan Viles-Gonzalez

Nileshkumar J Patel, Dhaval Pau, Nikhil Nalluri, Deepak Asti, Ritesh Kanotra, Azfar Sheikh, Cardiovascular Division, Staten Island University Hospital, Staten Island, NY 10305, United States

Aashay Patel, Cardiovascular Division, Lankenau Institute of Medical Research, Wynnewood, PA 19096, United States

Kanishk Agnihotri, Nilay Patel, Cardiovascular Division, Saint Peters University Hospital, New Brunswick, NJ 08901, United States

Samir Patel, Cardiovascular Division, Western Reserve Health Education, Yougstown, OH 44504, United States

Badal Thakkar, Cardiovascular Division, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA 70112, United States

Sabeeda Kadavath, Cardiovascular Division, Lincoln Medical and Mental Health Center, Bronx, NY 10451, United States

Shilpkumar Arora, Cardiovascular Division, St. Lukes Roosevelt Hospital, New York, NY 10025, United States

Achint Patel, Neil Patel, Cardiovascular Division, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States

Apurva O Badheka, Cardiovascular Division, Yale School of Medicine, New Haven, CT 06519, United States

Abhishek Deshmukh, Cardiovascular Division, Mayo Clinic, Rochester, MN 55905, United States

Hakan Paydak, Cardiovascular Division, University of Arkansas for Medical Sciences, Little Rock, AR 72205, United States

Juan Viles-Gonzalez, Cardiovascular Division, University of Miami Miller School of Medicine, Miami, FL 33136, United States

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Correspondence to: Nileshkumar J Patel, MD, Cardiovascular Division, Staten Island University Hospital, 475 Seaview Avenue, Staten Island, NY 10305, United States. dr.nilesh.j.patel@gmail.com
Telephone: +1-201-7449111
Fax: +1-610-3941787

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Abstract

Atrial fibrillation (AF) is the most common type of sustained arrhythmia, which is now on course to reach

epidemic proportions in the elderly population. AF is a commonly encountered comorbidity in patients with cardiac and major non-cardiac diseases. Morbidity and mortality associated with AF makes it a major healthcare burden. The objective of our article is to determine the prognostic impact of AF on acute coronary syndromes, heart failure and chronic kidney disease. Multiple studies have been conducted to determine if AF has an independent role in the overall mortality of such patients. Our review suggests that AF has an independent adverse prognostic impact on the clinical outcomes of acute coronary syndromes, heart failure and chronic kidney disease.

Key words: Atrial fibrillation; Heart failure; Chronic kidney disease; Acute coronary syndromes; Prognostic impact

Core tip: Atrial fibrillation (AF), the most common type of arrhythmia, is on course to reach epidemic proportions in the elderly. AF is a commonly encountered comorbidity in patients with acute coronary syndromes, heart failure and chronic kidney disease. Multiple studies have been conducted to determine if AF has an independent role in the overall mortality of such patients. Our review suggests that atrial fibrillation has an independent adverse prognostic impact on the clinical outcomes of acute coronary syndromes, heart failure and chronic kidney disease.

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INTRODUCTION

Atrial fibrillation (AF) is a commonly encountered arrhythmia in clinical practice[1] with an increased prevalence being reported with advanced age[2]. It is estimated that more than 8 million patients over the age of 80 will be affected by the year 2050[1,3]. Consequently, the associated healthcare expenses are also rising and have reached an all-time high of 16 to 26 billion dollars annually[4]. The major contributors to the burgeoning healthcare costs of AF include outpatient care and testing which accounted for nearly $1.5 billion of the total costs, prescription drugs that cost an approximate of $235 million, and also high costs associated with inpatient interventional procedures[4-6].

With a rising prevalence and economic burden, there is concern in the medical community regarding the temporal effect of cardiovascular conditions including atrial fibrillation on the clinical outcomes of associated comorbidities. In addition to its deleterious health consequences, cardiovascular disease is the number one cause of death in the United States and globally[7]. It is important to understand the role of other comorbidities in cardiovascular disease to prevent and reduce this mortality. In this article we focus on atrial fibrillation and commonly associated comorbidities. Atrial fibrillation is commonly encountered in the setting of acute coronary syndromes, heart failure and chronic kidney disease. The purpose of this article is to review the prognostic impact of atrial fibrillation on these comorbid conditions.

ACUTE CORONARY SYNDROMES

Acute coronary syndrome (ACS) is commonly associated with concomitant or incident AF. Most of the studies conducted have noted that the incidence of AF in ACS ranges from 2.3% to 23%[8]. Multiple factors explain this wide range of variation. The Cooperative Cardiovascular Project by Rathore *et al*[9] reported a higher incidence of AF in ACS patients, as the subjects were primarily elderly patients. Eldar *et al*[10] reported a lower incidence as they studied only paroxysmal AF. Some randomized controlled trials like TRACE and OPTIMAAL which studied the efficacy of angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) in acute myocardial infarction (AMI) have also reported lower incidences; the efficacy of these drugs in preventing atrial fibrillation had however been proven in earlier studies[11,12].

Broadly there has been a downward trend in the incidence of AF in AMI in studies done over time. This can be explained possibly by more widespread use of thrombolytic therapy and percutaneous coronary interventions (PCI) over the years. Advanced age, tachycardia at the time of admission, and advanced stage of heart failure were found to be the major clinical predictors of atrial fibrillation in patients with AMI[9,13,14].

Early studies done to assess the independent prognostic impact of atrial fibrillation on ACS outcomes were found to have contrasting results. A number of studies after multivariate analyses found atrial fibrillation to have no independent impact and concluded that it was more the coexisting comorbidities that contributed to the mortality[10,14-19]. However, a greater number of studies have reported that atrial fibrillation in the setting of AMI, results in a worse prognostic outcome[9,11-13,19-22].

However, two major meta-analyses done by Jabre *et al*[23] and Angeli *et al*[24] proved conclusively the independent impact atrial fibrillation had on AMI. In the analysis of 43 studies by Jabre *et al*[23] where 278854 patients were studied, it was observed that AF was associated with a 40% increase in risk mortality as compared to patients with normal sinus rhythm. While the impact of atrial fibrillation on both in hospital

mortality and long term mortality was noted, the timing of atrial fibrillation development, *i.e.*, new onset or pre-existing AF was not a contributor to the poor outcome as per this meta-analysis. Angeli *et al*[24], on the other hand, found that new onset atrial fibrillation had worse outcomes with an 87% higher risk compared to pre-existing atrial fibrillation. The study however only assessed in hospital mortality and not long-term outcomes.

Atrial fibrillation leads to a number of hemodynamic effects such as loss of atrial contraction, rapid ventricular rates, loss of atrio-ventricular synchrony and an irregular RR interval. All of these factors lead to a decreased cardiac output, which in turn explain the higher mortality rates[25,26].

Many mechanisms have been proposed to explain how AF is commonly encountered in the setting of ACS. Although many theories exist, the pathophysiological mechanism of the onset of AF after ACS is still not clearly understood. Conclusions drawn from experimental models and clinical investigations have shown different factors accounting for new-onset AF in ACS; it can be explained either by myocardial infarction causing atrial ischemia or atrial stretch[27]. Role of inflammation, autonomic nervous system activity, BNP and other hormone activation cannot be excluded as possible mechanisms for AF development in this patient subset[28,29]. Thus, proper understanding of the role of new onset AF complicating ACS can provide us with a new approach in formulating therapeutic guidelines.

Consensus has been reached on the independent role of AF on mortality in ACS. Treatment targeting the pathophysiological mechanism of AF development in ACS remains an area that needs to be explored. It therefore remains imperative to develop strategies to prevent AF onset and initiate aggressive treatment in case of a new onset AF in ACS.

HEART FAILURE

Heart failure (HF) and AF are closely linked cardiovascular diseases that often coexist and share a complex pathophysiological relationship. Both have continuously increasing prevalence, and the presence of AF in HF patients has been reported as being anywhere between 10% and 50%[30]. The difference in coexistence of this two-disease condition can be attributed to the different study settings, study design, severity of heart failure and other factors[30,31]. The prevalence of AF correlates directly with the severity of HF, as about 5% of patients with New York Heart Association (NYHA) class Ⅰ HF have AF and this prevalence increases to about 50% in NYHA class IV HF[32,33]. Regardless of the study design, a few factors like hypertension, prior history of ACS, diabetes, and obesity were commonly observed to be associated with an increasing prevalence of AF and HF.

Recent large heart failure trials have demonstrated the adverse prognostic influence of AF on HF[34]. A study conducted by Dries *et al*[35], in which data was obtained from SOLVD trial, showed AF was associated with an increased risk of all cause mortality in patients with symptomatic and asymptomatic left ventricular systolic dysfunction[35]. On the other hand, the COMET trial analysis by Swedberg *et al*[36] showed AF did increase mortality risk and HF hospitalizations but it was not identified as an independent risk factor for mortality when adjustment for other prognostic indicators was made[36].

AF also increases re-hospitalization rates, hospital stays, and has an overall adverse prognosis in HF patients that is very clearly evident in many studies. Mountantonakis *et al*[37] analyzed data obtained from 99810 patients enrolled in the Get with the guidelines - Heart failure Registry and concluded that AF independently was associated with adverse hospital outcomes and a longer length of in-hospital stay. Mentz *et al*[38] showed presence of AF on initial electrocardiogram in patients hospitalized with HF was associated with higher readmission, higher mortality and lower use of evidence-based therapies.

Corell *et al*[39] proved an adverse prognostic impact of AF in HF patients. Olsson *et al*[40] reviewed results from Candesartan in Heart failure-Assessment of Reduction in Mortality and morbidity (CHARM) program and showed that AF is associated with an increased risk of poor cardiovascular outcomes. In the meta-analysis by Mamas *et al*[41] which included 16 studies involving 53969 patients; the conclusion was that irrespective of left ventricular systolic function, AF has an overall adverse prognosis in HF patients.

Pathophysiological changes that explain the increased prevalence of AF in HF patients are not very well understood. It is difficult to ascertain in most cases if HF leads to AF or changes due to AF leads to worsening of the underlying HF. Studies have different conclusions on the cause - effect process but there is a general agreement about the vicious cycle of deterioration when both conditions co-exist. According to one thought process, HF results in specific electrophysiological changes in the atrium like prolonging the atrial refractory period or increasing heterogeneity of repolarization that leads to the development of AF[42]. On the other hand, HF also plays a part in concurrent worsening of AF through mechanical and hemodynamic changes. Atrial tissue stretching occurs as a result of the increased pressure and volume in HF patients, which in turn triggers AF by increasing automaticity and altering atrial repolarization[43]. Activation of the renin-angiotensin system secondary to HF and other neurohormonal changes also promotes the development of AF[43]. Further studies need to be conducted to understand the impact AF has on HF, especially in regards to the dynamic pathophysiological interplay and therapy should be aimed at correcting the predisposing factors.

Although beyond the scope of this article, the op-

timal management approach of AF in HF remains unclear. Pharmacological therapy remains the mainstay of choice in AF, and includes rate control and rhythm control. A recent meta-analysis involving 2486 patients suggested no significant difference in terms of mortality and thromboembolic events between both modes of pharmacological management. However, hospitalizations appear to be less frequent with rate control than with rhythm control[44]. Also there are data that suggest role of cardiac resynchronization therapy (CRT) in non-ischemic dilated cardiomyopathy and severe heart failure, which has favorable outcome on incidence of AF[45,46]. Further studies are warranted to determine the optimal management approach for AF in patients with HF.

CHRONIC KIDNEY DISEASE

It is a well-established fact that there is a high occurrence of cardiovascular disease in patients with chronic renal insufficiency. The overall prevalence of AF is higher among patients with end-stage renal disease (ESRD)[47]. Studies examining the prevalence of AF in cohorts pooled from the Dialysis Outcomes and Practice Patterns Study (DOPPS) and the United States Renal Data System (USRDS) estimated the occurrence of AF to range from 6% to 27% among patients with ESRD on dialysis[48-50]. This high rate of occurrence in ESRD patients is nearly two times higher than that reported in the general population[49]. Dissimilarity of the individual study pattern, study population, sample size, disease definition and diagnostic methods of AF can account for the difference between the prevalence of AF in this population.

Wetmore *et al*[50] and Wizemann *et al*[48] concluded that a significantly higher prevalence of AF exists in ESRD patients on dialysis. On the other hand, recent studies have found a higher incidence and prevalence of AF among patients with chronic kidney disease (CKD) who have not been started on dialysis, as is clearly evident in the ARIC study and CRIC study done by Alonso *et al*[51] and Soliman *et al*[52]. In the latter study by Soliman *et al*[52] where a multicenter cohort with a wide range of kidney function was studied, it was estimated that the prevalence of AF was at 18%.

Moreover, AF is an independent risk factor for ischemic stroke and death among patients with ESRD on dialysis[53]. A large cross sectional cohort study conducted by Winkelmayer *et al*[49], analyzed data from 1992 to 2006 for the prevalence of AF in hemodialysis patients from the United States Renal Data System (USRDS). According to this study, the prevalence of AF increased 3 fold from 3.5% in 1992 to 10.7% in 2006. A one-year mortality rate among patients with AF was twice that of those without AF and was as high as 72% after demographic variant adjustment was made.

Several mechanisms have been proposed to explain the increased risk of death in CKD patients with AF. Systemic inflammation could be responsible for the fibrotic changes seen in the kidney and myocardium and by could worsen cardiovascular outcomes such as heart failure, thromboembolic risk and stroke which in turn increases the risk of morbidity and mortality[35,54-57].

A large cohort study on adults with AF by Go *et al*[58] concluded that a lower level of GFR was associated with an increased risk of thromboembolism independent of the known AF risk factors. A higher rate of thromboembolic events was observed among individuals with a lower estimated GFR.

The cumulative effect of AF and CKD together has been shown to not only increase mortality but also the rate of cardiovascular events, as has been observed in two separate studies done by Nakagawa *et al*[59] in Japan and Genovesi *et al*[60] in Italy. From the findings of the study by Nakagawa *et al*[59], it was determined that a lower eGFR (< 60 mL/min per 1.73 m^2) with CHADS2 score > 2 was associated with a higher all-cause (12.9% *vs* 1.4% per year, $P < 0.001$) and cardiovascular (6.5% *vs* 0.2% per year, $P < 0.001$) mortalities compared to preserved eGFR (> 60 mL/min per 1.73 m^2) combined with CHADS2 score < 2. Also cardiovascular events, which include cardiac death, nonfatal myocardial infarction, or hospitalization for worsening of heart failure and ischemic stroke risk, were much higher in the same group (13.6% *vs* 1.5% per year, $P < 0.001$). The study concluded that a combined eGFR and CHADS2 score could be an independent powerful predictor of cardiovascular events and mortality in patients with nonvalvular AF[59].

Although there is a substantially increased risk of thromboembolism in patients with CKD and AF, there are no distinct guidelines to follow for thromboembolism prophylaxis in AF patients with CKD when compared to patients without CKD. Patients with severe renal impairment have been excluded from a vast majority of trials studying stroke prevention in AF, including trials that have formed the landmark for risk factor scoring schemes and guidelines. It therefore, poses a huge challenge to healthcare providers to treat this subset of patients. The available data suggests that the benefit from warfarin in terms of stroke reduction in CKD patients is not as clear as in the general population, and there is also an increased risk of bleeding complications[61].

One of the few studies that show a favorable outcome of anticoagulation for prevention of stroke in renal failure patients is the study by Hart *et al*[62]. Efficacy of adjusted-dose warfarin in prevention of stroke in atrial fibrillation patients with stage 3 CKD was demonstrated by this study. The study by Chan *et al*[63], a large retrospective cohort study of patients with AF on hemodialysis, suggests that warfarin use is associated with an increased risk for ischemic (HR = 1.81; 95%CI: 1.12-2.92) and hemorrhagic (HR = 2.22; 95%CI: 1.01-4.91) stroke. The data however is influenced by lack of appropriate monitoring and

difficulties in maintaining the international normalized ratio (INR) target[63].

Thus, it remains a dilemma to refer to the benefits of warfarin administration as has been determined by anticoagulation guidelines in the general population, to a group of people that have been actively excluded from clinical trials; the prediction rules for bleeding risk would be inaccurate and oversimplified and probably not suitable for clinical practice. In reality, there appears to be no large randomized controlled trials that evaluate the real risk *vs* benefit of full intensity anticoagulation including newer novel anticoagulants in patients with severe renal impairment. Information about management is limited and in the future there might be an opportunity to look into these patients and form risk stratification guidelines that can be followed.

LIMITATIONS

Although we have searched a wide range of appropriate literature from online data sources for our article, sometimes such studies are potentially susceptible to vary in conclusion due to different populations, settings, interventions, or outcome measures. All the studies we included have different limitations. Despite the limitations, the present article has important strengths, including a real-world large sample size from different studies and the absence of selection bias associated with clinical trials.

CONCLUSION

In conclusion, atrial fibrillation is a commonly encountered arrhythmia in clinical practice that has a rising prevalence and significant adverse prognostic implications on other comorbidities. In this article we concluded that AF, with its rising prevalence increases the economic burden on healthcare, and has an independent adverse prognostic impact on comorbidities like ACS, HF and CKD. A thorough understanding of AF prevalence and its pathophysiology, including the role of genetics, can serve as a potential biomarker for the prevention and treatment of AF[64,65]. Along with it, factors associated with AF and its increased association with other comorbidities, outcomes of these comorbidities in the setting of AF, prospective data and appropriate guidelines are needed to define more precisely how to treat these patients. Individual risk stratification may represent the best possible approach and provide opportunities for improvement in the future. Further studies need to be conducted to determine risk stratification for decision making and to develop an optimal management approach.

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Cholesterol confusion and statin controversy

Robert DuBroff, Michel de Lorgeril

Robert DuBroff, Department of Medicine, Division of Cardiology, University of New Mexico School of Medicine, MSC 10-5550, 1 University of New Mexico, Albuquerque, NM 87131, United States

Michel de Lorgeril, PRETA-TIMC-IMAG, Equipe Cœur and Nutrition, CNRS UMR 5525-UJF-INP, Faculté de Médecine, Domaine de la Merci, Université de Grenoble, 38700 La Tronche, France

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Correspondence to: Michel de Lorgeril, MD, PRETA-TIMC-IMAG, Equipe Cœur and Nutrition, CNRS UMR 5525-UJF-INP, Faculté de Médecine, Domaine de la Merci, Université de Grenoble, 38700 La Tronche,
France. michel.delorgeril@ujf-grenoble.fr
Telephone: +33-476-637471
Fax: +33-476-637152

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Abstract

The role of blood cholesterol levels in coronary heart disease (CHD) and the true effect of cholesterol-lowering statin drugs are debatable. In particular, whether statins actually decrease cardiac mortality and increase life expectancy is controversial. Concurrently, the Mediterranean diet model has been shown to prolong life and reduce the risk of diabetes, cancer, and CHD. We herein review current data related to both statins and the Mediterranean diet. We conclude that the expectation that CHD could be prevented or eliminated by simply reducing cholesterol appears unfounded. On the contrary, we should acknowledge the inconsistencies of the cholesterol theory and recognize the proven benefits of a healthy lifestyle incorporating a Mediterranean diet to prevent CHD.

Key words: Cholesterol; Statins; Coronary heart disease; Mediterranean diet; Cardiovascular disease; Mortality

Core tip: Traditional efforts to prevent cardiovascular disease have emphasized the benefits of cholesterol lowering and statin drugs. Often overlooked is the fact that numerous studies of cholesterol lowering have failed to demonstrate a mortality benefit and the benefits of statins may have been overstated. The Mediterranean diet has consistently lowered cardiovascular events and mortality in numerous studies and does not typically lower cholesterol levels. Alternative theories of atherosclerosis are independent of cholesterol metabolism and may provide the key to future preventive strategies.

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INTRODUCTION

Nearly twenty years ago two landmark randomized clinical trials appeared in *The Lancet* which forever changed the course of medicine for patients with coronary heart disease (CHD). The 4S study employed a cholesterol-lowering statin drug and reported a 30% mortality reduction[1]. The Lyon Diet Heart Study utilized the Mediterranean diet and reported a 70% mortality reduction[2]. Subsequent studies of the Mediterranean diet have confirmed these findings and also shown a reduced risk of cancer, diabetes, and Alzheimer's disease[3-6]. Subsequent statin studies have led the United States Food and Drug Administration to issue warnings regarding the increased risk of diabetes and decreased cognition with statin drugs. Paradoxically, statins have gone on to become a multi-billion dollar industry and the foundation of many cardiovascular disease prevention guidelines while the Mediterranean diet has often been ignored. We believe this statin-centric cholesterol-lowering approach to preventing CHD may be misguided.

ASSOCIATION DOES NOT EQUAL CAUSATION

The cholesterol hypothesis links cholesterol intake and blood levels to cardiovascular disease. Because cholesterol is considered a risk factor for atherosclerosis many believe that lowering cholesterol in the blood is the best way to prevent CHD. Ideally, risk factors should help us distinguish those who will develop a disease from those who will not. However, if one examines the original Framingham Heart Study data (as an example) it is clear that the cholesterol levels of those who developed CHD and those who did not overlap except when the total cholesterol level exceeded 380 mg/dL or was less than 150 mg/dL (Figure 1). Moreover, cholesterol may be associated with CHD but that does not prove causation. Despite the fact that high triglycerides and low HDL have long been associated with CHD, studies designed to raise HDL or lower triglycerides have failed to reduce CHD mortality. Similarly, cholesterol should not automatically become a treatment target. It may be a leap of faith to assume that lowering cholesterol is the best way to prevent CHD.

LOWERING CHOLESTEROL MAY NOT LOWER CARDIOVASCULAR MORTALITY

The rare occurrence of CHD in isolated, rural societies such as Tukisenta, New Guinea has been attributed to low cholesterol levels[7]. However, it is equally plausible that the diets and lifestyles of these individuals may protect them from CHD. While we may never be certain if low cholesterol or a healthy lifestyle (or both) are responsible for preventing CHD in these societies, there is ample evidence that lowering cholesterol does not consistently lower CHD mortality. Reducing

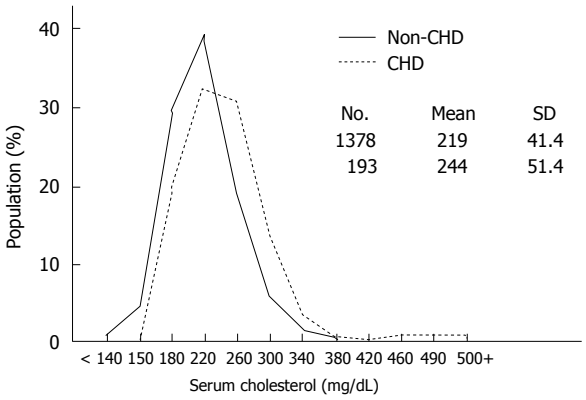

Figure 1 Serum cholesterol distribution among coronary heart disease and non-coronary heart disease patients in the Framingham Heart Study[43]. Reprinted with permission of the publisher. CHD: Coronary heart disease.

cholesterol blood levels by reducing dietary saturated fats is commonly recommended, but an exhaustive review and meta-analysis of 72 dietary studies concluded that reduced consumption of saturated fat does not reduce cardiovascular mortality[8]. Many drugs such as niacin, fibrates, and bile acid sequestrants can lower cholesterol levels, but the recent AHA/ACC guidelines on cholesterol concluded that these drugs do not lower CHD mortality rates[9]. Moreover, the results of cholesterol-lowering statin trials, as will be discussed and analyzed later, do not consistently lower mortality rates[10]. Consider also the dramatic mortality benefit of the Mediterranean diet in the Lyon Diet Heart Study which was achieved without a reduction in cholesterol levels[2-4]. Thus, the hypothesis that lowering cholesterol lowers mortality from CHD is not supported by many clinical research studies.

EARLY STATIN TRIALS MAY HAVE BEEN FLAWED

Early statin trials reported significant mortality benefits, yet serious concerns have been raised in some studies regarding biased results, premature trial terminations, under reporting of adverse events, high numbers of patients lost to follow-up and oversight by the pharmaceutical company sponsor[10]. Heightened awareness within the scientific community regarding problems in clinical trial conduct and analysis - exemplified by the unreported risk of heart attacks in patients taking the pain killers Vioxx and Celebrex - led to new regulatory rules for clinical trials in 2005[11]. Curiously, statin trials conducted after 2005 have failed to demonstrate a consistent mortality benefit[10].

MORTALITY RESULTS ARE MORE IMPORTANT THAN COMBINED CLINICAL ENDPOINTS

Cholesterol-lowering statin trials are often viewed as supporting the cholesterol hypothesis by reporting

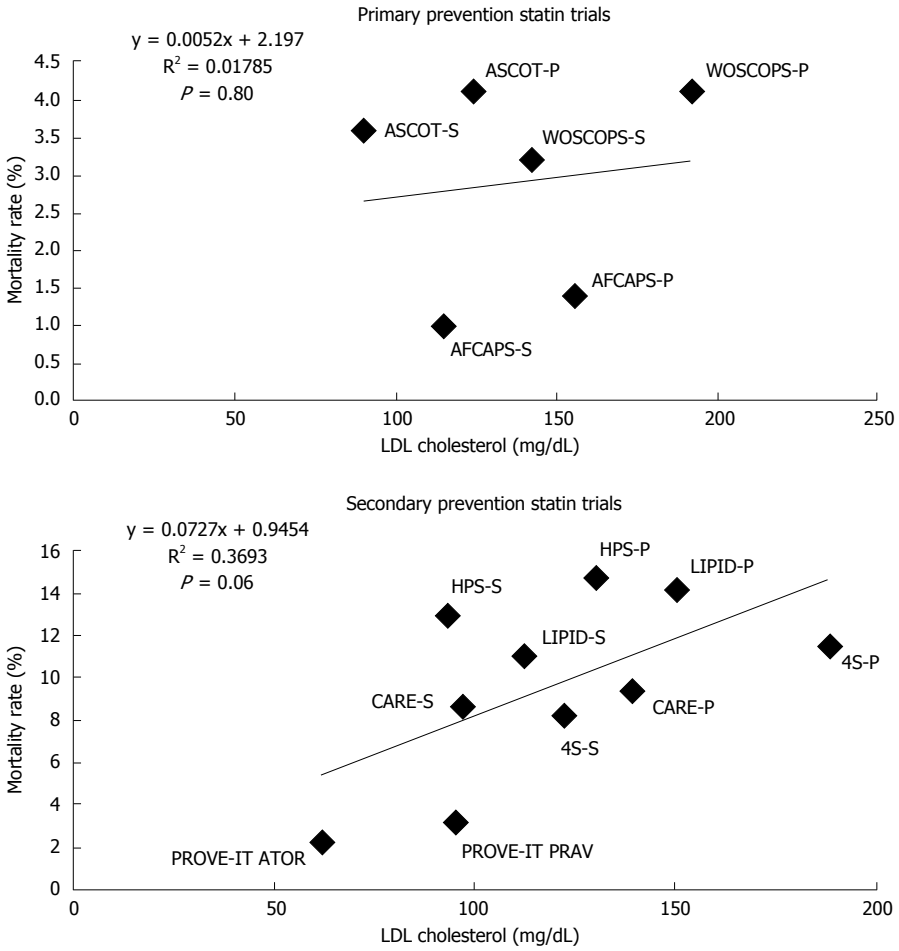

Figure 2 Comparison of mortality rates to low-density lipoprotein cholesterol levels using the randomized clinical trials cited in reference 14 (taken as an example).

significant reductions in combined clinical endpoints. Clinical endpoints are valuable and should not be ignored, but the ultimate measure of efficacy is total mortality that reflects both the treatment effect and potentially fatal side effects. Utilizing combined endpoints may lead to an exaggeration of perceived benefit by assigning equal importance to disparate clinical events such as a hospital admission for angina and death from a heart attack[12,13]. Some have argued that there is a linear relation between low-density lipoprotein (LDL) levels and CHD events[14]. This analysis may be inaccurate because it combines different types of CHD events from diverse studies into one endpoint even though each study defines CHD events differently. A more meaningful analysis compares total mortality rates to LDL cholesterol levels. When we performed such an analysis on these same statin trials - those analyzed in reference 14 - we found no statistically significant relationship (Figure 2).

MORTALITY BENEFITS OF STATINS ARE INCONSISTENT

Although a number of statin trials have reported a mortality benefit, quite a few have not. A corollary to the cholesterol hypothesis posits that patients at highest risk should derive the greatest benefit from cholesterol lowering. However, statin trials in the elderly (PROSPER), in patients with heart failure (CORONA, GISSI-HF), and in patients with renal failure (4D, AURORA, SHARP) have all failed to demonstrate a mortality benefit[10,15]. A Cochrane meta-analysis of 18 cholesterol-lowering trials (some with statins) in patients with peripheral arterial disease also failed to demonstrate a mortality benefit[16]. A separate meta-analysis of 11 statin trials for high-risk primary prevention similarly failed to demonstrate a mortality benefit[17]. Another Cochrane meta-analysis of statin usage after acute coronary syndromes concluded there was no mortality benefit[18]. The Cholesterol Treatment Trialists (CTT) performed a meta-analysis of 27 statin trials and concluded that statins were clearly beneficial in reducing cardiovascular events[19]. However, when the same 27 trials were assessed for mortality outcomes, no benefit was seen[20]. The coronary calcium score is considered to be one of the best predictors of cardiovascular risk, yet the St. Francis Heart Study showed no clinical benefit in asymptomatic patients with coronary calcium scores > 80th percentile randomized to statin therapy[21]. Finally,

diabetes mellitus is considered a CHD risk equivalent, but the three randomized controlled trials specifically designed and powered to assess the effect of statins in diabetes all failed to demonstrate a mortality benefit (CARDS, 4D, ASPEN)[22-24].

ALTERNATIVE THEORIES OF ATHEROSCLEROSIS AND CHD COMPLICATIONS ARE CHOLESTEROL INDEPENDENT

The dramatic benefits of the Mediterranean diet are likely due to multiple mechanisms which do not directly involve cholesterol. Independent of cholesterol metabolism are the true fatal complications of coronary atherosclerosis - thrombotic coronary occlusion, acute myocardial ischemia, left ventricular dysfunction, and malignant arrhythmias. The hemostatic system appears to be a principal modulator of atherosclerotic plaque formation and progression and the Mediterranean diet can favorably alter elements of the coagulation cascade[25,26]. Plaque rupture and intra-plaque hemorrhage leads to progressive atherosclerosis, thrombosis causes acute coronary syndromes, and sudden cardiac death is the main cause of cardiac mortality. At the genetic level large scale, genome-wide association studies have identified 46 loci directly linked to CHD, yet a majority of these loci have no apparent relation to cholesterol or traditional risk factors[27]. Although we can't change our genes, epigenetic studies have shown that the Mediterranean diet can favorably alter the expression of atherogenic genes[28], whereas a recent cholesterol-lowering statin trial failed to demonstrate a similar effect[29]. At the cellular level we now know that atherosclerosis is an inflammatory disease where macrophages and T lymphocytes likely play a dominant role. Whether or not specific anti-inflammatory therapies will be successful remains to be determined, but prior experience with Vioxx and Celebrex, which unexpectedly increased cardiovascular deaths, emphasizes the importance of proceeding cautiously. Recent studies have demonstrated that the Mediterranean diet can reduce markers of inflammation[26]. Accumulating evidence also implicates sugar in the pathogenesis of atherosclerosis. Diabetes is considered a coronary artery disease equivalent yet diabetics typically have average cholesterol levels. Other studies indicate that those who drink sugar-sweetened beverages are at much higher risk for CHD[30]. How elevated levels of blood glucose lead to atherosclerosis and why cholesterol lowering statins increase the risk of diabetes remains enigmatic, yet the totality of evidence suggests molecular mechanisms of atherosclerosis that are independent of cholesterol metabolism. The Mediterranean diet has been shown to reduce the risk of developing diabetes and the metabolic syndrome[31,32]. Elegant research into the gut microbiota is also providing an alternative theory of atherosclerosis[33]. Consider that L-carnitine, a component of red meat, is metabolized by the gut microbiota into trimethylamine oxide (TMAO). TMAO, in turn, promotes atherosclerosis and has been associated with a higher risk of cardiovascular events independent of traditional risk factors such as cholesterol. The gut microbiota can also adapt to changes in diet, which may explain why some vegans do not produce any TMAO after an L-carnitine challenge and how the Mediterranean diet may exert its anti-inflammatory and anti-atherosclerotic effects[34].

STATIN DRUGS HAVE UNINTENDED CONSEQUENCES

If statins have failed to consistently reduce mortality one must ask if statins improve the quality of life. Serious or fatal statin adverse events are rare, but side effects are not. The incidence of muscular aches and weakness in statin trials is highly variable, and real world experiences may differ from clinical trial reports. Consider that the adherence rates for statins in the elderly are poor with nearly 75% of primary prevention patients stopping the drug within the first two years[35]. More recently a cohort study of statin users reported a 53% discontinuation rate although a very high percentage were able to continue statin therapy after being rechallenged[36]. In the largest statin survey ever conducted, the National Lipid Association observed that roughly 30% of statin patients reported experiencing muscle pain and weakness and 57% of surveyed patients reported stopping the drug due to side effects[37]. One may debate the relationship of statins to diabetes and dementia, but the fact remains that the FDA now requires disclosure of these warnings. Most distressing is the recent report of gluttonous behavior among statin users who mistakenly believe they are "protected" by taking statins and can eat whatever they want[38].

CONCLUSION

The debate over the cholesterol hypothesis and statins has raged for decades. Some may point to the recent decline in cardiovascular deaths in the United States as proof of statin effectiveness, but this view fails to incorporate the impact of smoking cessation, lifestyle changes, and dramatic improvements in heart attack survival rates due to timely reperfusion and the availability of external and implantable defibrillators. Others may argue that statins are started too late in life to be effective (the horse may already be out of the barn) and reference Mendelian randomization studies which show that rare individuals with genetically low cholesterol levels have a much lower incidence of CHD[39].

However, this concept should not be extrapolated to the 99.99% of us who lack these genes and also fails to explain how the Mediterranean diet reduces mortality within months of initiation[2-4]. In 1996 Nobel laureates Brown and Goldstein anticipated the eradication of coronary disease in their *Science* editorial, "Exploitation of recent breakthroughs - proof of the cholesterol hypothesis, discovery of effective drugs, and better definition of genetic susceptibility factors - may well end coronary disease as a major public health problem early in the next century"[40]. History has proven otherwise, and the global prevalence of CHD, despite worldwide statin usage and cholesterol lowering campaigns, has reached pandemic proportions. Coronary heart disease is an extremely complex malady and the expectation that it could be prevented or eliminated by simply reducing cholesterol appears unfounded. After twenty years we should concede the anomalies of the cholesterol hypothesis and refocus our efforts on the proven benefits of a healthy lifestyle incorporating a Mediterranean diet to prevent CHD[2-4,41,42].

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MINIREVIEWS

Cardiac involvement in Duchenne and Becker muscular dystrophy

Sophie Mavrogeni, George Markousis-Mavrogenis, Antigoni Papavasiliou, Genovefa Kolovou

Sophie Mavrogeni, George Markousis-Mavrogenis, Genovefa Kolovou, Cardiology Department, Onassis Cardiac Surgery Center, 17674 Athens, Greece

Antigoni Papavasiliou, Department Pediatric Neurology, Pentelis Children Hospital, 15236 Athens, Greece

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Correspondence to: Sophie Mavrogeni, MD, FESC, Cardiology Department, Onassis Cardiac Surgery Center, 50 Esperou Street, 175-61 P Faliro, 17674 Athens, Greece. soma13@otenet.gr
Telephone: +30-210-9882797
Fax: +30-210-9882797

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Abstract

Duchenne and Becker muscular dystrophy (DMD/BMD) are X-linked muscular diseases responsible for over 80% of all muscular dystrophies. Cardiac disease is a common manifestation, not necessarily related to the degree of skeletal myopathy; it may be the predominant manifestation with or without any other evidence of muscular disease. Death is usually due to ventricular dysfunction, heart block or malignant arrhythmias. Not only DMD/BMD patients, but also female carriers may present cardiac involvement. Clinically overt heart failure in dystrophinopathies may be delayed or absent, due to relative physical inactivity. The commonest electrocardiographic findings include conduction defects, arrhythmias (supraventricular or ventricular), hypertrophy and evidence of myocardial necrosis. Echocardiography can assess a marked variability of left ventricular dysfunction, independently of age of onset or mutation groups. Cardiovascular magnetic resonance (CMR) has documented a pattern of epicardial fibrosis in both dystrophinopathies' patients and carriers that can be observed even if overt muscular disease is absent. Recently, new CMR techniques, such as postcontrast myocardial T1 mapping, have been used in Duchenne muscular dystrophy to detect diffuse myocardial fibrosis. A combined approach using clinical assessment and CMR evaluation may motivate early cardioprotective treatment in both patients and asymptomatic carriers and delay the development of serious cardiac complications.

Key words: Muscular dystrophies; Electrocardiography; Heart failure; Echocardiography; Cardiovascular magnetic resonance imaging

Core tip: Duchenne and Becker muscular dystrophy are the commonest X-linked muscular diseases. Death is usually due to cardiac disease including ventricular dysfunction, heart block or malignant arrhythmias. Female carriers may also present cardiac involvement. Overt heart failure may be delayed or absent. Electrocardiography findings include conduction defects, arrhythmias and myocardial necrosis. Echocardiography assesses a marked

variability of left ventricular dysfunction. Epicardial fibrosis in both patients and carriers has been documented by Cardiovascular Magnetic Resonance (CMR), even if overt muscular disease is absent. A combined approach using clinical and CMR assessment may motivate early cardioprotective treatment and delay serious cardiac complications.

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INTRODUCTION

Duchenne and Becker muscular dystrophy (DMD/BMD) includes a group of X-linked muscular diseases responsible for over 80% of all cases of muscular dystrophy[1]. The incidence of DMD is 1 in 3500 male newborns with a prevalence of 6 in 100000 males[1] and is characterized by weakness of leg, pelvic and shoulder girdle muscles starting in early childhood. BMD is a milder variant of dystro-phinopathy with a better prognosis. The incidence of BMD is 1 in 18450 males and prevalence 2.4 per 100000 in the general population[2,3]. First symptoms appear between ages of 3-21 years with a mean age of onset at 11 years. Age at death is at 21-89 years with an average age of about 45 years[4-7].

In DMD, boys are diagnosed as toddlers and most are wheelchair bound by age 15. Death usually occurs at the age of 20 years, due to respiratory complications or cardiomyopathy. Currently more patients survive until the age of 30 years, due to home ventilation and corticosteroids, which can prolong ambulation by 2-3 years, reduce risk of scoliosis and postpone pulmonary and heart failure after the age of 20 years[1]. Despite this documented efficacy, more than 25% of DMD boys are not treated with corticosteroids, either due to side-effects or lack of response[1]. In BMD, the disease is milder and more heterogenous, compared to DMD. Muscle weakness often is first noticed in adolescence or young adulthood. Cardiac involvement in BMD may precede the skeletal muscle decline, with death due to cardiomyopathy often occurring before the age 60 years[1].

Mutations leading in the absence of a functional dystrophin protein cause DMD, whereas mutations leading in a reduced amount or shortened dystrophin protein cause BMD[8,9]. Dystrophin is a large (427 kDa) subsarcolemmal protein that represents a physical link between the intracellular actin cytoskeleton and the extracellular matrix[10]. Loss or abnormal dystrophin destabilizes the sarcolemma, making the muscle fibers susceptible to contraction injury[11]. The repeated episodes of necrosis followed by regeneration finally lead to replacement of muscles by fat and connective tissue that is clinically manifested as progressive muscle weakness[10]. Dystrophin is also a scaffold protein that localizes other proteins to the sarcolemma and forms a highly organized multimeric dystrophin-associated glycoprotein complex (DGC)[10]. Dystrophin deficiency disrupts the DGC, resulting in downregulation and/or mislocalization of the dystrophin-associated proteins.

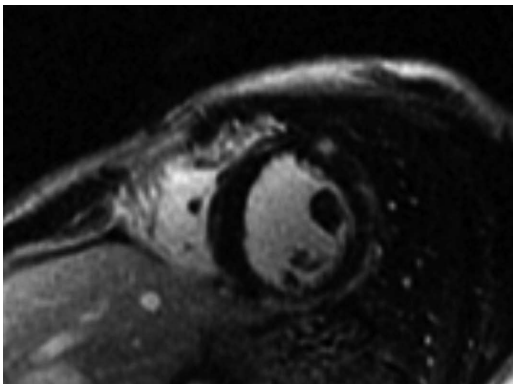

Figure 1 Fibrosis of the left ventricle in ecker muscular dystrophy patient, presented as late gadolinium enhancement in the inferolateral wall of left ventricular.

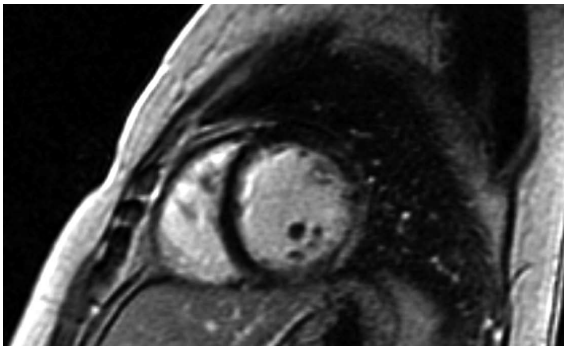

Figure 2 Fibrosis of the left ventricle in a mother Duchenne muscular dystrophy carrier, presented as late gadolinium enhancement in the lateral wall of left ventricular.

CARDIAC DISEASE IN DMD/BMD

Cardiac disease in DMD is progressive and finally leads to ventricular dysfunction, usually accompanied by ventricular dilation[12]. Pathology examination during the late stages of the disease shows cardiomyocytes' hypertrophy, atrophy and fibrosis[13-15]. Fibrosis of the left ventricle in DMD, BMD and DMD/BMD carriers has been observed at autopsy[13-15] and after evaluation with cardiovascular magnetic resonance (CMR) using late gadolinium enhancement (LGE)[16-19] (Figures 1 and 2).

The majority of DMD after the third decade of their age have established cardiomyopathy[20]. Although clinically overt heart failure may be delayed or absent, due to relative physical inactivity, cardiomyopathy is

the leading cause of death in DMD and myocardial damage precedes decline in left ventricular systolic function. Neither the age of onset nor the severity of cardiomyopathy was correlated with the type of mutation[21]. It was recently documented that in DMD with pre-served ejection fraction, the addition of eplerenone to background ACE inhibitors or ARB attenuates the progressive decline in left ventricular systolic function[22].

Cardiomyopathy is the main clinical complication in patients affected by subclinical or mild BMD. The clinical presentation is usually characterized by early right ventricular dysfunction and is later associated with left ventricular impairment. In mild BMD, myocardial damage may develop because the patients, who are unaware of a possible cardiac disease, can still perform strenuous muscle exercise and, through pressure or volume overload, may induce mechanical stress, which is harmful for dystrophin-deficient myocardial cells[23].

Cardiac disease in female carriers of dystrophinopathies may present with hypertrophy, arrhythmias or dilated cardiomyopathy[24]. The percentage of clinically overt cardiac involvement increases significantly with age, from 15% in carriers < 16 years to 45% in carriers > 16 years. By contrast, significant cardiac disease is unlikely in female carriers < 16 years[25]. In a cross-sectional study of 85 DMD and 44 BMD carriers aged 18-58 years, left ventricular dilatation and dilated cardiomyopathy were assessed in 18% and 8%, respectively[26]. Electrocardiography (ECG) abnormalities were found only in 47% of this population[27]. Another series of 56 adult, female carriers did not present any ECG abnormalities, but ventricular dilatation or hypertrophy was documented in 14% and dilated cardiomyopathy in 7% of them[28]. Nevertheless, severe heart failure may develop in some women necessitating heart transplantation to survive[29,30]. Exercise may unmask left ventricular (LV) systolic dysfunction in female carriers[31]. In a study by our group, CMR documented myocardial fibrosis in the majority of DMD and BMD mother-carriers, although the clinical presentation and the usual noninvasive assessment were mildly abnormal[19]. Therefore, detailed cardiac evaluation, at least once after the teenage years, should be recommended in all female carriers in order to start early cardiac treatment[32].

DMD is associated with increased R/S ratio in the right precordial leads, deep Q waves in the lateral leads, conduction abnormalities and arrhythmias (mainly supraventricular but also ventricular). In a study of 131 DMD, ECG was abnormal in 78.6%. All were in sinus rhythm and the following percentages were found for the main variables studied: short PR interval = 18.3%; abnormal R waves in V1 = 29.7%; abnormal Q waves in V6 = 21.3%; abnormal ventricular repolarization = 54.9%; abnormal QS waves in inferior and/or upper lateral wall = 37.4%; conduction disturbances in right bundle branch = 55.7%; prolonged QTc = 35.8% and wide QRS = 23.6%[33]. According to the study by Petri *et al*[34], ECG abnormalities were non-progressive in BMD and asymptomatic SVT and NSVT were present in 21% and 14%, respectively. Both ECG and Holter monitoring are necessary for DMD/BMD assessment. Serial clinical evaluation, including routine monitoring of electrocardiograms may detect early cardiomyopathy in DMD/BMD, even if left ventricular function is still preserved[35].

Echocardiography has already documented marked differences in LV function of DMD patients, independently of age of onset or mutation groups[21]. It has also proved a high prevalence of LV dysfunction in DMD, with frequent evidence of systolic ventricular asynchrony, particularly in patients with EF < 35%[36]. New echocardiographic techniques, using transmural strain profile (TMSP), can detect subclinical LV dysfunction in patients with DMD without wall motion abnormalities by conventional echocardiography[37]. The application of myocardial strain imaging in DMD patients was characterized by decreased peak systolic strain of the posterior wall, despite normal standard echocardiographic findings[38]. However, these studies were not universally accepted for the routine assessment of DMD/BMD.

Cardiovascular magnetic resonance (CMR) a non-invasive, non-radiating technique has been proved the most robust tool for detection of early myocardial fibrosis in DMD, BMD and female carriers, using late gadolinium enhancement (LGE). The pathology of cardiomyopathy in dystrophinopathies includes the presence of subepicardial fibrosis in the inferolateral wall[39], similar to that observed in viral myocarditis. The application of CMR in DMD/BMD and female carriers, in addition to the standard monitoring is of great value because: (1) Early start of heart failure treatment may delay the progression of LV dysfunction[22,40]; (2) Myocardial fibrosis, assessed by LGE, may be observed, even if the echocardiographic evaluation remains normal[16-18,40] and can potentially be used as an early sensitive index to start cardioprotective treatment; (3) It can be also applied as a screening tool to detect patients at high risk for ventricular arrhythmias, more advanced disease, adverse LV remodelling and death[41]. An impaired LV systolic function (LV-EF $\leqslant$ 45%) and a "transmural" pattern of myocardial fibrosis independently predict the occurrence of adverse cardiac events in DMD/BMD patients. Even in DMD/BMD patients with relatively preserved LV-EF (> 45%), the simple and visually assessable parameter "transmural LGE" is of additive prognostic value[42]; (4) in mutation carriers, CMR revealed a pattern of fibrosis similar to that observed in DMD[36], but without any correlation with genotype-phenotype[43], even in the absence of overt muscular disease; and (5) new CMR techniques, such as postcontrast myocardial T1 mapping, have been used in DMD to detect diffuse myocardial fibrosis. It was documented that post-contrast T1 obtained from the Look-Locker sequences (T1LL) ratio is abnormally shortened in DMD compared

with controls, even in DMD patients with otherwise normal CMR study. It is assumed that the application of more aggressive therapy for DMD with shorter T1LL may improve morbidity and mortality in DMD cardiomyopathy[44].

CONCLUSION

To conclude, heart involvement is common in both DMD/BMD and female carriers. Serial cardiac evaluation, including clinical examination, ECG, Holter monitoring, echocardiographic and CMR study, is the "sine qua non" for this population. Early detection of heart involvement should motivate early cardiac treatment with ACE inhibitors and b-blockers to delay serious cardiac complications.

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ORIGINAL ARTICLE

Basic Study

Feasibility of real-time magnetic resonance imaging-guided endomyocardial biopsies: An *in-vitro* study

Dirk Lossnitzer, Sebastian A Seitz, Birgit Krautz, Bernhard Schnackenburg, Florian André, Grigorios Korosoglou, Hugo A Katus, Henning Steen

Dirk Lossnitzer, Sebastian A Seitz, Birgit Krautz, Florian André, Grigorios Korosoglou, Hugo A Katus, Henning Steen, Department of Cardiology, University Hospital Heidelberg, 69120 Heidelberg, Germany

Bernhard Schnackenburg, Philips Medical Systems, 20099 Hamburg, Germany

Author contributions: Lossnitzer D, Seitz SA, Krautz B and André F performed the research; Lossnitzer D, Seitz SA and Steen H designed the research and wrote the paper; Schnackenburg B, Korosoglou G and Katus HA contributed to the new analytic tools and reviewing the paper.

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Institutional review board statement: The study was not reviewed by the University of Heidelberg Institutional Review Board since there were no patients enrolled and only phantoms and explanted pig hearts which were bought by a regional butcher were used.

Institutional animal care and use committee: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Regierungspräsidium Karlsruhe Case Number 35-9185.81/G-79/12.

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Data sharing statement: No additional data are available.

Correspondence to: Dirk Lossnitzer, MD, Department of Cardiology, University Hospital Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany. dirk.lossnitzer@medma.uni-heidelberg.de
Telephone: +49-6221-650412
Fax: +49-3212-1067858

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Abstract

AIM: To investigate if magnetic resonance (MR)-guided biopsy can improve the performance and safety of such procedures.

METHODS: A novel MR-compatible bioptome was evaluated in a series of *in-vitro* experiments in a 1.5T magnetic resonance imaging (MRI) system. The bioptome was inserted into explanted porcine and bovine hearts under real-time MR-guidance employing a steady state free precession sequence. The artifact produced by the metal element at the tip and the signal voids caused by the bioptome were visually tracked for navigation and allowed its constant and precise localization.

RESULTS: Cardiac structural elements and the target regions for the biopsy were clearly visible. Our method allowed a significantly better spatial visualization of the bioptoms tip compared to conventional X-ray guidance. The specific device design of the bioptome avoided inducible currents and therefore subsequent heating. The novel MR-compatible bioptome provided a superior cardiovascular magnetic resonance (imaging) soft-tissue visualization for MR-guided myocardial

biopsies. Not at least the use of MRI guidance for endomyocardial biopsies completely avoided radiation exposure for both patients and interventionalists.

CONCLUSION: MRI-guided endomyocardial biopsies provide a better than conventional X-ray guided navigation and could therefore improve the specificity and reproducibility of cardiac biopsies in future studies.

Key words: Endomyocardial biopsy; Cardiovascular magnetic resonance (imaging); Magnetic resonance imaging-guided interventions; Real-time imaging

Core tip: Myocardial biopsy is the method of choice for assessing tissue pathologies. Cardiac magnetic resonance imaging (MRI) provides a 3D visualization and discrimination of soft-tissue and could therefore enable a targeted specimen sampling. We developed a novel MR-compatible bioptome which was evaluated by *in-vitro* experiments in a 1.5T MRI system under real-time MR-guidance. MRI-guided endomyocardial biopsies provide a superior soft-tissue visualization, a better than conventional X-ray guided navigation and could therefore improve the specificity and reproducibility of cardiac biopsies in future studies. Not at least the use of MRI guidance for endomyocardial biopsies completely avoided radiation exposure for both patients and interventionalists.

Lossnitzer D, Seitz SA, Krautz B, Schnackenburg B, André F, Korosoglou G, Katus HA, Steen H. Feasibility of real-time magnetic resonance imaging-guided endomyocardial biopsies: An *in-vitro* study. *World J Cardiol* 2015; 7(7): 415-422 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i7/415.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i7.415

INTRODUCTION

Endomyocardial biopsy (EMB) is the "gold" standard diagnostic tool in the detection and classification of myocardial pathologies. It is recommended in the management and diagnosis of cardiomyopathies and inflammatory myocardial diseases[1-4].

Despite the benefits, EMB indications and their procedural management are controversially discussed. Reasons include EMB's low sensitivity when conducted under fluoroscopic guidance[5,6] as well as its potential complications. Thus, EMB may cause pericardial tamponade, severe arrhythmias or structural damages of the tricuspid valve[1]. Since sensitivity of EMB is low, clinicians are forced to increase the amount of samples (> 6, according to the AHA/ACCF/ESC scientific statement[1]) while focal pathologies like fibrosis in different forms of myocarditis may remain undetected.

In the clinical routine, fluoroscopy is used to guide the EMB procedure, offering a high frame rate (⩽ 30 fps) and high spatial resolution (2-3 line pairs/mm). However, this technique provides only two-dimensional projection images containing overlays of all anatomic structures in the X-ray beam in combination with a low soft-tissue contrast. Furthermore, it exposes both patient and interventionalist to a substantial radiation burden[7].

Cardiac magnetic resonance imaging (CMR) is a non-invasive modality which offers superior soft-tissue contrast, enables tissue characterization and allows the capture of specific slices with any required spatial orientations. Furthermore, contrast-enhanced CMR itself is able to visualize distinct pathologies like myocardial fibrosis, necrosis or inflammation and additionally provides macroscopic information, which may be complementary to that acquired by EMB[8]. Consequently, targeted myocardial biopsies under CMR guidance could reduce the number of required samples, increase specificity and sensitivity of the retrieved tissue samples and obviate radiation exposure. However, due to the very high static magnetic and radiofrequency (RF) electromagnetic fields, conventional metal bioptomes cannot be used in the MRI environment. First, the RF signals induce significant heating, mainly at the tip of metal wires leading to the necrosis of tissue[9,10], and secondly, metal devices lead to massive CMR image disturbance that could impair visualization of target areas. However, such problems can be overcome by MRI-compatible needles, which are already in use for real-time MR-guided tissue biopsies in static organs like breast, brain, liver, kidney or the prostate[11-14].

The localization and navigation of an invasive interventional instrument in the MRI system can be achieved with either active or passive tracking. While active tracking is more precise and faster since the bioptome's position is always known, it requires substantial modifications and cost-intensive miniaturized electrical extensions within the instrument. Conversely, passive tracking requires only minor modifications of the device to assure an appropriate visualization because it is implemented solely on the MRI system or additional computer systems and uses only the acquired real-time images to determine the instrument's position. This can be carried out manually by the operator, possibly supported by a software solution, but it requires a significant amount of operator training.

Therefore, we sought to develop and assess the feasibility of a novel MR-compatible bioptome for passive tracking in an *in-vitro* model to address these technical issues. Subsequently, we applied this method and performed endomyocardial biopsies in explanted animal heart models.

Table 1 Magnetic resonance imaging imaging sequences used during the real-time guidance procedures

Name	Type	SAR (W/kg)	Frame rate (fps)	Flip angle (degrees)	TR/TE (ms)	Resolution (mm)
Interactive	Balanced SSFP	0.765	8	45	3.10/1.55	1.79 × 1.79 × 6

SSFP: Steady state free precession.

MATERIALS AND METHODS

MRI system

All experiments were carried out in a cylindrical 1.5T MRI system (Achieva 1.5T, Philips Medical Systems, Best, The Netherlands) using the built-in birdcage coils for excitation and a cardiac 32-channel receive coil. The images were obtained with standard real-time sequences which provides continuous scanning and tracking of the bioptome's position in every possible angulation of three imaging planes within space (Table 1).

Bioptome

The major safety concern when using conventional metallic instruments that have dimensions in the range of the RF field's wave length is the possibility of tissue heating. The B_0-field of a 1.5T system correlates with a RF frequency of 64 MHz that corresponds then to a wavelength λ_{Air} of approx. 0.78 m in saline (0.9% NaCl), which is comparable to human body conditions. Therefore, characteristic RF wave lengths like $\lambda/2$ and $\lambda/4$ are well in the range of an outstretched bioptome, which could then lead to significant heating at the forceps of conventional endomyocardial bioptomes.

The shaft and the tip of the distal end of bioptome were constructed by using non ferromagnetic metals, synthetics and ceramic. The mechanical properties of the sample extraction mechanism at the tip of the MR bioptome were identical to a conventional device. It consisted of two spoon-shaped parts with sharp edges that could be pressed together and opened with a handle at the grip part. The spoons were coated with an MRI-visible marker to help determining the opening state. The device was engineered in close collaboration with H. + H. Maslanka GmbH, Tuttlingen, Germany.

The MR bioptome in the tested version did not have the capability of deflecting the tip for navigation purposes, which was compensated with a separate deflectable sheath (see below).

Deflectable sheath

The sheath prototype we used was a guiding catheter developed for electrophysiology applications and provided by Imricor (Burnsville, MN). It had the ability to deflect and steer the MR bioptome during the procedure and was fully MR-compatible causing neither heating nor disturbing imaging artifacts[15]. It was open at the distal end and a port prevented the outflow of blood at the proximal side. Its inner lumen provided a tight fit of the MR bioptome, effectively preventing a reverse flow of blood. The level of deflection (up to 150 degrees) at the tip could be adjusted and maintained in two directions with a mechanism at the grip. The diameter was approximately 3.7 mm.

Tracking and visualization of the instrument

In this study, we focused on a passive tracking approach with MRI visible markers at the spoons and the distal end of the device but without the need of an additional coil at the bioptome's tip and electronics inside the bioptome to evaluate the possibility of an affordable, easily available biopsy system as established in conventional EMB while benefiting from the imaging capabilities of MRI.

The necessary procedural adjustments of the corresponding image plane were conducted by the technician at the console while an MRI in-room monitor allowed the interventionalist to instruct the technician and navigate the bioptome. The MRI-control software provided an interface for real-time image visualization and parameter manipulation to allow a real time tracking of the bioptome within the phantom or heart.

Heart model

All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Regierungspräsidium Karlsruhe Case Number 35-9185.81/G-79/12.

The most beneficial aspect of the MR-guided myocardial biopsy was the targeted and specific retrieval of tissue samples. To reproduce the complex anatomical structures present in a human heart, explanted porcine and bovine hearts were employed. With their anatomical properties being comparable to human hearts, they provided an appropriate test environment to evaluate the navigation features and the new MR biopsy system.

When a catheter is pushed forward in a living heart, crossing the valve plane can normally be achieved in ventricular diastole (right ventricular biopsy) or systole (left ventricular biopsy). In an explanted, *ex-vivo* heart, the valves are static and might permanently obstruct the anatomic path of the instrument. Since their presence was not of primary concern for the actual experiment, the valve cups were removed during the preparation of the models.

A transparent plastic tube was attached at the orifice of the superior vena cava (SVC) into the right atrium (ostium venae cavae superioris) to mimic the venous vasculature normally guiding the instrument *in-vivo* into the right atrium and ventricle (Figure 1). The blood flow was not simulated.

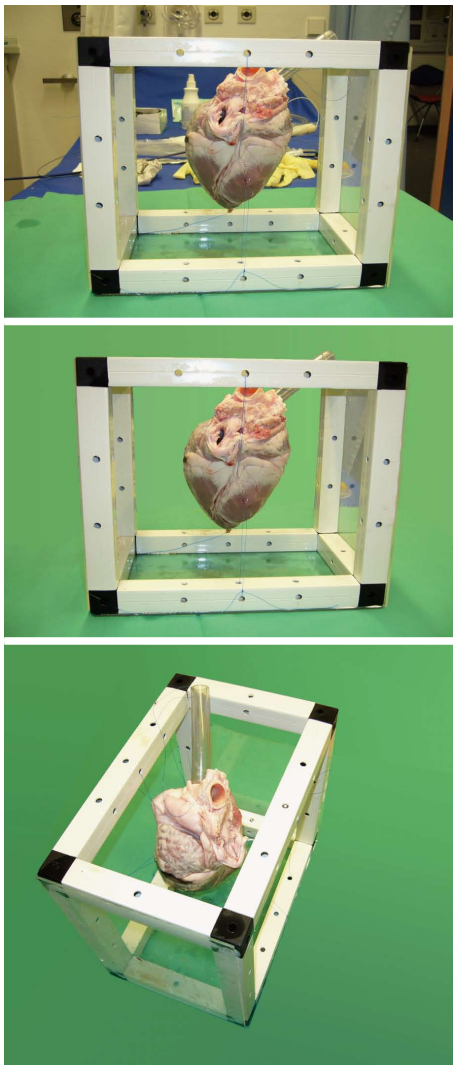

Figure 1 Model of swine heart with plastic tube attached to the trunk of the vena cava. The heart is fixated in a plastic frame to maintain form and position when submerged into the saline filled phantom.

To be able to evaluate the success of the biopsy later, several target areas of the endocardium were marked with an injection of a mixture of india ink and gadolinium. The first served as a visual marker in the specimen, the latter mimicked the elevated levels of gadolinium deposition in scar or fibrotic tissue areas eligible for an EMB. In the employed interactive sequences, a gadolinium marked area would appear as slightly brighter spot compared to the surrounding tissue.

Monitoring of heating

Due to the design and material composition of the MR bioptome, a heating problem was not expected. Nevertheless, the temperature was constantly monitored with a fiber optic-based thermometer (Fotemp, Optocon, Dresden, Germany) during the first experiment. One sensor was placed in the vicinity of the tip of the MR bioptome[16,17], the second recorded the overall temperature of the phantom filling.

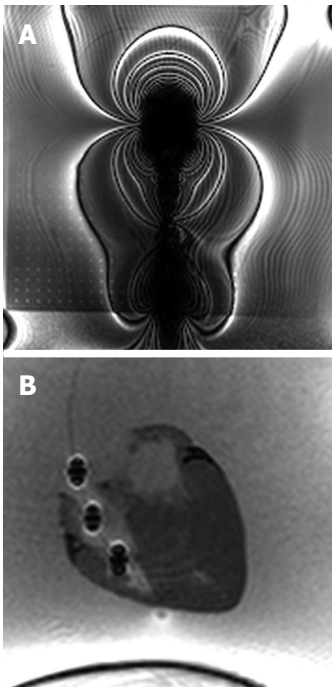

Figure 2 Cardiac magnetic resonance imaging-Images of conventional bioptome for endomyocardial applications (A), novel bioptome with three metal markers at the distal end inserted into a swine heart model (B).

RESULTS

Safety aspects/heating

During the course of the *in-vitro* experiments, the metal parts of the MR bioptome induced no heating. Only a general heating (< 1 ℃) of the phantom due to the constant exposure to the RF field and the heat dissipated by the MRI system during operation was detected.

Imaging and tracking

The new design of the MR bioptome effectively prevented the device from a negative interaction with the RF fields present during the MRI examination in terms of excessive artifacts. Only the coating of the sample cutting mechanism caused a small artifact at the tip (size approx. 2 mm × 2 mm, Figure 2), but this did not overlay the target structures in the heart (Figure 3). The shaft of the MR bioptome and the sheath caused a signal void compared to the surrounding tissue. At the same time signal voids supported the visual tracking of the tip of the MR bioptome when being pushed forward inside the sheath. The size of the artifact slightly increased when the tip left the isolating sheath and was exposed directly to the saline (Figure 3).

The opening and closing states of the sample cutter were slightly visible on the MRI images (Figures 4 and 5) when the bioptome was not moving. All maneuvers were carried out (1) in a saline (0.9% NaCl) filled but otherwise empty plastic container; and (2) in a porcine

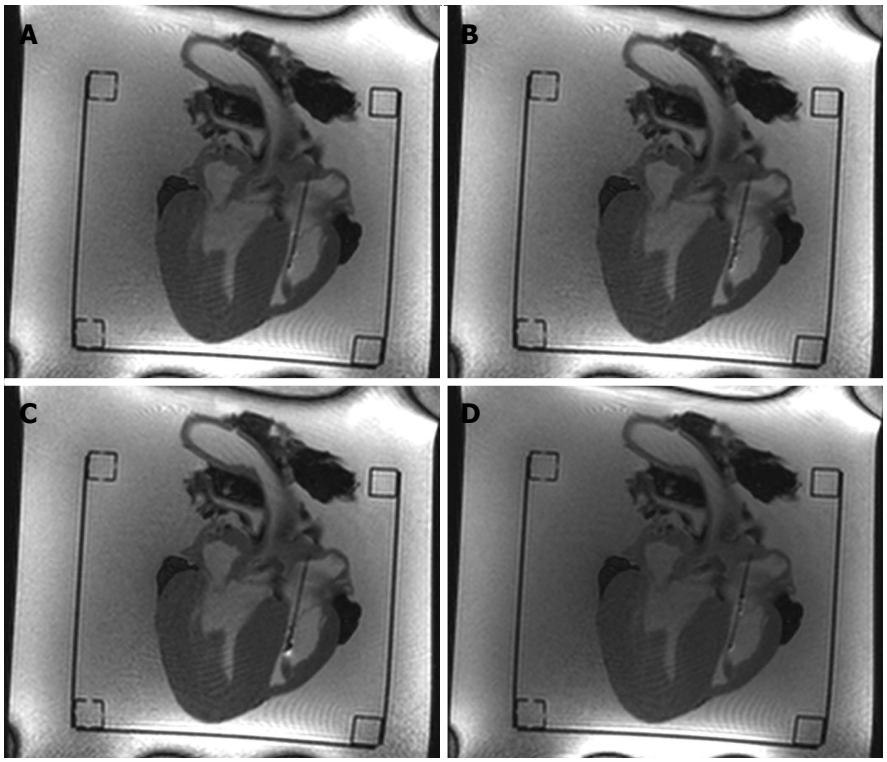

Figure 3 Real-time magnetic resonance image frames showing bioptome inside bovine heart. The bioptome is pushed forward through a plastic tube (A, B), is bare in the heart model (C) and then pulled back (D).

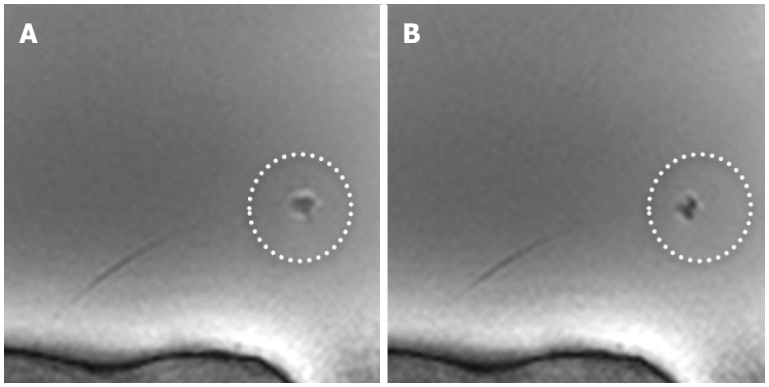

Figure 4 Real-time magnetic resonance imaging image frames with closed (A) and opened (B) cutter.

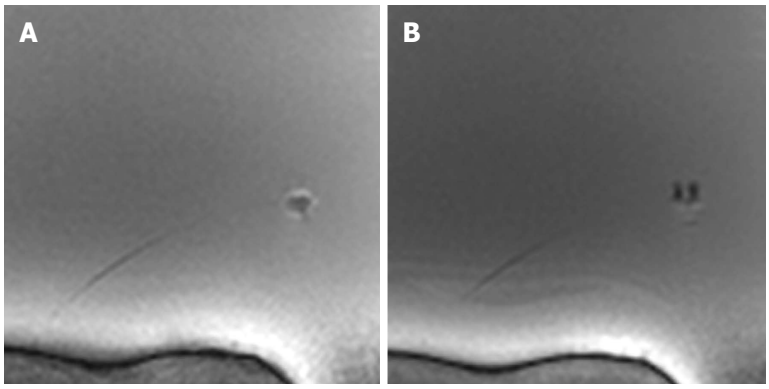

Figure 5 Real-time magnetic resonance imaging image frames with opened (A) and moving (B) tip.

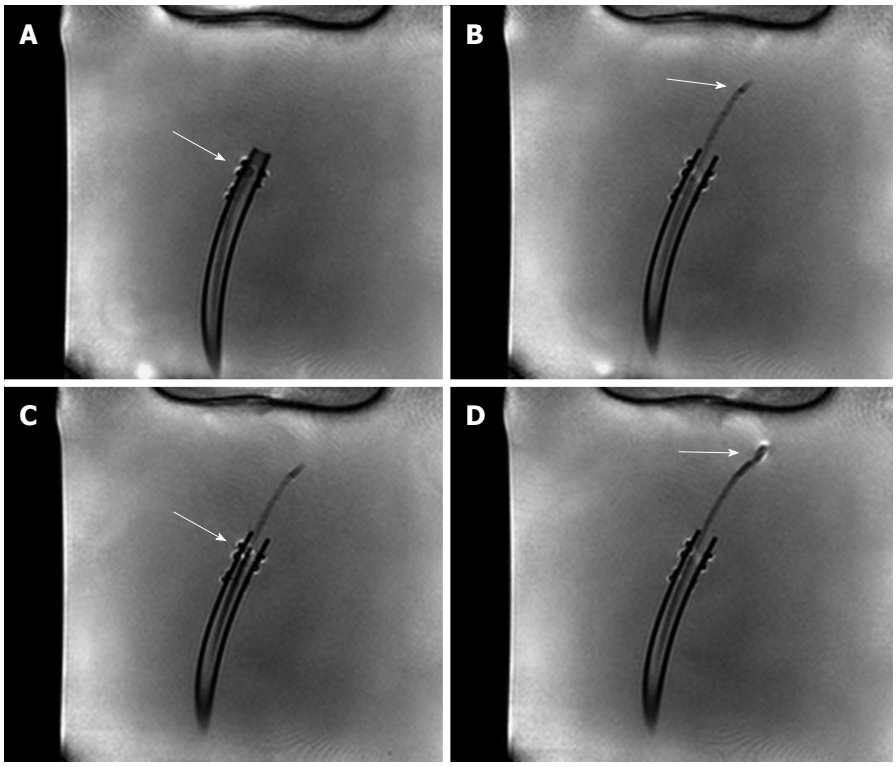

Figure 6 Real-time magnetic resonance imaging image frames showing bioptome and sheath inside saline filled phantom. The empty sheath is pushed forward in a plastic tube (A, B), later the bioptome is guided inside the sheath (C) until the tip is bare in the phantom (D).

heart (Figure 6) submerged in the saline.

The passive, visual tracking of the MR bioptome/sheath system and especially the tip was easily feasible due to the static design of the experiment. As shown in Figure 5, a moving tip caused turbulences in its vicinity which resulted in an artifact similar to the opened sample cutting mechanism. As a consequence, only for a non-moving tip, the opening/closing state could be reliably determined.

The control interface of the scanner supported a quick re-adjustment of the current imaging plane into parallel and orthogonal planes to follow the bioptome's movement. The achieved frame rate was approx. 4 fps for one slice when using an automatic continuous imaging mode. The visualization of orthogonal slices was only manually possible with manual plane re-adjustment causing the frame rate to decrease to approx. 0.3 fps to 0.25 fps.

Handling

In case of a jugular venous insertion, the interventionalist's access to the MR bioptome would be realized from the rear end of the scanner bore. Even the limited inner bore diameter of the MRI system (60 cm) left enough space to maneuver the bioptome. The handling was acceptable, but required a leaned-forward position of the interventionalist that was less comfortable than when carrying out a fluoroscopy.

When an access into the femoral venous system was simulated, the interventionalist stood in front of the CMR system. Here, the handling was better because it allowed a more upright position during the procedure.

Biopsy

The new MR bioptome retrieved tissue samples of appropriate size and quality (Figure 7). As with a conventional X-ray bioptome, the samples were derived from the endocardium and a visual inspection of the lesion area showed no irregular defects of the tissue. The samples were all taken at the targeted areas (Figure 8) and no rupture or substantial damage to the free lateral wall of the right ventricle was induced. Histological examinations of the specimen were abandoned because of the expected tissue degradation due to the time delay of about 12 h between the slaughter of the animals and the actual experiments.

DISCUSSION

To our knowledge, this is the first *in-vitro* study using MR-guided endomyocardial biopsy. The main findings of this study are as follows: (1) The visualization of the anatomical soft-tissue structures inside the heart is superior compared to fluoroscopy and allowed a good localization of the MR bioptome; (2) The biopsies could be performed successfully without any unintended damages to the endomyocardial tissue; and (3) No dangerous heating of the introduced instruments and surrounding environment was observed.

In the study presented here, we could show the feasibility of MRI-guided myocardial biopsies. During

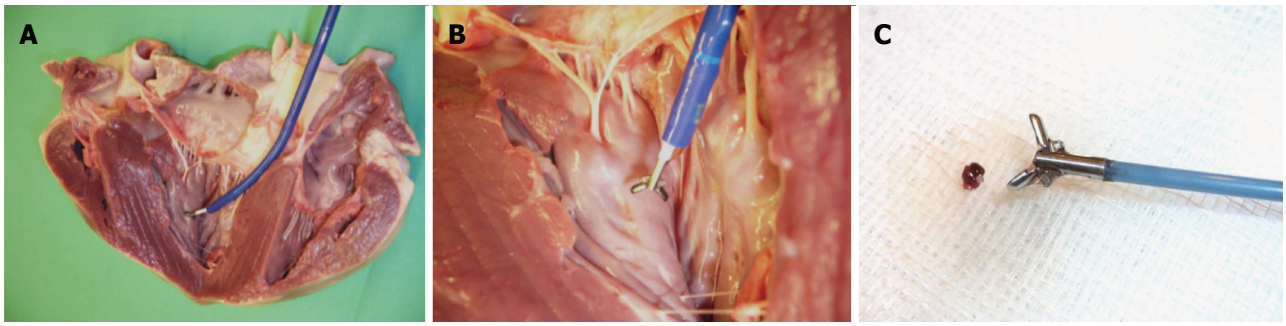

Figure 7 Close-up images of experimental *in-vitro* setup as shown in Figure 1: Opened porcine heart model with magnetic resonance bioptome (white) advanced through sheath (blue) (A), opened magnetic resonance bioptome forceps (B), magnetic resonance bioptome with tissue sample retrieved from endocardium (C).

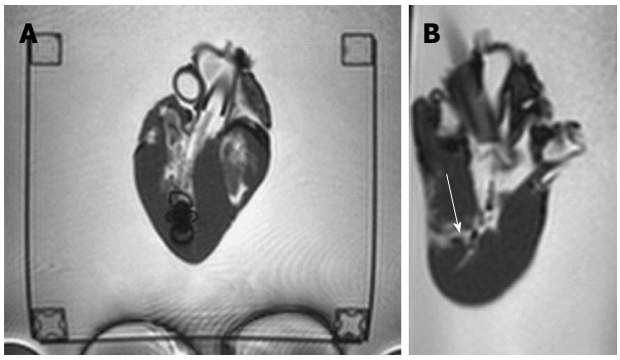

Figure 8 Side by side comparison of initial (A) and improved (B) version of magnetic resonance imaging compatible bioptome. Note the huge artifact in (A). The tip is highlighted in the circle. The targeted region is marked by previous Gadolinium injection (arrow).

the development of the novel device, we were able to reduce the size and the amount of artifacts to a level where no relevant areas in the vicinity of the instrument where obstructed. The visualization by MRI imaging allowed a clear and reliable distinction of the myocardial structures and therefore a safe and precise navigation to the targeted location previously marked with gadolinium injections. This targeted approach can potentially provide a higher sensitivity and specificity of each individual biopsy tissue sample and therefore reduce the number of required biopsy samples as well as the likelihood of complications.

The decision to use a separate bioptome and sheath system, as opposed to an all-in-one design provided substantial advantages. It allowed the maintenance of the position of the sheath while retrieving the individual samples from the myocardium. This could reduce the risk of valvular damage caused by repeated passages for sample acquisitions.

In conclusion, the complete absence of ionizing radiation is an important benefit of MRI based procedures, especially in younger patients with the need of repeated biopsies or multiple interventional procedures, *i.e.*, in cardiac transplant recipients.

Despite the significant advantages of MR-guided compared to fluoroscopically guided endomyocardial biopsies, MRI guidance also provides a number of challenges: (1) An overestimation of the image quality might be caused by the absence of motion artifacts of the static *ex-vivo* heart models. Further *in-vivo* experiments are required to evaluate the imaging capabilities when the heart as well as the instrument are constantly shifting and twisting during a cardiac cycle. Additionally, blood flow artifacts around the instrument could further impair the tracking performance; and (2) Since only one spatial slice is scanned, the operator can lose the tip's location of a passively tracked object when the instrument leaves the actual slice, whereas fluoroscopy with its projected image will always display the instrument as long as it is in the X-ray beam. This issue was successfully compensated by adding multiple passive markers at the distal end of the device. It could be further compensated with an active tracking system.

COMMENTS

Background

The aim of this study was to demonstrate the feasibility of magnetic resonance imaging (MRI)-guided endomyocardial biopsy in an *in-vitro* study. The motivation to use MRI was its superior soft tissue visualization and three-dimensional imaging capabilities when compared to the traditionally used fluoroscopy providing only two-dimensional projection images. Furthermore, the complete absence of ionizing radiation.

Research frontiers

As conventional bioptoms comprise multiple metallic components that prevent their use in an MRI environment. Furthermore, the navigation is more complex in MRI as this modality can only visualize objects in the imaging plane, whereas fluoroscopy creates projection images containing all objects in the X-ray beam.

Innovations and breakthroughs

In this study, a novel and fully MRI compatible instrument was developed. It could be successfully evaluated in a cylindrical 1.5T MRI system using bovine heart models. The bioptome was well visible and allowed precise discrimination from the surrounding tissue.

Applications

The new instrument allowed us to show the feasibility of MRI-guided interventional procedures, enabling radiation free procedures while benefitting from the widely accepted soft-tissue visualization and characterization capabilities of cardiac MRI.

Peer-review

The article under review represents the authors to present an *in-vitro* study to explore the feasibility of real-time MRI-guided endomyocardial biopsies. They found that MRI-guided endomyocardial biopsies provide a better than conventional X-ray guided navigation and could therefore improve the specificity and reproducibility of cardiac biopsies in future studies. The issue is interesting.

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ORIGINAL ARTICLE

Observational Study

Electrocardiographic changes during induced therapeutic hypothermia in comatose survivors after cardiac arrest

Pablo Salinas, Esteban Lopez-de-Sa, Laura Pena-Conde, Ana Viana-Tejedor, Juan Ramon Rey-Blas, Eduardo Armada, Jose Luis Lopez-Sendon

Pablo Salinas, Ana Viana-Tejedor, Instituto Cardiovascular, Hospital Clínico San Carlos, 28040 Madrid, Spain

Esteban Lopez-de-Sa, Laura Pena-Conde, Juan Ramon Rey-Blas, Eduardo Armada, Jose Luis Lopez-Sendon, Acute Cardiac Care Unit, Division of Cardiology, Hospital Universitario La Paz, 28046 Madrid, Spain

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Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at estebanlopezdesa@secardiologia.es. Consent was not obtained but the presented data are anonymized and risk of identification is very low or absent.

Correspondence to: Esteban Lopez-de-Sa, MD, FESC, Head, Acute Cardiac Care Unit, Division of Cardiology, Hospital Universitario La Paz, Paseo de la Castellana 261, 28046 Madrid, Spain. estebanlopezdesa@secardiologia.es
Telephone: +34-917-277199
Fax: +34-917-277135

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Abstract

AIM: To assess the safety of therapeutic hypothermia (TH) concerning arrhythmias we analyzed serial electrocardiograms (ECG) during TH.

METHODS: All patients recovered from a cardiac arrest with Glasgow < 9 at admission were treated with induced mild TH to 32-34 ℃. TH was obtained with cool fluid infusion or a specific intravascular device. Twelve-lead ECG before, during, and after TH, as well as ECG telemetry data was recorded in all patients. From a total of 54 patients admitted with cardiac arrest during the study period, 47 patients had the 3 ECG and telemetry data available. ECG analysis was blinded and performed with manual caliper by two independent cardiologists from blinded copies of original ECG, recorded at 25 mm/s and 10 mm/mV. Coronary care unit staff analyzed ECG telemetry for rhythm disturbances. Variables measured in ECG were rhythm, RR, PR, QT and corrected QT (QTc by Bazett formula, measured in lead v2) intervals, QRS duration, presence of Osborn's J wave and U wave, as

well as ST segment displacement and T wave amplitude in leads Ⅱ, v2 and v5.

RESULTS: Heart rate went down an average of 19 bpm during hypothermia and increased again 16 bpm with rewarming (P < 0.0005, both). There was a non-significant prolongation of the PR interval during TH and a significant decrease with rewarming (P = 0.041). QRS duration significantly prolonged (P = 0.041) with TH and shortened back (P < 0.005) with rewarming. QTc interval presented a mean prolongation of 58 ms (P < 0.005) during TH and a significant shortening with rewarming of 22.2 ms (P = 0.017). Osborn or J wave was found in 21.3% of the patients. New arrhythmias occurred in 38.3% of the patients. Most frequent arrhythmia was non-sustained ventricular tachycardia (19.1%), followed by severe bradycardia or paced rhythm (10.6%), accelerated nodal rhythm (8.5%) and atrial fibrillation (6.4%). No life threatening arrhythmias (sustained ventricular tachycardia, polymorphic ventricular tachycardia or ventricular fibrillation) occurred during TH.

CONCLUSION: A 38.3% of patients had cardiac arrhythmias during TH but without life-threatening arrhythmias. A concern may rise when inducing TH to patients with long QT syndrome.

Key words: Cardiac arrest; Therapeutic hypothermia; Post-cardiac arrest síndrome; Cardiac arrythmias; QT interval

Core tip: Induced, therapeutic hypothermia is a treatment for post-cardiac arrest syndrome with a potential survival benefit; however it is not widely used. We aimed to assess the safety of this therapy regarding cardiac arrhythmias through a systematical evaluation of electrocardiograms (ECG) changes during hypothermia and telemetry data. Our conclusions are that therapeutic hypothermia according to current practice is safe with arrhythmias in one third of the patients (38.3%) but no life-threatening arrhythmias. Bradycardia and reversible prolongation of ECG intervals are common findings. A concern may rise when inducing hypothermia to patients with arrhythmias related to long QT syndrome.

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INTRODUCTION

In 2002, two randomized clinical trials demonstrated that inducing mild therapeutic hypothermia (TH) between 32 ℃ and 34 ℃ Celsius during 12 to 24 h improve survival and neurologic outcome in comatose adults recovered from ventricular fibrillation (VF) cardiac arrest (CA)[1,2]. Thenceforth, the recommendation to induce TH has been extended to non-VF cardiac arrest, and in-hospital CA[3-6]. Today, TH is the only in-hospital treatment that improves survival in comatose patients recovered from a CA[7]. Despite the evidence, TH is still underused nowadays. Some causes have been proposed: technical difficulties, lack of experience with cooling methods, safety concerns and the many gaps on various issues such as optimal target temperature, duration of TH or rewarming rate[8,9].

While there are reports about complications and side effects of hypothermia from more than 50 years ago[10-12], the vast majority of the information on cardiovascular and side effects comes from case reports, accidental deep hypothermia or induced deep hypothermia in cardiac surgery. Known side effects of TH are shivering[13], increased risk of infection[2,14], increased diuresis, electrolyte abnormalities such as hypokalemia, hypophosphatemia and hypomagnesaemia[15], hyperglycemia, coagulopathy with increased risk of bleeding, bradycardia and complex effects in hemodynamics, with small reduction in cardiac output that balances with the decrease of metabolic rate[16]. The randomized clinical trials of TH did not show any differences in arrhythmias between patients assigned to TH or normothermia, but there is paucity of data regarding electrocardiographic abnormalities in humans recovered from a cardiac arrest under controlled mild hypothermia.

In this prospective, observational study we performed a systematic analysis on serial electrocardiograms (ECG) and arrhythmias during TH, in order to describe changes and assess the safety of TH concerning ECG alterations and rhythm disturbances.

MATERIALS AND METHODS

We prospectively collected data about every CA admission in the Coronary Care Unit of a Spanish tertiary hospital during a period of 3 years. TH was performed according to current guidelines to those patients recovered from CA with any initial rhythm and Glasgow ⩽ 8 at admission. Sedation was obtained with midazolam, fentanyl and muscular relaxation with cisatracurium. All drugs adjusted to body weight and administered by intravenous infusion through a central venous line. Patients where cooled to a target temperature of 32 ℃ to 34 ℃, as soon as possible, with cold fluid infusion. TH was maintained with physical measures (ice packs, isolating blankets) during 24 h in the first 20 patients. The rewarming process was passive, withdrawing cooling measures, during 12 to 24 h. In the last 34 patients an intravascular cooling device (Coolgard 3000®, Zoll medical Corp, Chelmsford, MA) was used to induce, maintain (33 ℃ for 24 h) and withdraw TH, set to fastest cooling speed at induction

Table 1 Baseline characteristics of study population

Patients	47
Age (median, range)	65.9 (19-85)
Male	40 (85.1%)
Cardiogenic shock at admission	15 (31.9%)
Urgent coronary angiography	28 (59.6%)
Left ventricular ejection fraction	43.2 (15.3%)
Initial Rhythm, *n* (%)	
Ventricular Fibrillation	30 (63.8)
Asystole	14 (29.8)
Pulseless Electrical Activity	3 (6.4)
Rhythm at admission, *n* (%)	
Sinus rhythm	31 (66)
Atrial fibrillation	8 (17)
AV block/nodal rhythm/paced rhythm	8 (17)
TH protocol	
Temperature at admission	35.7 (0.7)
Induction time (from admission to TH, h)	4.8 (2.6)
Time in TH (median, range, h)	20.8 (5-28.5)
Temperature during TH	32.8 (0.5)
Rewarming time (from TH to 36 ℃, h)	11.3 (7.4)
Cause of CA, *n* (%)	
Acute coronary syndrome	21 (44.7)
Chronic coronary disease[1]	8 (17.0)
Chronic heart failure	4 (8.5)
Others/unknown[2]	14 (29.8)

[1]This group represents those patients with known preexisting coronary disease but without an acute coronary syndrome diagnosis at admission. Presumed cause were ventricular arrhythmias secondary to chronic coronary disease; [2]No final diagnosis of the cardiac arrest could be made for this group, all of these patients died during admission. TH: Therapeutic hypothermia. Data are number (percentage) or mean (standard deviation).

and slow rewarming at a rate of 0.08-0.17 ℃/h, to slowly rewarm the patient in 12-24 h. Core temperature was measured with a Swan-Ganz catheter or urinary catheter.

During TH, all patients were under mechanical ventilation, muscular relaxation and sedation. Inotropics or vasodilators were used if necessary to maintain a target mean arterial pressure of 80-90 mmHg. Patients underwent urgent coronary angiogram (and percutaneous coronary intervention if necessary) if ST elevation acute coronary syndrome (ACS) or clinical indication. Echocardiogram was performed at admission. Complete 12 lead ECG were recorded at admission (ECG A), during peak hypothermia or minimum stable temperature (ECG B) and after rewarming (but before sedation was withdrawn, ECG C). Continuous ECG telemetry was recorded during TH. The ethical board of the hospital approved TH protocol.

For the present study we selected all consecutive patients (n = 54) that underwent TH. Baseline characteristics of the patients, cooling rates and temperatures of TH protocol, clinical outcome data, ECG telemetry data and original ECG were recorded. ECG analysis was blinded and performed with manual caliper by two independent cardiologists from blinded copies of original ECG, recorded at 25 mm/s and 10 mm/mV. Coronary care unit staff analyzed ECG telemetry for rhythm disturbances. Variables measured in ECG were rhythm, RR, PR, QT and corrected QT (QTc by Bazett formula, measured in lead v2) intervals, QRS duration, presence of Osborn's J wave and U wave, as well as ST segment displacement and T wave amplitude in leads II, v2 and v5. Quantitative data was obtained through arithmetical mean of 2 measured values. If there was any discordance in rhythm analysis or categorical variables, a final joint decision was reached with a third cardiologist.

Statistical analysis of measured intervals was performed with paired t-tests for related samples. Statistical significance was considered at $P < 0.05$ (two sided). Continuous variables are represented as means and standard deviation in brackets and categorical variables as percentages. Statistical analysis was performed with SPSS 15 (SPSS Inc, Chicago, IL).

The statistical methods of this study were reviewed by Pablo Salinas, MD, PhD, and bachelor degree in biostatistics.

RESULTS

A total 54 post-CA patients were included in the TH protocol. Of this 54 patients, 7 had one ECG missing (4 of them died before rewarming, 3 had unsatisfactory quality or were missing), therefore a total of 47 patients make the study population. PR interval changes were only considered when the 3 ECG were in sinus rhythm, 29 patients (61.7%).

Baseline characteristics of study population are shown in Table 1. Twenty one percent of the patients were under intraaortic balloon counterpulsation and 10% had a temporary transvenous pacemaker implanted, all of them during coronary angiogram. Two patients (4%) received continuous veno-venous hemofiltration therapy. Three patients (6.4%), already at TH target temperature, required premature protocol termination because of clinical indication, two because of hemodynamical instability and one because of emergent surgery of intraperitoneal hemorrhage, spleen and hepatic lacerations due to traumatic resuscitation. Median hospital stay was 11.5 d, ranging from 2 to 71 d. Mechanical ventilation was maintained for a median of 5.1 d. In-hospital survival rate was 53.2%. Implantable defibrillator was implanted in 23% of survivors.

Comparison of heart rate, QRS duration and RR, PR, and QTc intervals among ECG at admission (ECG A), during hypothermia (ECG B) and in normothermia after rewarming (ECG C) are shown in Table 2 and Figure 1. Changes form ECG A to ECG B were a statistically significant increase in RR interval (decrease of heart rate of 19.5 bpm, $P < 0.0005$); a non-significative prolongation in PR interval; a minor significant prolongation of QRS duration of 9.9 ms, $P = 0.041$; and a significant increase in QTc interval of 57.5 ms ($P < 0.0005$). Changes from ECG B to ECG C were a statistically significant decrease in RR interval (increase of heart rate of 15.9 bpm, $P < 0.0005$); a small

Table 2 Changes in electrocardiograms intervals, represented as means, standard deviation (in brackets) and *P* for difference

	Admission (ECG A)	During MTH (ECG B)	After MTH (ECG C)	*P* for difference (A to B)	*P* for difference (B to C)	*P* for difference (A to C)
RR interval (ms)	653.8 (174.6)	818.1 (222.6)	656.9 (114.4)	< 0.0005[a]	< 0.0005[a]	0.9
Heart rate (bpm)	97.9 (24.9)	78.3 (19.8)	94.2 (17.4)	< 0.0005[a]	< 0.0005[a]	0.3
PR interval (ms)	169.2 (42.7)	179.3 (37.5)	161.2 (37.0)	0.090	0.003[a]	0.2
QRS duration (ms)	108.8 (23.2)	118.7 (37.9)	102.0 (22.9)	0.041[a]	< 0.0005[a]	0.029[a]
QT interval (ms)	353.8 (58.1)	448.1 (106.1)	374.9 (72.0)	< 0.0005[a]	< 0.0005[a]	0.042[a]
QTc interval (ms)	441.7 (50.7)	499.2 (95.5)	463.9 (76.4)	< 0.0005[a]	0.017[a]	0.046[a]

Indicates statistical significance ([a]*P* < 0.05); ms: Milliseconds; ECG: Electrocardiograms.

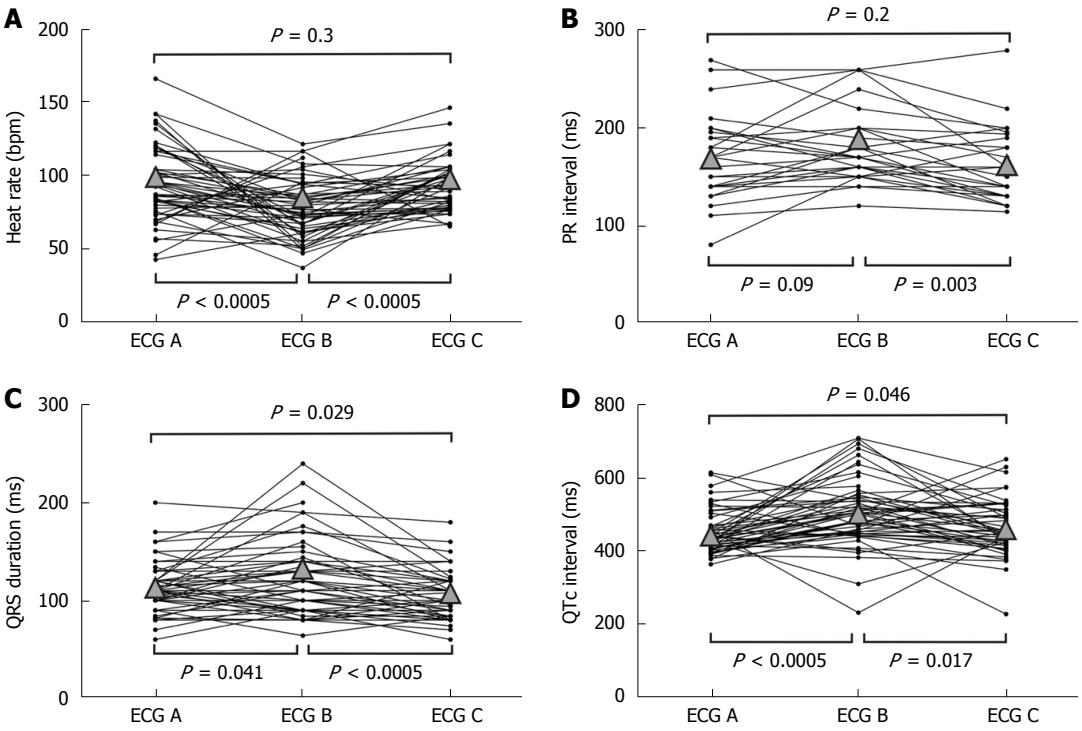

Figure 1 Graphics and statistical significance for paired *t*-test analyses for related samples. A: Heart rate (bpm); B: PR interval (ms); C: QRS duration (ms); D: QTc interval (ms). The dark dots are individual values of each single patient. Triangles represent mean values, shown in Table 2. Electrocardiograms (ECG) A represents ECG at admission; ECG B was performed at peak hypothermia and ECG C was recorded after rewarming.

significant decrease in PR interval of 18.1 ms (*P* = 0.003); a significant but small shortening of QRS duration of 16.7 ms, *P* < 0.0005; and a significant shortening of QTc interval of 35.3 ms (*P* = 0.017). Comparing basal ECG (A) with post-TH ECG (C), there were no significant difference in heart rate or PR interval; but we found a slight significant shortening in QRS duration of 6.8 (*P* = 0.029) and a significant increase in QTc of 22.2 ms (*P* = 0.046), with a final mean QTc interval above the upper limit of normal QTc interval (463.9 ms).

Comparison of T wave amplitude and ST segment deviation are shown in Table 3. On the whole there were no significant changes, except for a progressive decrease in amplitude of T wave in lead v5 through the TH process, a minor descent in ST from ECG B to ECG C in lead v5, and a slight decrease in amplitude of T wave in lead II. Osborn or J wave was observed in 21.3% of the patients in ECG B (Figure 2, arrow) with average amplitude of 0.2 millivolts. All of them appeared with cooling and reverted when patient was rewarmed. No U wave was detected in any ECG.

Arrhythmia analysis is shown in Table 4. Any new arrhythmia occurred in 38.3% of the patients during TH. The most frequent arrhythmia (50% of the patients with arrhythmias) was non-sustained monomorphic ventricular tachycardia (VT), 55% of them in patients with ACS. A 10.6% had severe bradycardia (< 50 bpm) or paced rhythms. An 8.5% had rapid nodal rhythms and 6.4% atrial fibrillation. Neither polymorphic VT, nor sustained VT, nor VF (considered as life-threatening arrhythmias) happened during TH. Twelve percent of the population changed to sinus rhythm after TH induction: half of them were in atrial fibrillation and the other half in accelerated nodal rhythm. Two patients (4.2%) had a reversible change of rhythm with TH: one in sinus rhythm developed an atrial fibrillation during TH and then relapsed to sinus rhythm and the other with an atrial fibrillation at admission had an accelerated nodal

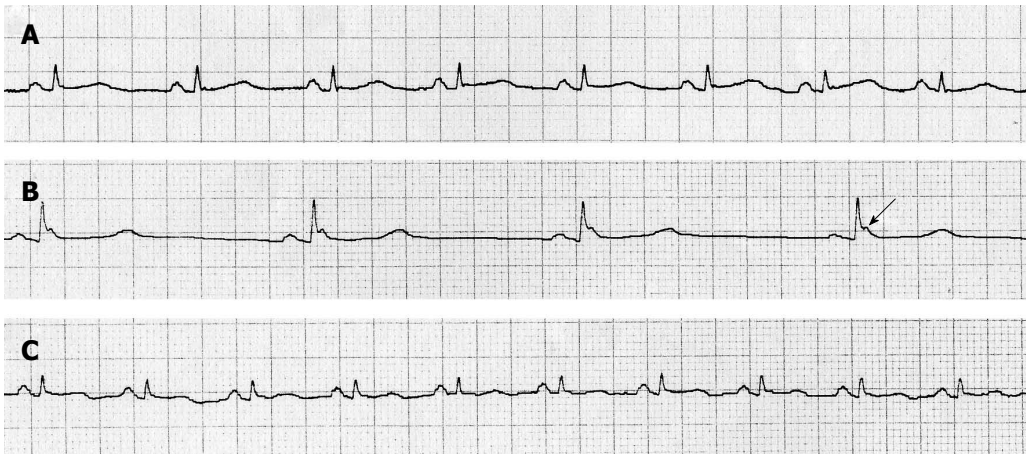

Figure 2 Electrocardiograms in lead Ⅱ from the same patient. Reversible prolongation of all electrocardiograms (ECG) intervals may be observed. A: ECG at admission, core temperature was 35.9 ℃; B: ECG at peak hypothermia, 33 ℃. Osborn or J wave is marked with a black arrow; C: ECG after rewarming, core temperature was 36.4 ℃.

Table 3 Changes in ST segment and T wave, represented as means, standard deviation (in brackets) and *P* for difference

	Admission (ECG A)	During MTH (ECG B)	After MTH (ECG C)	*P* for difference (A to B)	*P* for difference (B to C)	*P* for difference (A to C)
ST deviation lead II	+ 0.05 (1.4)	- 0.20 (1.4)	- 0.12 (0.7)	0.2	0.3	0.4
ST deviation lead v2	+ 0.39 (3.2)	+ 0.08 (0.6)	+ 0.32 (1.0)	0.4	0.06	0.9
ST deviation lead v5	- 0.25 (1.9)	- 0.38 (0.8)	- 0.16 (0.8)	0.6	0.036[a]	0.7
T wave lead II	+ 1.0 (1.8)	+ 0.63 (1.1)	+ 0.38 (1.2)	0.2	0.1	0.036[a]
T wave lead v2	+ 2.0 (3.6)	+ 2.10 (3.2)	+ 1.62 (2.4)	0.8	0.3	0.5
T wave lead v5	+ 1.60 (2.9)	+ 0.84 (2.3)	+ 0.04 (2.4)	0.1	0.013[a]	< 0.0005[a]

Indicates statistical significance ([a]$P < 0.05$). Units are millivolts. +: ST segment elevation or T-wave positive deflection; -: Descent in ST segment or T-wave negative deflection.

Table 4 Incidence of arrhythmias or rhythm changes during hypothermia

New arrhythmias during TH	38.3%
Non sustained monomorphic VT	19.1%
Bradycardia < 50 bpm/paced rhythm	10.6%
Accelerated nodal rhythm	8.5%
Atrial fibrillation	6.4%
Sustained VT	0%
Polymorphic VT or VF	0%
Change to sinus rhythm with TH	12.8%
Atrial fibrillation to sinus rhythm	6.4%
Accelerated nodal rhythm to sinus rhythm	6.4%

TH: Therapeutic hypothermia; VT: Ventricular tachycardia; VF: Ventricular fibrillation.

rhythm in TH and then reverted to atrial fibrillation with rewarming. No patient needed pacemaker implantation or chronotropic drugs as a result of bradycardia during TH. Supraventricular tachycardias were treated following current guidelines if considered necessary. No treatment was given to non-sustained VT.

DISCUSSION

In the 1950's there was a growing interest in hypothermia as a protective measure in the beginnings of open-heart surgery. Some reports from intraoperative ECG obtained during circulatory occlusion and profound hypothermia (reaching 21-23 ℃), described a decrease in heart rate and a prolongation in PR, QRS and QT intervals. Arrhythmias were common and were related to core temperature. During mild hypothermia most frequent arrhythmias were ectopic atrial rhythms and nodal rhythms. A remarkable incidence of atrial fibrillation occurred below 30-32 ℃[10,11]. VF appeared associated with circulatory occlusion. Other studies in dogs suggested temperature thresholds for VF below 26 ℃ and asystole below 18 ℃[10,11,17]. Changes in ECG and arrhythmias in these reports are subject to multiple confounding factors: very low temperatures, myocardial ischemia, circulatory occlusion, cardioplegic solutions and the open-heart surgery itself. Because of those factors, previously described changes can hardly be applicable to current mild controlled TH.

Since the beginning of our decade, and after a gap in the literature of 40 years, hypothermia has regained interest, partly because the mechanism involved in its therapeutic effect were progressively clarified. Most information about complications and side effects come from old reports, animal experimentation and case reports. In the present study we provide a systematized analysis of cardiac arrhythmias and

temperature-dependent, sequential ECG changes during TH performed to an unselected post-CA population.

According to our findings, during TH to a target temperature of 32-34 ℃ in post-CA patients, some ECG changes may be expected: a considerable decrease in heart rate, a minimum prolongation of PR interval, a slight prolongation of QRS duration and a significant prolongation in QTc interval. All of these changes were reversible, except the prolongation of QTc interval (at least in the first 24 h after rewarming). Temperature-related changes in ST segment and T wave were not conclusive, but there was a trend towards flattening of T waves through TH process. ST segment and T wave changes may be interfered by previous cardiac disease and cause of CA, as almost half of the patients had an ACS.

The Osborn wave, or J wave, first observed in 1938 and fully described in 1953[18], is a frequent ECG feature in deep hypothermia. It can be seen as a notch or hump-like deflection in the terminal forces of QRS or between QRS and ST segment, more visible in precordial leads[19]. The amplitude and duration correlates with temperature, and although literature rarely describes it in mild hypothermia, we found a J wave in 21.3% of the patients (Figure 2, arrow). It is caused by a temperature dependent, transmural voltage gradient of a transient potassium current, more intense in epicardium than endocardium.

On the whole, ECG changes found in our study are concordant with those described previously in deep hypothermia[20-23]. Medical staff as well as nurses working with patients treated with induced TH should be aware of the possible arrhythmias and ECG changes that may occur. An example of ECG changes is shown in Figure 2. These changes are secondary to low body temperature and should not be considered pathological. Prolongation of action potential and decrease of myocardial conduction velocity has been proposed as physiopathological explanations for these phenomena[16]. These changes were reversible with rewarming and did not deteriorate hemodynamic status or clinical situation.

Bradycardia is one of the most disturbing effects of hypothermia because CA-recovered patients are often in cardiogenic shock and cardiac output decreases along with heart rate. In our series, patients with low initial heart rates did not decrease further, but maintained or increased their heart rates (Figure 1A). Besides, some studies suggest that the relation between heart rate and cardiac output inverses with hypothermia and that allowing mild TH to reduce heart rate could actually improve myocardial contractility. This is explained because hypothermia worsens diastolic function in the myocardium, and this is partially balanced by bradycardia[24,25]. External pacing or administration of chronotropic drugs is not recommended during TH to increase cardiac output[16].

The use of TH in post CA patients was safe with no life-threatening arrhythmias that worsened hemodynamic stability or required withdrawing the TH protocol. Non life-threatening arrhythmias were found in less than a half of the patients (38.3%).

The behavior of QTc interval in our TH series was remarkable (Figure 1D). We found a mean baseline QTc interval in the upper normal limits (mean 441 ms), it increased with TH (mean 499 ms), and partially reverted with rewarming, but final QTc interval was still lengthened when compared to initial QTc interval (463 ms) and was above upper normal limits. In spite of that, we had no arrhythmias related to prolongation of QT interval, like polymorphic ventricular tachycardia. We presumably (some patients died before a cause of the CA could be elucidated) did not have any patient with arrhythmic CA caused by long QT, but as QT and QTc intervals lengthen with TH, and remain lengthened afterwards, a concern may raise about safety of hypothermia in patients with long QT CA. Further investigation about this issue is warranted.

There is a concern about whether TH may increase the risk for arrhythmias and that the hypothermic myocardium can be somewhat resistant to antiarrhythmic drugs during hypothermia. It is well known that deep hypothermia under 30° increments the risk for atrial fibrillation and progressively with cooling under 28° the risk for life-threatening arrhythmias as VT and VF is increased[26]. Conversely, controlled mild TH is associated with higher rates of ROSC in animal CA models and is successfully used as a treatment for junctional ectopic tachycardia in infants[27-29]. Our study supports all previous reports that controlled, mild TH, is a safe technique with no increased risk for malignant arrhythmias and a relatively small number of minor arrhythmias that on the other hand can not only be attributed to TH but also to post-CA situation and previous cardiac disease.

Our study has some limitations. Accuracy of manual calipers is limited but represents day-by-day clinical practice. Arrhythmias and ECG changes could be interfered by several confounding factors like electrolyte disturbances. We had no control group, so this point cannot be ruled out in our study. However, our findings are congruent with those previously described in hypothermia and the fact that the changes were reversible with rewarming supports that TH was the cause of these changes. Recent trials show conflicting evidence regarding optimal target temperature, one of them suggests a benefit from deeper hypothermia (32 ℃ *vs* 34 ℃), while other found no benefit of 33 ℃ over normothermia (36 ℃)[30,31]. It would be relevant to know the "arrythmical" safety of different temperature levels, however our study did not analyzed different target temperatures.

In summary, therapeutic hypothermia according to current practice is safe with a 38.3% of patients having cardiac arrhythmias during TH but without life-threatening arrhythmias. Main ECG changes were bradycardia and prolongation of PR, QRS and QT

intervals. A concern may rise when inducing TH to patients with long QT syndrome.

COMMENTS

Background

Induced therapeutic hypothermia is currently recommended by most cardiac arrest guidelines, to improve the prognosis of the so-called post-cardiac arrest syndrome. However it is not widely used and has some controversies. Some of the main concerns that prevent intensive care physicians from inducing therapeutic hypothermia are the potential pro-arrhythmic effects of hypothermia. A study regarding cardiac arrhythmias is relevant to reassure patient's safety, especially for patients with heart disease.

Research frontiers

The influence of hypothermia over cardiac rhythm and cardiac conduction system is unknown and main data comes from case reports of accidental deep hypothermia.

Innovations and breakthroughs

This study allows a more comprehensive understanding of the influence of mild hypothermia in cardiac conduction. It shows a reversible prolongation of all cardiac intervals measured by electrocardiograms, suggesting that mild hypothermia slows cardiac conduction speed. The absence of life-threatening arrhythmias is reassuring for using this therapy in cardiac patients.

Applications

This study must be interpreted with caution due to the relatively small sample and its observational nature. However, it supports the "electrical" safety of therapeutic hypothermia for cardiac patients. Future lines of research suggested by the study are the potential influence of QT prolongation by hypothermia in long-QT syndromes, and the need for experimental (most probably in animal models) studies on the influence of hypothermia and cardiac conduction speed.

Terminology

Hypothermia: any temperature below 35.5-36 ℃. It may be accidental (cold exposure in winter) or induced (cold fluid or specific devices); Target temperature: The desired temperature in induced hypothermia. Usually 32-34 ℃. Some groups are investigating 32 ℃ *vs* 34 ℃, while others advocate for only preventing hyperthermia (≤ 36 ℃).

Peer-review

It is an important topic and well written and well presented.

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CASE REPORT

Giant and thrombosed left ventricular aneurysm

Jose Alberto de Agustin, Jose Juan Gomez de Diego, Pedro Marcos-Alberca, Jose Luis Rodrigo, Carlos Almeria, Patricia Mahia, Maria Luaces, Miguel Angel Garcia-Fernandez, Carlos Macaya, Leopoldo Perez de Isla

Jose Alberto de Agustin, Jose Juan Gomez de Diego, Pedro Marcos-Alberca, Jose Luis Rodrigo, Carlos Almeria, Patricia Mahia, Maria Luaces, Miguel Angel Garcia-Fernandez, Carlos Macaya, Leopoldo Perez de Isla, Instituto Cardiovascular, Hospital Universitario San Carlos, Profesor Martin Lagos s/n, 28040 Madrid, Spain

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Correspondence to: Jose Alberto de Agustin, MD, PhD, Instituto Cardiovascular, Hospital Universitario San Carlos, Profesor Martin Lagos s/n, 28040 Madrid, Spain. albertutor@hotmail.com
Telephone: +34-91-3303394
Fax: +34-91-3303290

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Abstract

Left ventricular aneurysms are a frequent complication of acute extensive myocardial infarction and are most commonly located at the ventricular apex. A timely diagnosis is vital due to the serious complications that can occur, including heart failure, thromboembolism, or tachyarrhythmias. We report the case of a 78-year-old male with history of previous anterior myocardial infarction and currently under evaluation by chronic heart failure. Transthoracic echocardiogram revealed a huge thrombosed and calcified anteroapical left ventricular aneurysm. Coronary angiography demonstrated that the left anterior descending artery was chronically occluded, and revealed a big and spherical mass with calcified borders in the left hemithorax. Left ventriculogram confirmed that this spherical mass was a giant calcified left ventricular aneurysm, causing very severe left ventricular systolic dysfunction. The patient underwent cardioverter-defibrillator implantation for primary prevention.

Key words: Myocardial infarction; Echocardiography; Coronary artery disease; Left ventricular aneurysm; Coronary angiography

Core tip: Early diagnosis of ventricular aneurysms following acute transmural myocardial infarction is vital due to the serious complications that can occur. We report the case of a 78-year-old male with history of previous anterior myocardial infarction and currently under evaluation by chronic decompensated heart failure. Subsequent investigation revealed a huge thrombosed and calcified anteroapical left ventricular aneurysm. The peculiar findings of echocardiography, fluoroscopy and left ventriculography are shown with demonstrative images.

de Agustin JA, Gomez de Diego JJ, Marcos-Alberca P, Rodrigo JL, Almeria C, Mahia P, Luaces M, Garcia-Fernandez MA, Macaya C, Perez de Isla L. Giant and thrombosed left ventricular aneurysm. *World J Cardiol* 2015; 7(7): 431-433 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i7/431.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i7.431

INTRODUCTION

True left ventricular aneurysms are a frequent complication following acute extensive myocardial infarction. Early diagnosis is crucial due to the serious complications that can potentially occur, including heart failure, thromboembolism, or tachyarrhythmias.

CASE REPORT

A 78-year-old male with history of previous anterior myocardial infarction and currently under evaluation by chronic decompensated heart failure (NYHA functional class Ⅲ), underwent transthoracic echocardiogram revealing the presence of a huge and peripherally calcified anteroapical left ventricular aneurysm with a giant mural thrombus (Figures 1-3). Elective coronary angiography was performed which demonstrated that the left anterior descending artery was chronically occluded (Figure 4) and nonsignificant lesions in the other coronary arteries. Fluoroscopic imaging revealed a complete oval calcified image enclosed within an abnormal cardiac silhouette (Figure 5). Left ventriculogram confirmed that this image corresponded of a giant calcified and thrombosed left ventricular aneurysm, causing severe left ventricular systolic dysfunction (Figure 6). The calculated left ventricular ejection fraction was only 7%. The patient underwent cardioverter-defibrillator implantation for primary prevention.

DISCUSSION

Left ventricular aneurysms are a frequent complication of acute extensive myocardial infarction and are most commonly located at the ventricular apex[1,2]. A timely diagnosis is vital due to the serious complications that can occur, including heart failure, thromboembolism, or tachyarrhythmias. The benefits of surgical repair of left ventricular aneurysm have long been debated. Although a large amount of studies have showed that aneurysmectomy might improve the outcome[3], the results from the STICH trial have questioned the benefit of this treatment[4]. Therefore, indication for aneurysmectomy depends on the decision of individual surgeons, and should be based on the assessment of the left ventricular dimensions, mitral valve regurgitation severity, extent of myocardial scar tissue and viability of the other regions of the left ventricle, and surgery should be performed in centers with a high surgical experience.

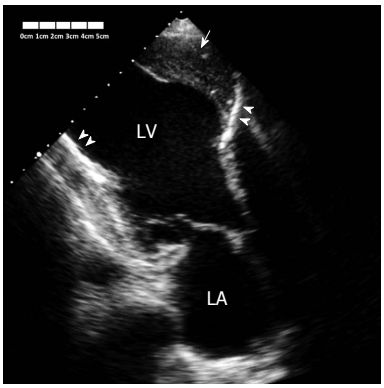

Figure 1 Transthoracic echocardiogram using apical three chamber view showing the big anterior left ventricular aneurysm (arrow). The wall of the aneurysm was calcified (arrowheads), and the aneurysm was covered with thrombus (arrow). LA: Left atrium; LV: Left ventricle.

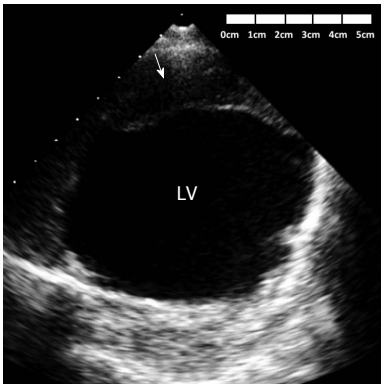

Figure 2 Transthoracic echocardiogram using parasternal short axis view at the midventricular level showing the thrombus (arrow) covering the anterior wall aneurysm. LV: Left ventricle.

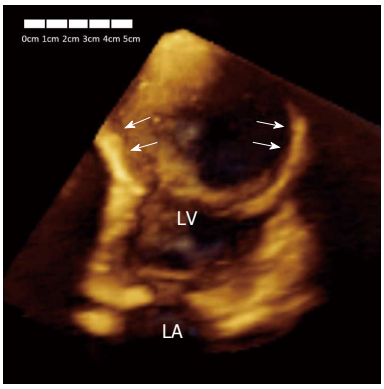

Figure 3 Three-dimensional echocardiography in apical four chamber view showing the big size of the aneurysm (arrows). LA: Left atrium; LV: Left ventricle.

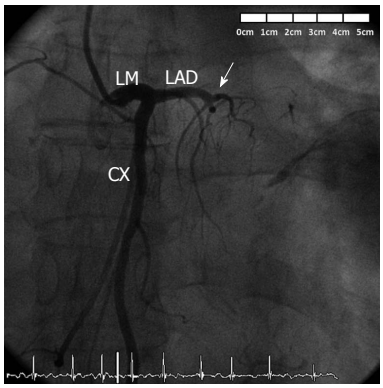

Figure 4 Left coronary angiography demonstrating a proximal occlusion of the left anterior descending artery (arrow). CX: Circumflex coronary artery; LAD: Left anterior descending coronary; LM: Left main coronary artery.

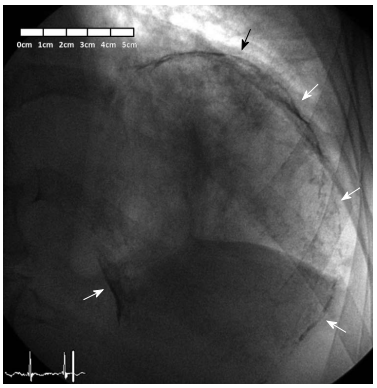

Figure 5 Fluoroscopic imaging in right anterior oblique projection showing a complete oval calcified mass (arrows), corresponding with the left ventricular aneurysm.

COMMENTS

Case characteristics

A 78-year-old male with history of previous anterior myocardial infarction and currently under evaluation by chronic decompensated heart failure.

Clinical diagnosis

Giant thrombosed left ventricular aneurysm.

Differential diagnosis

Intrathoracic mass.

Imaging diagnosis

Echocardiography and coronary angiography were used for the diagnosis of left ventricular aneurysm.

Treatment

The patient received an implantable cardioverter-defibrillator for primary prevention and was referred for consideration of cardiac transplantation.

Related reports

True left ventricular aneurysms are widely recognized as a common and serious complication following acute transmural myocardial infarction. However, this case is particular because of the huge size of the aneurysm.

Experiences and lessons

The recognition of ventricular aneurysms is of great importance due to the numerous complications that can potentially occur. Echocardiography and catheterism are fundamental tests for diagnosis.

Peer-review

It is a interesting case and well described.

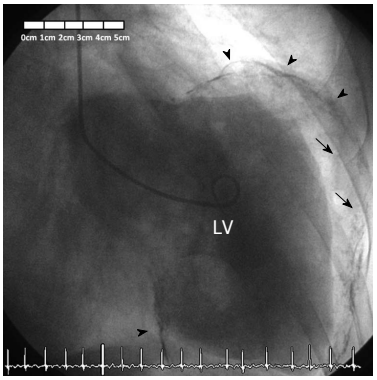

Figure 6 Left ventriculogram confirming diagnosis of a giant calcified and partially thrombosed left ventricular aneurysm, with severe left ventricular systolic dysfunction. The wall of the aneurysm is calcified (arrowheads), and the aneurysm is covered with thrombus (arrows). LV: Left ventricle.

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Jin-Lei Wang, Director
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: editorialoffice@wjgnet.com
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EDITORIAL

Thrombosis: Novel nanomedical concepts of diagnosis and treatment

Iwona Cicha

Iwona Cicha, Cardiovascular Nanomedicine Unit, Section of Experimental Oncology and Nanomedicine, ENT-Department, University Hospital Erlangen, 91054 Erlangen, Germany

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Correspondence to: Dr. Iwona Cicha, PhD, Cardiovascular Nanomedicine Unit, Section of Experimental Oncology and Nanomedicine, ENT-Department, University Hospital Erlangen, Glückstr 10a, 91054 Erlangen, Germany. iwona.cicha@yahoo.com
Telephone: +49-9131-8543953
Fax: +49-9131-8534828

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Abstract

Intravascular thrombosis, a critical pathophysiological feature of many cardiovascular disorders, leads to the formation of life-threatening obstructive blood clots within the vessels. Rapid recanalization of occluded vessels is essential for the patients' outcome, but the currently available systemic fibrinolytic therapy is associated with low efficacy and tremendous side effects. Additionally, many patients are ineligible for systemic thrombolytic therapy, either due to delayed admission to the hospital after symptom onset, or because of recent surgery, or bleeding. In order to improve the treatment efficacy and to limit the risk of hemorrhagic complications, both precise imaging of the affected vascular regions, and the localized application of fibrinolytic agents, are required. Recent years have brought about considerable advances in nanomedical approaches to thrombosis. Although these thrombus-targeting imaging agents and nanotherapies are not yet implemented in humans, substantial amount of successful *in vivo* applications have been reported, including animal models of stroke, acute arterial thrombosis, and pulmonary embolism. It is evident that the future progress in diagnosis and treatment of thrombosis will be closely bound with the development of novel nanotechnology-based strategies. This Editorial focuses on the recently reported approaches, which hold a great promise for personalized, disease-targeted treatment and reduced side effects in the patients suffering from this life-threatening condition.

Key words: Thrombosis; Thrombus imaging; Nanomedicine; Targeted nanoparticles; Thrombolytic drug-delivery systems

Core tip: The prevalence of thrombosis, the formation of life-threatening clots obstructing vital blood vessels, continues to rise. Accurate diagnosis and rapid recanalization of an occluded artery is essential to improve outcomes and reduce the mortality in acute myocardial infarction or stroke. The current thrombolytic therapy often fails to diminish the occlusion and is associated with a high rate of hemorrhagic complications. Develop-

ment of directed nanosystems for local thrombolysis, characterized by a strong fibrinolytic effect and low bleeding risk, is therefore one of the most urgent tasks in the prevention and the therapy of acute thrombotic events.

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INTRODUCTION

Intravascular thrombosis, the formation of life-threatening obstructive blood clots within the vessels, underlies a number of cardiovascular disorders such as heart attack, ischemic stroke, pulmonary embolism, and deep vein thrombosis[1,2]. Among these, atherothrombotic diseases (ischemic heart disease and stroke) are collectively responsible for 25% of all deaths worldwide[3]. In contrast, the burden of venous thromboembolism (VTE) is not well documented. According to the recent analyses[4], its incidence ranges between 1-2 per 1000 individuals in most of the studies, resembling the frequency of myocardial infarction[5], and the deaths due to VTE are estimated at 300000 per year in the United States[6]. Globally, the prevalence of thrombotic disorders continues to increase, particularly in the developing countries. However, despite significant advances in understanding of the disease mechanisms, which led to the development of more effective anti-thrombotic and thrombolytic drugs[7], the effect of these therapies on the patients' outcomes remains disappointing: According to the published data, less than 25% of high cardiovascular risk patients receiving antiplatelet therapy avoided a fatal thrombotic event[8]. The inherent problem with the conventional antithrombotic approaches is the increased risk of bleeding, as the existing therapeutics destabilize hemostatic processes. Among the most urgent challenges in this field are thus (1) identification of patients at increased risk for thrombosis and precise estimation of individual disease burden, as well as (2) development of safe and effective strategies to prevent thrombotic events and/or rapidly diminish vascular occlusion. These challenges have been intensively addressed by the researchers across the globe, resulting in a number of innovative approaches to diagnosis and treatment of thrombosis, as outlined below.

DIAGNOSIS OF THROMBOTIC DISEASE

Individual burden of thrombosis

In order to improve the diagnosis, risk stratification, and management of thrombotic syndromes, reliable methods of *in vivo* assessment of the thrombotic risk in patients with cardiovascular diseases are needed. Although thrombin is the most important serine protease within the coagulation cascade[9], thus far the diagnostic tests are lacking that are able to rapidly and reliably assess its activity in clinical settings. To address this need, a novel urinary nanomarker assay based on thrombin-sensitive iron oxide nanoparticles was recently developed that allows detection of thrombin activity and thus quantitative estimation of thrombosis burden *in vivo*[10]. The nanomarkers were produced by coupling iron oxide nanoworms with thrombin-cleavable peptides linked to a synthetic reporter system, composed of the protease-resistant peptide, glutamate-fibrinopeptide B, which was modified at the termini with ligands detectable by an immunoassay (fluorescein, or Alexa488, and biotin). In a mouse model of pulmonary embolism induced by thromboplastin[11], the circulating nanomarkers sucessfully accessed the local sites of thrombosis and released the reporters upon cleavage by thrombin. The urinary clearance of these reporters was detectable by ELISA with high sensitivity and significantly correlated with the thrombosis burden estimated by the histochemically analyzed amount of fibrin deposited in the lungs[10]. Given the need of rapid and reliable *in vivo* assessment of the thrombotic burden in cardiovascular patients, this urine analysis-based assay represents a very promising platform for use in clinical practice.

Imaging of thrombosis in vivo

During the thrombotic event, initially smaller clots form larger obstructive thrombi, which require a long time for recanalization, and a high dose of thrombolytic agents, or a high rate of mechanical clot disruption[12]. Both the burden and the localization[13,14] of thrombi are known to affect clinical outcomes and mortality[12,15]. By providing essential information about the size and localization of the thrombi, direct thrombus imaging would have an immense impact on clinical practice: Without a tool for *in vivo* imaging in the clinical settings, individualisation of the thrombolytic therapy is impossible. The recommended fixed dose of intravenous tissue plasminogen activator (tPA; 0.9 mg/kg) is thus insufficient in some patients, resulting in resistance to thrombolysis, or excessive in others, leading to increased risk of hemorrhagic complications.

Intravascular thrombus formation therefore represents a target for novel nanoparticle-based diagnostics. As early as in 2001, thrombus detection *in vivo* by magnetic resonance imaging (MRI) was accomplished in dogs using anti-fibrin monoclonal antibodies conjugated to lipid-encapsulated perfluorocarbon nanoparticles containing gadolinium-chelate[16]. More recently, *ex vivo* optical imaging of atherothrombosis in ApoE-deficient mice fed a high-fat diet was reported with lipopeptide nanoparticles carrying a fluorescently-labeled pentapeptide Cys-Arg-Glu-Lys-Ala (CREKA), which binds to clotted plasma proteins in the blood vessels[17]. Intravascular fibrin detection by MRI in this mouse model was also described by Makowski *et al*[18]

using a commercially available gadolinium-based fibrin-binding peptide EP-2104R. Another fibrin-targeting peptide (GPR, Gly-Pro-Arg) has been utilised in studies by Obermeyer *et al*[19] and McCarthy *et al*[20]. The former group applied bacteriophage MS2 capsids functionalized with GPR peptide on the exterior of each protein shell for fibrin imaging. The GPR-modified capsids were evaluated *in vitro* with regard to the fibrin imaging. Near-infrared fluorophores on the interior surface of the capsids enabled optical detection of their binding to fibrin clots with improved signal-to-background ratio as compared with non-targeted nanoagents[19]. Furthermore, in a mouse model of ferric chloride injury to jugular vein, McCarthy *et al*[20] utilised fluorescently labeled cross-linked iron oxide nanoparticles functionalized with GPR or FXIIIa-targeting peptides to obtain multimodal nanoagents exhibiting either covalent or noncovalent binding to thrombi. These nanosystems allowed *in vivo* detection of thrombus by both MRI and optical imaging modalities.

Apart from fibrin, activated platelets represent an important target for detection of intraluminal thrombi and endothelial activation, a marker of ongoing atherothrombotic disease. Therefore, the development of contrast agents for imaging of P-selectin, expressed by activated platelets, has been the aim of numerous efforts. In particular, the research group of Bachelet *et al*[21] and Manzo-Silberman *et al*[22] developed several nanosystems for *in vivo* P-selectin detection based on polysaccharide fucoidan (a mimic of sialyl Lewis X, the natural ligand of P-selectin) derived from brown seaweed. Radiolabeled fucoidan was demonstrated a suitable P-selectin targeting agent for *in vivo* single-photon emission computed tomography imaging of platelet-rich thrombi in rat models of infective endocarditis and elastase-induced aortic aneurysms, as well as endothelial activation in a model of myocardial ischemia-reperfusion[23]. Very recently, this group tested ultrasmall superparamagnetic iron oxide nanoparticles (USPIO) coated with fucoidan for molecular MRI of intraluminal thrombus: In a rat model of elastase-induced aortic aneurysms, all thrombi detected in MRI by USPIO-fucoidan particles were correlated with P-selectin immunostaining and USPIO detection by electron microscopy, whereas no intraluminal thrombi were detectable upon control USPIO[24]. In a study by Ta *et al*[25], iron oxide nanoparticles were coupled with a single-chain antibody that specifically binds to ligand-induced binding sites (LIBS) on glycoprotein IIb/IIIa (CD41/CD61), the most highly expressed molecules on the surface of activated platelets. LIBS-targeting nanocontructs showed a strong and specific binding to activated platelets *in vitro*, as well as *in vivo* by intravital microscopy and MRI of mouse carotid arteries[25].

Iron oxide nanoparticles allow *in vivo* visualization of thrombi with MRI. However, MRI is rarely the test of choice for the management of patients with acute thrombotic events, due to the time restrictions of clinical management, or contraindications for MRI in some patients. For most clinical decision making involving the administration of tPA, computed tomography (CT) is the current standard[26] due to its speed and convenience. However, non-contrast CT often does not allow a precise assessment of extent and distribution of thromboemboli, because the density of the thrombus is often not much different from that of the surrounding blood. Therefore, efficient and safe contrast agents are needed to achieve the enhancement of thrombus imaging with the clinical CT. Addressing this issue, thrombus detection using microCT has been recently tested in a mouse model of ferric chloride-induced carotid thrombosis utilising glycol chitosan (GC)-gold nanoparticles as contrast agents[27]. The study showed that these nanoparticles became trapped in the blood clots proportionally to thrombotic insult, and allowed the quantitative characterization and serial monitoring of thrombus evolution, embolization, and *in situ* recurrent thrombosis, as well as the assessment of therapeutic efficacy of tPA-induced thrombolysis. Due to a long circulating half-life, GC-gold nanoparticles remained available for entrapment into fibrin matrix for up to 3 wk, allowing repetition or ongoing monitoring of thrombogenesis and thrombolysis with microCT[27].

Collectively, the above-discussed studies demonstrate that nanosystems which target fibrin or activated platelets can enhance the detection and the diagnosis of intravascular thrombi by means of existing imaging modalities. Thus far however, little is known about their safety and efficacy in humans. Provided low toxicity and a good therapeutic index, these nanosystems should improve risk stratification when translated into the clinical practice, and allow personalized therapeutic regimen in thrombosis-related diseases.

PREVENTION AND TREATMENT OF THROMBOSIS

Current therapies

Platelet activation and aggregation are the key processes involved in thrombosis and thromboembolic disorders. The best preventive measures for the thrombotic events in the risk patients are thus antiplatelet or anticoagulation therapy. Although aspirin still plays an essential role in the primary and secondary prevention of atherothrombosis, new generation antithrombotic therapies are rapidly evolving. In combination with aspirin, ADP $P2Y_{12}$ receptor antagonists are used in the management of acute coronary syndromes and percutaneous coronary interventions in order to prevent adverse cardiac events and stent thrombosis[28]. Oral anticoagulation used for the treatment of VTE and for the prevention of emboli in patients with atrial fibrillation has advanced with the use of direct thrombin and factor Xa inhibitors that do not require therapeutic monitoring[29]. Although antiplatelet and anticoagulant therapy is essential for the primary and secondary prevention of atherothrombosis, systemic pre-treatment

with antithrombotic agents is associated with increased risk of hemorrhagic complications after intravenous thrombolysis in patients with acute ischemic stroke[30].

Acute management of stroke focuses on stabilizing the patient and ensuring the maximal reperfusion of the ischemic brain tissue. Hence, intravenous thrombolysis remains the mainstay treatment for acute ischemic stroke: A statistically and clinically significant improvement in outcomes is observed for carefully selected patients treated with tPA within 4.5 h of stroke onset[31]. While no other medication has demonstrated comparable efficacy, tPA remains the only drug for acute ischemic stroke approved by Food and Drug Administration since 1996[32]. However, its use is very limited both by the narrow eligibility and administration window, and by the risk of hemorrhagic complications[33], so called thrombolysis-related symptomatic intracerebral haemorrhage, which occurs in about 6% of patients and is associated with nearly 50% mortality[34,35]. Additionally, in some patients eligible for tPA treatment, the outcome is poor, when occlusion occurs in large arteries (internal carotid artery, middle cerebral artery or basilar artery). For these subgroups of stroke patients, an endovascular (intraarterial) administration route has been developed, but its clinical benefit remains unproven. Randomized controlled clinical trials did not show any added benefit of endovascular treatment over intravenous tPA alone in intravenous tPA-eligible patients, even in patients with persistent large-artery occlusion, nor have these trials provided evidence of clinical benefit in patients who were ineligible for intravenous tPA because of being > 4.5 h from symptom onset[36].

Alternative means of reperfusion, ideally based on individual thrombus burden estimation are therefore needed. For this purpose, thrombus-targeted nanosystems could serve as carriers for direct delivery of therapeutic agents to the occlusive thrombi in order to increase the effective local concentrations of antithrombotic drugs.

Antiplatelet and anticoagulant medications for prevention of thrombosis

Current antiplatelet drugs are only partially effective in preventing thrombus formation and thromboembolic events. Consequently, much interest is drawn both to the discovery of novel antiplatelet medications and to the optimization of the existing ones. The group of Chen *et al*[37] reported the synthesis of novel, self-assembly anti-platelet aggregation peptides containing L-arginine and L-aspartic acid, that were complexed with Cu(Ⅱ) to form stable nanoparticles. In a rat model of thrombus formation, these peptides at 5 μmol/kg achieved anti-thrombotic activity comparable to 110 μmol/kg aspirin, whereas the peptide-Cu(Ⅱ)-nanocomplexes were equally effective in reducing the thrombus weight already at 100-fold lower concentrations (0.05 μmol/kg). More recently, the same group reported a successful approach to overcome the low response to aspirin observed in some patients, which severely decreases its efficacy at the tolerated doses[38]. In that study, aspirin was conjugated to the Arg-Gly-Asp-Val (RGDV) tetrapeptide, resulting in a nano-assembly targeting glycoprotein Ⅱb/Ⅲa, the receptor for RGD peptide on the surface of the activated platelets. *In vitro*, aspirin-RGDV particles inhibited platelet aggregation induced by thrombin or arachidonic acid more effectively than free aspirin. A very strong antithrombotic effect of aspirin-RGDV was also observed in a rat model of thrombosis - whereas aspirin exhibited no antithrombotic activity at 16.7 μmol/kg, aspirin-RGDV significantly and dose-dependently inhibited thrombus formation in the treated rats already at doses of 0.1 and 1 nmol/kg[38]. Targeted delivery of aspirin to thrombus and its local release to activated platelets thus resulted in an extraordinarily potent inhibition of thrombus formation, overcoming the apparent non-response to aspirin. Formation of thrombi was also effectively prevented by novel heparin-conjugated carbon nanocapsules in a mouse model of acute hindlimb thromboembolism[39]. Compared to the injection of heparin alone, those heparin-functionalized carbon nanocapsules displayed superior antithrombotic activity *in vitro* and *in vivo*, representing a promising nanocarrier system for anticoagulant delivery.

Some of the most common cardiovascular interventions, including stent implantation or prosthetic heart valve replacement, are associated with increased risk of thrombosis, necessitating prolonged or even lifelong antiplatelet therapy. Particularly after cessation or premature discontinuation of the therapy, the incidence of thrombosis is high. Gene therapy is considered a safe strategy to increase the local expression of thrombolytic agents over an extended period of time, in parallel reducing the systemic risk of hemorrhagic complications. Ji *et al*[40] used a chitosan nano-*tPA* gene plasmid to locally transfect dog cardiomyocytes at the time of mechanical heart valve replacement. The transfected gene significantly increased the survival of animals and prevented thrombus formation on mechanical valves, without affecting systemic hemostasis. In a further study by the same group[41], the *tPA* gene plasmid was packaged in albumin nanoparticles crosslinked to ultrasonic microbubbles. Following intravenous administration, a local therapeutic ultrasound treatment of the heart after valve replacement had been performed, which resulted in increased myocardial expression of *tPA* and prevented thrombosis for 8 wk after operation.

Thrombolytic therapies

Rapid recanalization of thrombus-occluded arteries is essential to improve outcomes and reduce the mortality in acute myocardial infarction or stroke. Development of delivery systems for local thrombolysis is therefore one of the most urgent tasks in the prevention and the therapy of acute thrombotic events. Within the coagulation cascade, thrombin represents the most important target of direct anticoagulants. As an

example, hirulog, an analogue of the natural thrombin inhibitor hirudin was locally delivered to the thrombus using lipid nanoparticles containing a fibrin-binding peptide. Upon administration of the fibrin-targeting hirulog-carrying particles, significantly higher levels of antithrombin activity were achieved in the aortic tree of ApoE-deficient mice as compared with non-targeted particles[17].

The effects of another potent thrombin inhibitor, d-phenylalanyl-l-prolyl-l-arginyl-chloromethyl ketone (PPACK) were investigated the group of Myerson *et al*[42] and Palekar *et al*[43] in a mouse model of acute arterial thrombosis due to photochemical injury of the carotid artery. Perfluorocarbon nanoparticle-bound PPACK outperformed both heparin and uncomplexed PPACK in inhibiting thrombosis, and formed a local clotting barrier that remained effective even as systemic effects rapidly diminished[42]. Similarly, PPACK-liposomes administered prior to the arterial injury significantly delayed the time to arterial occlusion as compared to free PPACK. Whereas systemic anticoagulant profiles returned to control levels within 50 min, the inhibition of thrombus formation was maintained at the injury site beyond 2 h[43]. The establishment of a potent and long-acting anticoagulant surface over a newly forming clot with the use of thrombin targeted nanoparticles offers an alternative site-targeted approach to the management of acute thrombosis.

As described in detail above, intravenous infusion of tPA is characterized by several drawbacks, including low efficacy combined with a high risk of bleeding complications[35]. Therefore, several innovative strategies aiming at targeted and/or local applications of plasminogen activators have been designed. The possibility of magnetic-targeting of tPA for local thrombolysis was investigated by Ma *et al*[44] in a rat embolic model. Magnetite nanoparticles bound to tPA (tPA equivalent of 0.2 mg/kg) were administered intraarterially under guidance of an external magnet moving along the iliac artery. Magnetic tPA-nanoparticles accumulated in the thrombus-affected region and achieved an effective target thrombolysis with < 20% of a regular dose of free tPA. Another tPA delivery nanosystem comprising basic gelatin and zinc acetate was tested by Kawata *et al*[45] in a swine acute myocardial infarction model. Within this nanosystem, tPA activity was reduced *in vitro* to approximately 50% of free tPA and was fully recoverable by transthoracic ultrasound application. In comparison to treatment with free tPA (0.447 mg/kg), which recanalized the occluded coronary artery in only 1 of 10 swine, nanoparticles containing the same dose of tPA with ultrasound activation achieved recanalization in 9 of 10 swine within 30 min, suggesting that this nanosystem bears promising potential for improved intravenous thrombolysis.

In attempt to create a theranostic construct with fibrinolytic activity, McCarthy *et al*[46] synthesized a multimodal nanoagent using magnetofluorescent crosslinked dextran-coated iron oxide nanoparticles conjugated to tPA. Thrombus-targeting was achieved by nanoparticle functionalization with an activated FXIIIa-sensitive peptide. In murine models of arterial and venous thrombosis, the FXIIIa-targeted fibrinolytic nanoagent efficiently bound the margin of intravascular thrombi as detected by intravital fluorescence microscopy. The fibrinolytic activity of the nanoagent compared to free tPA was subsequently evaluated in a murine model of pulmonary embolism, showing that the FXIIIa-targeted agent lysed pulmonary emboli with similar efficacy as free tPA[46].

Targeted tPA delivery to stenotic arteries by employing universal hemodynamic phenomena was described by Korin *et al*[47]. Since occlusions in blood vessels result in local increases in shear stress, the authors designed micro-aggregates of poly-lactic-glycolic acid nanoparticles coated with tPA. Under physiologic flow conditions with shear stress values up to 70 dyn/cm^2, these micro-aggregates remained stable, but the exposure to abnormally high shear stress in the regions of vascular occlusion/stenosis resulted in their rapid break up followed by local drug release. As compared with free drug, the shear-activated tPA-coated nanoparticles rapidly dissolved the ferric chloride-induced arterial thrombi in mouse mesenteric arteries, with complete clearance of occluding thrombi within 5 min after application[47]. Moreover, upon infusion of lethally large fibrin clots, the immediate application of the shear-activated tPA-coated nanoparticles increased survival by 80%. The doses of shear-activated tPA-nanoparticles required for clot dissolution were about 100-times lower than the doses required for achieving comparable effects with free drug[47]. This strategy, utilizing a universal hemodynamic phenomenon of increased shear stress upon reduction in vessel diameter should result in a broad applicability for all occlusive vascular conditions, including *e.g.,* treatment of stenotic atherosclerotic plaques, pulmonary emboli, and ischemic stroke.

Venous thromboembolism, including deep vein thrombosis and pulmonary embolism, remains a common and potentially life-threatening disease[48]. Standard treatments aim to minimize acute morbidity and mortality by preventing the potentially fatal embolization of the initial thrombus and to reduce the long-term complications of post-thrombotic syndrome. For patients with VTE, catheter-based revascularization therapy [catheter-directed thrombolysis (CDT)] has emerged as favoured means of administration replacing systemic thrombolysis. Urokinase-type plasminogen activator (uPA) is commonly used for CDT in the clinical settings of VTE. However, similar to arterial thrombosis treatment, strict eligibility criteria are necessary to reduce the risk of bleeding complications, which limit the applicability of this therapy. To minimize the adverse effects and increase therapeutic benefits, Jin *et al*[49] produced uPA-coated, self-assembled chitosan and tripolyphosphate nanoparticles. In a

rabbit model of thrombosis, a significant improvement in the thrombolytic effect compared with free uPA was observed upon administration of uPA-carrying nanoparticles. Additionally, the study confirmed the superiority of CDT for improving clot lysis and minimizing adverse effects over drug-induced systemic thrombolysis.

CONCLUSION

The potential clinical impact of nanotechnology in terms of thrombosis prevention and management is enormous. But in spite of the promising results obtained in the vast number of bench investigations that have been published in the recent years, the thrombus-targeting imaging nanoagents and fibrinolytic nanotherapies are not yet implemented in humans. To ensure clinical safety and feasibility, the intravascular diagnostic and drug-delivery systems must be subject to a close toxicologic and pharmacologic scrutiny prior to their application in patients. Thus, substantial amount of *in vivo* studies will be necessary before the successful basic research can be translated into clinical trials. Despite multiple safety and regulatory constraints, the future progress in diagnosis and treatment of thrombosis is expected to benefit strongly from the development of novel nanotechnology-based strategies.

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EDITORIAL

Reduction of radiation exposure in catheter ablation of atrial fibrillation: Lesson learned

Roberto De Ponti

Roberto De Ponti, Department of Heart and Vessels, Ospedale di Circolo e Fondazone Macchi, University of Insubria, 21100 Varese, Italy

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Correspondence to: Roberto De Ponti, MD, FHRS, Department of Heart and Vessels, Ospedale di Circolo e Fondazione Macchi, University of Insubria, Viale Borri 57, 21100 Varese, Italy. rdeponti2015@alice.it
Telephone: +39-332-278564
Fax: +39-332-393644

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Abstract

Over the last decades, the concern for the radiation injury hazard to the patients and the professional staff has increased in the medical community. Since there is no magnitude of radiation exposure that is known to be completely safe, the use of ionizing radiation during medical diagnostic or interventional procedures should be as low as reasonably achievable (ALARA principle). Nevertheless, in cardiovascular medicine, radiation exposure for coronary percutaneous interventions or catheter ablation of cardiac arrhythmias may be high: for ablation of a complex arrhythmia, such as atrial fibrillation, the mean dose can be > 15 mSv and in some cases > 50 mSv. In interventional electrophysiology, although fluoroscopy has been widely used since the beginning to navigate catheters in the heart and the vessels and to monitor their position, the procedure is not based on fluoroscopic imaging. Therefore, non-fluoroscopic three-dimensional systems can be used to navigate electrophysiology catheters in the heart with no or minimal use of fluoroscopy. Although zero-fluoroscopy procedures are feasible in limited series, there may be difficulties in using no fluoroscopy on a routine basis. Currently, a significant reduction in radiation exposure towards near zero-fluoroscopy procedures seems a simpler task to achieve, especially in ablation of complex arrhythmias, such as atrial fibrillation. The data reported in the literature suggest the following three considerations. First, the use of the non-fluoroscopic systems is associated with a consistent reduction in radiation exposure in multiple centers: the more sophisticated and reliable this technology is, the higher the reduction in radiation exposure. Second, the use of these systems does not automatically lead to reduction of radiation exposure, but an optimized workflow should be developed and adopted for a safe non-fluoroscopic navigation of catheters. Third, at any level of expertise, there is a specific learning curve for the operators in the non-fluoroscopic manipulation of catheters; however, the learning curve is shorter for more experienced operators compared to less experienced operators.

Key words: Catheter ablation; Atrial fibrillation; Radiation exposure; Fluoroscopy time; Dose area product; Electro-anatomic mapping

Core tip: After 25 years from the formulation of the ALARA principle, the awareness of the potential hazard related to radiation exposure has greatly increased in medicine. Non-fluoroscopic three-dimensional systems, introduced in interventional electrophysiology to support complex procedures, have the potential to significantly decrease the use of fluoroscopy. In interventional electrophysiology, the clinical perspective is to perform procedures with minimal use of fluoroscopy without endangering the safety and efficacy. However, to achieve this task the use of the non-fluoroscopic system has to be optimized and a learning curve is necessary even for operators experienced in fluoroscopy-based electrophysiology.

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NEED FOR REDUCTION OF RADIATION EXPOSURE IN ELECTROPHYSIOLOGY PROCEDURES

Over the last years, the awareness of the risk related to the use of ionizing radiation in medicine has progressively increased. Cardiac imaging procedures lead to substantial radiation exposure in many patients: in a population-based analysis[1], the median cumulative effective dose over 3 years was 15.6 mSv and, among patients receiving a high annual dose (> 20-50 mSv), repeat cardiac catheterization procedures are the largest contributors to the radiation dose. The potential risks related to this radiation exposure are expected to be vastly outweighed by the benefits, especially if the procedure is appropriately justified and carefully optimized[2]. Although it is difficult to assess the consequences of the deterministic (dose-dependent) and stochastic (non dose-dependent) effects for the exposure to low-dose ionizing radiations used in cardiovascular imaging, the estimate of lifetime additional risk of cancer spans between 1/2000 and 1/1000 per single cardiovascular procedure[3]. It should be also taken into account that several patients undergo repeat procedures and that a younger patient population is more sensitive to the induction of cancer than an older patient population[4]. Similarly, the risk related to radiation exposure is not negligible for the medical staff. Noteworthy, according to a survey undertaken in Tuscany, Italy[5], interventional cardiologists and electrophysiologists represent more than 60% of the medical staff receiving the highest annual radiation exposure (> 6 mSv), with no statistically significant difference between physicians and nurses/technicians. This radiation exposure is by far greater than the one of the urologists, radiologists, and personnel of nuclear medicine. Moreover, according to the same source[5], the median lifetime professional exposure is 54 mSv, leading to an estimate lifetime attributable risk of cancer of 1 out of 200.

A decade ago, a document[6] endorsed by the main American scientific societies in cardiovascular medicine was published. This document states the clinical competence required for physicians performing fluoroscopically-guided invasive cardiovascular procedures to optimize patient safety and image quality. Importantly, it also highlights the ALARA principle, previously proposed by the United States National Council on Radiation Protection and Measurements[7]: due to both the stochastic and deterministic effect of radiation, there is no magnitude of radiation exposure that is known to be completely safe and, therefore, the use of ionizing radiations should be As Low As Reasonably Achievable. This principle confers to physicians the responsibility for reducing as much as possible the dose of radiation during cardiovascular procedures, in order to minimize the radiation injury hazard to the patients, to the professional staff and to themselves. The dose delivered to the patient depends on the following three factors: (1) type and setting of the X-ray equipment; (2) patient size; and (3) physician conduct. Consequently, all these three factors should be considered and optimized to comply with the ALARA principle. Importantly, opposite to what is commonly thought, fluoroscopy time poorly expresses the dose delivered to the patient. In fact, this value only reflects the operator's attitude to use radiation during a given procedure. Moreover, the same value of fluoroscopy time may correspond to a very different radiation exposure, depending on the predominant use of low-dose fluoroscopy or high-dose cine loop acquisition. Therefore, a reliable surrogate measurement for the total amount of X-ray energy delivered to the patient is the dose-area product (DAP), expressed usually in Gy·cm^2 and automatically measured by X-ray systems[6].

In the real world, after the dissemination of the ALARA principle, the process of optimization is still ongoing. Optimization depends on several factors, some of which are difficult to identify and control. Considering again the data by Chen *et al*[1], based on a population enrolled between 2005 and 2007, after the ALARA principle was diffused, percutaneous coronary interventions or electrophysiologic procedures were the main determinants of radiation exposure in the population receiving the highest radiation dose (> 20 mSv). As mentioned above, one of the determinants of the dose to the patient is the physician's conduct, which may be very much dependent on the physician's experience in a given procedure. In fact, in the very early phase of a physician's learning curve, the workflow can be far from being optimized and this can result in an excessive use of fluoroscopy. In this context, newer methodologies of teaching and learning can be effectively used. One small study performed in our

center[8] shows that the training implemented by a high-fidelity hybrid simulator reduces from 10 to 5 min, on average ($P < 0.0001$), the fluoroscopy time per patient spent by fellows novice in electrophysiology to position catheters in the conventional sites at the beginning of the procedure. In the future, if this or similar training modalities are not considered, we may face a new paradox: while the more experienced operators minimize radiation exposure in complex procedures using established techniques and technologies, the less experienced physicians use a higher dose for a standard and relatively simple procedure.

Recently, the European Society of Cardiology published two position papers on the appropriate and justified use of medical radiation in cardiovascular imaging[9] and on the practical ways to reduce radiation dose for patients and staff during electrophysiology procedures[10]. Focusing on the field of interventional electrophysiology, these papers report the radiation dose to the patients for electrophysiology procedures. This dose may vary from 3.2 mSv for a simple diagnostic electrophysiology study to a higher value for complex procedures, such as atrial fibrillation ablation, for which the median dose is 16.6 mSv, ranging from 6.6 to 59.6 mSv[10]. Another review of 17 studies, 12 of them published after the year 2000, reports an effective dose even higher (20.3 mSv) for catheter ablation of cardiac arrhythmias, in general, including ablation of less complex arrhythmias[11]. As suggested by the consensus document[10], this situation still requires further improvement, once optimization of X-ray equipment and shielding of the laboratory personnel are obtained. In fact, non-fluoroscopic three-dimensional systems, namely the Ensite-NavX (St.Jude Medical, United States) and the CARTO (Biosense Webster, United States), widely used since the late nineties for ablation of complex arrhythmias, can be used effectively to reduce radiation exposure during electrophysiology procedures. In a randomized study[12], the use of these systems for catheter ablation of cardiac arrhythmias reduced X-ray exposure with a similar efficacy and safety compared to the conventional approach. However, it should be highlighted that the use of these systems does not *per se* reduce radiation exposure, but the operators should develop procedural workflows to rely on non-fluoroscopic guidance as much as possible without compromising safety[10]. Especially for complex left atrial procedure during which the operator may face different anatomic variants, integration in these systems of pre-acquired three-dimensional imaging from computed tomography or magnetic resonance scan has the potential to drastically reduce the radiation exposure during the procedure[9].

The following sections will focus on reducing radiation exposure in catheter ablation of atrial fibrillation. This is an increasingly used procedure especially in patients with paroxysmal forms and, moreover, the use of fluoroscopy in such a complex and demanding procedure can be high. Therefore, reduction of radiation exposure in this procedure is expected to increase the net benefit of the procedure, minimizing the risks, which can be also related to the radiation exposure especially in case of repeat procedures.

ZERO OR NEAR-ZERO FLUOROSCOPY FOR ATRIAL FIBRILLATION ABLATION?

Unlike percutaneous coronary interventions, electrophysiologic procedures are based on recording and interpretation of intracavitary electrograms. Therefore, although fluoroscopy is very useful to maneuver and check the position of catheters, imaging based on ionizing radiations is not an integral part of the electrophysiologic procedure. In fact, ablation of various types of supraventricular and ventricular tachycardia with no use of fluoroscopy is feasible both in children[13-18] and adults[19-22] using non-fluoroscopic three-dimensional systems. Also a complex procedure, such as pulmonary vein isolation to treat atrial fibrillation, is feasible with no use of fluoroscopy[23,24]. Although these studies certainly demonstrate the feasibility of zero-fluoroscopy procedures, this issue deserves several considerations, especially in the case of complex procedures such as atrial fibrillation ablation. First, the majority of the reported series and in particular those on catheter ablation of atrial fibrillation are small and from very experienced centers. Even for senior electrophysiologists there may be a learning curve in the transition from fluoroscopically based procedures to zero-fluoroscopy procedures[17]. Second, even in the best scenario of published data on procedure planned to be with no fluoroscopy, very limited radiations are used in some cases[23] to assist a part of the procedure. Extrapolating these data to a wider population, it is unlikely that in the near future electrophysiologists will be able to work in laboratories not equipped with X-ray systems. Therefore, the zero-fluoroscopy strategy does not seem to bring any benefit in term of laboratory costs. Third, in ablation of atrial fibrillation with no fluoroscopy, some technologies, which require specific expertise and add costs in centers in which they are not routinely used, become necessary. In fact, to safely navigate catheters in the heart with no fluoroscopy, intracardiac ultrasounds is mandatory and imaging integration with pre-acquired computed tomography or magnetic resonance imaging very useful to obtain a high resolution anatomy of the left atrium and pulmonary veins[23,24]. The use of the recently introduced contact force sensing technology should be also considered mandatory to avoid excessive tissue/catheter contact when catheters are maneuvered with no fluoroscopy[22]. Fourth, the workflow of a zero-fluoroscopy procedure requires accurate cardiac chamber reconstruction before non-fluoroscopic catheter navigation. This can be done correctly only by experienced operators and, in any case, may significantly prolong the procedure duration, especially at the beginning of the specific learning curve in zero-

Table 1 Techniques and technologies for catheter ablation of atrial fibrillation in the four patient cohorts considered in our center

	1st cohort	2nd cohort	3rd cohort	4th cohort
No. of patients	30	30	30	30
Procedure technique	Double TSP-C Circular mapping catheter Imaging integration (CT scan)	Unchanged	Unchanged	Unchanged
NF technology	2nd generation NF 3-DS (CARTO XP)	3rd generation NF 3-DS (CARTO3)	Unchanged	CARTO3 + contact force sensing
Technology feature for NF use	NF visualization of the mapping/ablation catheter	NF visualization of all inserted catheter	Unchanged	Monitoring of the electrode/tissue contact added
NF 3-DS optimization	Yes	No	Yes (Table 2)	Yes (Table 2)
Timing	Last 30 cases with CARTO XP	First 30 cases with CARTO3	After 12 mo	After 12 mo

3-DS: Three dimensional system; CT: Computed tomography; NF: Non-fluoroscopic; TSP-C: Transseptal catheterization.

fluoroscopy procedures.

After these considerations, it can be concluded that zero-fluoroscopy procedures are a very interesting perspective for the future, but they are not common practice at present. Certainly, children and pregnant women are ideal candidates for zero-fluoroscopy catheter ablation, when other treatments fail or are not feasible. On the other hand, currently, every effort should be made by every operator to decrease as much as possible the use of radiation without endangering the procedure safety and efficacy until near-zero fluoroscopy procedures become routine.

LESSON LEARNED IN THE REDUCTION OF RADIATION EXPOSURE FOR ATRIAL FIBRILLATION ABLATION

Even in very experienced hands, catheter ablation of atrial fibrillation without a non-fluoroscopic three-dimensional system is associated with a fluoroscopy time of approximately 60 min[25] and, consequently, with a relatively high radiation exposure. However, as already mentioned[10], a non-fluoroscopic system without a workflow aimed at optimizing its use does not necessarily reduce the radiation exposure. In fact, in catheter ablation of atrial fibrillation, the sporadic use of non-fluoroscopic systems may paradoxically double the fluoroscopy time and radiation exposure when the system is used, due to the complexity of the procedure[26]. In a retrospective analysis[27] spanning 6 years (2004-2009) and including four cohorts of patients who showed comparable clinical characteristics and underwent catheter ablation of atrial fibrillation by using in a non-randomized way fluoroscopy or one of the non-fluoroscopic systems (Ensite NavX, CARTO XP, CARTO3), a third generation non-fluoroscopic system (CARTO 3) was associated with the shortest fluoroscopy time with no difference with the other 3 groups in term of procedural data and clinical outcomes. Although the reduction was statistically significant, the average fluoroscopy time using CARTO 3 in this study was still close to one hour (52 ± 21 min). This underlines the complexity of the variables that may determine the reduction of radiation exposure, which is not merely due to the use of a specific non-fluoroscopic system. Another study[28] further supports this concept. In this study, over six months, 120 patients were randomly assigned to use fluoroscopy only, a second generation (CARTO XP), or a third generation (CARTO3) non-fluoroscopic system to support catheter ablation of atrial fibrillation. The procedure was performed by operators with a specific experience in reduction of radiation exposure. While there was no difference in the clinical and anatomic variables among the three groups, the fluoroscopy time was shorter and less than 3 min for the whole procedure when the third generation non-fluoroscopic system was used with an optimized procedural workflow.

We evaluated the process of reduction of radiation exposure in catheter ablation of atrial fibrillation using a non-fluoroscopic three-dimensional electroanatomic system both in a single- and multicenter experience[29,30]. In our center, the procedural data of four cohorts of patients, sampled sequentially, were considered[29]. Each cohort included atrial fibrillation patients undergoing the first procedure of pulmonary vein isolation. The technologies and techniques used in each cohort are reported in Table 1. Among the four cohorts there was no significant difference in the clinical characteristics of the patients, in term of age, sex, body mass index, type and duration of atrial fibrillation, which reflects the homogeneous criteria used to select candidates for atrial fibrillation ablation in the considered time interval. The procedure was standardized as described elsewhere[31] and it was alternatively performed by two operators with a similar experience in atrial fibrillation ablation (> 400 procedures each), although the background in interventional electrophysiology was different (23 years *vs* 10 years, respectively). Importantly, the radiation exposure for the pre-procedure computed tomography scan was very low (< 1 mSv) due to an optimized acquisition protocol[31]. In the 3rd and 4th cohort, the use of a third generation non-fluoroscopic three-dimensional system was optimized by adopting the features listed in Table 2, including in the 4th cohort the recently introduced

Table 2 Features of the third generation non-fluoroscopic system CARTO 3 useful to minimize fluoroscopy during an electrophysiology procedure

Feature	Function
Imaging integration with pre-acquired CT or MRI image	Allows high resolution visualization of the LA and PVs; once registered in the system, the mapping/ablation catheter can be navigated with minimal use of fluoroscopy
Display in stable mode of the icon of the mapping/ablation catheter	Allows stable visualization of the mapping/ablation catheter on the system, similar to the one visualized on fluoroscopy
Colors on the distal part of the mapping/ablation catheter	Indicate the direction of the deflection of the distal part of the catheter
Catheter projection	Estimates the distance from the catheter tip to the surface of the electroanatomic map or to the surface of the CT/MRI image
Contact force sensing	Measures in grams the contact between the catheter tip and the tissue; used to avoid excessive contact during catheter manipulation and to optimize contact during ablation
Real time display of the circular mapping catheter	Allows real time visualization of the circular mapping catheter during positioning into the PVs
Highlight of the circular mapping catheter electrodes	Identify the position of the electrodes of the circular mapping catheter; used to identify the site of a conducting gap during circumferential PV ablation
Catheter snapshot	Shows a memorized position of a catheter (*e.g.*, circular mapping catheter); used to precisely re-navigated a previous catheter positioning

CT: Computed tomography; LA: Left atrium; MRI: Magnetic resonance imaging; PV: Pulmonary vein.

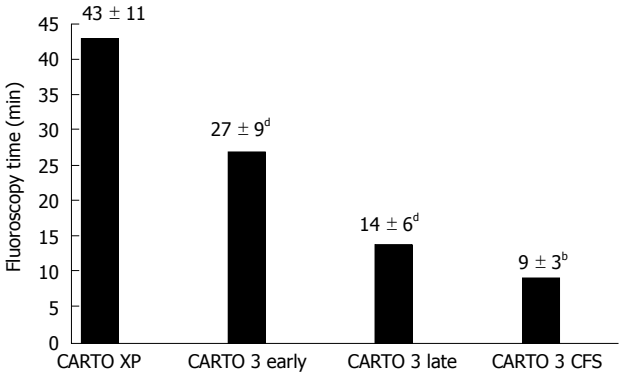

Figure 1 Histogram of fluoroscopy time (in minutes) for the whole procedure of pulmonary vein isolation in four cohorts of patients with atrial fibrillation, using the non-fluoroscopic CARTO system with progressively new technologies and protocols. There is a progressive and significant reduction in fluoroscopy time, but the greatest percent reduction (-48%) is observed between the second and third cohort, CARTO 3 early *vs* CARTO 3 late. In these two cohorts the technology was the same, but in the second one the system was used with an optimized protocol to reduce fluoroscopy. $^{b}P < 0.001$ *vs* the previous cohort; $^{d}P < 0.0001$ *vs* the previous cohort. CFS: Contact force sensing.

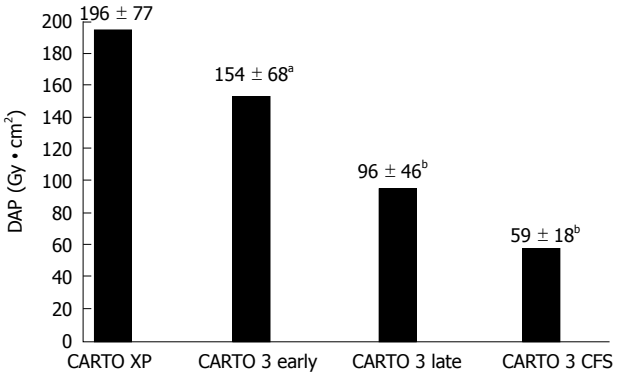

Figure 2 Histogram of dose area product values (in Gy•cm^2) for the whole procedure of pulmonary vein isolation in the same cohorts shown in Figure 1. As in Figure 1, there is a progressive and significant reduction in radiation exposure, expressed by the dose area product value. $^{a}P < 0.05$ *vs* the previous cohort; $^{b}P < 0.0001$ *vs* the previous cohort. DAP: Dose area product; CFS: Contact force sensing.

contact force sensing technology[32]. This was mainly used to avoid excessive contact force between the tip of the mapping/ablation catheter and the endocardium when the catheter was advanced non-fluoroscopically. Importantly, during non-fluoroscopic navigation of the catheter, its position was continuously monitored on the CARTO, to avoid events at risk for complications, such as entrapment of the circular mapping catheter in the mitral valve apparatus. While the procedural data, in term of procedure duration, number of pulmonary vein isolated, radiofrequency energy time, acute success and complication, was not significantly different among the four cohorts, there was a progressive decrease in fluoroscopy time and DAP values, as shown in Figures 1 and 2, respectively. Sub-analyzing data per operator, there are interesting findings when the 1st cohort is compared to the 2nd and the 2nd to the 3rd. In the first comparison, the more experienced operator obtained a 46% reduction (from 41 ± 9 to 22 ± 6 min, on average; $P < 0.0001$) in fluoroscopy time compared to only a 22% reduction (from 43 ± 13 to 33 ± 9 min, on average; $P = 0.0012$) obtained by the less experienced operator. Interestingly, an opposite phenomenon was observed in the second comparison: the more experienced operator, who had already obtained a greater reduction in the use of fluoroscopy, had a 36% reduction in fluoroscopy time (from 22 ± 6 min to 14 ± 5 min, on average; $P < 0.001$), definitely smaller than the 54% reduction obtained by the second operator (from 33 ± 9 min to 15 ± 7 min, on average, $P < 0.001$).

These data deserve two considerations, on the technology and the learning curve, respectively. First, the ability to reduce significantly radiation exposure towards a near zero-fluoroscopy procedure depends on the type and quality of the non-fluoroscopic system. A third generation non-fluoroscopic system, able to reliably visualize all the catheters inserted in the heart,

allows catheter manipulation with minimal or no use of fluoroscopy leading to an immediate improvement in radiation exposure compared to the older system. This was confirmed in the multicentric study in 240 consecutive patients undergoing catheter ablation of atrial fibrillation[30]. In this study, the average fluoroscopy time decreased from 26 ± 15 min to 16 ± 12 min ($P < 0.001$) and the positive effect of adopting the third generation system was significant in all the participating centers. The importance of the technology is further confirmed by the observation in our center of a still significant reduction in radiation exposure when the newer contact force sensing technology was introduced. The second consideration is on the need for a specific learning curve. Although in the multicenter study[30] the reduction in the use of fluoroscopy is observed in all centers, the percent reduction spans from 25% to 56% among centers. This is likely to be related to a specific learning curve in reduction of radiation exposure. In fact, considering again the data from our center, a more experienced electrophysiologist may exhibit a shorter learning curve in the reduction of radiation exposure, while a less experienced one eventually reaches the same level of ability in non-fluoroscopic maneuvering of catheters after a longer learning curve.

CONCLUSION

Over the last years, the awareness of the radiation injury hazard to the patients and the professional staff has greatly increased. Reduction in the radiation exposure in a complex electrophysiology procedure, such as atrial fibrillation ablation, should be considered. This is an increasingly used procedure with usually longer fluoroscopy times. Therefore, the decrease in radiation exposure is expected to improve the net benefit of the procedure for the patient and to minimize the radiation injury hazard for the professional staff. The lesson learned so far tells us that sophisticated technologies have to combine with a specific know-how to achieve this task. In fact, non-fluoroscopic three-dimensional systems with their constant updating in the technology content have a key role, but minimization in the use of radiations is obtained if these technologies are used with an optimized protocol and after a specific operators' learning curve. This may last several months and be longer for less experience operators.

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EDITORIAL

Glycated hemoglobin and its spinoffs: Cardiovascular disease markers or risk factors?

Jumana Saleh

Jumana Saleh, Department of Biochemistry, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat 123, Oman

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Correspondence to: Dr. Jumana Saleh, Department of Biochemistry, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, Muscat 123, Oman. jumana@squ.edu.om
Telephone: +968-24-143534

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Abstract

Atherosclerosis is a major complication of diabetes, increasing the risk of cardiovascular related morbidities and mortalities. The hallmark of diabetes is hyperglycemia which duration is best predicted by elevated glycated haemoglobin A_{1c} (HbA_{1c}) levels. Diabetic complications are usually attributed to oxidative stress associated with glycation of major structural and functional proteins. This non-enzymatic glycation of long lived proteins such as collagen, albumin, fibrinogen, liver enzymes and globulins result in the formation of early and advanced glycation end products (AGEs) associated with the production of myriads of free radicles and oxidants that have detrimental effects leading to diabetic complications. AGEs have been extensively discussed in the literature as etiological factors in the advancement of atherogenic events. Mechanisms described include the effects of glycation on protein structure and function that lead to defective receptor binding, impairment of immune system and enzyme function and alteration of basement membrane structural integrity. Hemoglobin (Hb) is a major circulating protein susceptible to glycation. Glycated Hb, namely HbA_{1c} is used as a useful tool in the diagnosis of diabetes progression. Many studies have shown strong positive associations between elevated HbA_{1c} levels and existing cardiovascular disease and major risk factors. Also, several studies presented HbA_{1c} as an independent predictor of cardiovascular risk. In spite of extensive reports on positive associations, limited evidence is available considering the role of glycated Hb in the etiology of atherosclerosis. This editorial highlights potential mechanisms by which glycated hemoglobin may contribute, as a causative factor, to the progression of atherosclerosis in diabetics.

Key words: Glycated hemoglobin; Glycoxidative stress; Advanced glycation end products; Atherosclerosis; Diabetes mellitus

Core tip: Glycated hemoglobin is a useful marker for the diagnosis of diabetes progression. Many studies present glycated haemoglobin (HbA_{1c}) as an independent predictor of cardiovascular risk in diabetics. Although haemoglobin (Hb) is a major circulating protein, limited

information is available about the role of glycated Hb as such in the etiology of atherosclerosis. This editorial highlights potential mechanisms by which glycated hemoglobin may contribute, as a causative factor, to the progression of atherosclerosis in diabetics.

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EDITORIAL

Abundant evidence exists that patients with diabetes mellitus have an increased risk of atherosclerosis and are more vulnerable to its progression into cardiovascular disease[1]. Several mechanisms were proposed to describe the pathogenesis of atherosclerosis in diabetic patients. Vascular endothelial cell damage, as a result of blood flow shear stress, increased blood viscosity and oxidative stress were described in several studies[1-4]. The chronic hyperglycemic state in diabetes creates an environment of oxidative stress manifested as a glycoxidative state[5]. This state is characterized by the accumulation of glycated proteins that are further modified into advanced glycation end products (AGEs). The discovery of AGEs dates back to 1912 when Louis-Camille Maillard originally observed a chemical reaction between amino acids and reducing sugars that gave browned foods their desirable flavor[6]. Human proteins normally undergo spontaneous non-enzymatic glycation reaction forming low levels of glycated products[7]. However, chronic exposure to abnormally high glucose levels leads to further modifications. The aldehyde group of the glucose molecule combines with the amino group of a lysine molecule in a protein to form a Schiff base which is a double bond between the carbon atom of the glucose and the nitrogen atom of lysine. The Schiff bases form Amadori products that undergo further molecular rearrangements producing advanced glycated end products AGEs. The formation of AGEs is accompanied by the release of myriads of oxidants and free radicals that cause oxidative damage in the cells and extracellular matrix. Subsequent degradation of AGEs produces more reactive oxidant species and protein reactive aldehydes that contribute to further macromolecular alterations[1,8,9]. In diabetes, long-lived proteins such as collagen, elastin and many enzymes are affected by advanced glycation which disrupts their structure and function[10]. Accumulation of AGE products contribute to a variety of vascular complications through the formation of cross-links between molecules leading to hardening of the vascular extracellular matrix (ECM) and increasing vascular permeability[9-11]. Modification of the extracellular matrix by AGEs traps cholesterol rich lipoproteins promoting their oxidation and stimulates an inflammatory response that accelerates plaque formation and advancement of the atherogenic process. Evidence of the formation of AGEs and their detrimental role in the pathogenesis and development of cardiovascular disease is extensively reported in the literature[10-13].

GLYCATED HEMOGLOBIN AS A DIAGNOSTIC MARKER AND ADVANCED GLYCATION PRODUCT

The extent and duration of hyperglycemia is best predicted by increased levels of glycated hemoglobin (glycated Hb) of which HbA_{1c} is considered a reliable marker[14,15]. HbA_{1c} in the medical literature is commonly described as a useful measure to reflect the duration of increased blood glucose levels up to several months[14]. Numerous studies have shown positive associations between elevated HbA_{1c} levels and cardiovascular disease including acute coronary syndrome, acute myocardial infarction and heart failure[15,16]. Large prospective cohort studies showed that HbA_{1c} is not only a diagnostic marker of diabetes progression, but also an independent cardiovascular risk predictor[17]. As mentioned earlier, prolonged sugar exposure produces early and AGEs affecting different proteins. A major example of early glycated proteins is HbA_{1c} which is further modified, through a series of reactions, into Hb-AGE[18]. Under normal conditions Hb-AGE constitutes 0.42% of circulating Hb levels which increases to 0.75% in diabetic subjects[19]. In spite of extensive reports showing positive associations between increased HbA_{1c} levels and cardiovascular risk in diabetics, the role of HbA_{1c} and Hb-AGE as potential etiological culprits in diabetic disease progression has been rarely discussed. This editorial highlights mechanisms by which glycated Hb may contribute, as a causative factor, to the initiation and development of atherosclerosis in diabetics.

HB GLYCATION ACCENTUATES INTRACELLULAR OXIDATIVE STRESS AND INCREASES ERYTHROCYTE FRAGILITY

Besides albumin, hemoglobin comprises a major fraction of circulating proteins that are susceptible to early and advanced glycation events. Glycation is accelerated in diabetics[11] where glucose uptake by erythrocytes is insulin independent and highly uncontrolled. Furthermore, glycated Hb is more readily oxidized and degraded by erythrocyte proteolytic enzymes than unglycated Hb[20,21] enhancing oxidative stress by increasing the release of heme and free iron in association with free radicles[22-25]. The released ferrous iron (II) reacts with hydrogen peroxide *via* the

Fenton reaction forming ferric iron (II) and hydroxyl radicals[26]. These reactive species contribute to further oxidative stress damaging lipids and proteins that alter cell membrane properties and lead to increased erythrocyte fragility[27,28]. High exposure to oxygen during gas transport render erythrocytes even more vulnerable to oxidative damage. However, damage is normally prevented by anti-oxidant factors that maintain a balanced intracellular oxidation status. This balanced environment maintains an intact Hb structure which itself exerts a stabilizing effect on erythrocyte membrane structure. When Hb structure is altered due to persistent glyco-oxidative stress, Hb becomes more susceptible to degradation decreasing the life span of erythrocytes. Studies have shown a decreased life span of 6.9 d for 1% increase in glycated Hb levels[29].

HB GLYCATION AFFECTS BLOOD VISCOSITY AND CONTRIBUTES TO ENDOTHELIAL INFLAMMATION AND VASCULAR DYSFUNCTION

Intracellular glyco-oxidative stress may contribute to vascular endothelial damage through several mechanisms: (1) accumulation of intracellular free radicals alters erythrocyte membrane properties leading to erythrocyte aggregation, increased blood viscosity and impaired blood flow. Shear stress, due to thicker abrasive blood consistency, affecting the vascular endothelium and triggering an inflammatory response that contribute to subsequent atherogenic events[3,4,27,28,30]; (2) buildup of free radicles promotes the oxidation of ferrous Hb (Hb-Fe^{2+}) into ferric Hb (Hb-Fe^{3+}) (methemoglobin), which is further modified, through several oxidation steps, into ferryl hemoglobin (Hb-Fe^{3+}/Fe^{4+}). The ferryl iron (Fe^{4+}) is unstable and regains the Fe^{3+} state by reacting with specific amino acids in hemoglobin forming covalently cross-linked Hb multimers[31]. The altered Hb structure promotes cellular damage and releases ferryl Hb into the subendothelial matrix. Silva *et al*[32] demonstrated that ferryl Hb, rather than Hb, or methemoglobin, increased endothelial permeability and production of pro-inflammatory monocyte adhesion proteins that promote macrophage accumulation and a local inflammatory reaction preceding plaque formation; (3) Free Hb penetrates the vascular smooth muscle layer[33] and inactivates endothelium-dependent relaxation induced by acetylcholine[34] possibly through binding to nitric oxide (NO) which is a potent vasodilator which initiates vaso-relaxation in response to stimuli. Nitric oxide also inhibits formation of oxidized LDL[35] which detrimental to endothelial integrity. Inactivation of NO is a major marker of endothelial dysfunction manifested in impaired vasoactive responses[35]. Rodríguez-Mañas *et al*[36] demonstrated that highly glycosylated Hb inhibited nitric oxide mediated relaxation to a larger extent than low glycated and unglycated Hb. The authors suggested that Hb-AGEs may exacerbate this effect as abundant *in vitro* and *in vivo* evidence demonstrates that AGEs inhibit nitric oxide production and function[36]; and (4) Furthermore, accelerated degradation of erythrocytes releases heme which sensitizes endothelial cells to oxidative damage and promotes oxidation of endothelial proteins and low density lipoproteins (LDLs)[31].

Altogether, these adverse modifications trigger a proliferative inflammatory response in the subendothelial space which involves recruitment of a myriad of inflammatory and immune factors including monocytes, platelets, lymphocytes and increased production of various growth factors and cytokines such as IL-1 and TNF-α and adhesion molecules[37]. Oxidized LDL particles are subsequently scavenged by macrophages forming lipid rich foam cells that contribute to the formation of fatty streaks and subsequent build-up of plaque. As atherosclerotic plaque builds up, further insult to the endothelium activates a vicious cycle of inflammatory/oxidation events and further progression of atherosclerosis[38]. The list of endothelial mediators that contribute to this inflammatory/atherogenic process continues to grow. Interleukin-17 (IL-17), produced by T-helper cells, induces chemokines such as IL-6, IFN-γ and TNF-α to recruit monocytes and neutrophils to the site of inflammation. Recent evidence points to additional allergic/hypergic responses, induced by IL-17, which involve cytokines such as IL-8 and eotaxin believed to play a role in atherogenesis. IL-17 induces eotaxin secretion from smooth muscles, macrophages and fat tissue in the atheromatous plaque[39]. The recruitment of eosinophils by eotaxin during the inflammatory process was recently linked to vascular inflammation and cardiovascular disease[40]. Exploring the relation between these inflammatory mediators and oxidative modification of glycated Hb may provide new avenues for understanding the progression of atherogenic events.

In summary, accumulating evidence suggests that glycation of Hb and formation of Hb-AGE in diabetics exacerbate cellular oxidative stress releasing potent oxidants which contribute to endothelial oxidative damage and trigger a vicious cycle of oxidative/inflammatory responses. Recruitment of inflammatory mediators contributes to the progression of atherogenesis and the development of diabetic vascular complications. Designing preventive and therapeutic measures that target hemoglobin glyco-oxidative pathways may be useful tools for the management and control of atherosclerosis progression and cardiovascular disease in diabetics.

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REVIEW

Adipose tissue-derived stem cells as a therapeutic tool for cardiovascular disease

Etsu Suzuki, Daishi Fujita, Masao Takahashi, Shigeyoshi Oba, Hiroaki Nishimatsu

Etsu Suzuki, Institute of Medical Science, St. Marianna University School of Medicine, Kawasaki 216-8512, Japan

Daishi Fujita, Masao Takahashi, Shigeyoshi Oba, the Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo 113-8655, Japan

Hiroaki Nishimatsu, the Department of Urology, Faculty of Medicine, University of Tokyo, Tokyo 113-8655, Japan

Author contributions: Suzuki E, Fujita D, Takahashi M, Oba S and Nishimatsu H contributed to this paper.

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Correspondence to: Etsu Suzuki, MD, PhD, Institute of Medical Science, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki 216-8512, Japan. esuzuki-tky@umin.ac.jp
Fax: +81-44-9778361

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Abstract

Adipose tissue-derived stem cells (ADSCs) are adult stem cells that can be easily harvested from subcutaneous adipose tissue. Many studies have demonstrated that ADSCs differentiate into vascular endothelial cells (VECs), vascular smooth muscle cells (VSMCs), and cardiomyocytes *in vitro* and *in vivo*. However, ADSCs may fuse with tissue-resident cells and obtain the corresponding characteristics of those cells. If fusion occurs, ADSCs may express markers of VECs, VSMCs, and cardiomyocytes without direct differentiation into these cell types. ADSCs also produce a variety of paracrine factors such as vascular endothelial growth factor, hepatocyte growth factor, and insulin-like growth factor-1 that have proangiogenic and/or antiapoptotic activities. Thus, ADSCs have the potential to regenerate the cardiovascular system *via* direct differentiation into VECs, VSMCs, and cardiomyocytes, fusion with tissue-resident cells, and the production of paracrine factors. Numerous animal studies have demonstrated the efficacy of ADSC implantation in the treatment of acute myocardial infarction (AMI), ischemic cardiomyopathy (ICM), dilated cardiomyopathy, hindlimb ischemia, and stroke. Clinical studies regarding the use of autologous ADSCs for treating patients with AMI and ICM have recently been initiated. ADSC implantation has been reported as safe and effective so far. Therefore, ADSCs appear to be useful for the treatment of cardiovascular disease. However, the tumorigenic potential of ADSCs requires careful evaluation before their safe clinical application.

Key words: Adipose tissue-derived stem cells; Cardiovascular disease; Acute myocardial infarction; Ischemic cardiomyopathy; Hindlimb ischemia; Stroke

Core tip: Adipose tissue-derived stem cells (ADSCs) have been used for the treatment of cardiovascular disease with the efficacy of ADSC implantation demonstrated in animal models. However, the mechanisms under-

lying the capacity of ADSCs for regenerating the cardiovascular system remain controversial. ADSCs may differentiate into blood vessels and cardiomyocytes, fuse with other cell types, obtaining the characteristics of those cells, and secrete paracrine factors that have proangiogenic and/or antiapoptotic activities. This review also discusses recently initiated clinical trials using autologous ADSCs.

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INTRODUCTION

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality worldwide. Despite advances in the treatment of acute myocardial infarction (AMI) using percutaneous coronary intervention, the treatment of heart failure (HF), which occurs as a result of the death of myocardial tissues and subsequent tissue remodeling, is still a challenging problem. As cardiomyocytes are terminally differentiated cells with minimal regenerative capacity, heart transplantation is currently the only treatment option for end-stage ischemic heart disease. The development of new therapies for AMI and HF is required to meet this substantial clinical requirement. Thus, stem cell therapy for CVD has recently gained substantial attention.

Stem cells are defined as cells capable of self-renewal and differentiation into a variety of phenotypes[1]. Stem cells comprise embryonic stem cells (ESCs) and adult stem cells (ASCs). ESCs were originally isolated from the inner cell mass of blastocysts[2], and are pluripotent stem cells capable of giving rise to all three germ layers. However, several issues, including ethical concerns and teratoma formation, limit the clinical use of ESCs. Induced pluripotent stem (iPS) cells are also pluripotent stem cells that have very similar characteristics to ESCs[3,4]. As ethical problems can be avoided, iPS cells represent a potentially promising option for stem cell therapy. However, cancer formation is a major issue that needs to be overcome before widespread acceptance of the use of iPS cells in clinical settings. ASCs are multipotent stem cells that reside in various adult tissues. Among ASCs, bone marrow-derived mesenchymal stem cells (BMMSCs) and adipose tissue-derived stem cells (ADSCs) are the most extensively studied. BMMSCs are reported to have the potential to differentiate into various cell types including bone, cartilage, cardiac muscle, skeletal muscle, vascular endothelial cells (VECs), and vascular smooth muscle cells (VSMCs)[5,6]. BMMSCs have been used to treat CVD in clinical settings, with promising results reported in a number of studies[7-14], although other studies failed to demonstrate positive outcomes[15,16]. ADSCs have gained substantial attention recently as subcutaneous adipose tissues are abundant and can be easily harvested using liposuction, a procedure that is less invasive than bone marrow aspiration, with minimal donor discomfort. Adipose tissue contains a significantly greater proportion of stem cells than the bone marrow (5% *vs* 0.01%) and is therefore a convenient source of stem cells[17]. Furthermore, ADSCs reportedly do not express class II major histocompatibility complexes[18,19], suggesting that ADSCs may be suitable for allogenic transplantation in addition to autologous transplantation. In this review, we discuss the characteristics of ADSCs and their potential use in the treatment of CVD.

CLASSIFICATION OF ADSCS

ADSCs can be obtained from subcutaneous adipose tissues with the use of collagenase digestion. Freshly isolated ADSCs (fADSCs) are known to be heterogeneous and contain hematopoietic cells ($CD45^+$ and/or $CD34^+$) and VECs ($CD34^+/CD31^+$) in addition to stem cells ($CD44^+$ and $CD105^+$)[20]. fADSCs can be cultured on plastic dishes in the presence of fetal bovine serum (FBS). Non-adherent cells, those that do not attach to plastic dishes, can be removed to obtain cultured ADSCs (cADSCs), a relatively homogeneous population that expresses stem cell markers, such as CD44 and CD105, but not hematopoietic lineage markers, including CD11b, CD45, and CD34 or the VEC marker CD31[21,22]. Artificially-modified ADSCs (mADSCs) are a type of ADSCs produced through the introduction of specific genes[23,24] or pre-treatment with drugs[25] before administration. The purpose of artificial modification is to improve the function of ADSCs, such as proangiogenic and antiapoptotic activities. cADSCs have been the most widely used type, particularly in animal studies. However, fADSCs may be more suitable for clinical applications for several reasons. First, fADSCs can be rapidly prepared compared with cADSCs as cell culture is not required while preparing fADSCs. The rapid preparation and administration of stem cells may be required to achieve sufficient recovery from tissue ischemia when treating AMI or critical hindlimb ischemia. Second, the preparation of fADSCs is technically less challenging compared with that of cADSCs as it does not require the use of foreign materials such as FBS. ADSCs used in clinical settings must not contain any foreign materials derived from animals or humans other than the individual patient receiving the stem cell therapy. Therefore, it is desirable to avoid culturing in the preparation of ADSCs for clinical applications.

DIFFERENTIATION POTENTIAL OF ADSCS *IN VITRO*

ADSCs have the potential to differentiate into cartilage,

Table 1 Differentiation potential of adipose tissue-derived stem cells *in vitro*

Cell type	Expression of VEC markers	Expression of VSMC markers	Expression of cardiomyocyte markers	Production of paracrine factors	Ref.
Human fADSCs	CD31, vWF	NE	NE	NE	Miranville *et al*[30]
Human cADSCs	CD31, vWF	NE	NE	NE	Planat-Benard *et al*[31]
Human cADSCs	NE	SMA, calponin, caldesmon, myosin heavy chain, SM22α	NE	NE	Rodríguez *et al*[32]
Human cADSCs	NE	SMA, calponin, SM22α	NE	NE	Jeon *et al*[33]
Rabbit cADSCs	NE	NE	Myosin heavy chain, sarcomeric α-actinin, troponin I	NE	Rangappa *et al*[34]
Human cADSCs	NE	NE	Sarcomeric α-actinin, desmin, cardiac troponin	NE	Gaustad *et al*[35]
Murine fADSCs	NE	NE	GATA-4, Nkx2.5	NE	Planat-Bénard *et al*[36]
Human cADSCs	NE	NE	NE	VEGF, HGF, TGF-β	Rehman *et al*[44]
Murine cADSCs	ND	ND	NE	VEGF, HGF	Nakagami *et al*[45]
Human cADSCs	NE	NE	NE	VEGF, IGF-1	Sadat *et al*[46]

NE: Not examined; ND: Not detected; ADSCs: Adipose tissue-derived stem cells; VEC: Vascular endothelial cell; TGF: Transforming growth factor; VSMC: Vascular smooth muscle cell; HGF: Hepatocyte growth factor; IGF-1: Insulin-like growth factor-1; vWF: von Willebrand factor.

bone, tendon, and fat when cultured under lineage-specific conditions[26-29]. Furthermore, ADSCs have the potential to differentiate into VECs, VSMCs, and cardiomyocytes *in vitro* (Table 1), the main components of the cardiovascular system. Miranville *et al*[30] isolated and examined the characteristics of human fADSCs. Human fADSCs were found to express CD34 (27.6%-63.4%) with CD34 positive cells shown to be composed of two populations: $CD34^+/CD31^+$ cells (probably VECs) and $CD34^+/CD31^-$ cells. The authors demonstrated $CD34^+/CD31^-$ cells expressed CD31 and von Willebrand factor (vWF) when cultured in a medium containing vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF). Planat-Benard *et al*[31] used relatively fresh human cADSCs cultured on plastic dishes for 3 d without passaging. Approximately 90% of these cells were found to express CD34, and they expressed VEC markers, including CD31 and vWF, when cultured in a semisolid medium. Rodríguez *et al*[32] studied human cADSCs cultured in MCDB 131 medium supplemented with 1% FBS. The authors found these cells expressed VSMC markers, including α-smooth muscle actin (SMA), calponin, caldesmon, myosin heavy chain, and smooth muscle protein 22-α (SM22α). Furthermore, differentiated cells contracted in response to carbachol demonstrated contractile capacity. Jeon *et al*[33] demonstrated the use of sphingosylphosphorylcholine (SPC) to induce the differentiation of human cADSCs into VSMCs, as determined by SMA, calponin, and SM22α expression. They also found that SPC-induced differentiation of ADSCs into VSMCs depended on transforming growth factor-β (TGF-β), shown to be secreted by ADSCs in an autocrine manner. Rangappa *et al*[34] incubated rabbit cADSCs with 5-azacytidine. The authors demonstrated that these cells differentiated into spontaneously beating cardiomyocytes with expression of myosin heavy chain, sarcomeric α-actinin, and troponin I. Gaustad *et al*[35] incubated human cADSCs with rat cardiomyocyte extracts and demonstrated ADSC expression of cardiomyocyte markers, including sarcomeric α-actinin, desmin, and cardiac troponin I. Differentiated cells were also shown to beat autonomously. Planat-Bénard *et al*[36] cultured murine fADSCs in a semisolid methylcellulose medium without 5-azacytidine and found that ADSCs expressed cardiac-specific markers, such as transcription factors, GATA-4, and Nkx2.5. These cells demonstrated spontaneous beating with acceleration in response to isoproterenol, a β-agonist, and deceleration in response to carbamylcholine, an acetylcholine agonist.

DIFFERENTIATION POTENTIAL OF ADSCS *IN VIVO*

It has also been suggested that ADSCs express VEC, VSMC, and cardiomyocyte markers *in vivo* (Table 2). For example, cADSCs administered in a hindlimb ischemia model[31] and AMI model[37] were reportedly incorporated into tissues and were found to express VEC markers, such as CD31 and vWF. ADSC implantation has been shown to improve blood flow in a murine hindlimb ischemia model[31]. Jack *et al*[38] injected human cADSCs into the bladder and urethra and demonstrated the expression of SMA, a marker for VSMCs, by engrafted cells. Valina *et al*[37] injected porcine cADSCs into the coronary artery following the induction of AMI and found that a proportion of engrafted cells expressed SMA. The authors also found that left ventricular function recovered following administration of ADSCs. Strem *et al*[39] prepared fADSCs from Rosa 26 mice ubiquitously expressing β-galactosidase and injected these cells into the intraventricular chamber following myocardial cryoinjury. The authors demonstrated co-expression of β-galactosidase with myosin heavy chain, Nkx2.5, and troponin I. Yamada *et al*[40] transplanted the CD29 positive fraction of murine cADSCs into the infarct border zone of an AMI model and demonstrated

Table 2 Differentiation potential of adipose tissue-derived stem cells *in vivo*

Cell type	Animal model	Expression of VEC markers	Expression of VSMC markers	Expression of cardiomyocyte markers	Functional recovery	Ref.
Human cADSCs	Murine hindlimb ischemia	CD31	NE	NE	Yes	Planat-Benard *et al*[31]
Porcine cADSCs	Porcine AMI	vWF	SMA	NE	Yes	Valina *et al*[37]
Human cADSCs	Bladders and urethras of athymic rats and SCID mice	NE	SMA	NE	NE	Jack *et al*[38]
Murine fADSCs	Murine AMI	NE	NE	Myosin heavy chain, Nkx2.5, troponin I	Yes	Strem *et al*[39]
Murine fADSCs	Rat AMI	NE	NE	Sarcomeric actin, GATA-4	Yes	Yamada *et al*[40]
Conditioned medium from human cADSCs	Murine hindlimb ischemia	NE	NE	NE	Yes	Bhang *et al*[48]

NE: Not examined; VSMC: Vascular smooth muscle cell; ADSCs: Adipose tissue-derived stem cells; vWF: von Willebrand factor; VEC: Vascular endothelial cell; SMA: Smooth muscle actin; AMI: Acute myocardial infarction.

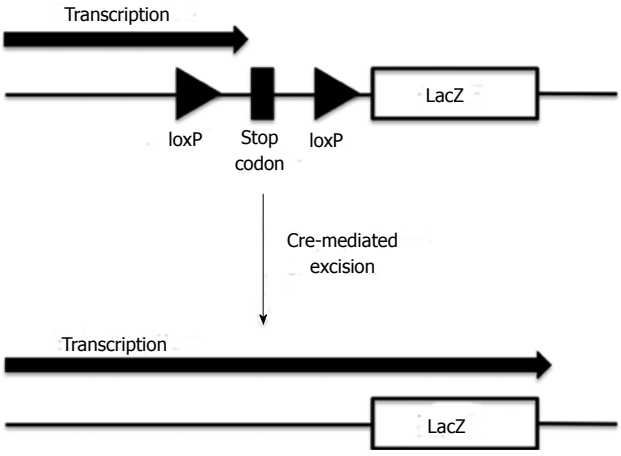

Figure 1 Schematic representation of LacZ expression following the excision of a floxed stop codon by Cre recombinase.

the expression of cardiomyocyte markers, such as sarcomeric actin and GATA-4. Furthermore, improved left ventricular function was observed in this study.

However, cell fusion should be considered carefully before concluding that ADSCs have the potential to differentiate into VECs, VSMCs, or cardiomyocytes *in vivo*. The *in vivo* fusion of administered ADSCs with tissue-resident VECs, VSMCs, and/or cardiomyocytes may lead to ADSCs acquiring the phenotypes of the corresponding fused cell types, making it appear as if ADSCs are directly differentiating into these cell types. In fact, cell fusion has been shown to occur with the *in vivo* administration of BMMSCs. Alvarez-Dolado *et al*[41] used R26R mice that contain a *lacZ* reporter gene downstream of a stop codon flanked by loxP sites (floxed). The *lacZ* reporter gene was therefore only expressed when the loxP-flanked stop codon was excised by Cre recombinase (Figure 1). The authors lethally irradiated these mice and transplanted BMMSCs from mice that ubiquitously express Cre recombinase and green fluorescent protein (GFP). If cells from the donor and recipient fused, the Cre enzyme would excise the Lox P-flanked stop codon, thereby allowing the expression of the *lacZ* gene. The results of this study revealed β-gal$^+$ (fused) and GFP$^+$ cells in the brain, heart, and liver of recipients, at 2 and 4 mo post-transplantation. Thus, BMMSCs potentially fuse with other cell types *in vivo*. There have been no reports so far clearly demonstrating the fusion of ADSCs with other cell types *in vivo*. Bai *et al*[42] injected both human fADSCs and cADSCs into murine hearts and examined the occurrence of cell fusion using fluorescence *in situ* hybridization to detect human X chromosomes and murine Y chromosomes. The authors did not detect co-localization of human X chromosomes with murine Y chromosomes in individual cells, excluding the possibility of cell fusion events. However, similar techniques used to detect cell fusion by BMMSCs (*e.g.*, the transplantation of ADSCs derived from transgenic mice expressing Cre recombinase into recipients expressing a *lacZ* reporter gene downstream of a floxed stop codon) should be used to conclusively determine whether ADSCs fuse with other tissue-resident cell types. Interestingly, Metzele *et al*[43] artificially fused human cADSCs with neonatal rat cardiomyocytes using hemagglutinating virus of Japan. The authors demonstrated spontaneous beating of fused ADSCs and the expression of human troponin I, suggesting fused ADSCs produced cardiomyogenic proteins. Furthermore, fused ADSCs were positive for the cell proliferation marker Ki67, suggesting proliferating capacity in marked contrast to terminally differentiated cardiomyocytes that are unable to proliferate. Therefore, ADSCs may stimulate the regeneration of heart muscles through *in vivo* fusion with cardiomyocytes.

PRODUCTION OF PARACRINE FACTORS BY ADSCS

ADSCs have been shown to produce a variety of proangiogenic and antiapoptotic factors. Rehman *et al*[44] examined the production of paracrine factors by human cADSCs. The authors showed that ADSCs produced VEGF, hepatocyte growth factor (HGF), and TGF-β. VEGF production increased five-fold when ADSCs were cultured under hypoxic conditions. Condi-

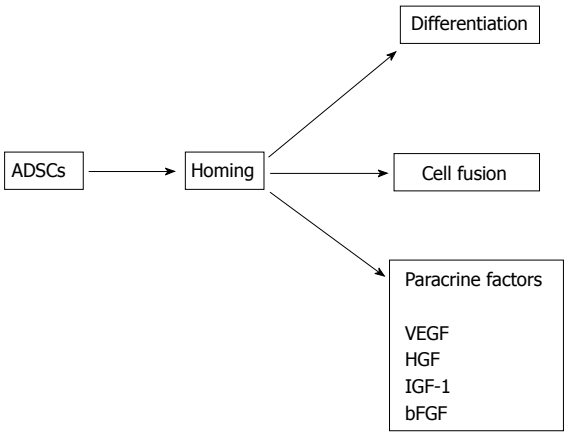

Figure 2 Possible mechanisms underlying the effect of adipose tissue-derived stem cells on regeneration of the cardiovascular system. ADSCs: Adipose tissue-derived stem cells; VEGF: Vascular endothelial growth factor; HGF: Hepatocyte growth factor; IGF-1: Insulin-like growth factor-1; bFGF: Basic fibroblast growth factor.

tioned medium (CM) obtained from hypoxic ADSCs significantly increased the proliferation and survival of VECs. Furthermore, the administration of these ADSCs significantly improved perfusion in a hindlimb ischemia model. Nakagami *et al*[45] reported murine cADSCs produce VEGF and HGF. The authors also administered ADSCs in a mouse hindlimb ischemia model and found transplanted ADSCs improved blood flow. However, transplanted ADSCs did not express VEC or VSMC markers, suggesting that ADSCs did not differentiate into vascular components in this study. Sadat *et al*[46] demonstrated human cADSCs produce VEGF and IGF-I and that these cytokines contribute to the antiapoptotic effects of ADSCs on cardiomyocytes. The authors implicated the secretion of VEGF by ADSCs in the ADSC-induced stimulation of tube formation by VECs. Yeghiazarians *et al*[47] administered BMMSCs and their lysates into the heart in a murine AMI model. The authors revealed that both BMMSCs and their lysates improved cardiac function and histology to similar extents, suggesting cytokines produced by BMMSCs, but not cells *per se*, are required for the recovery of cardiac function. Bhang *et al*[48] used a three-dimensional spheroid culture of human ADSCs to prepared CM. The authors injected CM into ischemic regions in a murine hindlimb ischemia model. They detected restoration of blood perfusion in this model. Albersen *et al*[49] injected rat cADSCs and their lysates into the penis in a rat model of cavernous nerve injury. The authors found that both ADSCs and their lysates restored erectile function to similar extents. These results suggest substances secreted by ADSCs, rather than cells *per se*, are critical for their regenerative function. Collectively, these results suggest that paracrine factors produced by ADSCs play a major, if not all, role in the regeneration of the cardiovascular system, although the differentiation and cell fusion of ADSCs may also be involved. The possible mechanisms underlying the regenerative effects of ADSCs on the cardiovascular system are summarized in Figure 2.

SURVIVAL OF ADSCS *IN VIVO*

The survival and engraftment of ADSCs *in vivo* have been examined within 30 d of ADSC implantation in the majority of studies[37,50-53]. Yin *et al*[54] injected swine cADSCs into the coronary artery following induction of AMI and examined the fate of ADSCs 8 wk after injection. The authors found that many ADSCs expressed troponin T and α-sarcomeric actin, indicating the ability of ADSCs to survive for 8 wk. Bai *et al*[42] introduced a luciferase reporter gene into human cADSCs and transplanted these cells into the murine heart muscle using an AMI model. The authors detected luciferase-positive ADSCs by bioluminescence imaging. Bioluminescence was observed 16 wk after ADSC transplantation, indicating that some ADSCs survived for 16 wk. However, murine cADSCs transplanted in a hindlimb ischemia model were found to barely remain in ischemic tissues 28 d after transplantation[45]. Therefore, the survival and engraftment of ADSCs in recipient tissues appear to vary according to the animal species and experimental models used.

APPLICATION OF ADSCS TO TREAT CVD

AMI and ischemic cardiomyopathy

Many studies have demonstrated the efficacy of ADSC administration in recovering cardiac function in AMI models. cADSCs have been predominantly used in animal models[37,39,40,51-57], although fADSCs[58] and mADSCs[23-25,59] have also been used. ADSCs have been transplanted *via* the coronary artery[37,53,54,57] and directly into cardiac muscles[23-25,39,40,51,55,56,58,59] in previous studies. Although ADSC implantation into the heart showed efficacy in recovering cardiac function in most studies, the underlying mechanisms remain controversial. Transplanted ADSCs expressed VEC, VSMC, or cardiomyocyte markers in numerous studies[37,39,40,52-55,57,59]; however, the "differentiation" of ADSCs was either not detected or examined in other studies[23-25,51,56,58]. Bai *et al*[42] transplanted both fADSCs and cADSCs in a murine AMI model and found both cell types recovered cardiac function to a similar extent. A proportion of transplanted fADSCs and cADSCs were found to express cardiomyocyte markers, including troponin I and connexin 43. These results are encouraging as fADSCs may be more suitable for clinical applications than cADSCs for reasons outlined above. ADSCs differentiated into specific cell types have been used to treat chronic MI. Okura *et al*[60] induced the differentiation of human cADSCs into cardiomyoblast-like cells (CLCs) *in vitro* and transplanted these cells into the swine coronary arteries 4 wk following the induction of MI. Cardiac function was

recovered by CLC implantation. Furthermore, implanted CLCs expressed human α-cardiac actin, Nkx2.5, and GATA-4. Several studies have used a monolayer sheet to transplant ADSCs into chronic MI models. Miyahara *et al*[61] cultured rat ADSCs on a temperature-responsive polymer to prepare a monolayer of ADSCs. The authors transplanted these cells onto scarred myocardium at 4 wk following coronary ligation. Transplanted cells grew *in situ* to form a thick stratum containing newly-formed blood vessels. The transplantation of monolayered cells prevented ventricular wall scarring and improved cardiac function. Okura *et al*[62] induced the differentiation of human cADSCs into CLCs *in vitro* and prepared monolayer sheets of human CLCs and ADSCs using a temperature-responsive polymer. The authors then transplanted these cells onto the infarcted areas of rats 4 wk following the induction of MI. The authors demonstrated that the implantation of CLCs, but not ADSCs, resulted in a long-term recovery of cardiac function and improved survival. Furthermore, CLCs, but not ADSCs, were found to express human troponin I.

Clinical trials of ADSCs in the treatment of AMI have recently been initiated. The AdiPOse-derived stem ceLLs in the treatment of patients with ST-elevation myOcardial infarction (APOLLO) trial is a double-blind, placebo-controlled, phase Ⅰ/Ⅱa trial[63]. Autologous fADSCs were transplanted into the coronary artery of AMI patients with ST-segment elevation following successful revascularization. During the 6-mo follow-up period, improvements in the left ventricular ejection fraction and myocardial perfusion and reductions in the infarct size were demonstrated. The subsequent phase Ⅱ/Ⅲ trial, called ADVANCE, is currently ongoing. In this trial, AMI patients with ST elevation are treated with intracoronary implantation of autologous fADSCs. The primary endpoint is reduction in the infarct size as measured by magnetic resonance imaging. The adiPose-deRived stEm Cells In the treatment of patients with non revaScularizable ischEmic myocardium (PRECISE) trial enrolled patients who had chronic ischemic cardiomyopathy (ICM) not amenable to any revascularization procedures[64]. Autologous fADSCs were transplanted into cardiac muscles from endocardial sites. Maximal oxygen consumption and total left ventricular mass were significantly improved by ADSCs implantation. The ATHENA trial is an ongoing clinical trial intending to treat patients who have chronic ICM with HF symptoms using autologous fADSCs. The endpoints of this trial include peak oxygen consumption, perfusion defects, HF symptoms, left ventricle end-systolic and diastolic volume, and ejection fraction.

Dilated cardiomyopathy

Several studies have demonstrated the efficacy of ADSC implantation in the recovery of cardiac function using dilated cardiomyopathy (DCM) models. Lin *et al*[65] used a rat DCM model induced by the injection of porcine myosin and implanted cADSCs into cardiac muscle. The effect of combination therapy with ADSCs and sildenafil, a phosphodiesterase type-5 inhibitor, was also evaluated. This study found that either ADSCs implantation alone or sildenafil treatment alone was effective for the recovery of cardiac function, with combination therapy being the most effective. Hamdi *et al*[66] transplanted a monolayer sheet of murine cADSCs onto the heart surface in a murine DCM model, in which a floxed serum response factor gene is conditionally deleted using the expression of Cre recombinase. The authors found many blood vessels in transplanted sheets and some transplanted ADSCs in the cardiac muscle, a proportion of which expressed CD31. The authors further demonstrated the recovery of cardiac function and significant reduction of cardiac fibrosis following ADSC transplantation. Pınarlı *et al*[67] transplanted cADSCs into a doxorubicin-induced HF model. They further examined combination therapy of ADSC transplantation with resveratrol, a polyphenolic compound found in red grapes with an antioxidant activity. This study found either ADSC implantation alone, or resveratrol administration alone, was effective in recovering cardiac function, although combination therapy was found to be most effective.

Hindlimb ischemia

A number of studies have demonstrated that ADSC implantation improves blood flow in animal hindlimb ischemia models. fADSCs[30,68], cADSCs[31,45,48,69-76], and mADSCs[77] have all been used in these studies. Although the efficacy of ADSC administration in the recovery of blood flow appears conclusive, the mechanisms underlying the ability of ADSCs to recover blood flow remain controversial. ADSCs have been shown to engraft and express VEC and/or VSMC markers in some studies[30,31,69,70,72,75,77]. However, other studies have shown that engraftment was either not observed or examined[68,76] or paracrine factors secreted by ADSCs appeared to predominantly mediate the recovery of blood flow[45,48,70,71,73,74,77]. Lee *et al*[76] performed the transplantation of autologous cADSCs in 15 patients with critical limb ischemia. Although this was a pilot study, ADSC implantation caused no complications during the follow-up period and clinical improvement was observed in 66.7% patients. Larger-scale clinical studies are required to conclusively evaluate the efficacy and safety of ADSC transplantation in the treatment of limb ischemia.

Stroke

Several studies have demonstrated ADSC implantation induces functional recovery following brain ischemia in animal models of cerebral infarction (CI)[78-81]. Kang *et al*[78] occluded the middle cerebral artery (MCA) to induce CI and transplanted human cADSCs into the lateral ventricle. Transplanted ADSCs migrated to the border zone of the injured area and intact brain tissue and into injured areas. A proportion of ADSCs were found to express microtubule-associated protein 2 (MAP2), a neuron marker, and glial fibrillary acid

protein (GFAP), an astrocyte marker. Furthermore, ADSC implantation improved motor and somatosensory behavior following CI, although no reduction in the area of CI was observed following ADSC implantation. Gutiérrez-Fernández *et al*[79] injected cADSCs intravenously following MCA occlusion in rats and found a significant recovery of motor function, although no reduction in the infarct size or ADSC engraftment into damaged tissues was observed. Furthermore, the expression of VEGF, synaptophysin, a neuron marker, and neurofilament was significantly increased following ADSC injection, although it was not examined whether ADSCs *per se* produced these molecules. Liu *et al*[80] transplanted human cADSCs into the right corpus striatum and cerebral cortex of rats following MCA occlusion. Neurological deficits were significantly attenuated by ADSC administration. Significantly increased expression of brain-derived neurotrophic factor (BDNF), nerve growth factor, and basic fibroblast growth factor mRNA and increased protein levels of BDNF and Bcl-2 were observed following ADSC transplantation, although it was not examined whether ADSCs produced these molecules.

Coronary artery restenosis

Balloon injury of the carotid artery and wire injury of the femoral artery have been widely used as models of coronary artery restenosis. We implanted rat cADSCs around the femoral artery from the adventitial side following wire injury of the femoral artery and found that ADSC implantation significantly inhibited neointimal formation and stimulated re-endothelialization[82]. We also demonstrated that ADSCs produced angiopoietin-1 (Ang-1) and that the effect of ADSC administration diminished when expression of Ang-1 was suppressed using small interfering RNA (siRNA) against Ang-1[83] (Figure 3), indicating that Ang-1 produced by ADSCs plays a critical role in the inhibition of neointimal formation. Although drug-eluting stents (DES) are widely used and they potently inhibit restenosis, the use of DES does not always improve patient outcomes, most likely due to increased risk of late thrombosis[84,85]. Because DES inhibit the proliferation of VECs as well as VSMCs by secreting antiproliferative drugs, DES may delay re-endothelialization, resulting in thrombus formation. Therefore, agents that stimulate re-endothelialization, such as Ang-1, may be more suitable for the suppression of neointimal formation than currently used inhibitors of cell proliferation. Systematic analysis of ADSC cytokine production is required to identify molecules that inhibit neointimal formation and stimulate re-endothelialization.

FUTURE DIRECTIONS

Careful examination of the following points is required before the safe and effective clinical application of ADSCs.

Tumorigenesis

Although ADSCs may be less prone to forming tumors than ESCs, it has been reported that BMMSCs form tumors *in vivo*[86]. Furthermore, several reports have suggested ADSCs promote the proliferation of cancer cells both *in vitro* and *in vivo*[87-89]. Therefore, ADSCs may stimulate the growth of pre-existing tumors even if ADSCs *per se* do not form tumors.

Effects of age and comorbid diseases on the function of ADSCs

Patients suffering from CVD are often older and have comorbid diseases, such as hypertension and diabetes. When considering the autologous transplantation of ADSCs in these patients, it is necessary to examine whether age and comorbid diseases affect the function of ADSCs. Several studies have demonstrated that ADSCs collected from aged patients have less capacity for proliferation and differentiation compared to those collected from young donors[90-92]. Furthermore, several reports have shown that ADSCs collected from diabetic mice, hemodialysis patients, and HF patients have less capacity for proliferation, differentiation, or proangiogenic cytokine production[93-95]. Therefore, patients requiring ADSC transplantation for the treatment of CVD may not have access to high-quality autologous ADSCs. Allogenic transplantation of ADSCs may be required in these patients.

Improved ADSC survival and function

The use of mADSCs may improve the survival and/or function of ADSCs. The incubation of ADSCs with chemical compounds, culture in hypoxic conditions, or the introduction of ectopic genes are all potential methods for the pre-implantation modification of ADSCs. It is noteworthy that ADSCs cultured under hypoxic conditions have demonstrated increased capacity for proliferation, proangiogenic cytokine production, and maintenance of stemness[96-98]. The incubation of fADSCs under hypoxic conditions prior to implantation into patients may be a feasible strategy for improving the results of ADSC implantation.

Identification of paracrine factors

ADSCs produce a variety of paracrine factors, as aforementioned, and these factors appear to play a major role in the regeneration of the cardiovascular system. Elucidation of cytokine combinations with the greatest efficacy in the regeneration of the cardiovascular system may remove the need for ADSC implantation in the future.

CONCLUSION

Evidence accumulated from animal studies has indicated that ADSCs show efficacy in the treatment of CVD including AMI, ICM, and critical limb ischemia. Clinical trials have reported the safety and efficacy of ADSC

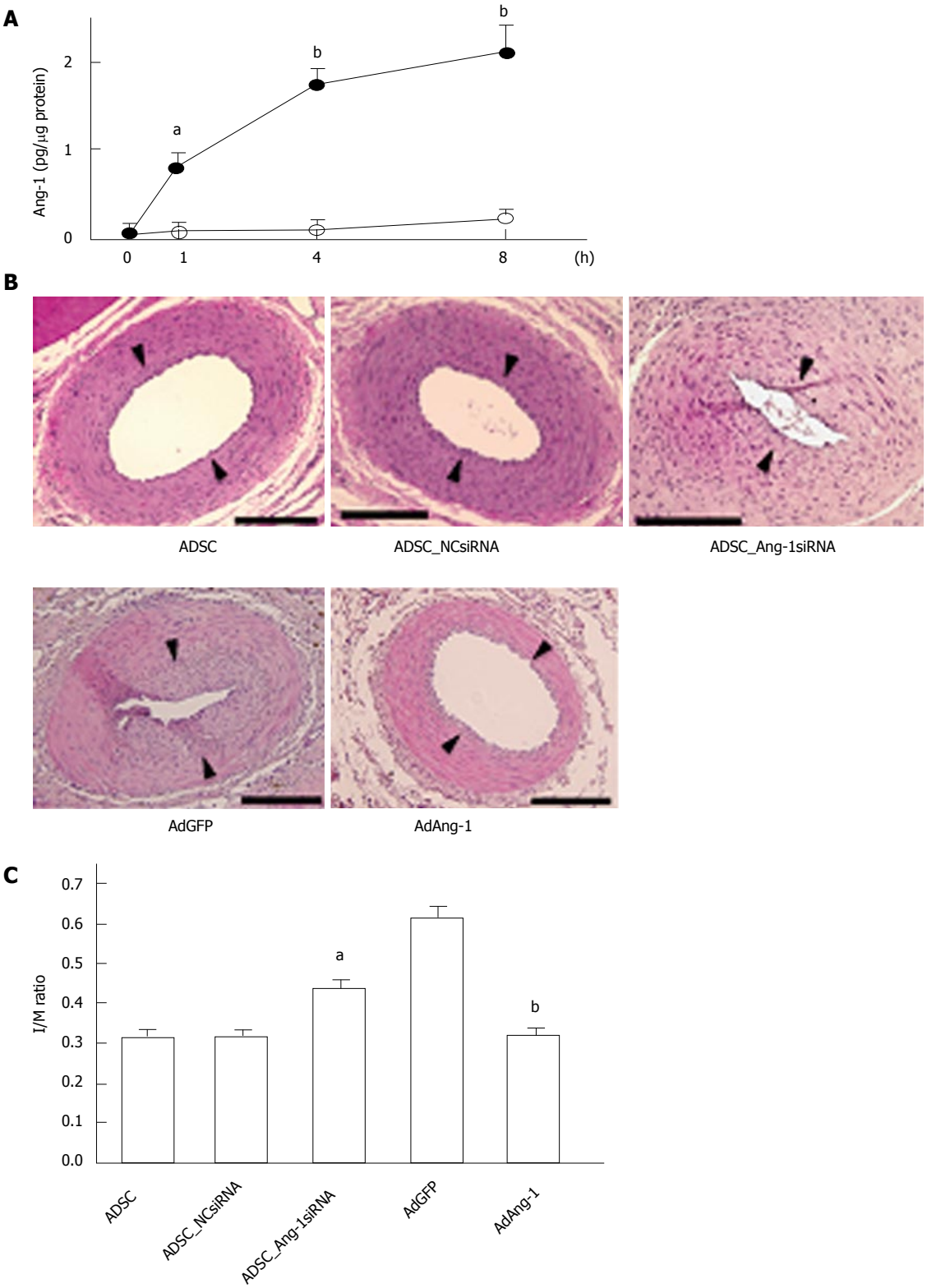

Figure 3 Ang-1 is implicated in Adipose tissue-derived stem cell-induced suppression of neointimal formation. A: ADSCs produce Ang-1, particularly when cultured in medium containing growth factors for VECs. Rat ADSCs were plated in 24-well plates and cultured in control medium (open circles) or medium containing growth factors for VECs (EGM: closed circles) for 1 wk. After washing with PBS, the medium was replaced with serum-free Dulbecco's modified Eagle medium and incubated for the indicated periods. Ang-1 accumulation was measured with an enzyme-linked immunosorbent assay kit. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* 0 h ($n = 6$ per group); B: Effect of knockdown of endogenous Ang-1 in ADSCs and forced expression of Ang-1 on neointimal formation. ADSCs were infected with lentivirus expressing negative control siRNA (NCsiRNA), which does not suppress the expression of mammalian mRNA, or lentivirus expressing Ang-1 siRNA (Ang-1siRNA). ADSCs not infected with lentivirus were used as positive controls (ADSC). ADSCs were cultured in EGM for 1 wk. ADSCs (10^6 cells) were seeded from the adventitial side immediately after wire injury of the rat femoral artery. Adenoviruses expressing green fluorescent protein (AdGFP) or Ang-1 (AdAng-1) were also injected into the femoral artery from the adventitial side following wire injury. Femoral arteries were harvested 14 d after injury for histological analyses. Arrowheads indicate the position of internal elastic lamina. Bars represent 100 μm; C: I/M ratios were compared among the groups ($n = 8$ per group). $^{a}P < 0.05$ *vs* NCsiRNA infection and $^{b}P < 0.01$ *vs* AdGFP infection. PBS: Phosphate-buffered saline; ADSCs: Adipose tissue-derived stem cells; VECs: Vascular endothelial cells.

implantation in the treatment of CVD. ADSCs may regenerate tissues through a number of mechanisms including direct differentiation into VECs, VSMCs, and cardiomyocytes, fusion with tissue-resident cells, and secretion of proangiogenic and antiapoptotic cytokines. The malignant potential of ADSCs should be carefully examined in the future.

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MINIREVIEWS

Early repolarization syndrome: A cause of sudden cardiac death

Abdi Ali, Nida Butt, Azeem S Sheikh

Abdi Ali, Nida Butt, Core Medical Trainees, Wrexham Maelor Hospital, Wrexham, Wales LL13 7TZ, United Kingdom

Azeem S Sheikh, Department of Cardiology, Wrexham Maelor Hospital, Wrexham, Wales LL13 7TZ, United Kingdom

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Correspondence to: Azeem S Sheikh, BSc, MBBS, FCPS, MRCP, Specialist Registrar in Cardiology, Wrexham Maelor Hospital, Croesnewydd Road, Wrexham, Wales LL13 7TZ, United Kingdom. drazeemsheikh@hotmail.com
Telephone: +44-7969-686217
Fax: +44-1978-725134

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Abstract

Early repolarization syndrome (ERS), demonstrated as J-point elevation on an electrocardiograph, was formerly thought to be a benign entity, but the recent studies have demonstrated that it can be linked to a considerable risk of life - threatening arrhythmias and sudden cardiac death (SCD). Early repolarization characteristics associated with SCD include high - amplitude J-point elevation, horizontal and/or down-slopping ST segments, and inferior and/or lateral leads location. The prevalence of ERS varies between 3% and 24%, depending on age, sex and J-point elevation (0.05 mV *vs* 0.1 mV) being the main determinants. ERS patients are sporadic and they are at a higher risk of having recurrent cardiac events. Implantable cardioverter-defibrillator implantation and isoproterenol are the suggested therapies in this set of patients. On the other hand, asymptomatic patients with ERS are common and have a better prognosis. The risk stratification in asymptomatic patients with ERS still remains a grey area. This review provides an outline of the up-to-date evidence associated with ERS and the risk of life - threatening arrhythmias. Further prospective studies are required to elucidate the mechanisms of ventricular arrhythmogenesis in patients with ERS.

Key words: Early repolarization syndrome; Early repolarization; Sudden cardiac death; J-wave

Core tip: Early repolarization syndrome (ERS), demonstrated as J-point elevation on an electrocardiograph, was formerly thought to be a benign entity, but the recent studies have demonstrated that it can be linked to a higher risk of ventricular arrhythmias and sudden cardiac death. The prevalence of ERS varies between 3% and 24%, depending on age, sex and J-point elevation (0.05 mV *vs* 0.1 mV) being the main determinants. ERS patients are sporadic and they are at a higher risk of having recurrent cardiac events. Implantable cardioverter-defibrillator implantation and isoproterenol are the suggested therapies in this set of patients. On the other hand, asymptomatic patients with ERS are common and have a better prognosis. The risk stratification in asymptomatic patients with ERS still remains a grey area. This review provides an outline of the up-to-date evidence associated with ERS and the

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INTRODUCTION

Sudden cardiac death (SCD) is defined as natural death due to cardiac causes in a person who may or may not have previously recognized heart disease but in whom the time and mode of death are unexpected[1]. In the context of time, "sudden" is defined for most clinical and epidemiologic purposes as 1 h or less between a change in clinical status heralding the onset of the terminal clinical event and the cardiac arrest itself[1]. The overwhelming majority of SCD cases are related to cardiac arrhythmias[2]. The commonest electrophysiologic mechanisms leading to SCD are ventricular arrhythmias. About 10% of the cases of SCD are related to primary electrophysiological disorders with known (*e.g.*, Brugada syndrome) or unknown (*e.g.*, idiopathic VF) ion-channel abnormalities[1,3-8].

Early repolarization (ER), also recognized as "J-waves" or "J-point elevation"' is an electrocardiographic abnormality consistent with elevation of the junction between the end of the QRS complex and the beginning of the ST segment in 2 contiguous leads[9,10]. Grant *et al*[11] are considered to be the first who used the term ER to describe ST-segment deviations and related T wave inversion and premature repolarization was thought to be the underlying aetiology.

The so - called "early repolarization syndrome (ERS)" was unanimously and indisputably regarded as "normal," a "normal variant", or a "benign early repolarization" until 2000[12]. However, numerous more recent reports have suggested a relationship between ER and an increased risk of death from cardiac arrhythmias[8,13-19].

ERS is an electrocardiographic (ECG) entity characterized by J-point elevation manifested either as either QRS slurring (at the transition from the QRS segment to the ST-segment) or notching (a positive deflection inscribed on terminal S wave), ST segment elevation with upper concavity and prominent T-waves in at least two contiguous leads[20] (Figure 1).

PREVALENCE

The ERS is commonly seen in athletes, cocaine users, hypertrophic obstructive cardiomyopathy and defects and/or hypertrophy of interventricular septal defects[21-24]. Prevalence of ERS varies between 3% and 24% in the general population, depending on the population studied and methods used for ECG interpretation. Young individuals, especially those predisposed to vagotonia, males, African Americans, and athletes are subpopulations known to have a higher prevalence of ERS[19,20]. Tikkanen *et al*[13] demonstrated that the location (inferior *vs* lateral leads) as well as J-point elevation of > 0.2 mV are linked to a significant risk of death from cardiac arrhythmias (adjusted relative risk, 2.98; 95%CI: 1.85-4.92; $P < 0.001$).

HISTORICAL PERSPECTIVE

The J-deflection presenting as either QRS slurring or notching was first described in 1936 by Shilpey *et al*[25] and was considered a normal ECG variant. In 1938, Tomaszewski[26] presented the case of an accidently frozen man whose ECG demonstrated a very slowly inscribed deflection between the QRS complex and the earliest part of the ST segment, representing a J wave. In 1953, Osborn[27] described a "current of injury" later named "the Osborn wave" in acidotic and hypothermic dogs at rectal temperatures < 25 ℃.

In 1961, Wasserburger *et al*[28] further defined ER as a 1-4 mm takeoff of the ST-segment at the end of the QRS complex with a distinct notch or slur on the downslope of the R wave in the mid to left precordial leads.

In 1999, Gussak *et al*[29] suggested that ER may be malignant in some cases, based on observations that an ER pattern in arterial perfused wedge preparations can easily convert to one which gives rise to polymorphic ventricular tachycardia.

In 2000, evidence supporting above hypothesis was provided by Kalla *et al*[30] and Takagi *et al*[31]; they reported VF in patients with prominent J-wave and ST segment elevation in inferior leads without structural heart diseases and postulated that idiopathic VF with an ER pattern in inferior leads may represent a variant of the Brugada syndrome. In 2008, Haïssaguerre *et al*[8] and Nam *et al*[32] described a strong relationship between J-waves and many different forms of ventricular arrhythmias in the absence of known heart disease.

CELLULAR, MOLECULAR AND GENETIC CONSIDERATIONS

The pathophysiologic basis of the ER is currently not fully understood. The most discussed hypothesis incriminates that this may be related to either an increased susceptibility or vulnerability to cardiac arrest in critical ischemic conditions such as acute coronary syndromes[33], or to subtle changes in the cardiac action potential[34]. ER in its simplest form occurs in early phase of the cardiac action potential and is caused by the cardiac transient outward potassium current (I_{to}). If a situation arises where there is a reduced density of the I_{to} channels in the endocardium compared with

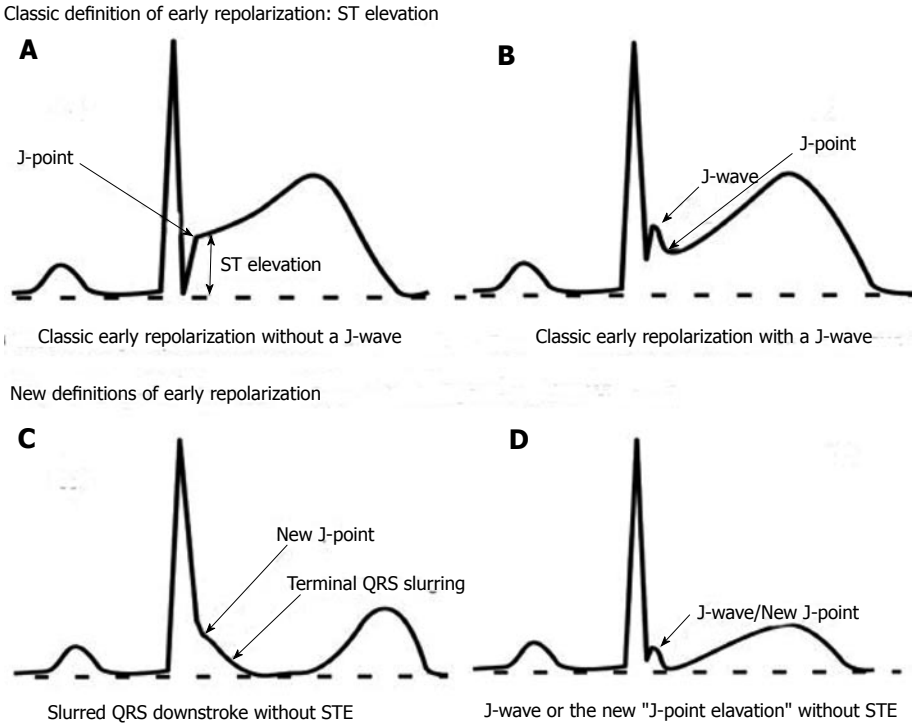

Figure 1 Examples of the classic and new definitions of early repolarization. Examples of the original (classic) and emerging (new) definitions of early repolarization (ER). A and B show the classic form of STE-type ER, which is the form identified by ECG software algorithms. Notice the presence of a J wave in (B), followed by an ascending/upsloping ST segment. Both forms are considered benign; C and D show the malignant form of ER demonstrated as slurring at the end of QRS complex (C) or a discrete notch/J wave (D) followed by a horizontal/downslopping ST segment (no ST elevation). Reproduced from ref.[49], with permission from the publisher. STE: ST elevation type ER; ECG: Electrocardiographic.

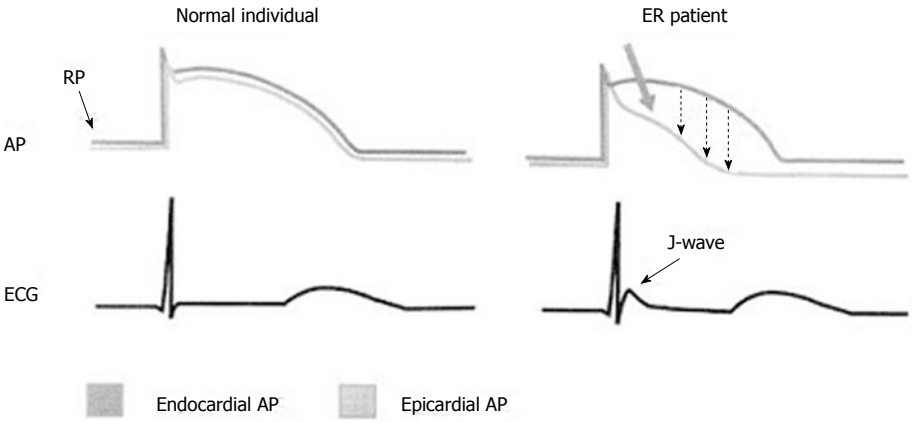

Figure 2 Schematic representation of the possible mechanisms underlying J-wave occurrence. Action potentials from epicardium and endocardium from normal individuals (left) and early repolarization (ER) patients (right) as well as the respective electrocardiograms are shown. A prominent phase I-notch and the loss of epicardial dome in phase - 2 (thick arrow) results in transmural dispersion of repolarization (dashed arrows) and appearance of the J-wave and ST-segment elevation on the surface ECG. AP: Action potential; ECG: Electrocardiogram; ER: Early repolarization; RP: Resting potention. Reproduced with permission, from ref.[67].

epicardium or mid-myocardium[35], a large Ito current can occur that results in electrocardiographic ER and large voltage gradients that may generate J wave elevation (Figure 2) and have the propensity to initiate life threatening arrhythmias[34,35].

Another hypothesis regarding the mechanism causing ER suggests an association of localized depolarization abnormalities with repolarization anomalies, as it happens in type 1 Brugada syndrome[36-39].

The genetic basis of ER syndrome continues to be elucidated, with the evidence restricted to either case reports or preliminary studies that fall short of clearly identifying the genetic basis of ER[40,41]. The reported implicated gene mutations involve the *KCNJ8* gene (responsible for the ATP sensitive potassium channel Kir6.1 - I_{KATP} current), *CACNA1C*, *CACNB2*, *CACNA2D1* genes (responsible for the cardiac L-type calcium channel - $I_{Ca.L}$ current), and the *SCN5A* gene (responsible for the sodium channel - I_{Na} current)[40-44]. All of these might enhance the underlying inward – outward current imbalance responsible for accelerated epicardial repolarization.

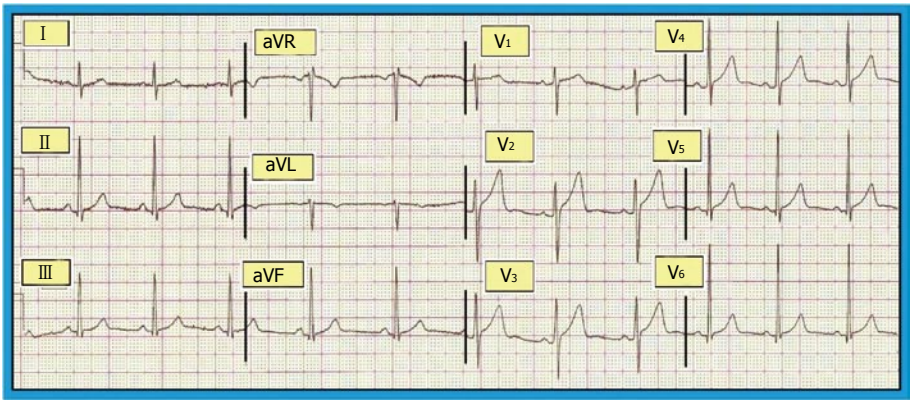

Figure 3 Benign early repolarization: Electrocardiogram showing ST segment elevation by at least 0.1 mV from the baseline. Reproduced with permission, from ref.[68].

CLINICAL MANIFESTATIONS OF ERS

The clinical presentation of patients with ERS can be subdivided into two main groups. The first includes those that manifest recognized symptoms of ERS, *i.e.*, high risk patients with syncope and survivors of cardiac arrest[45]. A study by Abe *et al*[46] demonstrated that the ER was noticed in 18.5% in patients with syncope compared to 2% in healthy controls, this equates to almost 10 - fold increase risk of syncope in patients with ERS. Although very rare, this group is highly likely to have recurrent cardiac events. In his study, Haissaguerre demonstrated 41% risk of arrhythmia recurrences in this cohort, when he followed up 64 ERS patients for a median of 51 mo[8].

The second and the most common group are asymptomatic patients who are incidentally noted to have an ER pattern on their ECG[8]. Overall, this group is less likely to have adverse cardiac events, and the challenge here lies in distinguishing those with risk of sudden cardiac death from those that are likely to run a benign course of the condition[47,48].

ECG DIAGNOSIS OF ERS

The electrocardiographic hallmark of ERS is elevation (> 1 mm above baseline) of the QRS - ST junction manifested as either QRS slurring or notching, ST-segment elevation with upper concavity, and prominent T-waves in two or more contiguous inferior and/or lateral leads in a patient resuscitated from otherwise unexplained ventricular arrhythmia[20]. Recent studies omitted ST segment elevation from the definition of ERS, and state that the J point changes described above is sufficient to diagnose ERS[49]. The inclusion or exclusion of right precordial leads is also an area for debate. Haïssaguerre *et al*[8] argue that in order to differentiate ERS from Brugada Syndrome, right precordial leads should be excluded, while others state that, the distinction is less straightforward. The latter group are backed by recent data pointing out the similarities in mechanism, overlapping genetic predisposition and the clinical findings of both conditions. Indeed, the term "J-Wave Syndrome" has been suggested to describe ERS and Brugada Syndrome as a spectrum of a clinical condition[36].

Antzelevitch *et al*[36] described three subtypes of ERS, and highlighted a pattern of risk profile: (1) type 1: It shows ER in the lateral precordial leads that is seen in healthy male athletes and has the lowest risk of malignant arrhythmias (Figure 3); (2) type 2: It shows ER in the inferior and inferolateral leads and is associated with a greater risk of malignant arrhythmias; and (3) type 3: It shows ER pattern in all ECG leads (Figure 4) and has the highest risk of malignant arrhythmias and electrical storms.

The Heart Rhythm Society/European Heart Rhythm Association/Asia Pacific Heart Rhythm Society (HRS/EHRA/APHRS) consensus statement on the diagnosis and management of primary inherited arrhythmia syndromes recommended criteria for the diagnosis of ER is shown in Table 1[50].

DIFFERENTIAL DIAGNOSIS

Early Repolarisation syndrome have a wide differential including Brugada Syndrome, short and long QT syndromes as well as other conditions causing ST segment elevation (ST segment elevation MI, acute pericarditis and idiopathic VF). Brugada sydnrome (BS), perhaps the closest clinical entity to ERS, is a primary repolarisation disorder characterized by a prominent J-wave causing a pattern of incomplete right bundle branch block and ST-segment elevation in the right precordial leads (V1-V3) (Figure 5) and significant risk of sudden cardiac death in individuals with no known structural heart disease[51]. BS, an autosomal dominant condition, is more common in males and has a variable penetrance[52,53]. Symptoms of BS include syncope with or without any warning signs, seizures and nocturnal agonal respiration; however, ECG remains the cornerstone of diagnosis of BS[54]. However, the Brugada ECG feature of provocation by sodium channel blocker is not observed in ER[55]. In fact, sodium channel blockers in most patients with ER

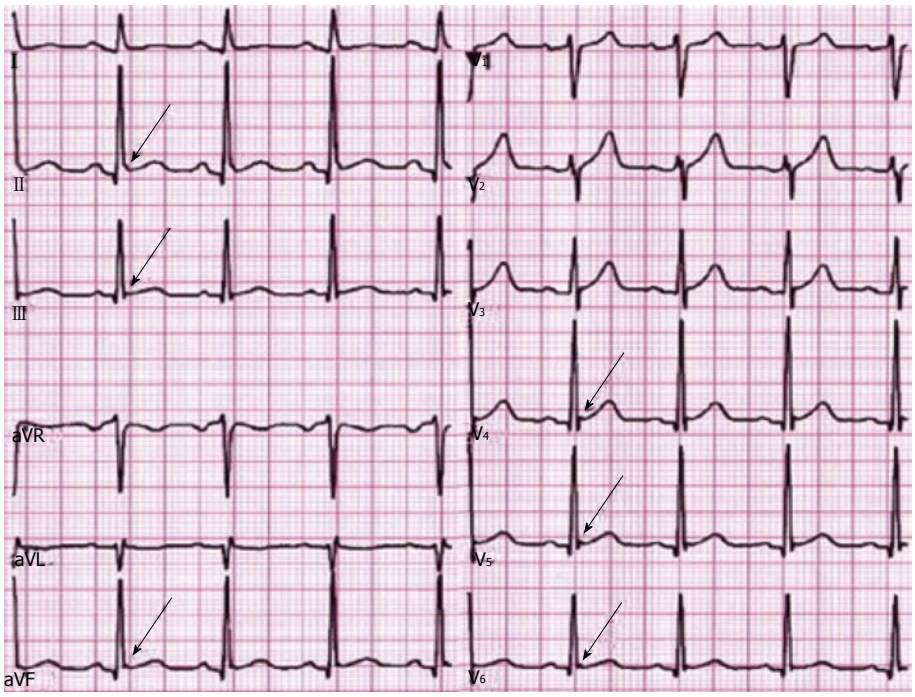

Figure 4 Malignant early repolarization: J-wave elevation (arrows) as slurring (lead II) and notching in the inferior and lateral leads and ascending ST segment in most leads. Reproduced with permission, from ref.[69].

Table 1 Heart Rhythm Society/European Heart Rhythm Association/Asia Pacific Heart Rhythm Society consensus statement on the diagnosis and management of primary inherited arrhythmia syndromes recommended criteria for the diagnosis of early repolarization

ER expert consensus recommendations on early repolarization diagnosis
ER syndrome is diagnosed in the presence of J-point elevation ≥ 1 mm in ≥ 2 contiguous inferior and/or lateral leads of a standard 12-lead ECG in a patient resuscitated from otherwise unexplained VF/polymorphic VT
ER syndrome can be diagnosed in an SCD victim with a negative autopsy and medical chart review with a previous ECG demonstrating J-point elevation ≥ 1 mm in ≥ 2 contiguous inferior and/or lateral leads of a standard 12-lead ECG
ER pattern can be diagnosed in the presence of J-point elevation ≥ 1 mm in ≥ 2 contiguous inferior and/or lateral leads of a standard 12-lead ECG

ER: Early repolarization; ECG: Electrocardiogram; SCD: Sudden cardiac death. Reproduced from ref.[50], with permission from the publisher.

attenuate the J-point, whereas the J-point is augmented by sodium-channel blockers in the right precordial leads in patients with a Brugada ECG.

In acute pericarditis, there is J-point elevation with resultant ST segment elevation, as seen in ER. Symptom presentation is distinctly different in the two conditions. Unlike ER, most patients with acute pericarditis have ST elevations diffusely in most or all limb and precordial leads. Additionally, patients with acute pericarditis often have deviation of the PR segment, which is not present in ER.

While patients with acute myocardial injury due to ST elevation myocardial infarction (STEMI) can initially have J-point elevation with concave ST segment elevation, the ST segment elevation typically becomes more pronounced and convex (rounded upward) as the infarction persists. However, the primary distinguishing factor between ER and acute myocardial injury is the presence of clinical symptoms such as chest pain or dyspnoea. ER and notching of the terminal QRS need to be considered in risk stratification for arrhythmias in patients with coronary artery disease and after coronary artery bypass grafting.

Table 2 gives a list of conditions with J-wave on the ECG.

BENIGN OR MALIGNANT

The identification of high-risk patients with ERS remains challenging. Currently, surface ECG is the only available tool in order to differentiate between the benign and the malignant forms of ERS. A horizontal or descending ST-segment elevation has been associated with adverse outcomes (compared with a rapidly ascending ST-segment elevation) following J-point elevation[56,57]. The extent of the J-point elevation may also have prognostic implication: a slurred or notched J-point elevation ≥ 2 mm (0.2 mV) appears to be associated with a higher risk[13,57]. Other abnormalities, such as localization of the ER pattern in inferior or inferolateral (compared with lateral) leads[3] or extension of ER into a BrS pattern, may also represent a worse prognosis[19,58,59].

The benign type of ERS is commonly associated with young age group, left ventricular hypertrophy on ECG,

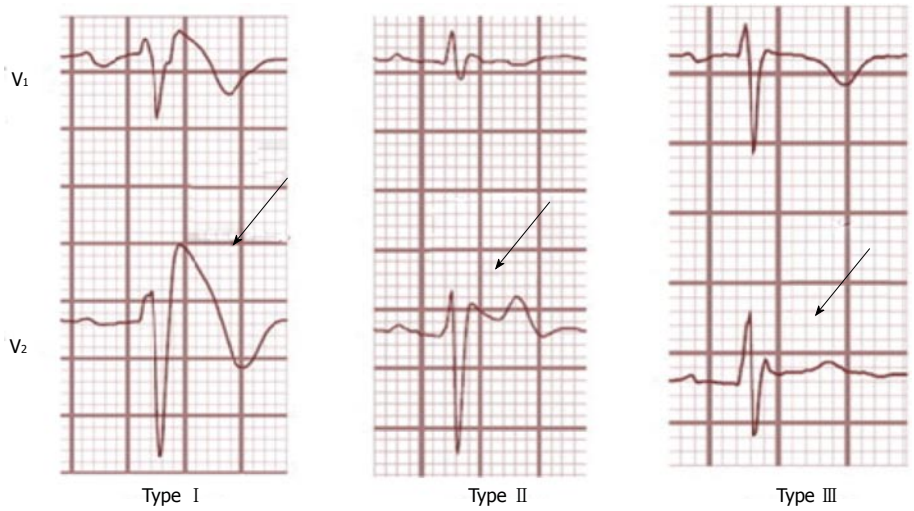

Figure 5 Brugada electrocardiogram-types. Type-1 is characterized by a complete or incomplete right bundle-branch block pattern with a coved morphology ST-segment elevation of ≥ 2 mm in the right precordial leads (V1-V3) followed by a negative T-wave. In type-2, ST-segment elevation has a saddleback appearance with a high takeoff ST-segment elevation of > 2 mm, a trough displaying > 1-mm ST-elevation followed by a positive or biphasic T-wave. Type-3 has an ST-segment morphology that is either saddleback or coved with an ST-segment elevation of < 1 mm. Reproduced with permission, from ref.[69].

Table 2 Conditions with J-wave on the electrocardiogram

Conditions with predominant J-waves
Hypothermia
Hypercalcaemia
Hyperkalaemia
Vasospastic angina
Brugada syndrome
Early repolarization syndrome
Short QT syndrome
Hypoxia
Acidosis
Pulmonary embolism
Arrhythmogenic right ventricular cardiomyopathy
Subarachnoid haemorrhage

Reproduced from ref.[50], with permission from the publisher.

lower blood pressure and lower heart rate, which are all features of healthy, physically active individuals. On the other hand, the malignant form of ERS, characterized by horizontal or descending ST-segment variation (Figure 6), is associated with older individuals and ECGs suggestive of ischaemic heart disease[60].

It appears that the morphology of the ST-segment could help in distinguishing "benign" from "malignant ER"[57]; nonetheless, there is no way to know who would be at considerable risk when presenting with slurring or notching of the QRS unless they have had a cardiac arrest[34].

TREATMENT

The ER pattern is a benign incidental finding, without any specific signs or symptoms attributed to it. There is no current risk stratification strategy for asymptomatic patients with ER pattern in general population and within families with ER pattern that would allow for identification of higher risk individuals with the ER pattern who might be candidates for treatment. The current consensus is that these patients do not require specific investigations or therapeutic interventions[28].

Among the survivors of SCD due to idiopathic VF, the reported rate of recurrent VF ranges between 22% and 37% at two to four years[6]. Because these patients have no structural heart disease, they have an excellent prognosis for long-term survival if VF is treated. As a result, such patients are best treated with an implantable cardioverter-defibrillator (ICD)[6,60-63]. HRS/EHRA/APHRS consensus statement on the diagnosis and management of primary inherited arrhythmia syndromes recommendations for therapeutic interventions in ERS are shown in Table 3[50].

It has been demonstrated that patients with VF and ER have a higher prevalence of recurrence of VF than VF patients without ER (43% *vs* 23%, $P < 0.001$) during a five years follow-up[8]. In a multicenter observational cohort study of 122 patients (90 males, mean age 37 ± 12 years) with ER in the inferolateral leads and more than three episodes of idiopathic VF (including those with electrical storm), isoproterenol was effective for the acute suppression of VF, immediately suppressing electrical storms in seven of seven patients[64]. In terms of long term therapy, VF recurrences have been demonstrated to be effectively suppressed by quinidine therapy[64]. Encouraging results recently emerged from a study by Gurabi *et al*[65], who demonstrated that in addition to quinidine, cilostazol, and milrinone suppress the hypothermia - induced VT/VF in a canine left ventricular model.

However, there exists a "gray area" in between the two ends of the spectrum, where no clear guidelines exist. Examples include patients with syncope who may have a "malignant" ER pattern and/or a significant family history of sudden cardiac death. The current guidelines suggest that ICD implantation may be

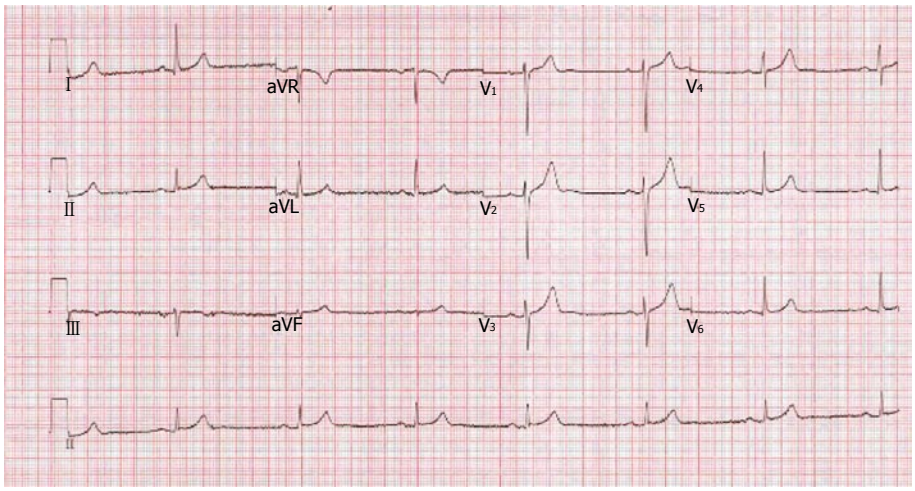

Figure 6 Malignant early repolarization: Horizontal ST-segment after early repolarization.

Table 3 Heart Rhythm Society/European Heart Rhythm Association/Asia Pacific Heart Rhythm Society consensus statement on the diagnosis and management of primary inherited arrhythmia syndromes recommendations for therapeutic interventions in early repolarization syndrome

Expert consensus recommendations on early repolarization therapeutic interventions		
Class Ⅰ	1	ICD implantation is recommended in patients with a diagnosis of ER syndrome who have survived a cardiac arrest
Class Ⅱa	2	Isoproterenol infusion can be useful in suppressing electrical storms in patients with a diagnosis of ER syndrome
	3	Quinidine in addition to an ICD can be useful for secondary prevention of VF in patients with a diagnosis of ER syndrome
Class Ⅱb	4	ICD implantation may be considered in symptomatic family members of ER syndrome patients with a history of syncope in the presence of ST-segment elevation > 1 mm in 2 or more inferior or lateral leads
	5	ICD implantation may be considered in asymptomatic individuals who demonstrate a high-risk ER ECG pattern (high J-wave amplitude, horizontal/descending ST segment) in the presence of a strong family history of juvenile unexplained sudden death with or without a pathogenic mutation
Class Ⅲ	6	ICD implantation is not recommended in asymptomatic patients with an isolated ER ECG pattern

ER: Early repolarization; ECG: Electrocardiogram; ICD: Implantable cardioverter-defibrillator.

considered in high-risk individuals with unexplained syncope[50].

SCREENING FAMILY MEMBERS

There are no current recommendations can be given to do ECG screening of the families of individuals with asymptomatic ER pattern or individuals with strong family history of ER or ER with VF. There are no recognized provocative tests that would help in diagnosing concealed ER in family members of patients with ERS, although preliminary observation advocate that concealed ER cases may be recognized by Valsalva maneurve[50,66].

CONCLUSION

In the recent years, ER syndrome has been associated with a significant risk of life - threatening arrhythmias and cardiac death. It is currently not possible to identify asymptomatic individual patients with ER who are at a higher risk of having cardiac arrhythmias with any clinically useful degree of accuracy. It is also not possible to identify asymptomatic individuals with a primary arrhythmogenic disorder attributable to ER. All patients with ER should continue to have modifiable cardiac risk factors addressed.

Until we have a better knowledge, physicians are left with the observation that in patients with ER in the inferolateral leads, life-threatening ventricular arrhythmias may occur and may lead to sudden cardiac death. Since there are a large number of patients who fit such a criteria but do not appear to have excess risk of arrhythmias, further data is needed to reveal how to identify the group of patients who would be at a significant risk and what measures can be taken to prevent it.

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MINIREVIEWS

Role of left ventricular twist mechanics in cardiomyopathies, dance of the helices

Floris Kauer, Marcel Leonard Geleijnse, Bastiaan Martijn van Dalen

Floris Kauer, Marcel Leonard Geleijnse, Bastiaan Martijn van Dalen, Erasmus University Medical Center Rotterdam, The Thoraxcenter, 's Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands

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Correspondence to: Bastiaan Martijn van Dalen, MD, PhD, Erasmus University Medical Center Rotterdam, The Thoraxcenter, 's Gravendijkwal 230, Room BA 302, 3015 CE Rotterdam, The Netherlands. b.m.vandalen@erasmusmc.nl
Telephone: +31-10-4633533
Fax: +31-10-4635498

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Abstract

Left ventricular twist is an essential part of left ventricular function. Nevertheless, knowledge is limited in "the cardiology community" as it comes to twist mechanics. Fortunately the development of speckle tracking echocardiography, allowing accurate, reproducible and rapid bedside assessment of left ventricular twist, has boosted the interest in this important mechanical aspect of left ventricular deformation. Although the fundamental physiological role of left ventricular twist is undisputable, the clinical relevance of assessment of left ventricular twist in cardiomyopathies still needs to be established. The fact remains; analysis of left ventricular twist mechanics has already provided substantial pathophysiological understanding on a comprehensive variety of cardiomyopathies. It has become clear that increased left ventricular twist in for example hypertrophic cardiomyopathy may be an early sign of subendocardial (microvascular) dysfunction. Furthermore, decreased left ventricular twist may be caused by left ventricular dilatation or an extensive myocardial scar. Finally, the detection of left ventricular rigid body rotation in noncompaction cardiomyopathy may provide an indispensible method to objectively confirm this difficult diagnosis. All this endorses the value of left ventricular twist in the field of cardiomyopathies and may further encourage the implementation of left ventricular twist parameters in the "diagnostic toolbox" for cardiomyopathies.

Key words: Left ventricular mechanics; Left ventricular twist; Cardiomyopathy

Core tip: Left ventricular twist is an essential part of left ventricular function. Nevertheless, knowledge is limited in "the cardiology community" as it comes to twist mechanics. It has become clear that increased left ventricular twist in for example hypertrophic cardiomyopathy may be an early sign of subendocardial (microvascular) dysfunction. Furthermore, decreased left ventricular twist may be caused by left ventricular

dilatation or an extensive myocardial scar. Finally, the detection of left ventricular rigid body rotation in noncompaction cardiomyopathy may provide an indispensible method to objectively confirm this difficult diagnosis. All this endorses the value of left ventricular twist in the field of cardiomyopathies and may further encourage the implementation of left ventricular twist parameters in the "diagnostic toolbox" for cardiomyopathies.

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INTRODUCTION

As early as the 16th century Leonardo da Vinci[1,2] wrote about the twisting deformation of the heart and Richard Lower compared the myocardial contraction with "the wringing of a linen cloth to squeeze out the water" in his observations of myocardial contraction in 1669[3,4]. A complex spiral architecture is the mechanical basis for this wringing motion[5,6]. The left ventricle comprises of obliquely orientated multiple layers of cardiomyocytes, transforming from a subendocardially located (smaller-radius) right-handed helix to a subepicardial (larger-radius) left-handed helix.

This helix generates a torsional motion pattern caused by rotation in a clockwise direction (as seen from the apex) at the level of the mitral valve (basal level) and counter clockwise rotation of the apex (apical level). This twisting deformation performs a fundamental part in the mechanical efficiency of the heart resulting in a 60% ejection fraction with only 15% fibre shortening[7]. Furthermore, left ventricular untwisting is essential in actively aiding diastolic filling[8]. The physiology of left ventricular twist and changes of left ventricular twist in different cardiomyopathies are reviewed in this paper.

ASSESSMENT OF LEFT VENTRICULAR TWIST

By his first description of left ventricular twist, Leonardo da Vinci[1,2] has been a constant inspiration for scientists in their pursuit to comprehend the functioning of the human heart. Nevertheless, reliable quantitative measurement of left ventricular twist in a non-invasive manner has not been possible until recently.

Speckle tracking echocardiography is based on automated tracking of a specific portion of myocardial tissue being visualized by a pattern of gray values, a speckle pattern, on an ultrasound image. These gray values are the result of the analysis of the reflection of ultrasound interfering with the myocardial tissue. Therefore, movement of speckle patterns represent motion of myocardial tissue[9].

In case a of suitably high frame rate, the pattern of speckles is conserved from frame to frame[10]. By following a specific pattern of speckles, the motion of the corresponding myocardial segment can be tracked, thus allowing to quantify deformation of the myocardium and, as a function of time, deformation rate (Figure 1). Several validation studies[11,12] showed good correlation between left ventricular twist assessment by commercially available speckle tracking software and magnetic resonance imaging. Also, speckle tracking derived left ventricular twist has been shown to be feasible and reproducible, and may thereby be used as a method to follow-up patients[13].

PHYSIOLOGY OF LEFT VENTRICULAR TWIST

After the first description of left ventricular twist by Da Vinci, it lasted until the late 1960s before a more detailed description of twist was provided by Streeter *et al*[5] in a study of post-mortem canine hearts. Generally, myofibre position changes gradually from +60 degrees (circumferential axis as a reference) subendocardially to -60 degrees at the subepicardium. The counter coiled helix of subepi - and subendocardial fibres generates twist. The direction of basal and apical rotation is dominated by the larger - radius fibres at the subepicardium, caused by their longer arm of movement[14]. The significance of the direction of fibres has been demonstrated in patient studies as well[15]. Left ventricular twist showed a linear relation with sphericity index (as a measure of change in left ventricle fibre orientation, because in more spherically shaped hearts fibres are supposed to be oriented more horizontally) in patients with a dilated cardiomyopathy, supporting the hypothesis on twist mechanics and the influence of the direction of fibres on the twisting left ventricular deformation[14].

The influence of aging on twist was studied by several groups[16-19]. In all these studies, aging appeared to be related to an increase of left ventricular twist. As the function of the fibres at the subendocardium deteriorates when getting older, even in normal hearts[20,21], the reduction of the opposing rotational forces of the subendocardium will result in an increase of apical rotation by the already dominant subepicardial fibres and consequently in an increase of left ventricular twist. This increase of left ventricular twist appears to be a part of "physiological cardiac aging". One may hypothesize that this increase of twist contributes to the conservation of left ventricular stroke volume with ageing.

Untwisting begins after left ventricular twist reaches its peak, usually shortly before end-systole. Systolic twisting leads to storage of potential energy in the compressed coil of twisted fibres of the left

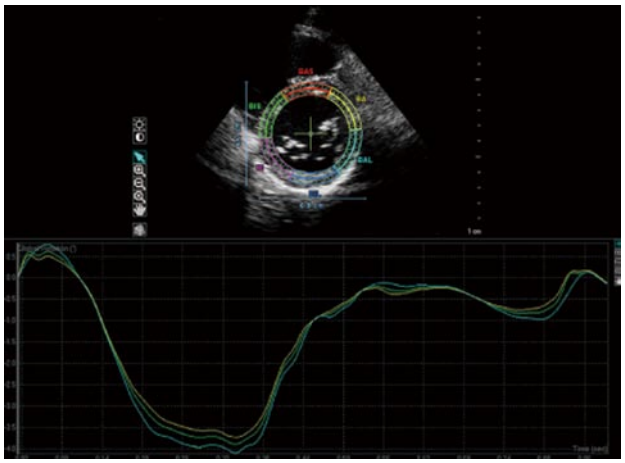

Figure 1 Example of QLAB workstation speckle-tracking analysis. Upper panel: 2D echo image of the basal left ventricular short axis. The software fully automatically draws the epicardial and endocardial contour of the myocardium. Lower panel: The software calculates the change in angle between the left ventricular (LV) wall and the virtual LV center during the cardiac cycle (green line). The blue and yellow line represent the angulation of the epicardial en endocardial angulation, respectively.

ventricular wall[19]. During isovolemic relaxation this coil springs open and releases this energy. Fibres at the subepicardium that are still depolarized and are at that time - in contrast to systole - not overruled by active contraction of the fibres located at the subepicardium, might dynamically reinforce this role of untwisting in diastole[19,22]. It was shown by magnetic resonance imaging that there is an important dissociation of time of untwist and filling, approximately 40% of the untwist takes place during isovolumic relaxation[23]. Furthermore, the extent of the diastolic intraventricular pressure gradient is strongly related to untwisting. Even more so, untwist precedes the development of this pressure gradient, thereby potentially being an important indicator of suction during early diastole[24,25].

TWIST MECHANICS IN CARDIOMYOPATHIES

Hypertrophic cardiomyopathy

Left ventricular twist in patients with hypertrophic cardiomyopathy is moderately increased, in particular the left ventricular basal rotation[26-29]. This augmented rotation at mitral valve level is probably caused by reduced counteraction of the subendocardial fibres, due to subendocardial ischemia caused by endocardial microvascular insufficiency and increased oxygen demand[30,31]. This is supported by the phenomenon of increased rotation being most pronounced in the hypertrophic segments[26]. A larger difference in radius between the subepicardium and subendocardium will increase the arm of effect on the already dominant fibres of the subepicardium and consequently will increase rotation at mitral valve level (basal rotation)[26].

There is a significant relation between the pattern of hypertrophy on apical rotation and twist. If the septum has a sigmoid curvature, rotation of the apex is more pronounced than in reverse septal curvature hearts. Outflow tract obstruction is more common in patients in whom the septum has a sigmoid curvature. The resulting intraventricular forces from these outflow tract gradients can lead to microvascular insufficiency and thereby to more (sub)endocardial ischemia. Subsequently, this lack of oxygen might cause impairment of the countereffect of contraction of the subendocardially located myocardial fibres on left ventricular twist.

The necessity to objectively demonstrate diastolic dysfunction in hypertrophic cardiomyopathy has caused an ongoing pursuit for a non invasive and load independent technique for quantifying the severity of diastolic dysfunction. For instance Takeuchi *et al*[32] examined the effect of left ventricular hypertrophy in hypertensive patients on untwisting of the left ventricle. In moderate to severe hypertrophy, untwisting was reduced and delayed as compared to healthy individuals, supposedly resulting in decreased function of left ventricular diastole. In hypertrophic cardiomyopathy[33] and also in aortic stenosis[34] the untwisting rate, being the mean untwisting velocity during the isovolumic relaxation phase, is decreased and as a result untwisting is delayed[27]. In hypertrophic cardiomyopathy this was most obvious in the affected segments[27,29]. Also, compromised elastic characteristics lead to suboptimal transformation of the potential kinetic energy stored in the twisted heart. Peak diastolic untwisting velocity is reduced in hypertrophic cardiomyopathy whereas it is augmented in aortic stenosis. In aortic stenosis twist is increased more severe. Release of the relatively high amount of potential energy results in increased untwisting, possibly compensating for otherwise diastolic dysfunction[27,35]. In hypertrophic cardiomyopathy twist is just discreetly increased, weakening this effect[27,33].

Dilated cardiomyopathy

Twist in non-ischaemic dilated left ventricles is known to be reduced. The abnormal shape of the left ventricle in dilated cardiomyopathy may cause a change in fibre orientation. This fibre orientation is of importance in left ventricular twist as described earlier. This influence was found as an independent linear relation between left ventricular sphericity index and peak systolic twist. The more dilated the left ventricle, the more decreased the left ventricular twist. Actually, also in patients with dilated cardiomyopathy and comparable left ventricular ejection fraction, sphericity index was still significantly related to left ventricular twist[15]. Nonetheless, derangement of myocardial fibre architecture is not the only cause for decreased left ventricular twist in patients with non-ischaemic dilated cardiomyopathy as fibrosis appeared to play a role in decreased left ventricular twist.

The extent of myocardial fibrosis in dilated cardiomyopathy has been evaluated by cardiac magnetic

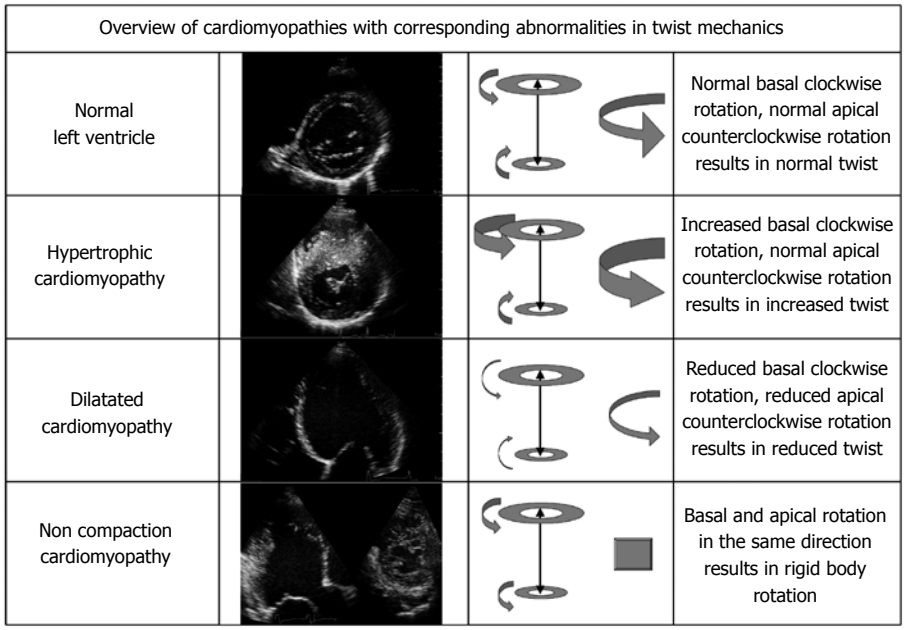

Figure 2 Overview of cardiomyopathies with corresponding abnormalities in left ventricular twist mechanics.

resonance imaging with late gadolinium enhancement. Fibrosis proved to be related to twist[36]. Reduction of twist indicated more extensive cardiac fibrosis.

As explained earlier, very rapid left ventricular untwisting is known to play a prominent part in fast filling in early diastole. However, in dilated hearts untwisting is delayed, leading to an apex-to-base-rotation delay. This will interfere with early diastolic suction and might harm filling of the left ventricle in dilated cardiomyopathy[27,37,38].

Noncompaction cardiomyopathy

Noncompaction cardiomyopathy is still subject to debate because of the shortcoming on consensus on its pathogenesis, diagnosis and treatment[39-41].

In the final embryonic development of the heart the myocardial tissue is transformed in a compact myocardium together with the formation of epicardial and endocardial fibre as oppositely wound helices[6,42]. As noncompaction cardiomyopathy is supposed to be caused by intrauterine arrest of cardiac embryogenesis during this transformation[43], distorted left ventricular twist features may be expected, even more than in the situation of reduced systolic function and a normally compacted myocardium. This was recognised in clinical studies[44-48] where noncompaction cardiomyopathy patients displayed a twist pattern with basal and apical rotation in the same direction, resulting in almost full absence of left ventricular twist. This rotation pattern is known as left ventricular rigid body rotation[44,49]. Rigid body rotation demonstrated, in a relative large study, to have a good predictive value for the diagnosis of noncompaction cardiomyopathy[47]. Even more interesting, all familial noncompaction cardiomyopathy patients showed rigid body rotation. The fact that noncompaction cardiomyopathy diagnosis is most definite in these patients underscores the good sensitivity of rigid body rotation in diagnosing noncompaction cardiomyopathy.

The clinical importance of left ventricular rigid body rotation was shown in more recent studies, where rigid body rotation was found in a majority of noncompaction cardiomyopathy patients as well[50,51], but the patients with rigid body rotation and noncompaction cardiomyopathy proved to have a lower NYHA functional status as compared to the patients without rigid body rotation[48].

Ischemic cardiomyopathy

An optical device attached to the apex was used in a canine model to study the early effects of myocardial ischemia[52]. Ischemia was inflicted by occluding the anterior descending coronary artery. Early after induction of ischemia, there was a paradoxical increase of apical rotation. This finding was ascribed to secluded ischemia of the subendocardium, resulting in a declined counteractive effect of the fibres located at the subendocardium[27,52].

Also, Moen *et al*[53] used speckle-tracking echocardiography on eight anesthetized pigs to define regional myocardial function in anterior wall ischemia. They discovered left ventricular twist remained normal until there was extensive impairment of perfusion of the left anterior descending artery.

Sun *et al*[54] induced a myocardial infarction in 7 pigs, leading to decreased twist, specifically in the area perfused by the occluded coronary artery[27,54]. Hence, it was suggested that twist might be used to assess wall motion abnormalities in order to localize cardiac ischemia. Conversely, in a clinical study using dobutamine stress echo in 125 patients with myocardial infarction or ischemia, the influence of myocardial infarction on left ventricular twist proved to be related

to size rather than localisation of infarction. In addition, stress-induced myocardial ischemia did not influence left ventricular twist[55]. Other studies on anterior myocardial infarction patients showed a decreased apical rotation in the infarcted left ventricle however with a preserved left ventricular basal rotation[56,57].

When left ventricular myocardial infarction was complicated by left ventricular aneurysm formation, rotation of the apex was lost or even reversed, consequently losing left ventricular twist. Restorative surgery as a treatment of this problem is rather complex: the aim is to reconstruct a near normal ventricular chamber after aneurysm formation and thus reducing left ventricular volume and improving ejection fraction[58]. Setser *et al*[59] did not see a significant improvement in their patients left ventricular twist after traditional left ventricular reconstruction. Much more interesting however; when an improved restoration technique was used, where residual myocardium around the defect was re-approached endeavouring to redirect fibre orientation displaced by infarct scar toward a more physiological gross disposition, left ventricular twist did improve in all patients[60]. This encouraging concept of fibre orientation based surgical reparative surgery, could expand the potential of repairing the failing heart.

CONCLUSION

Left ventricular twist is an essential part of left ventricular function. Nevertheless, knowledge is limited in "the cardiology community" as it comes to twist mechanics.

Fortunately, evolution of echocardiography, permitting speckle tracking to precisely assess left ventricular twist, has boosted the awareness of this fundamental feature of cardiac mechanics. The vital role of twist in the physiology of the heart is undisputable. Nevertheless, the significance of twist assessment in daily clinical practice in patients with a cardiomyopathy still has to be established[27]. On the other hand, twist analysis has contributed substantially to the understanding of pathophysiology in a diversity of cardiomyopathies (Figure 2). Increased twist in for example hypertrophic cardiomyopathy may be an early sign of subendocardial (microvascular) dysfunction. Furthermore, decreased twist might be initiated by left ventricular dilatation or an extensive myocardial scar. Finally, the detection of rigid body rotation in noncompaction cardiomyopathy could serve as an indispensible method to accurately diagnose this challenging entity. All this highlights the importance of left ventricular twist in the field of cardiomyopathies and may further encourage the implementation of left ventricular twist parameters in the "diagnostic toolbox" for cardiomyopathies[27].

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MINIREVIEWS

Clinical significance of lactate in acute cardiac patients

Chiara Lazzeri, Serafina Valente, Marco Chiostri, Gian Franco Gensini

Chiara Lazzeri, Serafina Valente, Marco Chiostri, Gian Franco Gensini, Intensive Cardiac Care Unit, Heart and Vessel Department, Azienda Ospedaliero-Universitaria Careggi, 50134 Florence, Italy

Gian Franco Gensini, Department of Experimental and Clinical Medicine, University of Florence, AOU Careggi, Fondazione Don Carlo Gnocchi IRCCS, 50100 Florence, Italy

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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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Abstract

Lactate, as a metabolite of easy and quick assessment, has been studied over time in critically ill patients in order to evaluate its prognostic ability. The present review is focused on the prognostic role of lactate levels in acute cardiac patients (that is with acute coronary syndrome, cardiogenic shock, cardiac arrest, non including post cardiac surgery patients). In patients with ST-elevation myocardial infarction treated with mechanical revascularization, hyperlactatemia identified a subset of patients at higher risk for early death and in-hospital complications, being strictly related mainly to hemodynamic derangement. The prognostic impact of hyperlactatemia on mortality has been documented in patients with cardiogenic shock and in those with cardiac arrest even if there is no cut-off value of lactate to be associated with worse outcome or to guide resuscitation or hemodynamic management. Therapeutic hypothermia seems to affect *per se* lactate values which have been shown to progressively decrease during hypothermia. The mechanism(s) accounting for lactate levels during hypothemia seem to be multiple ranging from the metabolic effects of reduced temperatures to the hemodynamic effects of hypothermia (*i.e.*, reduced need of vasopressor agents). Serial lactate measurements over time, or lactate clearance, have been reported to be clinically more reliable than lactate absolute value also in acute cardiac patients. Despite differences in study design, timing of lactate measurements and type of acute cardiac conditions (*i.e.*, cardiogenic shock, cardiac arrest, refractory cardiac arrest), available evidence strongly suggests that higher lactate levels can be observed on admission in non-survivors and that higher lactate clearance is associated with better outcome.

Key words: Lactate; Acute coronary syndrome; Cardiogenic shock; Cardiac arrest; Therapeutic hypothermia; Extracorporeal membrane oxygenation; Prognosis

Core tip: The present review is focused on the prognostic role of lactate levels in acute cardiac patients (acute coronary syndrome, cardiogenic shock, cardiac arrest). The prognostic impact of hyperlactatemia on mortality has been documented in cardiogenic shock and cardiac arrest even if there is no cut-off value of

lactate to be associated with worse outcome or to guide resuscitation or hemodynamic management. Lactate clearance was reported to be clinically more reliable than lactate absolute value in these patients. Despite differences in study design, timing of lactate measurements and type of acute cardiac conditions (*i.e.*, cardiogenic shock, cardiac arrest, refractory cardiac arrest), available evidence strongly suggests that higher lactate levels can be observed on admission in non-survivors and that a more favorable outcome is observed in patients with higher lactate clearance.

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INTRODUCTION

Hyperlactatemia is known to be associated with adverse outcome in critical illness[1-3]. However, the source, patho-physiology and metabolic function of lactate remain unclear probably because lactate is widely produced and it is cardinal to many energy-related pathways[4].

In recent years, available evidence strongly suggests that stress hyperlactatemia is due to increased aerobic lactate production with or without lactate clearance and that it is probably due to adrenergic stimulation. In other words, increased lactate levels are indicative of a stress response and lactate is a source of energy and not a waste product.

Lactate, since it is easy and quick to measure even at the bedside, has been widely investigated in critically ill patients to assess its prognostic role[5].

The present review is focused on the prognostic role of lactate levels in acute cardiac patients (acute coronary syndrome, cardiogenic shock, cardiac arrest). Post cardiac surgery patients are not included.

LACTATE IN THE HEART

In a normal heart, at rest, β-oxidation of fatty acids provides about 60%-90% of Adenosine triphosphate (ATP) while pyruvate produces 10%-40% of ATP[6]. However, fatty acids show lower production efficiency and increased intracellular free fatty acids activate uncoupling proteins, so that protones leak into the mitocondria without generating ATP[7]. That is why inhibition of β-oxidation is associated to an increased in mechanical efficiency of the left ventricle.

Lactate is an important fuel for the stressed heart[8]. During exercise the uptake of lactate by the myocardium and its use increase as well as during β-adrenergic stimulation and shock[4,9].

In presence of increased lactate concentrations, lactate might represent up to 60% of cardiac oxidative substrate. During shock, lactate is the most important fuel for the heart. Indeed, in laboratory animals, lactate depletion is associated with shock and mortality[10,11] while lactate infusion increased cardiac performance in cardiogenic and septic shock[12].

Hyperlactatemia can be viewed as part of the stress response including increased metabolic rate, sympathetic nervous system activation, accelerated glycolysis and a modified bioenergetic supply. In animals with cardiogenic shock[13] and in patients with cardiogenic shock, a marked increment in glycolysis and gluconeogenesis associated with hyperlactatemia was described[14]. In healthy subjects and in cardiogenic shock[12], it was observed, using an infusion of labelled lactate, that 50% of this lactate was oxidized and 20% used for glucose synthesis, without differences between the two subgroups. All these data strongly suggest that lactate is a source of energy in stress conditions.

PROGNOSTIC SIGNIFICANCE OF LACTATE

Acute coronary syndrome

Few investigations assessed whether lactate values are a diagnostic tool in patients with chest pain. In 129 patients with chest pain[15], lactate values measured on arrival identified those chest pain patients with critical cardiac illness (*i.e.*, severe congestive heart failure) while lactate concentrations within the normal range had a high negative predictive value for diagnosis of acute myocardial infarction (AMI). In patients arriving at the emergency department for suspected AMI[16], lactate values on arrival were highly sensitive for the diagnosis of AMI, mainly in those patients with more than 2 h of chest pain. In 229 patients admitted to coronary care unit[17] admission lactate showed the greatest predictive power for shock development.

To date, the prognostic significance of lactate in acute coronary syndrome (ACS), that is unstable angina, no ST elevation myocardial infarction and ST elevation myocardial infarction (STEMI), has been investigated in observational, mainly single-center, studies[18,19].

In 1176 STEMI patients[20], hyperlactatemia measured at arrival in the catheterization laboratory was associated with worse outcome measures [increased 30-d mortality, larger enzymatic infarct size and increased use of intra-aortic balloon pump (IABP)]. Among non survivors with admission lactates $\geqslant$ 1.8 mmol/L, the fifty percent died within a day after percutaneous coronary intervention (PCI). Hypotension, higher heart rate, poor Thrombolysis in myocardial infarction-flow, diabetes and non-smoking were independently associated with hyperlactatemia. In 253 STEMI non-diabetic patients with STEMI[21], lactate, measured after PCI, was an independent predictor of mortality together with C-peptide and homeostatic model assessment, an index of acute insulin resistance.

In 807 STEMI patients treated with PCI[22], our group observed that lactate values were independently associated with early mortality only in the subgroup of patients in advanced Killip classes. Lactate concentrations (measured in the early phase of STEMI) were influenced by the degree of hemodynamic impairment (as indicated by Killip class), of myocardial ischemia (as inferred by Tn I), and by glucose values.

Moreover, lactate represented an independent marker for complications (acute pulmonary edema and arrhythmia) observed during intensive cardiac care unit (ICCU) stay in 445 STEMI patients[19] and in 481 ACS patients with cardiogenic shock treated with IABP, lactate was an independent predictor of IABP-related complications (hemorrhagic, ischemic events) together with use of inotropes and nadir platelet count[22].

Overall, according to the available evidence hyperlactatemia in STEMI patients submitted to primary PCI identified a subset of patients at higher risk for early death and in-hospital complications, being strictly related mainly to hemodynamic derangement. Similarly in 754 consecutive patients with acute decompensated heart failure (ADHF) (ACS in the 52%)[23] admission lactate values > 3.2 mmol/L were associated with increased in-hospital mortality in ADHF patients either with or without ACS.

Cardiogenic shock

In 2006, in 38 patients with cardiogenic shock (CS) following acute myocardial infarction and retrospectively analyzed, interleukin-6 concentrations were independently associated with increased 30-d mortality while lactate values were not[24]. In the following years, increasing evidence supported the notion of lactate as a prognostic factor in circulatory shock[14,25]. In 45 CS patients complicating STEMI[26], increased lactate values (that is > 6.5 mmol/L) were independently associated with in-hospital death. Similar results were reported in other investigations[27,28].

The strict relationship relation between lactate and hemodynamic impairment was documented in 25 CS patients in whom a short-term increase in mean arterial pressure with norepinephrine was associated with a significant reduction in lactate levels, better cardiac performance and improved microcirculatory variables[29].

So far there is no cut-off value of lactate associated with worse outcome[30]. Lactate values higher than 2.0 mmol per liter was one of the diagnostic criteria for impaired end-organ perfusion (together with altered mental status; cold, clammy skin and extremities; oliguria with urine output of less than 30 mL/h) in a randomized multicenter trial, including 600 CS patients complicating AMI randomized to intraaortic balloon counterpulsation (301 patients) or no intraaortic balloon counterpulsation (299 patients). Intra-aortic balloon pump did not affect serum lactate concentration as well as the length of ICU stay, catecholamine therapy (dose and duration), and renal function and its use was not associated with a reduced 30-d mortality.

Cardiac arrest

The prognostic significance of lactate levels in cardiac arrest was investigated mainly in observational studies, not homogeneous for study design, inclusion criteria (cardiac arrest of cardiac/not cardiac origin) and time and number of lactate determination. The influence on lactate value of treatments such as mild hypothermia and support therapy like extracorporeal membrane oxygenation (ECMO) are so far not completely elucidated. Thus, there is no cut-off of lactate values in post-cardiac arrest patients to be associated with increased mortality and/or neurological impairment or to be use to guide resuscitation or post-resuscitation hemodynamic management.

Hyperlactatemia observed in the early phase in cardiac arrest patients may be related to both the ischaemia that occurs during arrest and to the inflammation resulting from ischemia-reperfusion injury[31-34].

Hyperlactatemia in post cardiac patients has been reported in several investigations[31-34]. In 128 out-of-hospital cardiac arrest patients[33] it was reported a progressive increased mortality associated with hyperlactatemia (39% lactate < 5 mmol/L, 67% lactate 5 mmol/L to 10 mmol/L, and 92% lactate ⩾ 10 mmol/L; $P < 0.001$). In out-of-hospital cardiac arrest (OHCA) patients[35] blood ammonia and lactate on arrival were independent prognostic factors and, when combining both biomarkers, the positive predictive value was nearly 100%.

An association between lactate levels and neurological outcome has been documented in recent investigations[36]. In 930 cardiac arrest patients who underwent therapeutic hypothermia (TH) collected from the Korean Hypothermia Network[37] high levels of lactate measured 1 h after return of spontaneous circulation were related to early mortality and poor neurological outcome. In 184 OHCA patients[38], lactate levels < 5 mmol/L and lower epinephrine doses (< 1.5 mg) were predictors of a normal Glasgow Coma Scale. Lactate concentrations measured at 6, 12, 24 and 48 h were significantly lower in the good neurological outcome group than in the poor neurological outcome group, while admission lactate values were comparable between the two subgroups. Moreover, in 76 OHCA patients submitted to TH, lactate clearance (6-h and 12-h) was related to good neurological outcome also when adjusted for confounding factors[39].

However, data on the effect of therapeutic hypothermia on lactate values are so far not uniform. In a prospective trial comparing moderate induced hypothermia with normothermia in OHCA survivors[40], during hypothermia it was reported an increment in lactate values, together with reduced pH values, reduced MAP and increased glucose levels. On the other hand, when comparing therapeutic hypothermia and normothermia[41], no significant difference in peak lactate values, arterial pressure, and need of vasopressors was reported in comatose survivors of ventricular fibrillation with STEMI. On the other hand,

in 20 CS patients after successful resuscitation[42], the initially increased lactate levels were lower in the hypothermic than the control group.

When measured serially during hypothermia in cardiac arrest patients[43], the lactate levels decreased from induction (6.68 ± 3.64; 0.5-1.7 mmol/L) to the maintenance phase (3.29 ± 2.44) and normalized in the rewarming phase.

Recently[44], in 33 cardiac arrest patients treated with TH, we observed that lactate values showed a progressive reduction during hypothermia, reduction which was independent of blood pressure variations, since mean arterial pressure showed no significant changes throughout hypothermia and of volemia (central venous pressure remained unvaried). It can be hypothesized that in patients submitted to TH lactate values are influenced by more complex mechanism(s) beyond perfusion (as indicated by mean arterial pressure) and/or volemia (as inferred by central venous pressure). We can suppose that the metabolic effect(s) of temperature may have contributed to lactate reduction, since hypothermia induces a reduction in metabolic rate (8% per degree centigrade drop in core temperature)[45] and in oxygen consumption (as previously observed when applying therapeutic hypothermia to critically ill febrile patients)[46]. In addition, pharmacological agents may have affected lactate values, since vasoactive pharmacological drugs influences the rate of glycolysis, where the rate of pyruvate utilization does not meet the rate of glycolysis, leading to lactate production[47]. As a matter of fact, in cardiac arrest patients[44] a decrease in vasopressor dose was observed during hypothermia.

In our series, lactate levels when measured during hypotermia were associated with in-ICCU death and, similarly, in 199 post cardiac arrest patients submitted to hypothermia[48], lactate (at 12 and 24 h, respectively) were significantly associated with adverse outcomes.

In the last years, a few reports analyzed the relation between hyperlactatemia and mortality in patients with refractory cardiac arrest treated with venous-arterial ECMO support. In 57 patients with refractory cardiac arrest who received ECMO during cardiopulmonary resuscitation, recruited over a six-year period[49], lactate values (measured on the first, third and seventh days, respectively) showed a significant correlation with weaning and survival. In 66 CA patients treated with ECMO, lactate concentration ⩾ 21 mmol/L (measured before cannulation) was associated with worse outcome together with fibrinogen ⩽ 0.8 g/L, and prothrombin index ⩽ 11%[50]. More recently, in 15 consecutive OHCA patients due to acute coronary syndrome submitted ECMO support, combination of base excess (less than -10 mmol/L) and lactate (> 12 mmol/L), measured 3 h after starting ECMO, can be used to predict multiorgan failure occurrence and mortality in the following 21 h[51].

LACTATE CLEARANCE

Lactate clearance have been reported to be more reliable on clinical grounds than absolute value of lactate for risk stratification in different critically ill conditions, ranging from sepsis to trauma[52-59].

Lactate clearance in acute cardiac patients has been investigated to date in few reports, all including observational single-center investigations performed in different populations of acute cardiac patients. Despite differences in study design, timing of lactate measurements and type of acute cardiac conditions (*i.e.*, cardiogenic shock, cardiac arrest, refractory cardiac arrest), available evidence strongly suggests that higher lactate levels can be observed on admission in non-survivors and that higher lactate clearance is related to more favourable outcome.

In 394 survivors from cardiac arrest[60], serum lactate levels, measured on admission and at 48 h, was retrospectively analyzed. Lactate values were lower in survivors at 6-mo after cardiac arrest than in non-survivors.

In 51 CS patients complicating STEMI[61], we observed that a 12-h lactate clearance < 10% was independently associated with early death and with poor survival rate at follow up. Since a more compromised renal failure (as indicated by a lower estimated glomerular filtration rate) was observed in patients with a low lactate clearance, associated with a lack of differences in haemodynamics (left-ventricular ejection fraction and mean arterial pressure) and transaminase values (as indexes of liver function), we supposed that a more compromised renal function may have a role in the development of persistent hyperlactataemia in these patients. Since no differences were observed in mean arterial pressure, left ventricular ejection fraction, and incidence of PCI failure between patients with 12 lactate clearance < 10% and those with 12 lactate clearance ⩾ 10%, it cannot be ruled out that microvascular alterations (despite global hemodynamic restoration) may be responsible for persistent increased lactate values in patients who exhibited a 12 lactate clearance < 10%.

Similarly, in 96 CS patients following AMI treated with percutaneous cardiopulmonary support, lactate clearance calculated at 48 h < 70% was one of the independent predictors for in-hospital mortality[62] (together with older age ⩾ 67 years and unsuccessful revascularization).

Data on the lactate clearance in patients with cardiac arrest supported by ECMO are quite scarce and not uniform.

In a heterogeneous series of 43 patients supported by ECMO for cardiogenic shock or cardiac arrest, hyperlactatemia at 6 h after ECMO implantation were observed in patients who died within 30 d[63].

In 51 patients who hadwitnessed out-of-hospital refractory cardiac arrest and were supported by ECMO upon arrival in the hospital[64], lactate clearance (values were measured before and 1-2 h after ECMO implantation) was greater in patients who survived. Conversely, in 24 patients with refractory cardiac supported by ECMO[65] lactate values, measured on

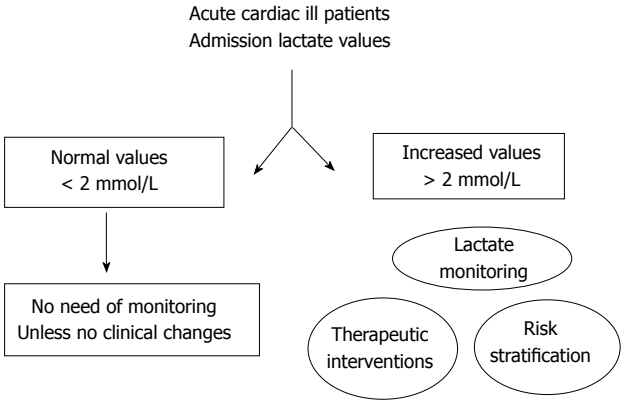

Figure 1 Admission lactate values.

admission, at 12 h and at 24 h, significantly decreased over time, with no differences between non-survivors and survivors and with no influence on outcome. A strict relation was documented between lactate and mean arterial (that is system perfusion) which increased both in survivors and in non-survivors. This relation probably explains why no difference was detectable in the dynamic behavior of lactate values during the first 24 h since admission between survivors and non survivors. Moreover, since lactate values can be related to mean arterial pressure (and not to renal function or gluycemia) it can be supposed that they may be considered a marker of perfusion, that is ECMO support efficacy in these patients.

CONCLUSION

In patients with acute coronary syndrome, cardiogenic shock and/or cardiac arrest, data on the prognostic impact of hyperlactatemia mainly stem from observational investigations. However, hyperlactatemia in these patients is associated with worse outcome, even if a cut-off value of lactate is so far not available.

Serial lactate measurements or lactate clearance have been reported to be more reliable for risk stratification in acute cardiac patients and, on a clinical ground, repeated measurement of lactate is highly advisable especially in those patients who showed increased values on admission (Figure 1). Further investigations are needed to identify the cut-off value of lactate to guide hemodynamic management.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Right ventricular septal pacing: Safety and efficacy in a long term follow up

Eraldo Occhetta, Gianluca Quirino, Lara Baduena, Rosaria Nappo, Chiara Cavallino, Emanuela Facchini, Paolo Pistelli, Andrea Magnani, Miriam Bortnik, Gabriella Francalacci, Gabriele Dell'Era, Laura Plebani, Paolo Marino

Eraldo Occhetta, Rosaria Nappo, Emanuela Facchini, Andrea Magnani, Miriam Bortnik, Gabriella Francalacci, Gabriele Dell'Era, Laura Plebani, Paolo Marino, Cardiology Department, AOU Maggiore della Carità, 28100 Novara, Italy

Gianluca Quirino, Cardiology Division, P.O. SS. Annunziata, 87100 Cosenza, Italy

Lara Baduena, Cardiology Division, Ospedale SS. Trinità, 28021 Borgomanero, Italy

Chiara Cavallino, Cardiology Division, Ospedale S. Andrea, 13100 Vercelli, Italy

Paolo Pistelli, Cardiology Division, Ospedale Civile, 10015 Ivrea, Italy

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Correspondence to: Dr. Eraldo Occhetta, Cardiology Department, AOU Maggiore della Carità, Corso Mazzini 18, 28100 Novara, Italy. eraldo.occhetta@maggioreosp.novara.it
Telephone: +39-321-3733413
Fax: +39-321-3733142

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Abstract

AIM: To evaluate the safety and efficacy of the permanent high interventricular septal pacing in a long term follow up, as alternative to right ventricular apical pacing.

METHODS: We retrospectively evaluated: (1) 244 patients (74 ± 8 years; 169 men, 75 women) implanted with a single (132 pts) or dual chamber (112 pts) pacemaker (PM) with ventricular screw-in lead placed at the right ventricular high septal parahisian site (SEPTAL pacing); (2) 22 patients with permanent pacemaker and low percentage of pacing (< 20%) (NO pacing); (3) 33 patients with high percentage (> 80%) right ventricular apical pacing (RVA). All patients had a narrow spontaneous QRS (101 ± 14 ms). We evaluated New York Heart Association (NYHA) class, quality of life (QoL), 6 min walking test (6MWT) and left ventricular function (end-diastolic volume, LV-EDV; end-systolic volume, LV-ESV; ejection fraction, LV-EF) with 2D-echocardiography.

RESULTS: Pacing parameters were stable during

follow up (21 mo/patient). In SEPTAL pacing group we observed an improvement in NYHA class, QoL score and 6MWT. While LV-EDV didn't significantly increase (104 ± 40 mL *vs* 100 ± 37 mL; P = 0.35), LV-ESV slightly increased (55 ± 31 mL *vs* 49 ± 27 mL; P = 0.05) and LV-EF slightly decreased (49% ± 11% *vs* 53% ± 11%; P = 0.001) but never falling < 45%. In the RVA pacing control group we observed a worsening of NYHA class and an important reduction of LV-EF (from 56% ± 6% to 43% ± 9%, P < 0.0001).

CONCLUSION: Right ventricular permanent high septal pacing is safe and effective in a long term follow up evaluation; it could be a good alternative to the conventional RVA pacing in order to avoid its deleterious effects.

Key words: Right ventricular septal pacing; Parahisian pacing; Resynchronization therapy; Left ventricular cardiac function; Permanent cardiac pacing

Core tip: We evaluated the safety and efficacy of the permanent high interventricular septal pacing in a long term follow up, as alternative to right ventricular apical pacing. We retrospective evaluated 244 patients with a narrow QRS implanted with a single/dual chamber pacemaker with ventricular screw-in lead placed at the right ventricular high septal (parahisian) site. Contemporary we checked the clinical evolution of two control groups of patients: without ventricular stimulation and with conventional right ventricular apical stimulation. In a long term follow up we observed stability of pacing parameters and ejection fraction, and improvement in New York Heart Association class, quality of life and exercise tolerance.

Occhetta E, Quirino G, Baduena L, Nappo R, Cavallino C, Facchini E, Pistelli P, Magnani A, Bortnik M, Francalacci G, Dell'Era G, Plebani L, Marino P. Right ventricular septal pacing: Safety and efficacy in a long term follow up. *World J Cardiol* 2015; 7(8): 490-498 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i8/490.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i8.490

INTRODUCTION

The treatment of atrioventricular block or sinus node disease is represented by artificial pacemaker implant; usually the ventricular catheter is placed in right ventricular apical (RVA) position. This therapy proved efficacious in long term follow up, granting improvement in life expectancy and quality of life (QoL). However, similarly to the negative hemodynamic and clinical effects of spontaneous left bundle branch block, new data have emerged showing negative effects of the left bundle branch block-like activation determined by RVA pacing[1-5].

Several published studies[6-10] demonstrated that more than 40% of the heart beats are paced from the right ventricular apex, an increase in the incidence of atrial fibrillation, heart failure, hospitalizations and even death is observed. When ventricular pacing is necessary permanently or for long periods of time, sites for a more physiologic pacing should be identified to avoid he occurrence of ventricular desynchronization[11-13].

A better way to pace the heart in case of intraventricular conduction delay (especially left bundle branch block) is biventricular pacing: comparing to RVA pacing, it can improve left ventricular ejection fraction and volumes and reduce mitral regurgitation and sympathetic nervous system activity[14-17]. His bundle pacing may be considered as a reliable and effective method to prevent mechanical desynchronization when intraventricular conduction is preserved and QRS is narrow[18,19]. However, it requires adjuntive skills and may be more challenging and time-consuming; it is not always applicable and higher pacing thresholds have to be accepted[20]. The right ventricular septal pacing in the parahisian area, early penetrating the His-Purkinje conduction system, produces a more physiological ventricular activation, very similar to the one that is achieved with direct His bundle pacing[21].

We aimed to evaluate feasibility, safety and long-term clinical efficacy of permanent right ventricular septal pacing in the parahisian area, performed to obtain a shorter QRS duration than that resulting from conventional right ventricular apical pacing.

MATERIALS AND METHODS

Population

From January 2001 to December 2011, we evaluated 244 patients implanted with a single or dual-chamber pacemaker with the ventricular lead positioned in the high interventricular septum (parahisian site): "SEPTAL pacing" group.

The patients were implanted at the Cardiology Clinic of Azienda Ospedaliero-Universitaria (AOU) Maggiore della Carità in Novara (School of Medicine, Study University of Piemonte Orientale, Italy) (181 patients), at the Division of Cardiology of the Ospedale Civile in Ivrea, Italy (50 patients), and at the Division of Cardiology of the Ospedale SS. Annunziata in Cosenza, Italy (22 patients).

The mean age of patients was 74 ± 8 years; 169 patients were men (69%) and 75 patients were women (31%). Inclusion criteria were: (1) permanent VVIR pacing after AV node ablation for permanent atrial fibrillation with uncontrolled (high) ventricular rate, despite negative dromothropic therapy comprising digoxin, beta-blockers and diltiazem as monotherapy or associated (51 patients; 21%); (2) permanent VVIR pacing in permanent atrial fibrillation with impaired AV conduction and low ventricular frequency (81 patients; 33%); (3) permanent DDD(R) pacemaker in patients

Table 1 Comparison of pre-implantation clinical features in the patient control groups (NO pacing and right ventricular apical pacing) and parahisian pacing group

	NO pacing	RVA pacing	PH pacing
Total patients	22 patients	33 patients	244 patients
Age (yr)	75 ± 7	77 ± 9	74 ± 8
Sex	13 M/9 F	21 M/12 F	169 M/ 75 F
NYHA class	1.09 ± 0.29	1.15 ± 0.36	2.13 ± 0.46
LV ejection fraction (%)	57 ± 5	55 ± 8	53 ± 11
LV end-dyastolic volume (cc)	89 ± 25	98 ± 22	100 ± 37
LV end-systolic volume (cc)	38 ± 13	47 ± 17	49 ± 27
Associated heart diseases	Ischemic heart disease: 6/22 (27%) Valvular heart disease: 2/22 (9%) Hypertensive heart disease: 2/22 (9%) No significant heart disease: 12/22 (55%)	Ischemic heart disease: 12/33 (37%) Valvular heart disease: 4/33 (12%) Hypertensive heart disease: 3/33 (9%) No significant heart disease: 14/33 (42%)	Ischemic heart disease: 80/244 (33%) Valvular heart disease: 29/244 (12%) Hypertensive heart disease: 90/244 (37%) No significant heart disease: 45/244 (18%)
Atrial fibrillation	1 (5%)	4 (12%)	132 (54%)
Sinus rhythm	21 (95%)	29 (88%)	112 (46%)

Comparison of pre-implantation clinical features in the patient control groups [NO pacing and right ventricular apical (RVA) pacing] and parahisian (PH) pacing group. NYHA: New York Heart Association; LV: Left ventricular.

with sinus rhythm and first and second degree AV block, symptomatic for syncope/dizziness (82 patients; 34%); and (4) permanent DDD(R) pacemaker in patients with sinus rhythm and complete AV block (30 patients; 12%).

All patients had a narrow spontaneous QRS complex (mean 101 ± 14 ms; always < 120 ms), detected at standard ECG; in patients with AV node ablation a narrow QRS was detected during junctional escape rhythm after radiofrequency (RF) AV ablation; in patients with atrial fibrillation not undergoing AV ablation, a narrow QRS was documented during 24-h Holter recording.

At the same time, we retrospectively evaluated two other "control groups" of patients (all implanted at the Cardiology Clinic of AOU Maggiore della Carità in Novara, School of Medicine, Study University of Piemonte Orientale, Italy): (1) 22 consecutive patients with ventricular apical pacing (single or dual chamber pacemakers) but percentage of permanent pacing < 20%, retrospectively detected by pacemaker telemetry, owing to the presence of spontaneous AV conduction and preserved intraventricular conduction (QRS < 120 ms): "NO pacing" control group; (2) 33 consecutive patients with a ventricular or dual-chamber pacemaker providing a high percentage of ventricular pacing (> 80%) in the apex of the right ventricle, always retrospectively detected by pacemaker memories: "RVA pacing" group.

Before the implantation procedure, all patients were planned to undergo a complete evaluation.

Following assessments were performed: (1) New York Heart Association (NYHA) functional class; (2) quality of life (QoL), evaluated with "Minnesota Living with Heart Failure" questionnaire[22]; (3) twenty-four hour Holter monitoring; (4) six-minute walking test; (5) standard 2D-echocardiogram with measurement of left ventricular end-diastolic (LV-EDV) and end-systolic (LV-ESV) volumes computed according to a biplane Simpson's method, and left ventricular ejection fraction (LV-EF).

Clinical characteristics of the population are presented in Table 1: enrolled patients presented LV-EF values close to the lower limit of the normal range, narrow QRS with normal electrical axis and moderate compromission of functional class.

Implant procedure

In patients with permanent atrial fibrillation and AV node ablation, pacing leads were placed after RF ablation procedure. A quadripolar RF catheter was used to map the His bundle and an active fixation bipolar lead was placed as near as possible to the hisian dipole of the catheter. A second conventional bipolar lead was placed at the right ventricular apex. The septal and the apical leads were then connected to the "atrial" and "ventricular" pacemaker channels, respectively. The pacemaker was programmed in "DDDR" mode with "short" atrio-ventricular delay (*i.e.*, 90 ms). Thus, if the parahisian stimulation was effective through the "atrial" channel, the following RVA pulse pacing was inhibited or delivered during the refractory period through the "ventricular" channel. While, in case of ineffective parahisian stimulation, the RVA pulse pacing ensured ventricular capture.

In patients with permanent atrial fibrillation and bradyarrhythmia (without indication to AV node ablation), a single chamber VVIR pacemaker was used and connected to the lead positioned in the parahisian area, without RVA back up lead.

In patients with sinus rhythm and advanced spontaneous AV block (first, second or third degree) a conventional atrial lead was placed in addition to the parahisian lead; both leads were connected to a DDD/

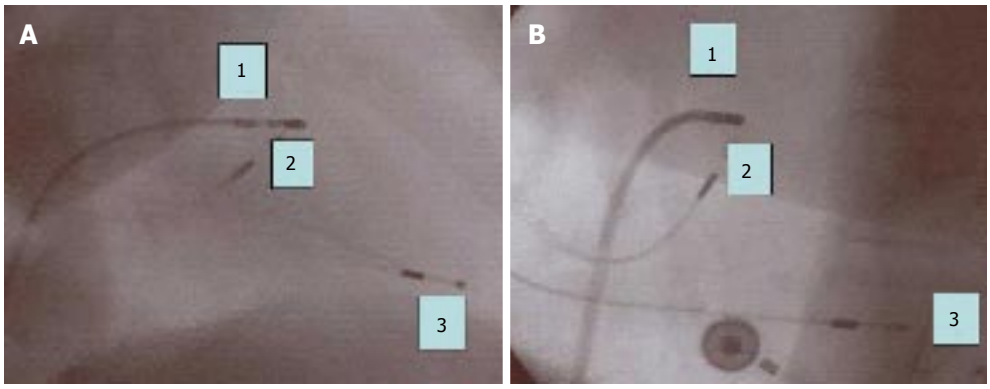

Figure 1 Antero-posterior (A) and left anterior oblique (B) fluoroscopic projections showing leads position after the "ablate and pace" procedure and parahisian pacing. 1 = quadripolar radiofrequency catheter mapping the Hisian site; 2 = screw-in bipolar lead positioned near the His-bundle; 3 = bipolar lead positioned in right ventricular apex.

DDDR pacemaker.

Following criteria were applied to obtain parahisian pacing[20]: (1) positioning of the tip of the screw-in lead as close as possible to the mapping dipole of the electrophysiological catheter (distance < 1 cm in left and right oblique projections) (Figure 1); (2) even if larger than the spontaneous QRS, the duration of the paced QRS had to be < 130 ms; (3) full concordance between electrical axis of the paced QRS and that of the spontaneous QRS; and (4) the pacing threshold had to be < 1 V (pacing of the muscular portion of the interventricular septum).

Control groups patients were implanted with a conventional apical right ventricular lead (and conventional atrial lead in dual chamber pacing).

Statistical analysis

Continuous variables with normal or Gaussian distribution of each group of patients were expressed in terms of average ± SD. Pre-implantation and follow-up data in the parahisian pacing group were analyzed and compared by means of the parametric Student *t* test for paired data. Similar parameters observed in the three groups were compared by means of the Student *t* test for numerically different samples with the same variance. A value of $P \leqslant 0.05$ was considered statistically significant.

The study was reviewed by our expert Biostatistic Gabriele Dell'Era, MD.

RESULTS

Implant data

To obtain the parahisian high septal pacing we used: (1) a bipolar catheter with 1.5 mm retractable screw lead (CapsureFix 4068/5068/5076; Medtronic Inc., Minneapolis, Minnesota) in 172 patients; (2) a bipolar catheter with 1.5 mm retractable screw lead (Cristalline ICQ09B, Vitatron BV, The Netherlands) in 10 patients; (3) a bipolar catheter with 1.5 mm retractable screw lead (Tendril 1488T/1888T; St.Jude Medical, Inc. St. Paul, Minnesota) in 12 patients; (4) a bipolar catheter with 3 mm retractable screw lead (10627 Medtronic Inc., Minneapolis, Minnesota) in 5 patients (controlled clinical evaluation); and (5) a bipolar, fixed screw, steroid eluting lead (Select Secure 3830, Medtronic Inc., Minneapolis, Minnesota) in 45 patients.

The total radiological exposure time was 15 ± 9 min (range from 3 to 68 min for the first implant with Select Secure system). Electrical parameters at the parahisian site were measured in bipolar configuration.

We excluded from the analysis 14 patients (6%), in which the criteria for parahisian pacing were not met, specifically the paced QRS was > 130 ms.

For patients in analysis, the average duration of the basal QRS was 101 ± 14 ms, and 122 ± 9 ms during parahisian pacing.

We obtained an average parahisian pacing threshold of 0.6 ± 0.3 V (at 0.5 ms pulse duration), pacing impedance 736 ± 238 Ω, endocavitary potential 10.1 ± 5.3 mV; we never recorded high-amplitude "far-field" type atrial potentials from the parahisian lead.

Parahisian pacing follow up

The average follow up of the 230 patients in analysis was 21 mo/patient, with a maximum of 70 mo for the first enrolled patient and a minimum of 12 mo for the last one.

In one patient a 3 cm dislodgment of the parahisian lead was reported. However, the paced QRS appeared superimposable to that recorded at the end of the implantation.

During long-term follow-up, the duration of the QRS during parahisian pacing remained comparable to that recorded at the implantation. Electrical measurements from the parahisian position remained stable and acceptable during time: pacing threshold was 0.6 ± 0.3 V at implantation and 0.8 ± 0.5 V at follow-up, mean endocardial potential was 10.1 ± 5.3 mV at implantation and 9.1 ± 4.4 mV at follow up, pacing impedance was 736 ± 238 ohms at implantationand 540 ± 116 ohms at follow up.

The clinical results at long-term follow-up were (Table 2): (1) In 167/230 patients (73%) we compared

Table 2 Long term follow up results of parahisian pacing

	Basal	Parahisian pacing	*P* value
NYHA class (167 pts)	2.15 ± 0.51	1.59 ± 0.55	< 0.001
6-min walk (m) (70 pts)	354 ± 90	400 ± 88	0.03
QoL (score) (70 pts)	29 ± 18	19 ± 7	0.02
LV-EDV (mL) (121 pts)	100 ± 37	104 ± 40	0.35
LV-ESV (mL) (121 pts)	49 ± 27	55 ± 31	0.05
LV-EF (%) (121 pts)	53 ± 11	49 ± 11	0.01

NYHA: New York Heart Association; QoL: Quality of life; LV-EDV: Left ventricular end diastolic volume; LV-ESV: Left ventricular end systolic volume; LV-EF: Left ventricular ejection fraction.

Table 3 New York Heart Association functional class before implantation and at follow-up, in patients with a low percentage of stimulation (NO pacing), with apical pacing (right ventricular apical pacing) and with parahisian pacing groups

	NO pacing (22 pts)	RVA pacing (33 pts)	PH pacing (167 pts)
Baseline	1.09 ± 0.29	1.15 ± 0.36	2.15 ± 0.51
Follow-up	1.22 ± 0.52	1.88 ± 0.99	1.59 ± 0.55
Significance	0.32 (ns)	*P* < 0.05	*P* < 0.001
	Unchanged	Worsening	Improvement

RVA: Right ventricular apical; PH: Para hisian.

NYHA functional class measured before implantation and at a mean follow-up of 18 ± 16 mo: the prolonged parahisian pacing led to a significant improvement from 2.15 ± 0.51 to 1.59 ± 0.55; $P < 0.001$ (Figure 2); (2) The quality of life score and exercise performances (6 min walk), performed in a sub-group of 70/230 patients (30%), significantly changed after a mean follow up of 14 ± 2 mo (QoL score from 29 ± 18 to 19 ± 17, $P = 0.02$; 6 min walk distance from 354 ± 90 m to 400 ± 88 m, $P = 0.03$) (Figure 2); (3) In 121/230 patients (53%) we compared echocardiographic volumes and ejection fraction before and after parahisian pacing (Figure 3): LV-EDV went from 100 ± 37 to 104 ± 40 mL, $P = 0.35$; LV-ESV from 49 ± 27 to 55 ± 31 mL, $P = 0.05$; LV-EF from 53% ± 11% to 49% ± 11%, $P = 0.01$. Medium-long term evaluation of the LV-EF showed values superimposable to enrollment values, confirming that parahisian pacing can prevent deterioration of the left ventricular function.

Control groups comparation

In RVA-paced patients QRS duration increased significantly (average 165 ± 10 ms, with values always > 130 ms).

In the "NO pacing" control group, the NYHA functional class was good both at the baseline and during follow-up; the conduction system disease did not significantly affect the functional class, which did not change during follow-up in the absence of ventricular pacing. By contrast, in "RVA pacing" patients there was a trend toward worsening NYHA functional class, though the upper classes of overt heart failure were not reached. Thus, during follow-up the NYHA functional class was better in patients without stimulation and those on PH stimulation (no significant difference between these two groups) and worse in patients stimulated at the apex ($P < 0.05$ *vs* unstimulated patients and *vs* PH-stimulated patients) (Table 3). QoL scores did not significantly differ among the three groups: 21 ± 19 score in NO pacing patients; 29 ± 13

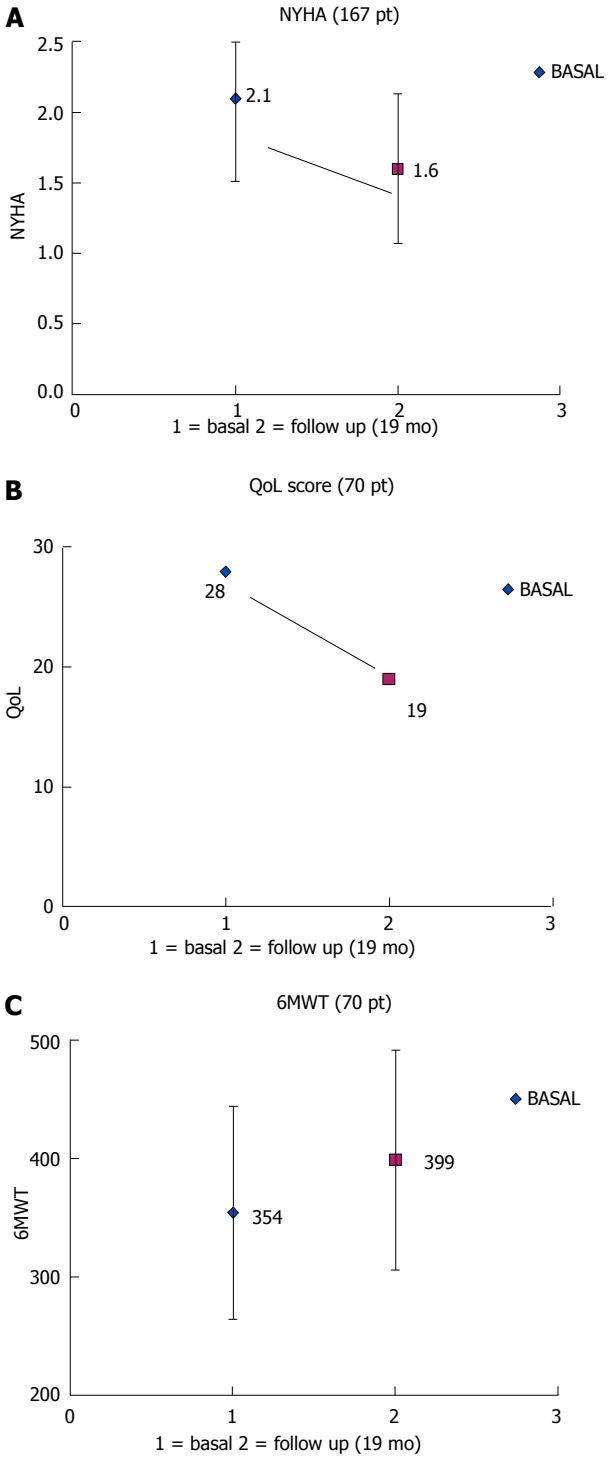

Figure 2 Clinical data before pacemaker implant (basal) and after septal pacing follow up. A: New York Heart Association functional class (NYHA); B: Quality of life (QoL) minnesota score; C: Six-minute walking test (6mwt) (meters).

Table 4 Evolution of echocardiographic parameters: end-diastolic volume, end-systolic volume and ejection fraction in the NO pacing group (22/22 patients), in the right ventricular apical pacing group (33/33 patients) and in the parahisian group (121/230 patients)

		Basal	Follow-up	*P*
Contr	EDV (mL)	88 ± 25	99 ± 46	0.23 (ns)
(22 pts)	ESV (mL)	38 ± 13	46 ± 29	0.11 (ns)
	EF (%)	57 ± 5	56 ± 5	0.1 (ns)
RVA	EDV (mL)	98 ± 23	139 ± 31	< 0.0001
(33 pts)	ESV (mL)	44 ± 14	79 ± 22	< 0.0001
	EF (%)	56 ± 6	43 ± 9	< 0.0001
PH	EDV (mL)	100 ± 37	104 ± 40	0.35 (ns)
(121 pts)	ESV (mL)	49 ± 27	55 ± 31	0.05
	EF (%)	53 ± 11	49 ± 11	0.01

in RVA pacing patients; 19 ± 17 in PH pacing patients (P < 0.06 RVA group *vs* NO pacing; P < 0.07 PH group *vs* NO pacing). Exercise tolerance, expressed in meters walked in 6 min, was better in patients without persistent pacing (448 ± 110 m) than in PH-stimulated patients (400 ± 88 m), but the difference was not significant; on the contrary it was worse in RVA-stimulated patients (338 ± 158 m) (P < 0.05 *vs* both "NO pacing" and PH-stimulated patients).

Left ventricular volumes and ejection fraction (EF) values in the controls and parahisian pacing groups of patients are shown in Table 4. In patients without significant ventricular pacing (NO pacing group), left ventricular function was almost unchanged during follow-up; indeed, no significant changes in volumes and EF were recorded (P = ns). All patients on ventricular pacing, however, presented some differences. The average end-diastolic and end-systolic volumes increased markedly in the RVA group, while in the PH group these volume increments were so modest as to be almost comparable to those observed in control patients. In RVA-paced patients, the increased left ventricular volumes led to a significant reduction in the mean EF to below normal values (post-pacing average of 43% ± 9% *vs* 56% ± 6% at the baseline; mean decrease of 13.2 percentage points, P < 0.0001); in PH patients, left ventricular function was fairly well preserved (post-pacing EF 49% ± 11%, *vs* 53% ± 11% baseline; mean change of 4 percentage points) (Figure 4).

DISCUSSION

Cardiac pacing aims at providing an adequate cardiac rhythm, restoring a physiological excito-conduction of the heart. Two elements are traditionally considered as cornerstone for "physiologic pacing": the maineinance of a correct atrioventricular sequence and the presence of chronotropic response (*via* rate-responsive sensors) during exercise or stress; till recent times, dual-chamber rate-response pacemakers were considered "physiological".

However, we know that conventional RVA pacing has the potential to induce electro-mechanichal desynchronization, causing potential harm (negative remodeling and worsening heart failure) in less than normal heart[23,24].

Therefore, a real physiological pacing must: (1) increase the cardiac frequency according to the metabolic needs; (2) keep correct atrioventricular sequence of activation; and (3) keep inter and intraventricular synchrony.

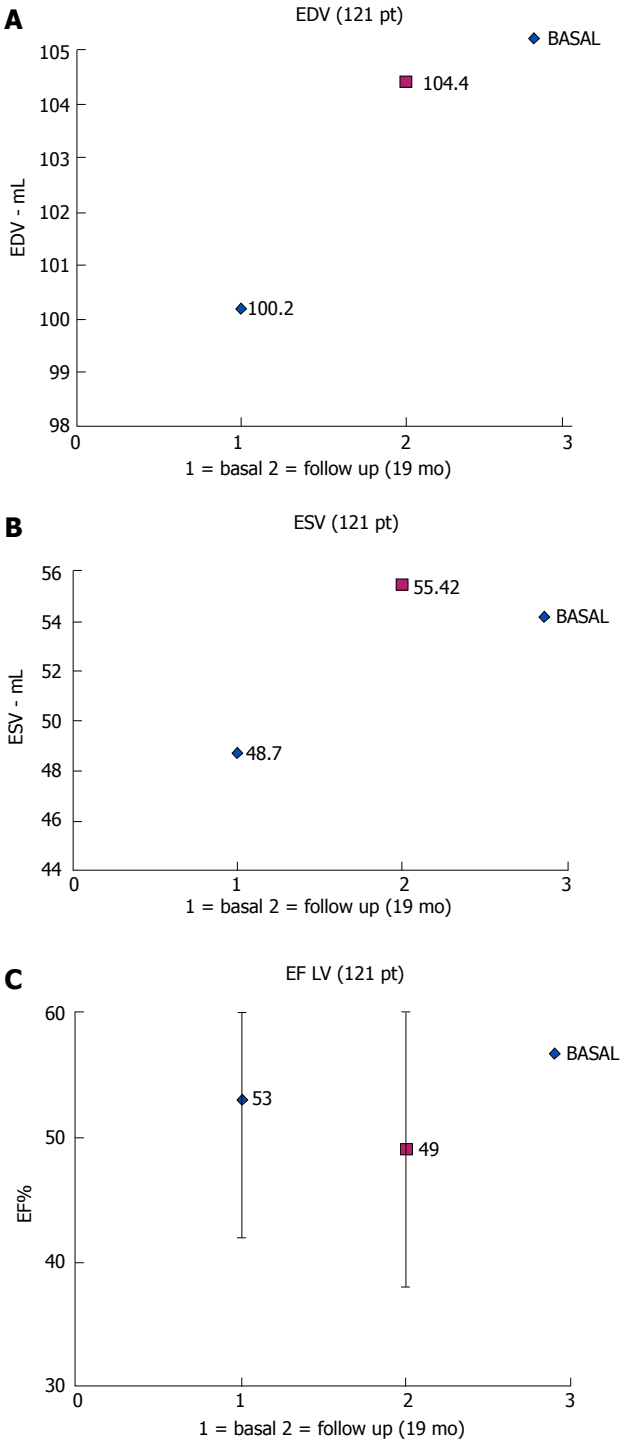

Figure 3 Echocardiographic data before pacemaker implant (basal) and after septal pacing follow up. A: End diastolic left ventricular volumes (EDV); B: End systolic left ventricular volumes (EDV); C: Left ventricular ejection fraction (EF LV).

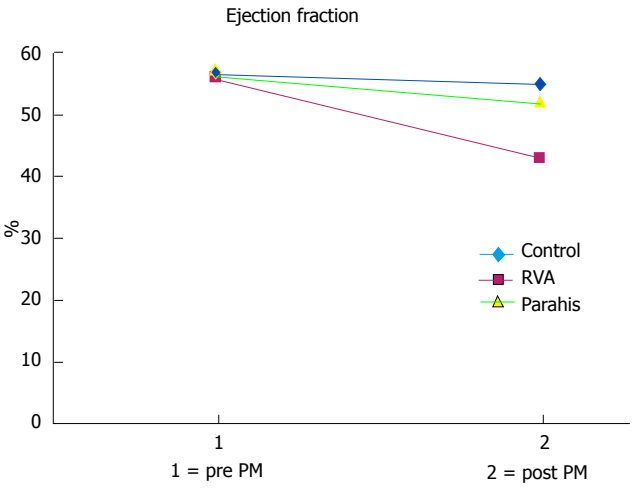

Figure 4 Average values of left ventricular ejection fraction at the baseline (1) and after two years of follow-up (2) in patients without significant stimulation (blue: control NO pacing), right ventricular apex paced patients (red: right ventricular apical) and parahisian paced patients (green: parahis). In control patients and PH patients, the ejection fraction remained essentially normal (values above 50%), while in RVA patients it declined significantly to average values of around 40. RVA: Right ventricular; PH: Parahisian.

Biventricular pacing proved effective in improving quality of life and cardiac function in patients with left bundle branch block (spontaneous electromechanical desynchronization)[14,17]. However, when intraventricular conduction is preserved and an atrioventricular block occurs, pacing must be as physiological as possible[25]. His bundle pacing has already established itself as an effective alternative to biventricular pacing for these patients. Indeed, it uses the His-Purkinje system without inducing intraventricular conduction delays[18,19]. Unfortunately, direct His bundle pacing may be challenging, needs high pacing electrical output and may pose the risk of traumatic (post-screwing of the pacing lead) His bundle block[20].

In our experience, a simpler and reliable method to achieve physiological intraventricular conduction is the so-called parahisian pacing: placing the tip of the catheter in the upper muscular part of the interventricular septum, activation is granted through the myocardium, but the His-Purkinje conduction system is activated at the same time[21]. With this technique, a fairly narrow (120-130 ms) QRS with an electrical axis concordant to the non-paced QRS can be obtained[26].

We already presented data about the improvement of hemodynamic and functional parameters obtained with parahisian pacing compared to conventional right apical pacing at a short follow up in patients undergoing AV node ablation for permanent atrial fibrillation with unsatisfactory rate control despite optimal therapy[27,28].

Long-term follow-up confirms these results, showing that parahisian pacing confers a durable improvement of quality of life, functional class and exercise tolerance. The improvement is sustained over time, modifying the expected natural progression of the underlying cardiopathy by means of a preserved atrioventricular and interventricular synchrony and by rate regularization; ejection fraction was positively affected, too, avoiding deterioration usually observed in paced patients.

Therefore, parahisian pacing should be considered easy to apply, reliable and effective in preventing the detrimental remodeling caused by non-physiological right ventricular apical pacing[29]. This kind of physiological pacing may be proposed as first line in patients needing high ventricular pacing percentage, presenting with preserved intraventricular conduction and mild systolic left ventricular dysfunction[30-32].

Limits of the study

The aim of the study was to evaluate the long term safety of septal parahisian permanent cardiac pacing and this has been definitively confirmed.

As for the long term efficacy of this pacing site, the main limitation of the study was the heterogeneity of our population: 54% of patients had atrial fibrillation (21% with concomitant AV node ablation) and VVIR pacing, 46% were in sinus rhythm with various AV block degrees and DDD(R) pacing.

This can surely affect the general prognosis, but all patients had an high percentage of ventricular pacing and a more "physiological" site of stimulation, respect to RVA pacing, could make the difference. In effect, basal NYHA functional class, higher in parahisian group than in control groups of patients, improved during the follow up; on the contrary, patients with high percentage RVA pacing had a NYHA class worsening (Table 3). Unfortunately, we could not collect definite informations about hospital readmission for heart failure and long-term mortality of our patients: this is another limitation to better establish the long term efficacy of parahisian septal permanent pacing.

The second main limit of the the study was the retrospective evaluation of patients; however, in every group (NO pacing, RVA pacing and SEPTAL pacing) the patients evaluated were consecutively enrolled and this could reproduce a real world situation.

Surely, the superiority of parahisian septal *vs* RVA permanent pacing should be evaluated and confirmed with a prospective multicenter study.

COMMENTS

Background

The usual way to treat symptomatic severe bradycardia is to implant an artificial pacemaker, with a stimulating lead in the apex of the right ventricle of the heart, providing electrical stimuli that generate the pulse. Unfortunately, that kind of stimulation can be detrimental in the long term, causing progressive heart failure in a number of patients. Alternative strategies were attempted: one of the most promising seems the placement of the stimulating lead in the upper region of the interventricular septum (parahisian site, near the division between right and left bundle branches), a position that can partly reproduce the physiological electrical activation of a normal heart. This kind of cardiac stimulation is called "septal parahisian pacing".

Research frontiers

The authors' group pioneered septal parahisian stimulation; the authors think

that this kind of cardiac pacing must have a wide diffusion (as an alternative to the usual way) and they provide support to their hypothesis with this paper, reporting safety and efficacy in a long term follow up.

Innovations and breakthroughs

In the past, some concerns arose about long-term safety and efficacy of septal pacing. In addition, some authors described it as difficult to perform for the traditionally trained interventional cardiologists. This paper shows that septal parahisian pacing can be easily obtained (some "tips and tricks" are provided to attempt the procedure) and that long term safety is guaranteed; in addition, better outcomes in term of exercise capacity, quality of life and cardiac function are obtained.

Applications

Patients with symptomatic severe bradycardia will benefit from a physiologic heart stimulation, if treated with septal parahisian pacing, avoiding unfavorable long term effect of the conventional electrical therapy.

Terminology

Septal parahisian (PH) pacing is a kind of cardiac stimulation that uses a transvenous lead placed in the upper region of the inter-ventricular septum, near the division between right and left bundle branches, to determine "physiological" ventricular electrical depolarization. The ejection fraction of the left ventricle is the measure commonly used to quantify cardiac function, and is negatively affected by conventional cardiac artificial pacemakers in a number of patients.

Peer-review

Very good work has been performed by Eraldo Occhetta *et al* comparing the safety, efficacy and benefits of right ventricular septal pacing *vs* right ventricular apical pacing. Congratulation to the authors for adding valuable data for the long-term superiority of septal pacing above apical stimulation.

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CASE REPORT

Difficult case of a trans-septal puncture: Use of a "SafeSept" guidewire

Martina Zucchetti, Michela Casella, Antonio Dello Russo, Gaetano Fassini, Corrado Carbucicchio, Eleonora Russo, Vittoria Marino, Valentina Catto, Claudio Tondo

Martina Zucchetti, Michela Casella, Antonio Dello Russo, Gaetano Fassini, Corrado Carbucicchio, Eleonora Russo, Vittoria Marino, Valentina Catto, Claudio Tondo, Cardiac Arrhythmia Research Centre, Centro Cardiologico Monzino, IRCCS, 20138 Milan, Italy

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Informed consent statement: The patient provided informed written consent prior to ablation procedure.

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Correspondence to: Martina Zucchetti, MD, Cardiac Arrhythmia Research Centre, Centro Cardiologico Monzino, IRCCS, Via Parea 4, 20138 Milan, Italy. martina.zucchetti@ccfm.it
Telephone: +39-2-58002721
Fax: +39-2-58002782

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Abstract

A 69-year-old man was admitted to our center to undergo catheter ablation of paroxysmal atrial fibrillation refractory to antiarrhythmic drug therapy. This procedure required access to the left atrium through the interatrial septum. During hospitalization, the patient performed routinely pre-procedure transthoracic echocardiography and gadolinium-enhanced cardiac magnetic resonance showing a normal anatomy of both the fossa ovalis and the interatrial septum. Access to the left atrium proved difficult and several unsuccessful attempts to perform the trans-septal puncture were made under both fluoroscopy and intracardiac echocardiography guidance, even with radiofrequency energy delivery. Finally, trans-septal puncture was successfully carried out using a novel nitinol J-shaped "SafeSept" trans-septal guidewire, designed to cross the interatrial septum through the trans-septal needle thanks to a special sharp tip. Moreover, thanks to its rounded J shape that reduces the risk of atrial perforation, the "SafeSept" guidewire, when advanced into the left atrium, becomes atraumatic.

Key words: Trans-septal puncture; "SafeSept" guidewire; Atrial fibrillation; Interatrial septum; Intracardiac echocardiography

Core tip: In recent years, the number of percutaneous therapeutic techniques requiring trans-septal catheterization has increased. We present the case of a 69-year-old man with a ten-year history of paroxysmal atrial fibrillation. Access to the left atrium proved difficult and several unsuccessful attempts to perform the trans-septal puncture were made under both fluoroscopy and intracardiac echocardiography guidance, even with radiofrequency energy delivery. Finally, trans-

septal puncture was successfully performed using a novel nitinol "SafeSept" trans-septal guidewire. If the interatrial septum is thickened, scarred, fibrous, too mobile and/or aneurismal, the use of the "SafeSept" guidewire may be a safe and effective option.

Zucchetti M, Casella M, Dello Russo A, Fassini G, Carbucicchio C, Russo E, Marino V, Catto V, Tondo C. Difficult case of a trans-septal puncture: Use of a "SafeSept" guidewire. *World J Cardiol* 2015; 7(8): 499-503 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i8/499.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i8.499

INTRODUCTION

Atrial fibrillation (AF) catheter ablation is a common therapeutic approach. The access to the left atrium, required to perform the procedure, is usually achieved through the interatrial septum. Trans-septal catheterization during catheter ablation results in high success rates with low complication incidence. Failure of the trans-septal approach is often related to unfavorable anatomical features of both the interatrial septum and the fossa ovalis[1,2]. In particular cases, trans-septal puncture can prove difficult even with the support of transesophageal or intracardiac echocardiographic imaging[3,4], or using radiofrequency energy to facilitate trans-septal puncturing[5,6]. In these cases, the use of the "SafeSept" trans-septal guidewire can be a valid alternative for achieving catheterization across the interatrial septum[7].

CASE REPORT

We present the case of a 69-year-old man with a ten-year history of paroxysmal AF. One year earlier, he had undergone catheter cryoablation of a common typical atrial flutter. After the procedure, several recurrences of paroxysmal AF refractory to antiarrhythmic drug therapy were recorded. The patient was then referred to our hospital for pulmonary vein disconnection by radiofrequency ablation. The patient had never undergone a previous procedure requiring trans-septal approach or heart surgery and did not have congenital heart defects.

During hospitalization, the patient underwent a baseline electrocardiogram that showed normal sinus rhythm and an echocardiogram that demonstrated a large left atrium (Ø 54 mm). The membrane of fossa ovalis was confirmed to be intact by gadolinium-enhanced cardiac magnetic resonance imaging, performed before ablation to assess left atrium and pulmonary vein anatomy and merge morphological and electroanatomic information during AF ablation.

At first a percutaneous trans-septal puncture was attempted. A decapolar catheter was inserted *via* femoral venous approach guided by fluoroscopy into the coronary sinus. A trans-septal needle (BRK, St Jude Medical Inc.) was advanced through a long sheath (SLO, 8 F, St. Jude Medical Inc., St. Paul, MN, United States) against the septum. By a percutaneous contralateral femoral venous approach, an ultrasound catheter (AcuNav, Siemens Healthcare, Mountain View, CA, United States) was advanced for intracardiac echocardiography monitoring. This technique was used to guide the needle to the correct position against the fossa ovalis. Normal interatrial septum anatomy was observed during the positioning.

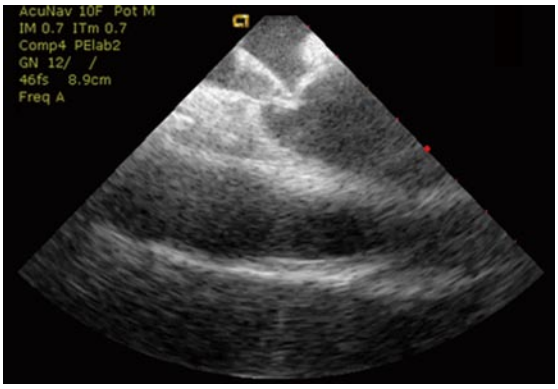

Figure 1 Intracardiac echo imaging. Correct localization of the puncture site with tenting of the fossa ovalis without crossing the interatrial septum.

Several unsuccessful attempts to obtain a trans-septal puncture were performed by two expert electrophysiologists despite changing site of puncture, needle orientation, needle types and different curved sheaths. During needle puncture, a strong resistance against the septum was encountered; both fluoroscopy and intracardiac echocardiography showed tenting of the fossa ovalis, but puncturing was not achieved despite the correct location of the needle (Figure 1).

An electrosurgical cautery generator was used to facilitate trans-septal catheterization. A standard cautery pen was placed upon the proximal portion of the trans-septal needle, then a radiofrequency pulse was delivered for few seconds at 45 W. Two unsuccessful attempts with this technique were performed.

The previous techniques were unsuccessful because of the anatomical features of the interatrial septum (*i.e.*, unusual thickness of the fossa ovalis).

In order to get a successful fossa ovalis puncture, a special trans-septal guidewire ("SafeSept" Pressure Products, Inc., United States) was considered as an option (Figure 2).

The "SafeSept" is a nitinol trans-septal guidewire designed to easily cross the interatrial septum through the trans-septal needle thanks to a special sharp tip that allows it to penetrate the fossa ovalis without the use of a particular hard contact. Moreover, the "SafeSept" is non-traumatic when advanced into the left atrium thanks to its rounded J shape, thus reducing the perforation risk of the atrial wall. The trans-septal guidewire's distal end can be easily visualized thanks to a radiopaque coil.

Figure 2 "SafeSept" trans-septal guide wire. Details of the radiopaque coil and the rounded J shape of the tip.

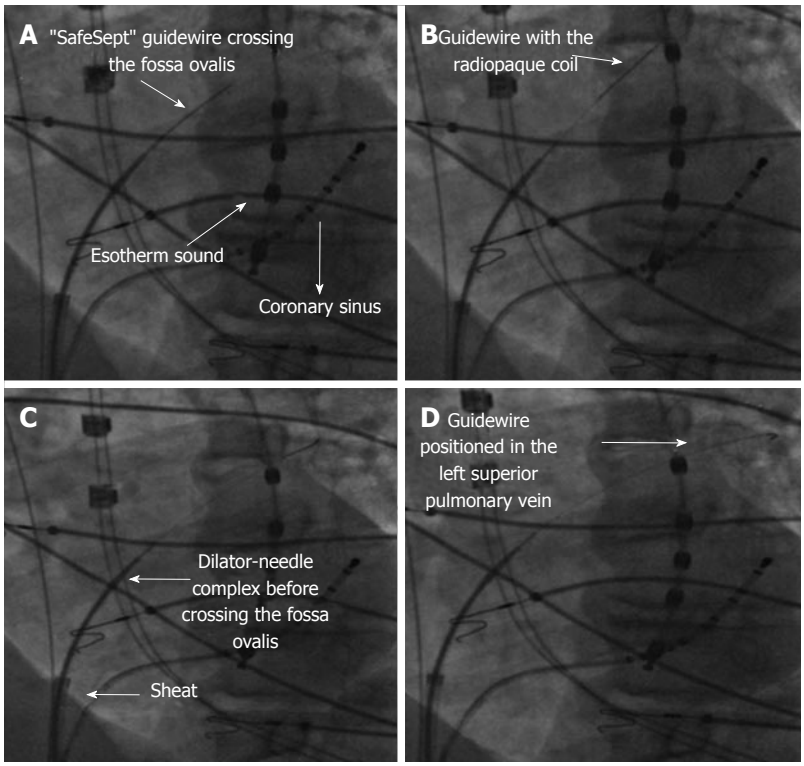

Figure 3 Sequence of fluoroscopy imaging of trans-septal puncture in the same projection (left anterior oblique view). A: The esotherm sound for the esophageal temperature control and coronary sinus catheter are in place. The "SafeSept" guidewire penetrates the fossa ovalis; B: The "SafeSept" guidewire is visible in the left atrium thanks to radiopaque coil; C: The trans-septal assembly (needle, dilator and sheath) is placed in the right atrium before crossing the fossa ovalis; D: The distal part of trans-septal guidewire is positioned in the left superior pulmonary vein.

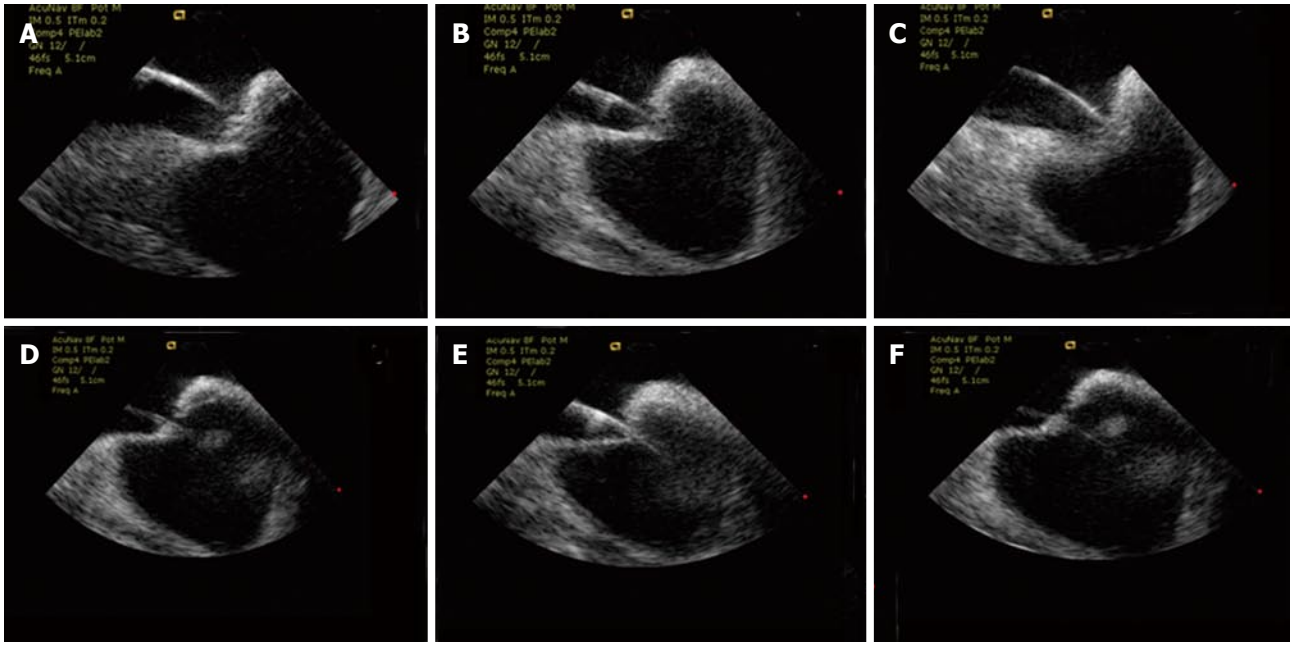

Figure 4 Sequence of intracardiac echo imaging of trans-septal puncture. The "SafeSept" guidewire is advanced through the trans-septal needle; while tenting constant force on the septum (A-C), the "SafeSept" easily crosses the fossa ovalis (D-F).

The trans-septal assembly (needle, dilator and sheath) was advanced over the guidewire. In particular, under fluoroscopic and intracardiac echo guidance, the "SafeSept" was advanced through the trans-septal needle; while tenting and maintaining constant force on the septum, the guidewire was advanced and easily crossed the interatrial septum (Figures 3 and 4). The wire (clearly visible thanks to its radiopaque coil) was further positioned in the left superior pulmonary vein.

The trans-septal needle was then advanced through the dilator and long sheath over the "SafeSept" across the fossa ovalis into the left atrium. Then the long sheath was placed in the left atrium and both trans-septal guidewire and dilator were pulled out.

Afterwards, unfractionated heparin was administered (100 U/kg) and pulmonary vein disconnection was successfully performed by radiofrequency ablation supported by the EnSite NavX electroanatomic mapping system.

DISCUSSION

In recent years the number of percutaneous therapeutic techniques requiring trans-septal catheterization has significantly increased[1,2]. The risks of complications related to perforation of the posterior atrial wall or aortic bulb remain limitations of this technique in the presence of anatomical alterations (distorted and/or thickened atrial septal tissue)[8].

Usually the trans-septal puncture is performed through fossa ovalis because it is the region offering the least resistance. This site can be located fluoroscopically and through the use of standard electrode catheters as anatomical landmarks (His region or, bulb of the aorta with a "pig-tail" catheter).

Intracardiac echocardiography was helpful to visualize the fossa ovalis in order to guide trans-septal puncture thus avoiding perforation of structures adjacent to the atrial septum or pericardial tamponade[3,4]. In the presence of unfavorable anatomy (*i.e.*, a thickened atrial septum, extremely elastic or aneurysmal fossa ovalis, presence of fibrosis due to a previous catheterization), trans-septal puncture may be challenging even with the use of these methods.

A brief application of radiofrequency to the septum, either through a dedicated radiofrequency catheter system or the use of an electrosurgical cautery pen, could be helpful in facilitating both fluoroscopy and imaging guidance[5,6].

In extremely difficult puncturing, "SafeSept" could be a valid option to cross the interatrial septum[7,9,10]. After crossing the septum, the guidewire immediately bends into a J shape, so as to be atraumatic when advanced into the left atrium. Furthermore, a radiopaque coil is positioned on the distal end of the wire to provide a fluoroscopic visualization during every step of the procedure.

In conclusion, trans-septal catheterization may be challenging if the interatrial septum is thickened, scarred, fibrous, too mobile and/or aneurysmal. The use of fluoroscopy, intracardiac ultrasound and RF energy are helpful, but may sometimes not be enough to achieve trans-septal catheterization. In these cases, the use of the "SafeSept" trans-septal guidewire may be a safe and effective option.

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COMMENTS

Case characteristics

A 69-year-old man affected by palpitations with diagnosis of paroxysmal atrial fibrillation.

Clinical diagnosis

The patient had several recurrences of paroxysmal atrial fibrillation after the cryoablation of a common typical atrial flutter.

Differential diagnosis

Difficult trans-septal puncture using fluoroscopy and intracardiac echocardiography, radiofrequency energy delivery and "SafeSept" trans-septal guidewire.

Laboratory diagnosis

The patient had no alterations of hematological values.

Imaging diagnosis

Transthoracic echocardiography and gadolinium-enhanced cardiac magnetic resonance imaging showed a normal anatomy of the fossa ovalis and interatrial septum.

Pathological diagnosis

Access to the left atrium proved difficult and several unsuccessful attempts to perform the trans-septal puncture were made under both fluoroscopy and intracardiac echocardiography guidance, even with radiofrequency energy delivery.

Treatment

Trans-septal puncture was successfully carried out using a nitinol J-shaped "SafeSept" trans-septal guidewire.

Related reports

Very few cases of unsuccessful trans-septal puncture that require the use of nitinol J-shaped "SafeSept" trans-septal guidewire have been reported in the literature.

Term explanation

The novel nitinol J-shaped "SafeSept" trans-septal guidewire is designed to cross the interatrial septum through the trans-septal needle thanks to a special sharp tip but simultaneously is a non-traumatic device due to its rounded J-shape that reduces the risk of atrial wall perforation.

Experiences and lessons

Trans-septal catheterization may be challenging if the interatrial septum is thickened, scarred, fibrous, too mobile and/or aneurysmal. The use of fluoroscopy, intracardiac ultrasound and radiofrequency energy are helpful, but may sometimes not be enough to achieve trans-septal catheterization. In these cases, the use of the "SafeSept" trans-septal guidewire may be a safe and effective aid.

Peer-review

This manuscript showed a case of paroxysmal atrial fibrillation in whom SafeSept was effective for trans-septal puncture. The case is peculiar and interesting.

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EDITORIAL

Central and peripheral testosterone effects in men with heart failure: An approach for cardiovascular research

Željko Bušić, Viktor Čulić

Viktor Čulić, Department of Cardiology, University Hospital Center Split, 21000 Split, Croatia

Željko Bušić, Viktor Čulić, University of Split School of Medicine, 21000 Split, Croatia

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Correspondence to: Viktor Čulić, MD, PhD, Professor, University Hospital Center Split, Šoltanska 1, 21000 Split, Croatia. viktor.culic@st.t-com.hr
Telephone: +385-21-557531
Fax: +385-21-557385

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Abstract

Heart failure (HF) is a syndrome recognized as a health problem worldwide. Despite advances in treatment, patients with HF still have increased morbidity and mortality. Testosterone is one of the most researched hormones in the course of HF. Growing interest regarding the effect of testosterone, on a variety of body systems, has increased the knowledge about its mechanisms of action. The terms central and peripheral effects are used to distinguish the effects of testosterone on cardiac and extracardiac structures. Central effects include influences on cardiomyocytes and electrophysiology. Peripheral effects include influences on blood vessels, baroreceptor reactivity, skeletal muscles and erythropoesis. Current knowledge about peripheral effects of testosterone may explain much about beneficiary effects in the pathophysiology of HF syndrome. However, central, *i.e.*, cardiac effects of testosterone are to be further explored.

Key words: Cardiomyocytes; Exercise; Electrophysiology; Heart failure; Vasodilation; Testosterone

Core tip: Patients with heart failure often have a lower endogenous testosterone level. Testosterone has a number of effects on cardiac and extracardiac structures *via* genomic and non-genomic mechanisms. We summarize current knowledge about the involvement of testosterone in heart failure syndrome.

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INTRODUCTION

Despite many advances in medicine, heart failure (HF) remains one of the leading causes of increased morbidity and mortality among adult population. In recent years, there has been growing interest in

Table 1 Effects of nonphysiological testosterone levels

	Supraphysiological	Subphysiological
Cardiomyocytes	Hypertrophy	Hypotrophy
QT interval	Shortening	Prolongation
Vasculature	Vasodilation	Not known
Skeletal muscles	Hypertrophy	Hypotrophy
Exercise capacity	Increased	Decreased
Baroreceptor sensitivity	Increased	Attenuated

hormonal disturbances that accompany HF. A body of evidence suggests that several hormones and a variety of metabolic signals may be altered in a way that instigates progression of the disease[1]. Within this framework, testosterone receives vivid research interest.

Many epidemiological studies have found a high incidence of comparably lower testosterone level in men with coronary heart disease, regardless of patient's age[2]. Moreover, population studies have found an association of increased all-cause and cardiovascular mortality with low testosterone levels in general population as well as within a subpopulation of men with coronary heart disease[3-7].

Testosterone deficiency has been implicated in the pathophysiology of HF, contributing to some characteristics of this syndrome such as reduced skeletal muscle mass, oxygen consumption, reduced exercise capacity and cachexia[8]. The association of serum testosterone levels with clinical severity of HF seems to be present only in non-obese HF patients[9]. In obese patients with HF, lower testosterone levels and a lack of correlation with the disease severity may suggest altered hormonal and hemodynamic mechanisms which could contribute to a better prognosis and the obesity paradox[9].

To distinguish and classify various cardiovascular, hormonal, muscular and other mechanisms the terms central, *i.e.*, cardiac and peripheral, *i.e.*, extracardiac effects are used to describe the effects of testosterone on cardiac and extracardiac structures (Figure 1). Those effects are particularly important under the circumstances of nonphysiological testosterone levels (Table 1).

CENTRAL EFFECTS OF TESTOSTERONE

Cardiomyocytes

Testosterone is responsible for protein synthesis and hypertrophy of the cardiac muscle of several investigated species, including humans, through a receptor-specific interaction which results in an increased amino acid incorporation into proteins[10]. In a post-infarction model of HF, testosterone supplementation led to a particular type of myocardial hypertrophy with a significant increase in left ventricular mass, but without increase in hypertrophy markers or collagen accumulation[11]. It appears that testosterone stimulates the expression of α-myosin heavy chain as opposed to β-myosin heavy chain which is usually seen in pathological cardiac hypertrophy, thus indicating a "physiological" type of cardiac hypertrophy with potentially long term improvement in cardiac function[10]. An animal study of ischemia-reperfusion injury showed that testosterone reduced cardiomyocyte injury by upregulating cardiac α1 adrenoceptor and possibly by activating cardiac mitochondrial ATP-sensitive potassium (K^+) channels[12].

It has been also suggested that testosterone has an influence on myocardial contractility. Gonadectomy in male rats changed the transcriptional and translational control of genes encoding the L-type calcium (Ca^{2+}) channel, the Na^+/Ca^{2+} exchanger, β1 adrenoceptors, and myosin heavy chain subunits which reduced cardiomyocyte contractile capacity[13,14].

Ventricular function

Among other clinical parameters, several studies have assessed the left ventricular ejection fraction in HF patients who received testosterone supplementation[15-19]. While some animal studies showed that androgens are important for cardiac contractility, such findings were not reported in humans. Despite improvement in exercise capacity and ventilatory efficiency in patients receiving testosterone supplementation, there was no improvement in left ventricular ejection fraction[15-19].

Higher serum levels of testosterone, most frequently found in athletes using prohibited anabolic androgen steroids, have been shown to cause myocardial hypertrophy[20,21]. However, in a study by Malkin *et al*[4], patients with HF that received testosterone supplementation had no increase in myocardial mass nor in wall thickness, thus suggesting that testosterone supplementation is safe if kept in physiologic doses.

Cardiac electrophysiology

Both endogenous and exogenous sex hormones have been shown to affect cardiac electrophysiology[22,23]. Changes in QT interval are associated with an increased risk of atrial and ventricular tachyarrhythmias, and of sudden cardiac death[24,25]. Several studies have been performed in order to explore the influence of testosterone on QT interval duration. It has been reported that ventricular repolarization was prolonged in castrated men compared with noncastrated men[26]. In addition, women with hyperandrogenism had shorter QT-interval duration than did their respective control[26]. Furthermore a negative linear correlation was found between the duration of QT interval and serum testosterone levels in hypogonadic men after receiving a single intramuscular administration of testosterone[27].

Low testosterone levels have been also associated with the incidence of atrial fibrillation, particularly in men over 80 years of age[28]. Hence, testosterone supplementation could possibly be beneficial for primary prevention of atrial fibrillation. However, an animal study from 2014, showed that testosterone supplementation in aging rabbits increased arrhythmogenesis by

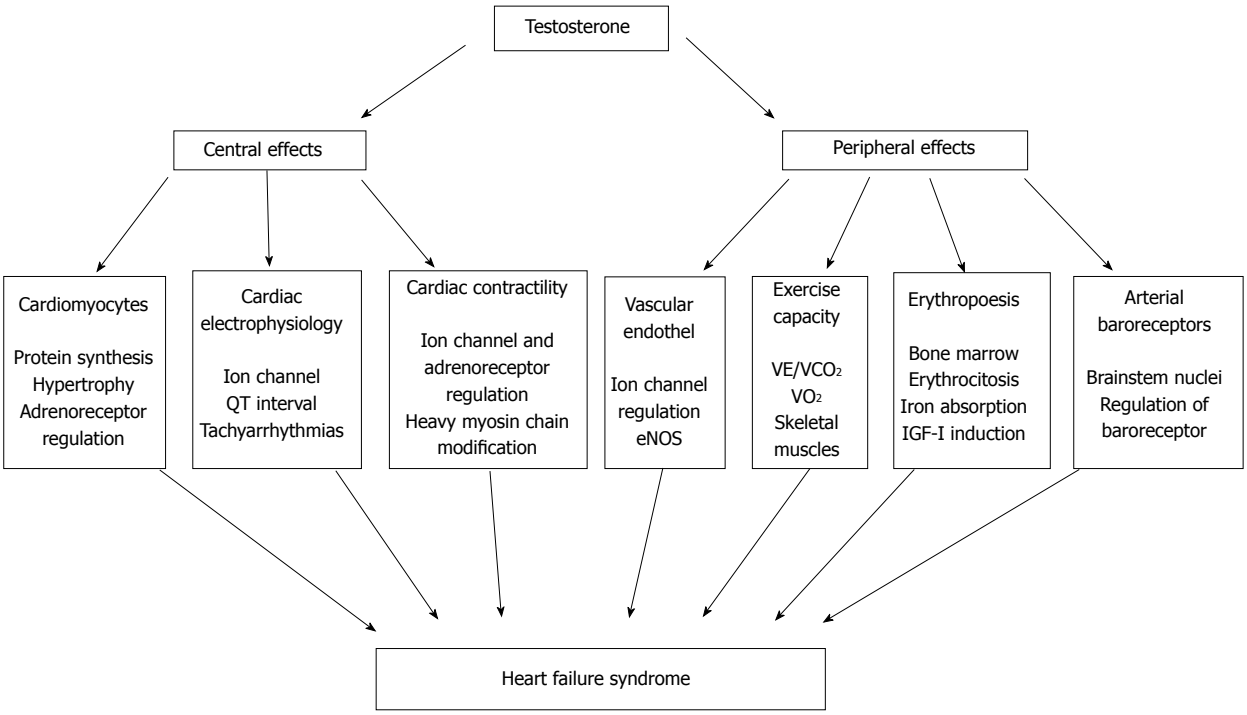

Figure 1 Testosterone effects that may be implicated in the pathogenesis of heart failure syndrome. eNOS: Endothelial nitric oxide synthase; VE/VCO_2: Ventilation to carbon dioxide production ratio; VO_2: Oxygen consumption; IGF-I: Insulin growth factor-I.

enhancing adrenergic activity which brought the previous hypothesis in question[29].

Several mechanisms, through which testosterone acts on cardiac electrophysiology, have been proposed. An animal study from 2005 found that testosterone induced a dose dependent shortening of action potential duration through non-genomic enhancement of slowly activating delayed rectifier K^+ current and suppressing the L-type Ca^{2+} current[30]. In another animal study, dihydrotestosterone, a metabolite of testosterone, induced QT interval shortening through an increased current density of inward repolarizing rectifier K^+ current and by rapidly activating delayed rectifier K^+ current[31]. Finally, in another animal study, repolarization of canine ventricular myocardium was significantly modified by testosterone, most likely due to increased expression of ion channel proteins[32]. However, those mechanisms are still being explored and at the moment there is not enough information about the effects of testosterone on cardiac electrophysiology.

PERIPHERAL EFFECTS OF TESTOSTERONE

Vascular effects

Basic cellular and molecular mechanisms through which testosterone regulates vascular responsiveness are not entirely understood. Animal studies suggest that testosterone affects vascular reactivity by both influencing endothelium-dependent and independent actions in a variety of vascular beds[33]. In HF, peripheral vasodilation produces a reduced cardiac afterload and increased cardiac output[34]. Coronary vasodilation improves myocardial oxygenation thereby achieving a beneficiary effect in HF patients[8,34].

Endothelium-dependent effects of testosterone include long term genomic and rapid non-genomic effects. Nitric oxide (NO) is a powerful vasodilator synthesized by the endothelial NO synthase (eNOS) and released, among other tissues, by the vascular endothelium[35]. Testosterone modulates NO release which in addition way is affecting vasoreactivity[36]. It is not fully understood whether this testosterone effect is genomic or non-genomic. There are several proposed mechanisms through which testosterone may act on NO synthesis and release. A study from 2012 showed that testosterone, *via* non-genomic activation of intracellular signaling pathways and Ca^{2+} influx, increases endothelial NO synthesis and additionally inhibits platelet aggregation[37]. Furthermore, in another study where vascular aging was explored, testosterone increased expression of genes that govern replicative life span which subsequently inhibited endothelial senescence *via* upregulation of eNOS activity[38].

In addition to well explored endothelium-dependent mechanisms, several studies investigated endothelium-independent effects of testosterone. The crucial endothelium-independent mechanism, which may underlie the vasodilatory effect of testosterone, involve ion channel function of the smooth muscle cells influencing K^+ channel opening and/or Ca^{2+} channel inactivation[39]. In an electrophysiological patch-clamp study, testosterone inactivated L-type voltage-operated

Ca^{2+} channels and consequently restricted Ca^{2+} influx and thereby inducing vasodilation[40]. Testosterone also shares the same molecular binding site as nifedipine on the subunit of L-type Ca^{2+} channels which causes channel blockade and may induce vasodilation[41]. Moreover testosterone blocks Ca^{2+} influx *via* store-operated Ca^{2+} channels by blocking their response to prostaglandin F2a[42]. As another option, a study from 2008 showed that testosterone activates voltage-operated K^+ channels and/or large-conductance Ca^{2+} activated K^+ channels, thereby increasing intracellular K^+ efflux and inducing vasodilation[43].

Baroreceptor sensitivity

It has been established that arterial baroreceptor sensitivity is attenuated in HF which is an important adverse prognostic indicator[44]. In light of this, Caminiti *et al*[18], sought out to investigate the effect of testosterone supplementation on baroreceptor sensitivity in patients with HF. Their results showed an increase in baroreceptor sensitivity in the testosterone treated group. Although they weren't able to identify the mechanisms through which testosterone enhances baroreceptor sensitivity, several animal studies have shown that testosterone administration improves arterial baroreceptor control of heart rate through an enhancement of cardiac efferent vagal activity[45-47]. It is possible that this effect takes place at central nervous system sites, because androgen receptors have been identified in brainstem nuclei that are involved in the baroreflex cardiac regulation[48].

Exercise

Patients with HF have poor exercise capacity test results. This is a consequence of poor left ventricular function, a poor ventilatory efficiency and muscle wasting which is enhanced in HF syndrome leading to early fatigue and limited exercise tolerance. Although peak oxygen consumption (VO_2) and ventilation to carbon dioxide production ratio (VE/VCO_2 slope) express different pathophysiologic segments of the cardiorespiratory response to exercise in HF, they both are facets of that response. Ventilatory efficiency, commonly assessed by the minute VE/VCO_2 and VO_2, is a powerful prognostic marker in the HF patients[49].

Another important segment in exercise capacity are skeletal muscles. Several morphological and functional irregularities, relatively independent of reduced blood flow, present in the skeletal muscle of HF patients contribute to early lactic acidosis and fatigue during exercise[50]. These changes are involved in the pathophysiology of HF and have been gathered under the term "the muscle hypothesis"[51]. According to this hypothesis, exaggerated ergoreflex activation occurs in exercising muscles of HF patient which leads, *via* activation of sympathetic system, to fatigue and an excessive ventilatory response in a form of dyspnea.

Recent studies have shown that testosterone supplementation improves exercise capacity, peak VO_2 and VE/VCO_2 slope[16-18]. The mechanism through which testosterone affects cardiorespiratory parameters in HF patients can be in part explained by the association of muscle ergoreflex overactivity with VE/VCO_2 slope[52]. Animal studies have indicated that anabolic androgens attenuate muscle fatigue in response to exercise, though the precise mechanism of this effect has not been identified[53,54]. Combination of exercise training and testosterone supplementation may beneficiary change muscle structure and function[50,55]. This may attenuate muscle ergoreflex activity and ventilatory response to exercise in HF patients and consequently improve exercise test results[50,55].

Erythropoesis

Further mechanism of testosterone that could explain improvement in exercise capacity and ventilatory response is the increase in hemoglobin level and oxygen delivery. A body of evidence suggests an association of lower hemoglobin levels with increased risk of hospitalization, poorer clinical status and death due to HF[56,57].

Testosterone has a strong stimulatory effect on erythropoiesis[58-60]. Suggested mechanisms of this effect are stimulation of intestinal iron absorption, erythrocyte iron incorporation and hemoglobin synthesis[60]. Although testosterone was found not to affect erythropoietin or soluble transferrin receptor levels, it is possible that testosterone has a direct effect on the bone marrow hematopoietic stem cells through the induction of insulin growth factor-I *via* androgen receptor-mediated mechanisms[61-63].

CLINICAL IMPLICATIONS

Testosterone deficiency is an independent risk factor of worse outcome in patients with HF of both sexes[64]. Testosterone supplementation results in positive physiological and biochemical changes in patients with HF and testosterone administration acutely increases cardiac output and reduces peripheral vascular resistance[15,65]. In addition, transdermal testosterone administration induces coronary vasodilation and increases coronary blood flow and improves angina threshold in patients with coronary artery disease[66,67].

An interesting question is whether testosterone may be helpful in women as it prove useful in men? As opposed to men, it seems that testosterone is not a significant factor of sudden cardiac arrest in women[68]. The only testosterone supplementation study that included female patients with HF showed no difference in effect on functional capacity and muscle strength therefore indicating no differences in possible mechanisms of action between male and female HF patients[69].

Another interesting issue is a possibility of the interplay among testosterone therapy and other endogenous anabolic hormones. Growth hormone and insulin growth factor-I levels are important for

preserving both cardiac morphology and performance in adult life[1]. Individuals with low insulin growth factor-I levels undergo cardiovascular alterations that are reminiscent of those observed in HF patients and are corrected by replacement therapy[70,71]. An interaction also exists between testosterone and insulin growth factor-1 through androgen receptor-mediated mechanisms[61-63]. Whether testosterone acts directly on insulin growth factor-I or indirectly by influencing the growth hormone is to be investigated.

FUTURE RESEARCH

Testosterone is currently one of the most investigated hormones in the course and prognosis of HF syndrome. Over the past decade, growing interest has widen research targets that could contribute to symptoms and pathophysiology of HF on all body systems. Studies have been performed in order to establish whether testosterone can be included in the standard therapy for HF patients with a low testosterone level.

Several unanswered questions should be addressed in future studies: (1) are the effects of exogenous testosterone on tissues, organs and body systems the same as the effects of endogenous testosterone? (2) is there a difference between the routs of testosterone administration which could be important for testosterone supplementation? (3) what is the role of testosterone on cardiac fibrosis and remodeling[34,71]? and (4) has testosterone adverse effects in the elderly, particularly in those with an advanced ischemic or other heart disease[34]?

In conclusion, current knowledge about peripheral effects of testosterone may explain much about beneficiary effects in the pathophysiology of HF syndrome. However, many fields of testosterone's central, *i.e.*, cardiac effects are to be further explored.

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REVIEW

Lean heart: Role of leptin in cardiac hypertrophy and metabolism

Michael E Hall, Romain Harmancey, David E Stec

Michael E Hall, Department of Medicine/Division of Cardiology, University of Mississippi Medical Center, Jackson, MS 39216, United States

Michael E Hall, Romain Harmancey, David E Stec, Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS 39216, United States

Michael E Hall, Romain Harmancey, David E Stec, Center for Excellence in Cardiovascular-Renal Research, University of Mississippi Medical Center, Jackson, MS 39216, United States

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Correspondence to: David E Stec, PhD, Associate Professor, Department of Physiology and Biophysics, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, United States. dstec@umc.edu
Telephone: +1-601-8151859
Fax: +1-601-9841817

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Abstract

Leptin is an adipokine that has been linked with the cardiovascular complications resulting from obesity such as hypertension and heart disease. Obese patients have high levels of circulating leptin due to increased fat mass. Clinical and population studies have correlated high levels of circulating leptin with the development of cardiac hypertrophy in obesity. Leptin has also been demonstrated to increase the growth of cultured cardiomyocytes. However, several animal studies of obese leptin deficient mice have not supported a role for leptin in promoting cardiac hypertrophy so the role of leptin in this pathological process remains unclear. Leptin is also an important hormone in the regulation of cardiac metabolism where it supports oxidation of glucose and fatty acids. In addition, leptin plays a critical role in protecting the heart from excess lipid accumulation and the formation of toxic lipids in obesity a condition known as cardiac lipotoxicity. This paper focuses on the data supporting and refuting leptin's role in promoting cardiac hypertrophy as well as its important role in the regulation of cardiac metabolism and protection against cardiac lipotoxicity.

Key words: Leptin; Leptin receptor; Lipotoxicity; Cardiotoxicity; Obesity; Diabetes

Core tip: Leptin is a hormone derived from adipocytes which regulates food intake and body weight. It is present at high levels in obese individuals where it can impact organs such as the heart. Leptin has been shown to both promote and protect the heart against

obesity induced heart disease. This review examines the controversial role of leptin in the development of cardiac hypertrophy as well as its important role in regulating cardiac metabolism and protecting the heart against obesity induced lipotoxicity.

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INTRODUCTION

Leptin is a hormone most abundantly produced by white adipocytes which then acts in the hypothalamus of the brain to decrease appetite and increase energy expenditure. Leptin was discovered in the early 1990's after genetic mapping of a mutation in the gene found in a specific strain of obese mice, the *ob/ob* mouse, which was originally described in the 1950's[1,2]. These mice are characterized by having no leptin which results in marked hyperphagia, decreased energy expenditure and obesity. Another strain of obese mice called *db/db* mouse was subsequently found to have a mutation in the *ObR* gene encoding the leptin receptor[3]. This strain of mice is characterized by having very high levels of circulating leptin due to lack of functional leptin receptors, marked hyperphagia, decreased energy expenditure and obesity. There are also several rat strains with defective leptin receptor such as the Zucker fatty (*fa/fa*) rat and the Koletsky fatty rat[4,5]. Recently, a zinc-finger approach was utilized to create a rat model of leptin receptor deficiency on a salt-sensitive hypertension background[6]. All of these models as well as development of cell type/tissue-specific knockouts of the *ObR* gene have greatly increased our knowledge about the physiological role of leptin[7].

While leptin is mainly expressed in adipose tissue, it is also expressed in peripheral organs such as the heart[8]. Leptin receptors are also highly expressed in the heart of several species including humans[9,10]. In the rat heart, the long form of the leptin receptor is expressed in addition to other shorter isoforms[11]. Reverse-transcriptase polymerase chain reaction of the mouse heart readily reveals expression of all isoforms of the leptin receptor similar to the expression pattern found in the brain[12] (Figure 1). The long form of the leptin receptor, ObRb, activates signaling through the Janus kinases (JAK)/ signal transducers and activators of transcription (STAT) pathway and other Src Homology 2 domain containing proteins such as suppressor of cytokine signalling and SHP-2 (Src-like homology 2 domain containing protein tyrosine phosphatase) and STAT[13]. Short leptin receptor isoforms (ObRa, ObRc, ObRd) contain a box 1 motif which is able to bind JAK and activate other signal transduction cascades[13]. The ObRe which is also referred to as the soluble leptin receptor can regulate serum leptin concentration and also serves as a carrier protein delivering the hormone to its membrane receptors[14]. Not only are leptin receptors expressed in the heart but they are also regulated by various stimuli. Cardiac ischemia has been reported to have varying effects on expression of leptin receptors, with studies demonstrating that a 30 min ischemic period was associated with a decrease in leptin receptor expression and another study reporting that a 40 min ischemic period increased leptin receptor expression[11,15]. The specific role of leptin in cardiac ischemia was addressed in an elegant study utilizing cardiac-specific deletion of leptin receptors. Cardiac-specific deletion of leptin receptors resulted in a decrease in contractile function and metabolism of glucose and in an increase in mortality and morbidity following cardiac ischemia[16]. These results highlight the important cardio-protective function of leptin in cardiac ischemia due in part to its role in the regulation of cardiac metabolism which will be discussed in greater detail below. Leptin receptors in the heart are also regulated by pressure and stretch. It has been reported that pressure-overload induced cardiac hypertrophy resulted in a significant increase in the long form of the leptin receptor (ObR-B) but not the short form (ObR-A) in the heart[17]. The controversial role of leptin in cardiac hypertrophy will be addressed in the following section below.

THE ROLE OF LEPTIN IN CARDIAC HYPERTROPHY

Leptin has several functions in the heart including stimulation of fatty acid and glucose metabolism, prevention of steatosis, and protection against apoptosis (Figure 2). It also can raise blood pressure and heart rate through central mechanisms and promotes cardiac inflammation (Figure 2). Although obesity is associated with hyperleptinemia, increased cardiac mass and left ventricular (LV) wall thickness, it is unclear if leptin can directly cause cardiac hypertrophy (Figure 2). Epidemiologic studies have demonstrated positive correlations between plasma leptin levels and LV hypertrophy[18]. However, most of these observations are confounded by the fact that increased body mass and plasma leptin levels are highly correlated[19]. Obesity is also usually accompanied by hypertension which is the most common cause of cardiac hypertrophy[20]. In addition to increased blood pressure, obesity may cause cardiac hypertrophy by several other mechanisms including neurohormonal (renin-angiotensin-aldosterone system) and sympathetic nervous system activation, insulin resistance and hyperglycemia, and increased blood volume[21,22]. The exact roles of leptin in regulating cardiac structural changes in obesity such as hypertrophy are not well understood. In fact, differential hypertrophic and antihypertrophic effects of leptin have

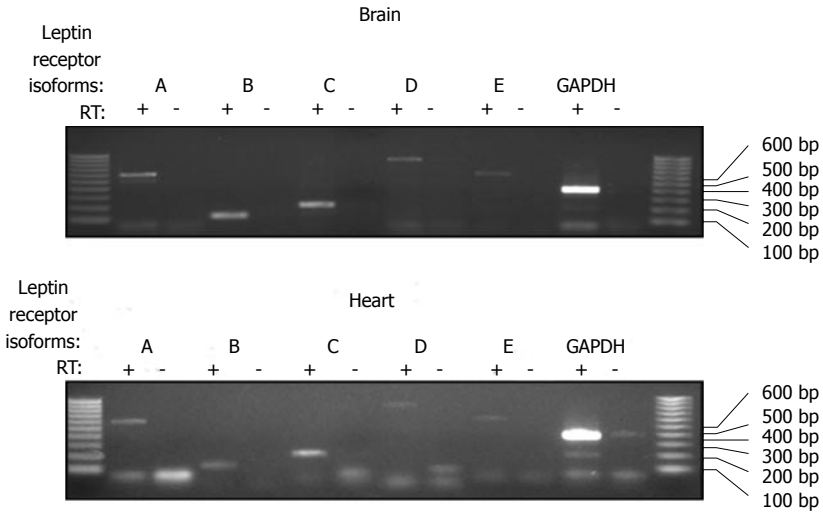

Figure 1 Comparison of leptin receptor isoforms in the mouse brain and heart. RNA was isolated from the brain and heart and reverse transcribed into cDNA. Polymerase chain reaction was then performed using primers specific for each mouse isoform of the leptin receptor as previously described[12]. All 5 of the leptin receptor isoforms were detected in the mouse heart as well as the brain.

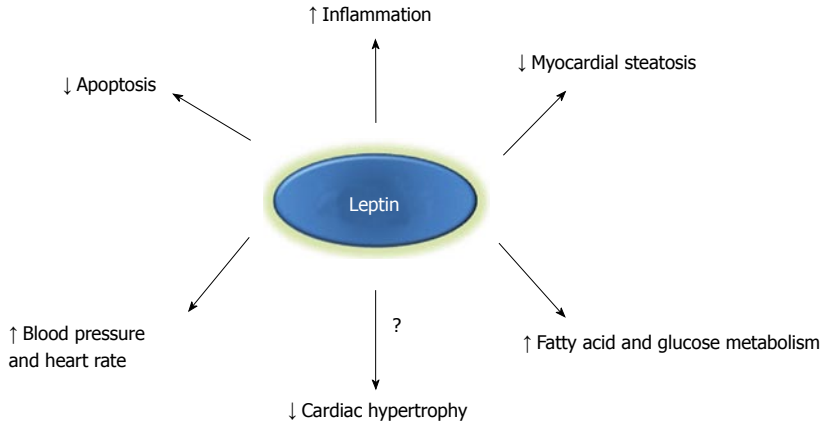

Figure 2 Potential mechanisms by which leptin may mediate cardiac function. Leptin may exert cardioprotective or maladaptive effects through hemodynamic factors such as increased heart rate and blood pressure, metabolic changes including augmented fatty acid or glucose utilization, reduced cardiac apoptosis, or structural cardiac changes such reduced cardiac lipid accumulation and possibly attenuated myocardial hypertrophy. Increased inflammation may be beneficial in some cardiac conditions (*i.e.*, post-myocardial infarction) depending on the timing and extent of the inflammatory response.

been reported and may be related to temporal effects or synergistic interactions with other obesity-associated factors.

Does leptin directly cause cardiac hypertrophy?

One of the earliest studies demonstrating a pro-hypertrophic effect of leptin comes from an experiment by Rajapurohitam *et al*[23] in which cultured neonatal rat ventricular myocytes were treated with varying concentrations of leptin. The authors observed a 42% increase in cell surface area 24 h after administration of 3.1 nmol/L leptin. Exposure to leptin also significantly increased cell size in cultured human and neonatal rat cardiomyocytes[24,25]. Leptin treatment increased matrix metalloproteinase-2 activity and collagen Ⅲ and Ⅳ mRNA expression but resulted in no change in total collagen synthesis. Tajmir *et al*[26] demonstrated hyperplasia of both murine and human cardiomyocytes in response to leptin treatment which appeared to be mediated by activation of extracellular signal-regulated kinase (ERK) 1/2 and phosphatidylinositol-3 kinase. However, studies by Piñieiro *et al*[27] did not observe any effect of Leptn to increase cell size of murine HL-1 cardiomyocytes while these *in vitro* results suggest leptin contributes to adverse cardiac remodeling and hypertrophy, the results from whole animal and human studies are not that clear regarding the direct role of leptin to cause cardiac hypertrophy.

Human patient studies have reported associations of plasma leptin levels with cardiac hypertrophy. In hypertensive insulin-resistant men, fasting plasma leptin levels were positively correlated with myocardial wall thickness, but not with LV mass. This relationship was significant even after controlling for BMI, waist-to-hip ratio and blood pressure suggesting an independent effect of leptin on cardiac structure[28]. In another study, LV mass was found to be positively correlated with leptin levels after controlling for body mass index (BMI)[29]. After gastric bypass surgery and profound weight loss, there were significant reductions in BMI, insulin resistance and leptin levels, but only leptin levels were significantly correlated with the decrease in LV mass on multivariable analyses. These clinical findings suggest that leptin may contribute to the LV hypertrophic process. In a study of 36 hypertensive men, plasma leptin was significantly predictive of echocardiographic wall thickness independent of 24 h ambulatory blood pressure. However, other significant predictors in this model included insulin sensitivity and night-time diastolic blood pressure[18].

The mechanisms by which leptin may contribute to myocardial hypertrophy are poorly understood. In addition to its powerful effects to regulate appetite and body weight, leptin also has a powerful effect to activate

the sympathetic nervous system *via* central nervous system pathways. Chronic leptin infusion increased arterial blood pressure which increases cardiac afterload and which would lead to increased cardiac hypertrophy over the long-term[30]. Leptin is also associated with increased heart rate which would also tend to increase myocardial workload and promote hypertrophy[31]. In addition to these effects, leptin may also contribute to endothelial dysfunction and vascular stiffness which could also contribute to cardiac hypertrophy[32]. It is important to note, however, that many of the reported effects of leptin are based on either short-term animal studies, *in vitro* experiments or epidemiologic data which makes it difficult to determine the direct role of leptin in regulating cardiac hypertrophy.

While it is clear that obesity is associated with cardiac hypertrophy, the role of leptin as a mediator or cause is still under investigation. Evidence strongly supporting an antihypertrophic role of leptin comes from an elegant experiment by Barouch *et al*[33] in which they evaluated LV structure and function, including LV wall thickness and mass, in *ob/ob* and *db/db* mice. To differentiate the direct effects of leptin on cardiac hypertrophy from the effects of obesity, the investigators subjected *ob/ob* mice to intravenous leptin infusion or caloric restriction. Administration of leptin significantly reduced wall thickness and reduced myocyte size by approximately 25%. While both the leptin-treated *ob/ob* mice and the calorie-restricted mice lost a similar amount of body weight, the pair fed group had no significant reduction in LV mass or wall thickness suggesting a leptin dependent effect in the reversal of myocardial hypertrophy. Additionally, the hypertrophic LV changes in the *ob/ob* mouse are not related to changes in blood pressure since these mice are normotensive[34]. Another important observation of this study was that the increase in myocardial wall thickness was not related to fatty infiltration of the heart muscle, as cardiac myocyte size was found to be increased in *ob/ob* mice[33].

Additional evidence for an antihypertrophic effect of leptin comes from experiments performed in our lab[35]. We evaluated the direct effect of leptin on myocardial lipid accumulation and LV hypertrophy in *db/db* mice and transgenic *db/db* "rescue" mice in which the normal rat leptin receptor was overexpressed or "rescued" in a cardiomyocyte-specific manner. After 30 wk of study including serial metabolic parameters and echocardiographic assessments, both the *db/db* and "rescue" mice were morbidly obese, hyperglycemic, and had high plasma triglycerides compared to lean control mice. The *db/db* mice developed significant cardiac hypertrophy and increased LV wall thickness. The "rescue" mice, in which cardiac leptin signaling was restored, had lower heart weights and LV wall thickness compared to *db/db* mice suggesting an antihypertrophic effect of leptin. If leptin had a direct hypertrophic effect, our *db/db* cardiac leptin receptor rescue mice would be primed for an increase in myocardial mass in this setting. *db/db* mice have very elevated circulating leptin levels, and our transgenic "rescue" mice had evidence of increased leptin signaling in the heart as indicated by elevated levels of phosphorylated STAT3. If increased leptin signaling directly leads to cardiac hypertrophy our transgenic "rescue" model would have developed an increase in myocardial mass and wall thickness due to high circulating leptin and augmented leptin receptor responsiveness. One limitation of this study was that we did not specifically evaluate myocyte sizes but instead measured wall thickness and heart weight[35].

In summary, the available data on the effects of leptin on cardiac growth and hypertrophy are conflicting and are summarized in Table 1. Hyperleptinemia is associated with cardiac hypertrophy but the presence of many confounding factors makes it difficult to establish a causal relationship. Furthermore, acute and chronic effects of leptin differentially regulate myocyte growth. Obesity and subsequent leptin resistance may play an important role in this relationship. Animal studies suggest that hyperleptinemia does not directly cause cardiac hypertrophy but may rather play an integral role in cardiac structural alterations that occur in response to obesity and the associated hemodynamic and metabolic changes. Additional, well controlled studies are warranted to better delineate the mechanisms by which leptin may regulate cardiac structural remodeling.

Leptin and cardiac function

In addition to its role in regulating cardiac structural changes, leptin may also be an important factor in regulating cardiac function. Leptin has been associated with pathophysiologic cardiovascular conditions including coronary artery disease and congestive heart failure[36,37]. Leptin has important effects on systemic hemodynamics and myocardial metabolism (as discussed in detail below) which may also have profound effects to regulate cardiac function. Similar to its potential implication in cardiac hypertrophy, the effects of hyperleptinemia on cardiac function have been difficult to assess given the number of confounding factors associated with obesity that all have detrimental effects on the heart. As obesity and its co-morbid conditions such as hypertension and diabetes are increasingly prevalent, understanding the relationships and mechanisms by which each of these conditions impacts the development and progression of congestive heart failure has important clinical implications for its prevention and treatment.

Elevated leptin levels have been observed in patients with dilated cardiomyopathy and have been suggested to be a marker of heart failure progression[38]. In a prospective study of 4080 older men followed for 9 years, increased BMI and circulating leptin levels were independent predictors of incident heart failure. After adjustment for BMI and other potential mediators, increased leptin levels remained significantly associated with an increased risk for heart failure in men without pre-existing coronary artery disease[39]. Leptin levels were also associated with incident congestive heart failure and cardiovascular disease in an elderly cohort

Table 1 Effects of leptin on cardiac mass and left ventricular hypertrophy

	Ref.	Findings	Effect of leptin on LVH
In vitro experiments	Rajapurohitam *et al*[23]	Exposure of cultured neonatal rat ventricular myocytes to leptin (0.31 to 31.4 nmol/L) increased cell area by 42%	Pro-hypertrophic
	Xu *et al*[25]	Exposure of cultured neonatal rat cardiomyocytes to leptin (1-1000 ng) for 4 h increased cell surface area	Pro-hypertrophic
	Piñieiro *et al*[27]	Exposure of murine HL-1 cells to leptin did not increase cell size of cardiomyocytes	Neutral
	Madani *et al*[24]	Treatment of human pediatric cardiomyocytes with 6 nmol/L leptin increased cell size by 60%	Pro-hypertrophic
	Tajmir *et al*[26]	Treatment of HL-1 cells with 60 nmol/L leptin increased cell numbers 2.3-fold	Pro-hypertrophic
In vivo experiments	Barouch *et al*[33]	6-mo-old leptin deficient *ob/ob* mice had increased myocyte diameters compared with wild-type mice. Leptin (*iv*) treatment in *ob/ob* mice completely reversed LVH and normalized wall thickness as well as reduced cellular hypertrophy by approximately 25%. Pair-feeding did not significantly reduce LV mass despite similar weight loss	Anti-hypertrophic
	Hall *et al*[35]	*db/db* mice developed LVH (increased wall thickness and heart weights). Transgenic *db/db* mice with cardiomyocyte-specific leptin receptor rescue did not cause LVH; in fact the heart weights were reduced	Anti-hypertrophic
Epidemiologic studies	Paolisso *et al*[28]	Plasma leptin level was correlated (n = 55 males) with interventricular wall (r = 0.34) and posterior wall (r = 0.38) thicknesses after adjusting for BMI and waist/hip ratio	Pro-hypertrophic
	Paolisso *et al*[18]	Study of 36 hypertensive patients demonstrating increased LV wall thickness (but not LV mass) measured by echo was associated with plasma leptin independent of BMI or waist/hip ratio (P = 0.001)	Pro-hypertrophic
	Perego *et al*[29]	Study of 31 obese subjects undergoing gastric bypass surgery demonstrated leptin was independently associated with LV mass (β = 10.66, P = 0.001). One year after surgery, decrease in LV mass only correlated with the decrease in leptin levels (P = 0.01)	Pro-hypertrophic
	Lieb *et al*[40]	Cross-sectional analysis of 432 aged (> 70 yr) participants in the Framingham Heart Study demonstrated leptin concentrations were inversely correlated with LV mass (β = -0.134, P = 0.02), left atrial size (β = -0.131, P = 0.04) and LV wall thickness (β = -0.134, P = 0.02) measured by echo	Anti-hypertrophic
	Martin *et al*[41]	In 1464 MESA Study participants who underwent cardiac magnetic resonance imaging, a 1-SD increment in leptin was associated with smaller LV mass (β = -4.66%, P < 0.01), LV volume (β = -5.87, P < 0.01), and reduced odds ratio for presence of LVH (OR = 0.65, P < 0.01) after adjustment for age, gender, race, height, and weight	Anti-hypertrophic

LVH: Left ventricular hypertrophy; BMI: Body mass index.

from the Framingham Heart Study. However, after adjustment for BMI the association with congestive heart failure was negated[40]. More recently, investigators from the Multi-Ethnic Study of Atherosclerosis demonstrated that leptin levels were not associated with incident cardiovascular events after adjustment for cardiovascular risk factors and BMI[41]. Based on these epidemiologic data, it remains unclear whether leptin is associated with development of heart failure, and if so, whether it plays a causal or compensatory role?

Leptin exerts physiologic effects that may be detrimental in states of cardiac dysfunction or heart failure. Leptin's hemodynamic effects generally increase myocardial workload *via* activation of the sympathetic nervous system. These effects include increasing resting heart rate and blood pressure[30]. Leptin may therefore act synergistically with other factors associated with obesity such as hyperglycemia, inflammation, and oxidative stress to accelerate the development of cardiovascular disease. However, it is possible that the chronic effects of leptin may have adverse consequences on myocardial function and the acute effects may provide a compensatory response to cardiac insults such as ischemia or heart failure.

Evidence for an acute beneficial effect of leptin comes from studies in experimentally-induced myocardial infarction and heart failure. McGaffin *et al*[16] induced anterior myocardial infarctions in control mice and in mice with cardiac-specific deletion of the leptin receptor. Mice lacking the leptin receptor specifically in the heart developed more LV dysfunction and had higher mortality after induction of myocardial infarction. The disruption of leptin signaling was associated with more LV dilation, hypertrophy, inflammation and adverse cardiac remodeling post-myocardial infarction. These investigators also demonstrated that many of the beneficial effects of leptin in this setting may be mediated *via* the AMP-activated protein kinase (AMPK) pathway.

We have studied the acute protective effects of leptin in a model of heart failure induced by Cre-recombinase activation[42]. Activation of Cre-recombinase is a widespread molecular tool used to conditionally delete or express genes in a tissue-specific and temporal manner. This technique has been somewhat limited due to observations by our lab and others that induction of Cre-recombinase activity in the heart can lead to transient LV dysfunction and a dramatic drop in ejection fraction[43,44]. Specifically, we reported that conditional deletion of the cardiac leptin receptor resulted in severe cardiogenic shock and death of the animals which was most likely related to impaired myocardial energy metabolism[42].

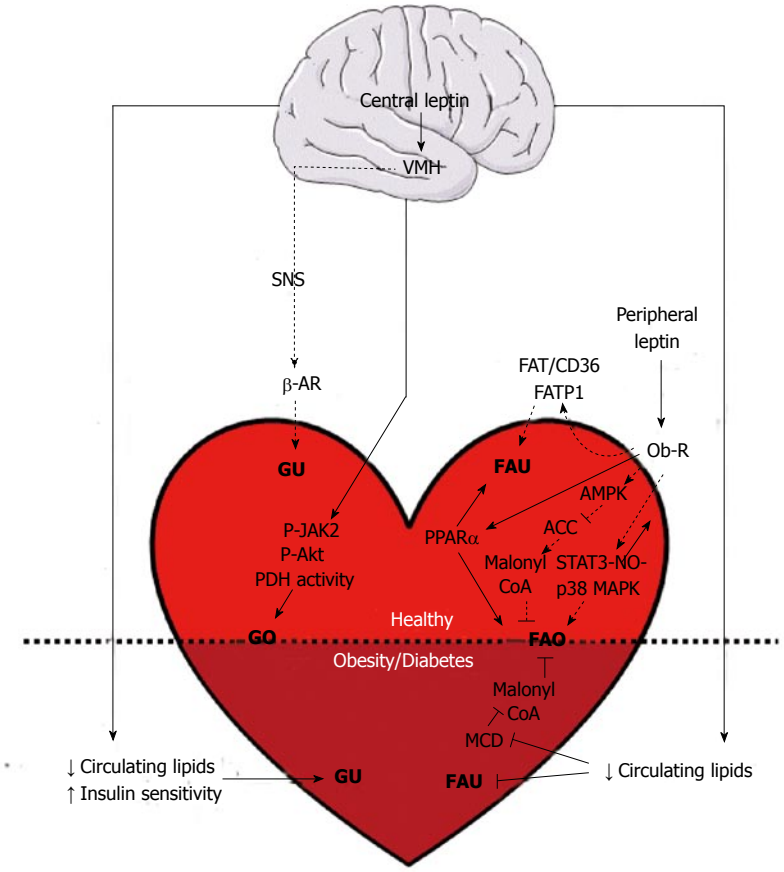

Figure 3 Cardiometabolic effects of leptin in health and obesity. Triangular and flat arrowheads represent stimulatory and inhibitory effects on the designed targets, respectively. Dotted lines indicate acute leptin effects (appearing between less than an hour and several hours of treatment), while plain lines represent chronic leptin effects (reported after several days or weeks of treatment). ACC: Acetyl-CoA carboxylase; AMPK: AMP-activated protein kinase; β-AR: Beta-adrenergic receptor; FAO: Fatty acid oxidation; FAT/CD36: Fatty acid translocase/cluster of differentiation 36; FATP1: Fatty acid transport protein 1; FAU: Fatty acid uptake; GO: Glucose oxidation; GU: Glucose uptake; MAPK: Mitogen-activated protein kinase; MCD: Malonyl-CoA decarboxylase; NO: Nitric oxide; Ob-R: Leptin receptor; P-Akt: Phosphorylated Akt kinase; PDH: Pyruvate dehydrogenase; P-JAK2: Phosphorylated Janus kinase 2; PPARα: Peroxisome proliferator-activated receptor alpha; SNS: Sympathetic nervous system; STAT3: Signal transducer and activator of transcription 3; VMH: Ventromedial hypothalamus. Horizontal black line demarcates differences between the healthy heart and the heart in obesity/diabetes.

These results emphasize the important role that leptin plays in cardiac metabolism which will be discussed in further detail in the next section.

Further evidence for a beneficial effect of leptin on cardiac function comes from experiments showing aged *ob/ob* and *db/db* mice have increased cardiac myocyte apoptosis and decreased survival compared with wild-type controls[45]. Leptin treatment significantly reduced apoptosis in *ob/ob* mice as well as in isolated myocytes. Although *ob/ob* and *db/db* mice generally have normal LV systolic function, they appear to have LV diastolic functional abnormalities[35,46]. These studies suggest that intact cardiac leptin signaling is important for normal cardiac function and may be protective against cardiac insults such as ischemia.

ROLE OF LEPTIN IN THE REGULATION OF CARDIAC METABOLISM

The role of leptin in the control of whole body energy homeostasis in humans is well established[47]. The effects of leptin on myocardial metabolism, and the consequences for cardiac adaptation in disease states, are far less well understood. Our knowledge in that area comes from studies performed on live rodents and isolated heart preparations. These studies have focused on the metabolism of glucose and free fatty acids, the main substrates for energy provision of the heart. Altogether, the investigations have revealed a dichotomy between central and peripheral actions of leptin (Figure 3). However, variations in the age or strain of animals, and in the experimental conditions employed, have made it difficult to identify with certainty the molecular regulatory pathways involved. It should also be noted that most studies used male animals only. Consequently, it is unknown whether the metabolic actions of leptin on the heart are characterized by a sexual dimorphism. This part of the review will focus first on the cardiometabolic effects mediated by leptin signaling in the brain. The metabolic consequence of leptin signaling activation in cardiac muscle will then be reviewed. Lastly, the effects of leptin on cardiac metabolism in disease states, and their impact on contractile function, will be discussed.

Centrally mediated effects of leptin on cardiac metabolism

Pioneering work performed with the leptin-deficient *ob/ob* mice demonstrated that chronic intraperitoneal leptin injection at a sub-active dose for the reduction of body weight gain or hyperinsulinemia was sufficient to normalize blood glucose levels[48]. It was later demonstrated in the same animal model that acute intravenous infusion of leptin increased glucose turnover and stimulated the uptake of glucose in peripheral organs, including the heart. The 6-fold increase in myocardial glucose uptake did not significantly impact heart rate[49]. Both intravenous and intracerebroventricular (*icv*) infusions of leptin were found to similarly increase glucose turnover in C57BL/6J wild-type mice[50]. A single bolus injection of leptin in the ventromedial

hypothalamus of young Sprague Dawley rats also resulted in a 4-fold increase in myocardial glucose uptake[51]. This increase in glucose uptake was shown to be additive to the one induced by an intravenous administration of insulin[42]. Based on these observations it was concluded that this rapid increase in peripheral glucose utilization is governed by a central mechanism, independent from insulin, and involving the activation of sympathetic nerves and the local activation of β-adrenergic receptors on target tissues[52]. A week of daily *icv* leptin administration in C57BL mice on a low-fat diet also resulted in an increase in myocardial glucose oxidation that was associated with increased phosphorylation of JAK2 and Akt kinases, and with increased pyruvate dehydrogenase activity (Figure 3). Rates of palmitate oxidation were not significantly altered, and here again, the switch toward higher glucose utilization did not modify mechanical function of the heart[53]. Thus, in rodents with normal leptin sensitivity, both acute and chronic activation of central leptin signaling favor myocardial glucose utilization through mechanisms stimulating the uptake and the oxidation of this substrate. Although the involvement of other hormonal signals cannot be ruled out, the effect seems to be independent from insulin secretion or from a change in insulin sensitivity.

Peripheral effects of leptin on cardiac metabolism

Unlike the reports on its central actions, there is little evidence to support a stimulatory effect of peripheral leptin on myocardial glucose metabolism: While a small but significant increase in glucose uptake was reported when hearts of Wistar rats were perfused in the Langendorff mode with a low dose of leptin (1 ng/mL), the effect may have been caused by the absence of fatty acids in the perfusate[54]. Indeed, in hearts of male Sprague Dawley rats perfused in the working mode with both glucose and palmitate, rates of glucose oxidation were unaffected by the presence of a pharmacological dose of leptin (60 ng/mL). Conversely, the total oxidation of fatty acids, from both exogenous (palmitate) and endogenous (triacylglycerol stores) origin, increased by 82%[55]. Using similar experimental conditions, Sharma and colleagues confirmed the existence of a leptin-mediated increase in exogenous palmitate oxidation that occurred in absence of changes for the rates of glucose oxidation[56]. Experiments performed with HL-1 cardiomyocytes partly corroborate these results: while a 1 h incubation with leptin failed to modify basal or insulin-stimulated glucose uptake and oxidation, it resulted in increased palmitate uptake and oxidation[57]. The increase in palmitate uptake was linked to the upregulation of the fatty acid transporters FATP1 and CD36. It is noteworthy that the incubation of neonatal rat ventricular myocytes with leptin for 72 h induced the expression of peroxisome proliferator-activated receptor alpha (PPARα), a key activator of fatty acid metabolism in the heart[58]. However, while increased fatty acid oxidation in HL-1 cells was traced to an increase in AMP-activated protein kinase activity and to the subsequent inhibition of malonyl-CoA production (a potent endogenous inhibitor of mitochondrial fatty acid uptake), this mechanism was not induced in the isolated rat heart[55]. Instead, in the intact heart, leptin was found to stimulate fatty acid oxidation by a STAT-3-nitric oxide-p38 MAPK-dependent mechanism (Figure 3)[56]. In conclusion, based on experimental settings where both glucose and fatty acids were present, the activation of myocardial leptin signaling rapidly stimulates fatty acid uptake and oxidation without affecting glucose oxidation. In the long term, leptin may also promote fatty acid oxidation through the upregulation of PPARα.

Metabolic effects of leptin in disease states

Obesity and diabetes are characterized by an increased reliance of the heart on fatty acid oxidation for energy provision. Sustained high rates of fatty acid uptake and oxidation inhibit both basal rates of glucose oxidation and insulin-stimulated glucose utilization, leading to a dramatic reduction in cardiac mechanical efficiency (work performed per unit of oxygen consumed)[59,60]. This metabolic remodeling has been observed in *ob/ob* mice, and persists even when the isolated hearts are perfused under low fatty acid condition[61]. However, this metabolic remodeling is reversible, and glucose intolerant patients undergoing a modest weight loss present with reduced myocardial fatty acid uptake and with improved cardiac mechanical function[62]. Sloan and colleagues elegantly demonstrated the importance of hypothalamic leptin signaling in the regulation of the balance of myocardial substrate selection during weight loss. By combining calorie restriction with leptin treatments in *ob/ob* mice, they showed that the hormone is necessary to normalize basal myocardial palmitate oxidation and to restore the insulin-mediated switch to glucose utilization[63]. The authors attributed their results to the leptin-mediated inhibition of the rise in circulating free fatty acids caused by calorie restriction, thereby leading to the normalization of myocardial fatty acid oxidation gene expression and to the improvement of myocardial insulin sensitivity. In accordance with these results, Keung *et al*[53] observed that chronic central leptin treatment (*via* intracerebroventricular infusion) of C57BL mice inhibited the increase in myocardial fatty acid oxidation caused by high-fat feeding. The effect was also linked to an improvement in insulin sensitivity and to a decrease in circulating lipid levels, with a subsequent reduction in the expression of cardiac malonyl-CoA decarboxylase, the enzyme that degrades malonyl-CoA (Figure 3). Although the absolute rates of myocardial glucose oxidation were unaffected, this resulted in an increased contribution of glucose metabolism to Krebs cycle activity[53]. Lastly, in a rat model of insulin-dependent diabetes, increased glucose uptake in cardiac muscle as well as in several other organs, together with the suppression of hepatic glucose output, is part of the mechanism by which the activation of central leptin signaling normalizes gly-

cemia[64].

Heart failure also elicits disturbances in the balance between fatty acid and glucose oxidation. Severe heart failure has generally been associated with increased glucose oxidation and decreased fatty acid oxidation, a switch in substrate meant to improve mechanical efficiency of the stressed heart[65]. The expression of leptin and of its receptor increases more than 4-fold in the failing human heart, suggesting increased activity of this signaling pathway as the condition progresses[66]. In a murine model with heart failure from ischemic origin, cardiomyocyte-specific deletion of the leptin receptor exacerbated the deterioration of myocardial structure and function. While myocardial metabolism was normal in the unstressed heart, cardiac specific loss of leptin receptors completely inhibited the switch toward increased glycolysis and glucose oxidation post myocardial infarction and enhanced the development of heart failure[16,67]. These results indicate that both central and cardiac leptin signaling play an important role in metabolic adaptation of the heart in heart failure. The beneficial effects of leptin are achieved either by favoring the return to a normal energy balance in dysregulated metabolic states, or by facilitating the transition toward a state of improved mechanical efficiency.

What is lipotoxicity?

Excess fatty acids as occurs in individuals who consume too many calories or expend too few calories are normally stored as triglycerides in white adipose tissue. However, when there is a defect in the amount of adipose tissue as seen in lipodystrophy or an excessive amount of fatty acids are consumed which exceed the ability of white adipose tissue to expand as seen in obesity, fatty acids can start to accumulate in organs such as the heart. With obesity, the substrate preference and utilization of the heart becomes altered such that substrate utilization is shifted towards fatty acids. This switch towards fatty acid metabolism is promoted by the increased expression of proteins involved in fatty acid oxidation such as carnitine palmitoyltransferase-1 (CPT1). These alterations in normal fatty acid oxidation can promote the formation of toxic lipids like ceramide and contribute to cardiac dysfunction observed in obesity[68-71]. Several studies have demonstrated that increases in ceramide production which arises by condensation of unoxidized palmitoyl-CoA and serine causes cells including cardiac myocytes to undergo apoptosis and die[72,73]. Diacylglycerol (DAG) is another lipid that can mediate fatty acid-induced toxicity. DAG acyl transferase (DGAT) is the enzyme responsible for the addition of the final fatty acid onto DAG to convert it to triglyceride. Transgenic mice which overexpress DGAT in the heart have increased lipid accumulation in the form of increased triglyceride levels but they are protected from lipotoxic induced cardiac dysfunction[74]. Thus, the specific role of increased triglyceride accumulation in the development of cardiac lipotoxicity remains controversial.

Role of lipotoxicity in heart disease

Descriptions of fat storage in the heart date all the way back to the 1800's and were thoroughly described by Smith *et al*[75] in the 1930's. Over 10 years ago Sharma and colleagues described intramyocardial lipid accumulation in human heart failure that was identical to that found in the zucker diabetic fatty (ZDF) rat[76]. These studies clearly demonstrated that increased myocardial lipid accumulation was associated with an upregulation of PPARα responsive genes and an increase in the inflammatory marker tumor necrosis factor-α (TNF-α) both of which are thought to contribute to the cardiac contractile dysfunction observed in both ZDF rats and human patients[76]. Recent advances in cardiac imaging techniques has resulted in the measurement of cardiac triglyceride levels in various patient populations with mixed results regarding the significance of increased cardiac triglyceride accumulation on cardiac function. Several studies in overweight and insulin resistant patients have positively correlated increased myocardial triglyceride levels with alterations in cardiac structure and function[77,78]. Myocardial triglyceride accumulation has also been found to contribute to the pathology of severe aortic stenosis[79]. While these studies have implicated cardiac triglyceride accumulation to alterations in cardiac function several studies have not reported such a correlation. McGavock *et al*[80] reported increased myocardial triglyceride accumulation in the absence of any changes in cardiac function in patients with type Ⅱ diabetes. Likewise studies by Nyman correlated increased epicardial and pericardial fats but not intramyocardial triglyceride accumulation with alterations of cardiac function in male patients with metabolic syndrome[81]. Lastly, studies by Liu *et al*[82] reported that although myocardial triglyceride levels correlated with increases in BMI, they failed to correlate with alterations in cardiac function in healthy African-American males. Although increases in cardiac triglyceride levels have been documented in several pathological conditions as well as in the metabolic syndrome and type Ⅱ diabetes, their specific role in altering cardiac function in these conditions is still unresolved.

There are several experimental models of cardiac lipotoxicity in rats and mice. The most studied are the models of leptin signaling deficiency such as the ZDF rat and the *ob/ob* and *db/db* mouse models[83]. Several other models of cardiac lipotoxicity have been developed in which myocardial fatty acid uptake is increased above normal by overexpression of fatty acid transport protein 1 (FATP1) or cardiac specific expression of long chain acyl CoA synthase 1 (ACS1)[84,85]. Models of cardiac lipotoxicity have also been created by overexpression of enzymes CPT1 and the transcription factor PPAR-α stimulating the β-oxidation of fatty acids. Interestingly, both cardiac specific deletion and overexpression of PPAR-α result in cardiac lipid accumulation with the deletion of PPAR-α decreasing expression of critical enzymes involved in β-oxidation

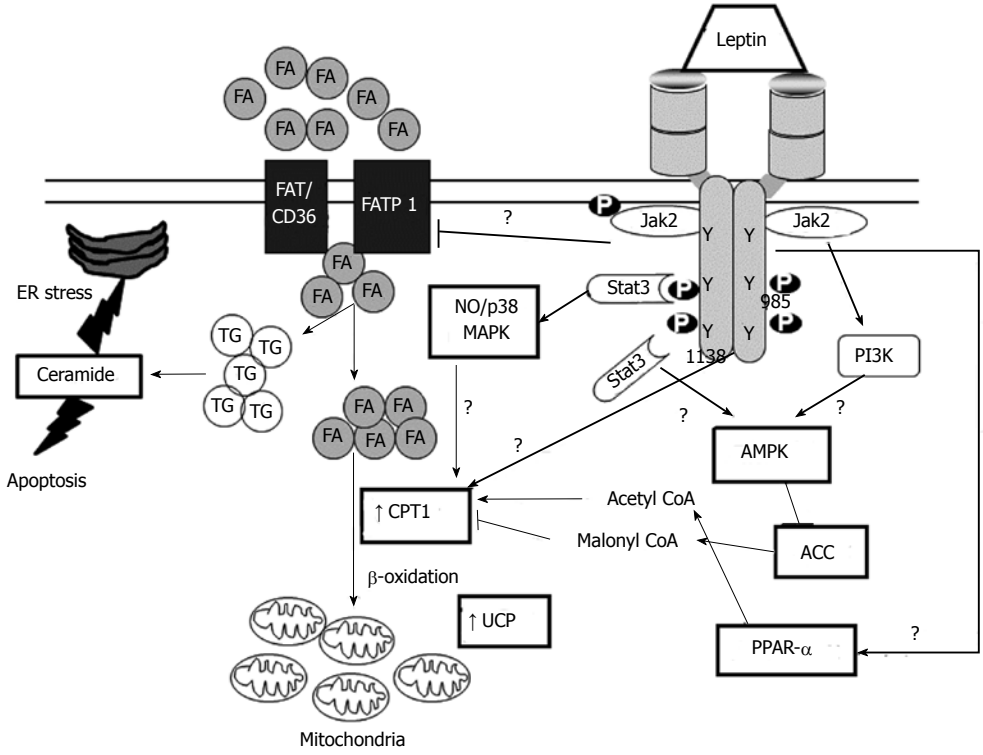

Figure 4 Leptin and cardiac lipotoxicity. Triangular and flat arrowheads represent stimulatory and inhibitory effects on the designed targets, respectively. Heavier lines represent proposed actions of leptin. ACC: Acetyl-CoA carboxylase; AMPK: AMP-activated protein kinase; CPT-1: Carnitine palmityl transferase-1; ER: Endoplasmatic reticulum; FA: Fatty acids; FAT/CD36: Fatty acid translocase/cluster of differentiation 36; FATP1: Fatty acid transport protein 1; NO: Nitric oxide; P-JAK2: Phosphorylated Janus kinase 2; PPARα: Peroxisome proliferator-activated receptor alpha; STAT3: Signal transducer and activator of transcription 3; TG: Triglycerides; UCP: Uncoupling protein.

of fatty acids and overexpression of PPAR-α resulting in increased transport of fatty acids into the heart[86,87].

While the mechanism of cardiac lipid accumulation in these various models may differ, the end result by which lipids cause cardiac dysfunction is similar in these various models. The accumulation of lipids such as ceramide and DAG to toxic levels promotes cardiomyocyte apoptosis *via* increases in reactive oxygen species production which induces non-coding RNAs such as growth arrested DNA-damage inducible gene (*GADD*)[88,89]. Accumulation of these toxic lipids also promotes endoplasmic reticulum stress which induces eukaryotic elongation factor and leads to cell death[90]. Excess lipid accumulation in the heart also interferes with insulin signaling resulting in cardiac insulin resistance and down-regulation of the IRS1/PI3K/Akt pathway which is a protective pathway in the heart[91].

Role of leptin in protection against cardiac lipotoxicity

Plasma leptin levels are elevated in individuals with obesity due to expansion of white adipose tissue mass. In obesity, it is hypothesized that there is central resistance to the effects of leptin on appetite and energy expenditure which results in increased adiposity and an increase in plasma leptin levels[7]. The increase in plasma leptin levels is also believed by some investigators to be an underlying cause for cardiovascular pathology in obesity[92,93]. However, an alternative hypothesis put forth by Unger proposes that increased plasma leptin levels are a protective mechanism preventing steatosis of organs such as the liver, pancreas, and heart in obesity[94].

Much of what is known about the role of leptin in protecting against lipotoxicity is derived from strains of leptin and leptin receptor deficient rodents. For example, leptin receptor deficient rats and mice are characterized by marked steatosis of peripherial organs such as the heart. Previous studies by Sharma *et al*[76] have reported that intracardial lipid accumulation and subsequent alterations in cardiac function and gene expression seen in the ZDF rat are remarkably similar to that observed in human patients with heart failure. Likewise studies in leptin receptor deficient *db/db* mice have also reported increased cardiac triglyceride accumulation that is associated with the development of cardiomyopathy and alteration of cardiac metabolism[95-97]. The leptin deficient *ob/ob* mouse also displays severely increased cardiac triglyceride levels associated with diastolic dysfunction[46]. Interestingly, the alterations in cardiac metabolism and cardiac lipid accumulation characterizing the *ob/ob* mouse appear to be totally dependent on leptin insufficiency as calorie restriction used to normalize body weight does not improve the increase in plasma fatty acid levels, the enhanced uptake of fatty acids by the heart or the increase in cardiac lipid accumulation[63]. Both central and peripheral administration of leptin restored myocardial insulin sensitivity and decreased myocardial fatty acid transport and lipid accumulation

independently of calorie restriction[63].

The important role of leptin to protect against lipotoxicity was first demonstrated in the liver and pancreas where adenoviral restoration of leptin receptor in these tissues in ZDF rats decreased triglyceride accumulation and protected against lipotoxic injury[98,99]. In the heart, increases in plasma leptin levels achieved by adenoviral overexpression of leptin in the liver were able to normalize cardiac triglyceride levels and restore normal cardiac function and histology in cardiac specific acyl CoA synthase transgenic mice which are a model of severe cardiac steatosis[100]. We recently reported that cardiac specific overexpression of leptin receptors normalized cardiac triglyceride levels and diastolic function in *db/db* "rescue" mice despite these mice being severely obese, hyperglycemic, and hyperlipidemic[35]. This effect was associated with enhanced STAT3 phosphorylation in the hearts suggesting that activation of this pathway is involved in the protection against lipid accumulation in the heart[35].

At the molecular level, leptin can protect against cardiac lipotoxicity through several pathways. One mechanism by which leptin protects against cardiac lipotoxicity is through induction of fatty acid oxidation. Fatty acid oxidation is highly regulated by the AMPK pathway. AMPK can phosphorylate acetyl-CoA carboxylase (ACC) and malonyl CoA decarboxylase (MCD). Phosphorylation of these proteins has opposite effects on their activity which results in decreased levels of malonyl CoA which is the first committed step in lipogenesis and a powerful inhibitor of carnitine palmityl transferase-1 (CPT-1)-mediated fatty acid oxidation (Figure 4). Previous studies in skeletal muscle have demonstrated that leptin can increase AMPK phosphorylation to promote fatty acid oxidation[101]. However, acute leptin treatment (60 min) in the isolated working rat heart stimulated fatty acid oxidation without any changes in AMPK phosphorylation state, ACC activity, or malonyl-CoA levels[55]. These results suggest that leptin may stimulate cardiac fatty acid oxidation through a mechanism that does not include AMPK activation; however, the effects of chronic leptin exposure *in vivo* have not been determined. Leptin may also promote fatty acid oxidation in the heart by decreasing the sensitivity of CPT-1 to malonyl CoA *via* an Akt related signaling pathway[102]. Uncoupling proteins are another potential pathway by which leptin may increase fatty acid oxidation. Leptin can act centrally to increase uncoupling protein levels *via* β-adrenergic mediated mechanism,and the hormone may also directly regulate uncoupling protein levels in skeletal muscle and the heart[103]. Leptin can also increase cardiac fatty acid oxidation through a STAT-3–nitric oxide–p38 MAPK-dependent mechanism (Figure 4)[56]. Leptin may also protect against cardiac lipotoxicity by its actions on fatty acid transport into cardiac myocytes. Leptin has been demonstrated to decrease the levels of both the fatty acid transport protein, fatty acid translocase (FAT/CD36), and plasma membrane-associated fatty acid-binding protein (FABPpm) in skeletal muscle; whereas, it has been reported to increase both FAT/CD36 as well as FATP1 in cultured mouse cardiomyocytes[57]. These results from cultured cells are in opposition to studies in leptin deficient *ob/ob* mice in which the levels of expression of genes that stimulate fatty acid uptake were increased in the heart[46]. Although the exact mechanism(s) need to be worked out, it is more than likely that the stimulation of fatty acid oxidation by leptin in the heart reduces the amount of lipid intermediates such as ceramide and DAG below toxic levels[104]. This in turn decreases lipid mediated apoptosis and protects cardiac function in obesity (Figure 4).

CONCLUSION

Leptin is a hormone derived from adipose tissue which undoubtedly plays an essential role in the regulation of body weight and appetite. However, emerging studies have demonstrated that leptin is also a critical hormone for the cardiovascular system which can regulate metabolism and function of the heart. It is clear that leptin exerts diverse functions in the heart (Figure 2). While some of leptin's effects can be deleterious to cardiac function primarily through its central actions on blood pressure and heart rate, its potential growth effects on the heart to promote cardiac hypertrophy are not clear. Leptin does have beneficial actions on myocardial fatty acid and glucose metabolism and the loss of cardiac leptin signaling can adversely affect the heart's response to stresses such as transient ischemia. Leptin may also directly protect the heart against excessive lipid accumulation in obesity. The development of leptin receptor antagonists has resulted in some investigators suggesting that blockade of leptin signaling in the heart may be beneficial in obesity to attenuate cardiac hypertrophy and improve cardiac function[105-107]. However, given leptin's beneficial actions on cardiac metabolism and lipid accumulation, any intervention on leptin's action in the heart must be considered very carefully. Clearly, more animal studies are needed to unravel the biological roles and mechanistic actions of leptin before any interventional studies specifically targeting leptin in the heart are undertaken.

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REVIEW

Coronary physiology assessment in the catheterization laboratory

Felipe Díez-delhoyo, Enrique Gutiérrez-Ibañes, Gerard Loughlin, Ricardo Sanz-Ruiz, María Eugenia Vázquez-Álvarez, Fernando Sarnago-Cebada, Rocío Angulo-Llanos, Ana Casado-Plasencia, Jaime Elízaga, Francisco Fernández Avilés Díaz

Felipe Díez-delhoyo, Enrique Gutiérrez-Ibañes, Gerard Loughlin, Ricardo Sanz-Ruiz, María Eugenia Vázquez-Álvarez, Fernando Sarnago-Cebada, Rocío Angulo-Llanos, Ana Casado-Plasencia, Jaime Elízaga-Corrales, Francisco Fernández Avilés Díaz, Instituto de Investigación Sanitaria Gregorio Marañón, Servicio de Cardiología, Departamento de Medicina, Universidad Complutense de Madrid, 28007 Madrid, Spain

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Correspondence to: Enrique Gutiérrez-Ibañes, MD, Instituto de Investigación Sanitaria Gregorio Marañón, Servicio de Cardiología, Departamento de Medicina, Universidad Complutense de Madrid, Calle Dr Esquerdo 46, 28007 Madrid, Spain. e-guti@hotmail.es
Telephone: +34-65-3992157

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Abstract

Physicians cannot rely solely on the angiographic appearance of epicardial coronary artery stenosis when evaluating patients with myocardial ischemia. Instead, sound knowledge of coronary vascular physiology and of the methods currently available for its characterization can improve the diagnostic and prognostic accuracy of invasive assessment of the coronary circulation, and help improve clinical decision-making. In this article we summarize the current methods available for a thorough assessment of coronary physiology.

Key words: Coronary heart disease; Coronary physiology; Endothelial dysfunction; Microvascular dysfunction; Fractional flow reserve; Coronary flow reserve; Index of microcirculatory resistance

Core tip: Assessment of the coronary circulation in the cathlab cannot be limited to angiography nowadays. The interventional cardiologist needs to be aware of current knowledge on coronary physiology and of the methods and measurements available for its characterization in clinical practice and research. In this article we review the main methods to assess the functional severity of coronary stenosis, myocardial blood flow, microvascular circulation, and endothelial function.

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INTRODUCTION

Physicians rely on angiography to assess the coronary vasculature of patients with symptoms of myocardial ischemia. However, angiography has a low interobserver agreement[1], and acknowledged limitations for the assessment of myocardial ischemia in a variety of settings, such as intermediate, eccentric or diffuse coronary stenosis[2]. Notably, intermediate lesions are the most frequently found in coronary angiography[3]. The importance of determining which lesions truly produce ischemia and thus require intervention is underscored by clinical trials showing that revascularization of non-ischemia inducing stable lesions does not improve patient outcomes, and may in fact be deleterious[4-6]. Also, a considerable percentage of patients referred to the catheterization laboratory for angina, or even myocardial infarction, have angiographically normal, or only mildly diseased, coronary arteries[7], which highlights the importance of factors beyond epicardial fixed stenosis in the development of myocardial ischemia. Among these factors, coronary microvascular disease and coronary tone dysregulation due to endothelial dysfunction are frequent causes of myocardial ischemia[8-10]. Furthermore, a normal coronary angiogram does not accurately predict prognosis in all patients, since patients with microvascular or endothelial dysfunction have an increased risk of major adverse cardiac events[11].

This article reviews the most important methods currently available to the invasive cardiologist for a comprehensive physiological assessment of the coronary circulation. After a brief reminder of the coronary structure and the physiology of flow regulation, we will discuss the contemporary methods used to assess coronary flow, flow reserve, epicardial stenosis, microvascular function, and, finally, endothelial function.

CORONARY STRUCTURE AND PHYSIOLOGY

From a physiological perspective, the coronary circulation is structured in three main compartments. The first compartment (R1) is formed by the large epicardial coronary arteries, with a size over 500 μm. These are the conduction vessels, which offer minimal resistance to flow under normal conditions, accounting for less than 10% of the overall resistance of the coronary circulation. Accordingly, blood pressure remains unaltered along these vessels. The second compartment (R2) is formed by extramyocardial prearterioles, 100-500 μm in diameter. The third coronary compartment is formed by arterioles (< 100 μm) and capillary vessels. The second and third compartments, generically known as "microcirculation", are accountable for over 90% of the total coronary resistance, and therefore are the main regulators of flow[3,12].

The microvascular compartments are responsible for coronary autoregulation of flow. Because myocardial extraction of oxygen is very high at rest, an increase in oxygen demands must be met with an increase in coronary blood flow. Intramyocardial arterioles respond to metabolic signals, directly diffused from the myocardium, with vasodilation or vasoconstriction. When arterioles relax to reduce resistance and increase myocardial flow, proximal prearterioles and large epicardial arteries respond with flow-mediated dilation, which happens mainly through the release of nitric oxide by the endothelium[12].

In pathological conditions, when a severe epicardial stenosis develops, epicardial resistance increases causing a pressure drop in the distal circulation. This is registered by the prearterioles, which respond with vasodilation to maintain a normal flow and pressure in the arteriolar compartment. It is through this autoregulation that the coronary circulation manages to maintain myocardial blood flow within a normal range in the face of moderate or even severe coronary atherosclerosis. It is also because of this mechanism that the functional significance of a coronary stenosis may be obscured to the interventional cardiologist at rest, and become evident only under conditions of maximal hyperemia.

CORONARY BLOOD FLOW MEASUREMENT

There are currently two methods available for measuring coronary blood flow in clinical practice: Doppler velocity and thermodilution. Both methods require engaging the coronary artery with a guide catheter, and introducing an intracoronary diagnostic 0.014 wire in the vessel. Heparin must be administered before the procedure, at the same doses as used during percutaneous coronary intervention.

Doppler-velocity coronary flow measurement

Coronary blood flow can be assessed by blood velocity measurement using an intracoronary Doppler wire (Flowire, Volcano Corp, San Diego, CA, United States)[13]. After engaging the coronary artery with a guiding catheter and administering heparin, the Doppler wire is positioned into the artery, usually at a proximal segment. Doppler-derived blood flow velocity is recorded in a dedicated console, and the average peak velocity (APV) is calculated with the use of integrated automatic software. Coronary flow is then estimated from the APV and the crossectional vessel area, 5 mm distal to the tip of the wire. The vessel area can be calculated from the angiographic vessel diameter, or directly measured by intravascular ultrasound or optical coherence tomography. Thus, the formula to calculate the coronary blood flow by Doppler is:

$$CBF = 0.5 \times APV \times (D^2 \pi)/4$$

where CBF is coronary blood flow (cm^3/s); APV is

Table 1 Substances used in the catheterization laboratory for coronary vascular function assessment

Substance	Doses	Site of action	Endothelium response	Effect
Adenosine	Iv: 140 μg/kg per minute Ic: 20-150 μg bolus	Microvascular	Independent	Direct vasodilation
Acetylcholine	Ic: 10^{-6} M/10^{-5} M/ 10^{-4} M	Micro and macrovascular	Dependent	Vasodilation if normal endothelial function; vasoconstriction if endothelial dysfunction
Nitroglycerin	Ic: 200 μg bolus	Macrovascular	Independent	Vasodilation
Nitroprusside	Ic 0.3-0.9 μg/kg bolus	Micro and macrovascular	Independent	Vasodilation
Papaverine	Ic: 8-20 mg bolus	Micro and macrovascular	Independent	Enzyme Phosphodiesterase inhibition Vasodilation
Regadenoson	Iv: 400 μg bolus	Microvascular	Independent	Adenosine receptor agonist vasodilation

average peak velocity (cm/s); and D is coronary diameter (cm).

Thermodilution coronary blood flow measurement

Coronary blood flow can be estimated by the indicator dilution method, using an intracoronary thermodilution wire (PressureWire, St Jude Medical Inc.; St. Paul, MN, United States)[14]. This wire has two temperature sensors, located at its proximal and distal parts. The wire is introduced into the coronary artery, until the more distal sensor is at least 50 mm away from the catheter tip. A bolus of 3 mL of saline injected through the guiding catheter produces a change in temperature that is recorded by both sensors, and a thermodilution curve is recorded. This is repeated 3 times and the results are averaged. Flow is derived from the thermodilution formula:

$$CBF = V/Tmn$$

were *CBF* is coronary blood flow (cm^3/s); *V* is vessel volume (cm^3) between the injection site and measuring site; and *Tmn* is mean transit time (s), which is calculated by the system console from the thermodilution curve.

As can be appreciated, both methods for measuring coronary blood flow require estimation of the vessel crossectional area or the vessel internal volume, which introduces a source of inaccuracy, and limits their actual use in clinical practice. Fortunately, however, both methods are well suited for a simple and reliable estimation of the most important flow-derived measurement: coronary flow reserve.

CORONARY FLOW RESERVE

Coronary flow reserve (CFR) is defined as the ratio between coronary blood flow at maximal hyperemia and at baseline condition[15]. It expresses the capacity of the coronary circulation to respond to a physiological increase in oxygen demands with a corresponding increase in blood flow. In animals and healthy subjects CFR is usually over 3, meaning their coronary circulation can triple the baseline flow when needed. In humans with chest pain and angiographically normal coronary arteries, however, the average CFR is lower, at 2.7 ± 0.6[16]. Therefore, a cutoff value of 2.0 has been widely accepted for CFR in the clinical practice[3]. Because it is a ratio of two flows, CFR is dimensionless.

Two different methods of assessing CFR invasively are available, based on the previously described systems for coronary flow measurement: Doppler and thermodilution. In both cases, flow determination is made at baseline, and then repeated under maximal hyperemia. Hyperemia can be achieved with several drugs, such as papaverine, nitroprusside or adenosine, but most often the latter is used. A complete description of the main substances used in the catheterization laboratory is shown in Table 1.

Adenosine produces a direct, endothelium-independent vasodilation of the coronary microcirculation, while having no appreciable effect on the epicardial vessel. It can be administered intravenously, at a dose of 140 μg/kg per minute[17], which usually achieves maximal stable hyperemia in 2-3 min. A central vein or a large brachial one must be used for the drug to reach sufficient concentration in the coronary circulation. Alternatively, hyperemia can be achieved by a single intracoronary bolus of adenosine administered through the guiding catheter. Doses for intracoronary injection range from 30-60 μg in the left coronary artery, and 20-30 μg in the right[3]. Higher doses, however, have been shown to be safe[18,19].

CFR can be assessed by coronary Doppler, using the method described above for flow measurement[13]. After engaging the artery with a guiding catheter, the wire is advanced and positioned at a segment where a stable Doppler signal is obtained, away from a coronary stenosis and branch ostia. The wire must not move between baseline and hyperemia measurements. Thus, since adenosine does not induce epicardial vasodilation, and the wire position does not change throughout the procedure, the crossectional area of the vessel can be assumed constant and removed from the flow equation, which leaves a simplified formula for CFR estimation:

$$CFR = APVh/APVb$$

where APVh is average peak velocity (cm/s) during maximal hyperemia, and APVb is average peak velocity

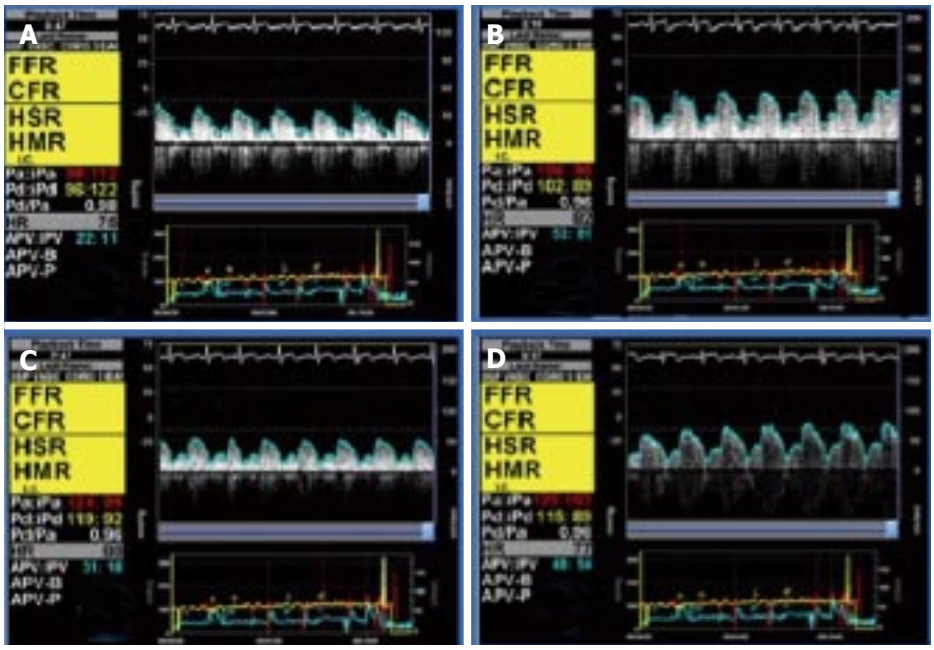

Figure 1 Microvascular function measured by intracoronary Doppler. A shows an intracoronary Doppler tracing at baseline condition, with an APV of 22 cm/s; B shows Doppler at the same position during maximal hyperemia, induced with an adenosine intracoronary bolus of 200 μg, with an APV of 53 cm/s. CFR is therefore 2.4, indicating a normal endothelium-independent microvascular function; in C and D, increasing doses of intracoronary acetylcholine have been administered; the APV is compared with the baseline tracing to determine the degree of microvascular vasodilation induced by acetylcholine, which in this case is normal, suggesting a normal endothelium-dependent microvascular function. CFR: Coronary flow reserve; APV: Average peak velocity.

(cm/s) at baseline condition. Figure 1 shows an example of CFR measurement with intracoronary Doppler.

Similarly, CFR can be calculated with the use of the thermodilution technique, by comparing mean transit time between hyperemia and baseline[17]. The pressure-temperature wire must be advanced distally into the artery, since mean transit time is more reproducible when the distal thermistor is at least 50 mm away from the catheter tip[14]. Care should be taken not to move the wire from the original position where baseline measurements are made. After three baseline thermodilution curves are obtained, hyperemia is achieved by intravenous infusion of adenosine.It is important to use intravenous administration, because the effects of an intracoronary bolus only last a few seconds, and will thus not suffice to obtain the thermodilution curves under stable maximal hyperemia. Finally, CFR is calculated with the equation:

$$CFR = Tmn.b/Tmn.h$$

where Tmn.b is mean transit time at baseline (s), and Tmn.h is mean transit time during hyperemia (s). Note that, because transit time is inversely related to flow, in this equation the hyperemia factor is in the denominator, and the baseline in the numerator, conversely to the Doppler method. An example of this technique can be found in Figure 2.

Pitfalls and contraindications

Since both thermodilution and Doppler techniques require the use of adenosine, the most common side effects associated are those described to this substance: bradycardia, hypotension, flushing, dyspnea and chest discomfort; however, the effects of adenosine disappear in seconds after the infusion is stopped or the bolus is administered, so concerning side effects are exceptional. Probably the only truly serious complication of adenosine administration is persistent bronchospasm, which is why it should be avoided in asthmatic patients[20]. If bronchospasm occurs, adenosine may be antagonized with theophylline[21].

The procedure is safe in experienced hands. However, physicians must be aware of the generic potential complications related to the insertion of guiding catheters and wires in the coronary arteries, such as coronary thrombosis, dissection, and spasm.

CFR has two main limitations. First, when an abnormal CFR value is obtained in a stenotic artery, it does not pinpoint the exact level at which flow is limited - that is, it does not help differentiate between epicardial and microvascular flow limitation. Second, given that CFR arises from the ratio of hyperemic to baseline flow, any disturbances in the baseline condition of the patient (tachycardia, stress, vasoactive drugs, abnormal loading conditions, *etc*.) will alter the ratio, thus possibly rendering a falsely abnormal CFR value.

Since CFR assesses the whole coronary vascular tree (*i.e.*, macrovascular and microvascular compartments), a normal CFR value reflects a basically healthy coronary circulation. The best CFR cutoff value for the detection of inducible ischemia is around 2.0, according to most studies[22-27]. The same cutoff value is useful to decide safe deferral of percutaneous coronary intervention when

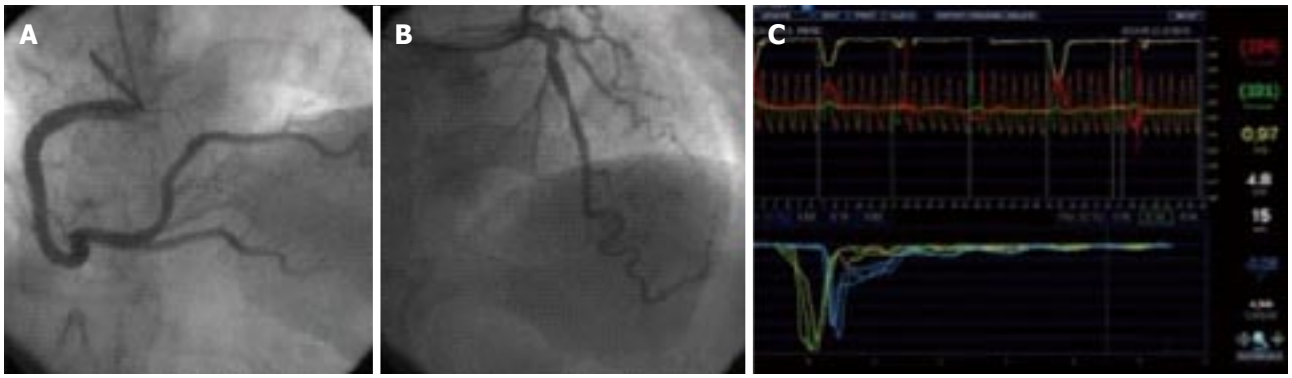

Figure 2 Coronary flow reserve measured by thermodilution. A 60-year-old woman with episodes of atypical chest pain was referred to the catheterization laboratory for coronary angiography. A and B: Show angiographically normal epicardial coronary arteries. A microcirculation study was performed, using a thermodilution PressureWire, and intravenous adenosine for hyperemia; C: Shows the thermodilution curves, with a mean transit time of 0.70 s at baseline and 0.15 s during hyperemia, and a resulting coronary flow reserve of 4.5. Index of Microvascular Resistance was 15, within normal range. We concluded that microvascular function was preserved.

assessing angiographically intermediate lesions[28,29]. A reduced coronary flow reserve, on the other hand, is an independent predictor of poor clinical outcomes in diverse settings, such as angina without severe coronary stenosis[30] and percutaneous coronary intervention[31].

Conclusion

In conclusion, CFR is a simple measurement of flow, which evaluates both the epicardial and microvascular compartments, and usually reflects normal physiology and good prognosis when it is found to be within normal limits. But other measures are needed in order to specifically investigate epicardial and microvascular disease. These measures are described and discussed below.

MEASURES OF EPICARDIAL CORONARY OBSTRUCTION

Fractional flow reserve

As we described earlier in this article, the small vessels of the heart, below 400 μm, are responsible for most of the coronary resistance, and therefore for the regulation of myocardial flow. These vessels autoregulate their resistance with the purpose of maintaining a constant myocardial blood flow independently of blood pressure, across a wide range of pressures. Similarly, when a coronary stenosis appears, the microvascular circulation regulates the resistance to flow to compensate for the stenosis.

In a state of steady maximal hyperemia, as can be achieved with adenosine, coronary autoregulation is abolished, and coronary blood flow is directly proportional to blood pressure. This principle has been used to substitute the measurements of flow by the more simple and reproducible measurements of pressure[32]. Because epicardial stenoses produce a pressure drop due to friction and separation of flow across the obstruction, different pressures can be obtained proximally and distally to a coronary stenosis, and, in a state of hyperemia, these pressures can be considered proportional to flow. Thus fractional flow reserve (FFR) arises from the ratio of the maximal flow achievable by the coronary artery with the epicardial stenosis, compared with the theoretical maximal flow of the same artery without the stenosis. Because in normal conditions the intracoronary pressure does not vary along the epicardial artery, the blood pressure at the tip of the guiding catheter is chosen to represent the theoretical pressure of the non-stenotic artery; this is compared to the pressure detected by a pressure wire distal to the stenosis. Although theoretically venous pressure should be taken into account, in clinical practice it is disregarded, and the simplified equation for FFR is used:

$$FFR = Pd/Pa$$

where Pd is the mean pressure distal to the stenosis (mmHg), recorded by the pressure wire, and Pa is the mean aortic pressure (mmHg), recorded by the tip of the guiding catheter.

To measure FFR, the coronary artery should be engaged with a guiding catheter, as with previous methods. Intracoronary nitroglycerin is administered to abolish epicardial reactivity, and a pressure wire (PressureWire, St Jude Medical Inc., St. Paul, MN, United States; or PrimeWire Prestige, Volcano Corp., San Diego, CA, United States) is advanced into the artery. Pressure is balanced against the fluid filled guiding catheter placing the wire transducer at the tip of the catheter, and then the lesion under investigation is crossed with the wire. Maximal hyperemia is induced, and the simultaneous pressure tracings of the wire and the catheter are recorded. FFR is automatically calculated with integrated software, using the mentioned equation.

Hyperemia is usually achieved with adenosine, either intravenous (140 μg/kg per minute)[33] or by intracoronary bolus. Adenosine bolus doses vary from 40 to 150 μg[33,34], although higher doses have been

proposed and deemed safe[18]. Alternatively, other drugs may be used, such as papaverine, at doses of 8-20 mg[33]; regadenoson, in a single intravenous bolus of 400 μg[35]; or nitroprusside, in intracoronary bolus of 0.3-0.9 μg/kg[36,37]. These three have the advantage of providing a longer hyperaemic plateau than intracoronary adenosine, but have been less extensively tested in the clinical setting.

The theoretical normal value of FFR in a coronary artery without stenosis is 1, since there should be no appreciable pressure drop along the vessel. A cutoff value of 0.75 (expressing a maximal-flow reduction of 25% attributable to the epicardial stenosis) accurately predicts inducible ischemia[38-40]. The most important clinical studies, however, set a cutoff of 0.8 for safe deferral of coronary intervention[41,42], and accordingly, the current European[43] and American[44] guidelines for revascularization recommend intervention in cases of coronary stenosis with FFR $\leqslant$ 0.8. As pointed out by Pijls *et al*[33], less than 10% of lesions fall into this grey area between 0.75 (almost certain ischemia) and 0.8 [safe deferral of percutaneous coronary intervention (PCI)]. In these cases, sound clinical judgement should be applied. It should also be noted that, while great emphasis has been made on cutoff points, the FFR values express a continuous rather than dichotomic function of risk[45], which highlights the importance of keeping a clinical perspective and evaluating the global risk/benefit profile all treatment options[46].

Pitfalls and contraindications

FFR is a feasible and reproducible technique, minimally modified by the baseline characteristics and hemodynamic status of the patient. Despite its reproducibility, some pitfalls and limitations have been reported.

Pharmacological side effects of adenosine are the same as those described in CFR. Accordingly, the patient must be in a stable hemodynamic condition, and adenosine should be avoided in patients with bronchospasm, severe hypotension, bradycardia or conduction disturbances.

In patients with sequential lesions or diffuse coronary disease, FFR does not provide precise information on which specific lesion is responsible for the ischemia. In such patients, the pressure pullback recording during stable hyperemia may allow identification of the culprit lesion. In order to perform this pullback recording, intravenous adenosine should be used, since an intracoronary bolus will not provide stable hyperemia.

Attention should always be paid to careful technique. The pressure wire signal must be carefully balanced at the tip of the catheter, and the balancing checked at the end of the procedure to rule out pressure drift. The tip of the catheter must be correctly positioned, to avoid damping of the pressure signal and obstruction of the coronary ostium. Submaximal hyperemia may occur, especially if a small peripheral vein is used. In this case, a central vein or an intracoronary bolus should be used to obtain maximal hyperemia.

Finally, FFR is not recommended to assess unstable thrombotic coronary lesions, *i.e.*, in acute myocardial infarction, or when thrombus or instability are evident, since pressure drop across the stenosis would provide an incomplete assessment of the risk associated with this kind of lesions.

Conclusion

In conclusion, FFR has become an indispensable tool in the catheterization laboratory to make decisions on revascularization of intermediate lesions. It can be performed in almost all elective clinical situations, providing functional information with relevant clinical implications and limited pitfalls. Figure 3 shows an example of an FFR procedure to decide revascularization in multivessel disease.

INSTANTANEOUS WAVE FREE RATIO

One of the few pitfalls of FFR is the necessity for maximal hyperemia. Without maximal microvascular vasodilation, the linear relation between pressure and flow that supports the use of FFR disappears. Coronary resistance, however, is not constant throughout the cardiac cycle; and we have already mentioned how, in some cases, stable maximal hyperemia is suboptimal or dubious, and in other rare cases adenosine may be contraindicated. To overcome these difficulties, a new physiologic measure of obstruction has been developed: instantaneous wave free ratio (iFR).

Using wave intensity analysis of simultaneous pressure-velocity recordings, Sen *et al*[47] identified a period of the cardiac cycle when there are no compression or expansion waves, and the coronary microvascular resistance at rest is minimal and stable, and very similar to the averaged resistance achieved with adenosine. The wave-free period is calculated beginning 25% of the way into diastole, and ending 5 ms before the end (covering around 75% of diastole).

A pressure wire (Verrata, Volcano Corp, San Diego, United States) is inserted in the coronary artery, following the same steps and precautions as with FFR, except that no hyperemia is induced. The wire is attached to a console with built-in proprietary software, which identifies the diastolic period of interest and automatically calculates iFR. The formula of iFR is identical to FFR, but instead of the averaged pressure of the whole cardiac cycle, it only uses the averaged pressure of the mentioned interval:

$$iFR = Pd/Pa$$

iFR is highly reproducible and has excellent correlation with FFR (r = 0.90; P < 0.001)[47]. A cutoff value of $\leqslant$ 0.90 has been set, which has an overall diagnostic accuracy of 80% to predict an FFR $\leqslant$ 0.80[48]. Because dichotomic agreement is logically lower around the cutoff values, a hybrid strategy has been proposed

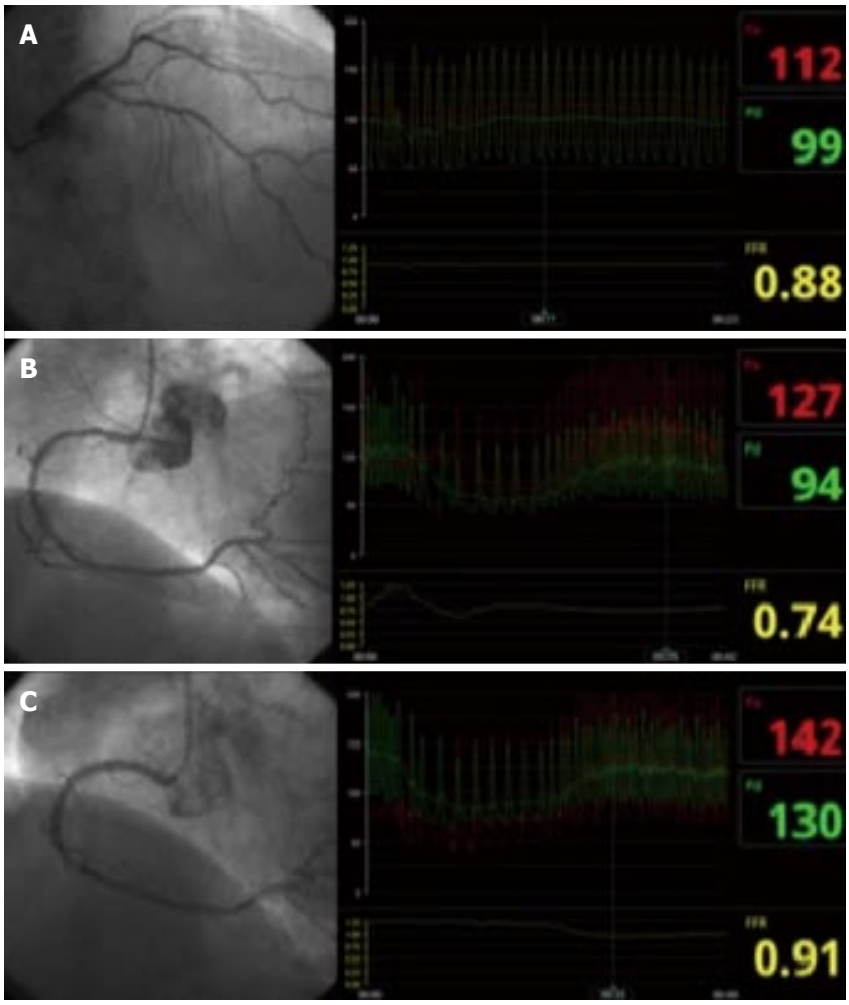

Figure 3 Functional assessment of epicardial stenosis. A 77-year-old woman with exertional angina was referred for coronary angiography. Two intermediate lesions were found in the proximal and distal segments of the left anterior descending artery (LAD) (A), and in the proximal and mid segments of the right coronary artery (RCA) (B). Fractional Flow Reserve was non-significant on the LAD (FFR 0.84) and significant on the RCA (FFR 0.74). Accordingly, percutaneous coronary intervention was performed with a drug-eluting stent on the RCA (C). A post-intervention FFR confirmed that the good angiographic result was also associated with functional improvement (FFR 0.91). Six months after the procedure the patient remains asymptomatic. FFR: Fractional flow reserve.

and tested[49,50], which would involve using an upper cutoff of 0.93, above which the coronary stenosis is considered non-significant, and a lower cutoff of 0.86, below which the stenosis is considered significant and PCI is indicated. When iFR falls between these two values (0.86-0.93, the "adenosine zone"), FFR is indicated, and the clinical decision is made according to the FFR value. This strategy may allow for two thirds of patients to be studied without hyperemia, maintaining a 95% agreement with an FFR-for-all strategy[50]. Benefits of this strategy would include reductions in cost and time, and assessment of patients who are unsuitable candidates for adenosine administration such as those with asthma, bradycardia and hypotension. Upcoming important clinical trials, such as the SYNTAX-II[51] should establish the clinical usefulness of this strategy.

HYPEREMIC STENOSIS RESISTANCE INDEX

Both CFR and FFR show good correlation with non-invasive testing for inducible ischemia. However, when both measurements are made, many patients exhibit a degree of discordance between CFR and FFR determination of ischemia[52,53]. In this context, an index of epicardial resistance that includes information from flow and pressure may have an additional value. The Hyperemic Stenosis Resistance index (HSR) is calculated from simultaneous pressure and Doppler intracoronary tracings, using a pressure-flow guidewire (Combowire, Volcano Corp, San Diego, United States). HSR is defined as the ratio of hyperemic stenosis pressure gradient (Pa-Pd) and hyperemic average peak velocity:

$$HSR = (Pa - Pd)/APV$$

where HSR represents hyperemic stenosis resistance (mmHg × s/cm); Pa represents mean aorta pressure (mmHg); Pd distal pressure (mmHg); and APV average peak velocity (cm/s). In normal epicardial arteries, no pressure gradient is expected, so the HSR should be 0. The best cutoff value has been identified at 0.8, and it

shows better diagnostic accuracy for ischemia than FFR and CFR[52]. The main limitation of HSR is that it requires the use of a Doppler-pressure wire, which increases the cost of the procedure. Also, it is less validated in clinical practice than FFR and CFR. An index of stenosis resistance derived from thermodilution flow calculations has not been validated to date.

MEASURES OF MICROVASCULAR RESISTANCE

As mentioned above, the main determinant of myocardial blood flow in physiological conditions is the microcirculation. FFR, iFR and HSR give specific information on the resistance to flow of epicardial stenoses; on the other hand, CFR provides an estimation of the overall resistance to flow in the coronary circulation. Thus, if a patient has a low CFR with normal FFR, this is usually attributed to microvascular dysfunction. This may not always be correct, however, as diffuse epicardial disease or altered resting conditions may have an impact of CFR independent of the microvascular circulation[53]. For this reason, resistance indices that provide specific information about the microcirculation have been developed.

HYPEREMIC MICROVASCULAR RESISTANCE

Using a Combowire for simultaneous determination of intracoronary pressure and Doppler velocity during adenosine-induced hyperemia, a resistance index called hyperemic microvascular resistance (HMR)[54], can be determined from this equation:

$$HMR = Pd/APV$$

where HMR is hyperemic microvascular resistance (mmHg×s/cm); Pd is the pressure in the distal part of the artery (mmHg); and APV is the average peak velocity at the same point (cm/s). Note that venous pressure is here disregarded to simplify the calculations. There are no clearly set cutoff values for HMR, but Meuwissen *et al*[54,55] have shown that the median HMR of patients with abnormal CFR is 2.4, compared with 1.9 of patients with normal CFR.

INDEX OF MICROCIRCULATORY RESISTANCE

Fearon *et al*[56] first described the index of microcirculatory resistance (IMR) in 2003, representing minimal microcirculatory resistance measured during conditions of hyperemia. IMR is calculated from the average pressure in the distal part of the coronary artery and the coronary blood flow measured by thermodilution, with the use of a specific pressure wire (PressureWire, Radi Medical Systems, St Jude Medical Inc.; St. Paul, Minn). Mean transit time correlates inversely with flow, so its inverse is used to substitute absolute flow. Theoretically, wedge coronary pressure and central venous pressure should be included in the equation to account for collateral flow and loading conditions. However, as this is usually impractical in the clinical setting, it is most common to disregard both measurements and use the simplified equation:

$$IMR = Pd \times Tmn$$

where IMR is index of microcirculatory resistance (mmHg×s); Pd is distal pressure (mmHg); and Tmn is mean transit time (s) - all measured during stable hyperemia. In practice, this simplified equation is usually correct, unless there is severe epicardial disease in the artery, in which case collateral flow may be a confounding factor. In such circumstances, the wedge coronary pressure can be measured during PCI, or alternatively an empirical corrected formula[57] may be used:

$$IMR = Pa \times Tmn \times [1.35 \times (Pa/Pd) - 0.32]$$

An IMR higher than 25 is considered abnormal[58], expressing a damaged coronary microcirculation.

Elevated IMR is related to adverse clinical outcomes in acute myocardial infarction[59], percutaneous intervention[60], and angina with apparently normal epicardial arteries[61].

In conclusion, when simultaneous measurement of pressure and flow indexes is performed, the calculation of a microvascular resistance index (either Doppler or thermodilution derived) adds specific information on the status of the microcirculation, and allows for a better diagnostic and prognostic assessment.

ENDOTHELIAL FUNCTION

The vascular endothelium is a monolayer of cells that covers the internal lumen of all the blood vessels, separating the blood from the vascular wall and organ tissues. The endothelium is a major determinant of coronary resistance and flow. In response to physiological triggers, the vascular endothelium regulates arterial smooth muscle tone through the release of vasodilators - mainly nitric oxide and prostacyclin - and vasoconstrictors, such as endothelin-1. When the vascular endothelium is damaged or dysfunctional, this function of coronary flow regulation is altered, which results in an insufficient vasodilation, or even paradoxical vasoconstriction, in response to a physiological increase in oxygen demands, such as exercise or stress. This flow dysregulation can be the cause of chronic angina or acute coronary syndromes, even in the absence of coronary epicardial stenosis.

The coronary vasomotion can be assessed directly and invasively by coronary angiography, using mainly

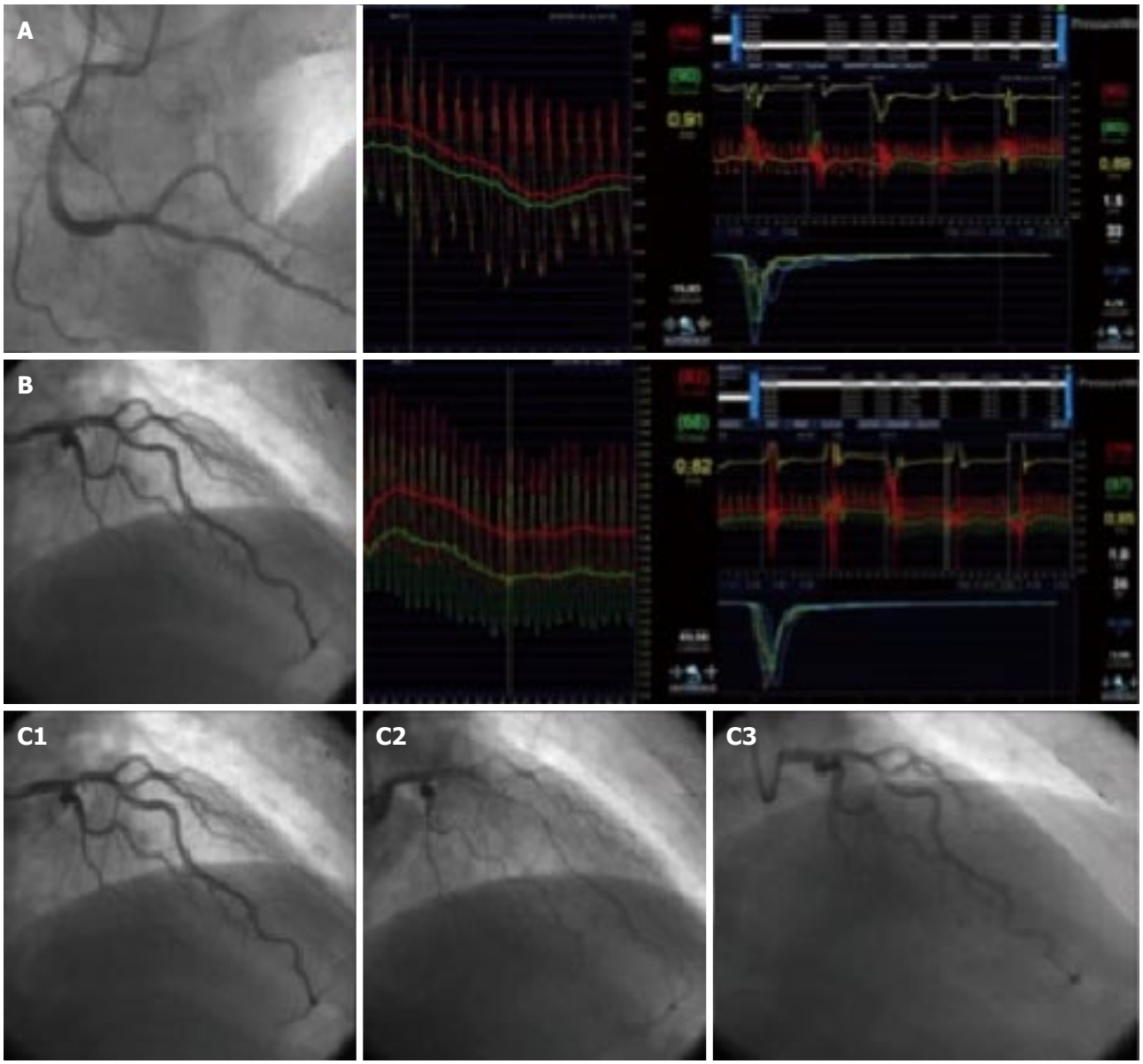

Figure 4 Thorough physiological assessment: Fractional flow reserve, microvascular and endothelial function. A 66-year-old man with typical angina, both resting and exertional, was referred for coronary catheterization. The coronary angiogram showed moderate lesions on the posterior descending artery of the RCA (50%) and on the middle LAD (40%). Panel A depicts the coronary stenosis of the RCA on the left box; FFR of this lesion on the centre box, with a value of 0.91; and microvascular study on the right box, with a CFR of 1.5 (low) and IMR of 33 (elevated); Similarly, Panel B shows the angiogram of the LAD (left), the FFR of 0.82 (centre), and the microvascular study (right), with a CFR 1 (low) and IMR 24 (borderline); Panel C shows the successive angiograms at baseline (C1), after 20 μg of acetylcholine (C2), and after 200 μg of nitroglycerin (C3). As can be appreciated, a severe diffuse spasm of the left coronary artery was induced by acetylcholine. We concluded that both coronary stenosis were non-significant, and decided on optimal medical therapy. The study also revealed microvascular endothelium-independent dysfunction, and macrovascular vasospasm due to endothelial dysfunction. CFR: Coronary flow reserve; FFR: Fractional flow reserve; IMR: Index of microcirculatory resistance; LAD: Left anterior descending artery; RCA: Right coronary artery.

acetylcholine as a trigger of endothelium-dependent vascular reactions[62-64]. In the presence of a healthy endothelium, acetylcholine at the doses used induces NO release, which results in coronary vasodilation, both epicardial and microvascular; conversely, if the endothelium is dysfunctional, NO release will be blunted and the predominant net effect will be vasoconstriction due to muscarinic stimulation of smooth muscle. Macrovascular vasodilation or vasoconstriction is evaluated by successive angiographies; the microvascular compartment, being the major determinant of flow velocity, is evaluated using intracoronary Doppler.

Before the endothelial function test, no nitroglycerin should be administered, to allow for epicardial reactivity. Ideally, the patient should be off vasoactive medication for 48 h, although in clinical practice this is not always feasible. The coronary artery - most often the left - is engaged with a guiding catheter, and a baseline angiography is performed to serve as a reference. A coronary microcatheter is advanced into the proximal part of a main vessel, usually the left anterior descending artery, and a Doppler wire (FloWire

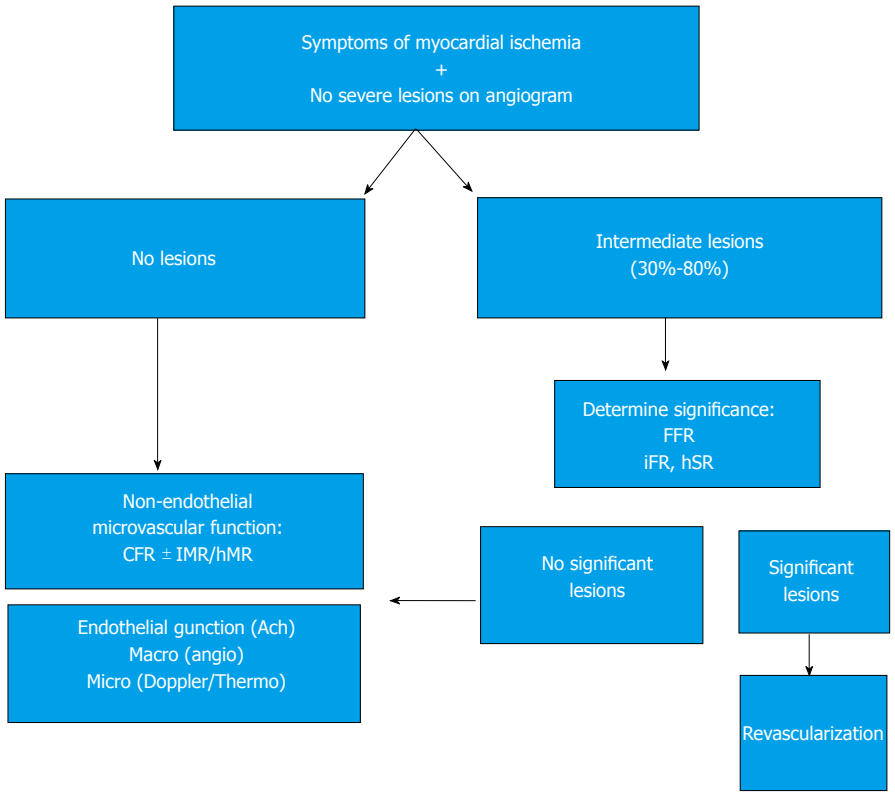

Figure 5 Summary of the proposed evaluation algorithm of patients with symptoms of ischemia without significant coronary lesions. CFR: Coronary flow reserve; IMR: Index of microcirculatory resistance; FFR: Fractional flow reserve; hMR: Hyperemic microvascular resistance; hSR: Hyperemic Stenosis Resistance index.

or ComboWire, Volcano Corp., San Diego, United States) is advanced through the microcatheter into the artery. After checking for signal quality, a baseline tracing of Doppler is recorded. Next, three consecutive infusions of acetylcholine are administered through the microcatheter, at concentrations of 10^{-6} mol/L, 10^{-5} mol/L and 10^{-4} mol/L, at a rate of 1 mL/min, 2-3 min per infusion. After each infusion, the Doppler APV and a coronary angiogram are recorded. Finally, 200 μg of nitroglycerine are administered intracoronary, to evaluate macrovascular endothelium-independent response.

The epicardial endothelial response is considered normal if vasodilation - or at least no vasoconstriction - is observed. The response is considered pathological if a reduction ⩾ 20% in coronary artery diameter occurs. A normal microvascular response to acetylcholine would be a 50% increase in APV. If a lower vasodilation, or even vasoconstriction occurs, the microvascular endothelial response is considered abnormal[64].

Most studies o endothelial function have followed this protocol approximately. It has the advantage of evaluating both the macrovascular and microvascular compartments, and the added safety of injecting the drug directly into the LAD. However, other protocols have been described and found safe. The study of the microvascular response by thermodilution, although seldom used, is feasible and has been validated[65]. Some groups[9,66] inject acetylcholine directly into the left main artery (at increasing doses ranging from 2 to 100 μg, for example 2-20-100 μg; each infusion over 3 min), and perform exclusively macrovascular angiographic assessment. This approach, although admittedly less complete than the first, still frequently offers valuable information.

Pitfalls and contraindications

Acetylcholine should be avoided in patients with severe intestinal and/or urologic obstructive disease, as it may enhance muscular contractions. Special attention should be paid to patients with bradycardia or hypotension. Generally, the coronary spasm related to macrovascular endothelial dysfunction is easily reverted with intracoronary nitroglycerin. In any case, vasospasm may cause serious complications, so the patient must be monitored and the procedure must be conducted with utmost care.

Conclusion

Endothelial dysfunction limits maximal coronary flow, and can be the cause of angina without epicardial stenosis[67,68]. It is an important risk factor for poor outcomes in this setting[11], as well as in stable coronary artery disease[69], acute myocardial infarction[70], heart failure[71], and heart transplant[72]. A more detailed account of the importance of endothelial function in coronary heart disease can be found in our recent review[73]. The finding of severe acetylcholine-induced spasm can also assist the physician in the optimization of the medical therapy.

Table 2 Main parameters available to assess coronary macrovascular and microvascular circulation

Technique	Cutoff value	Implications	Commentary
CFR	< 2	Unspecific macrovascular and microvascular inability to increase flow	Patients with CFR > 2 have favorable outcomes
FFR	≤ 0.8	Functionally significant epicardial stenosis	Extensive clinical validation Requires vasodilation
iFR	≤ 0.9	Functionally significant epicardial stenosis	Functionally significant epicardial stenosis Vasodilation-Independent
HSR	0.8 mmHg × s/cm	Functionally significant epicardial stenosis	Requires doppler-pressure wire Convenient in the presence FFR/CFR discordances
HMR	> 2 mmHg × s/cm	Microvascular dysfunction	Requires doppler-pressure wire
IMR	> 25 mmHg × s	Microvascular dysfunction	Thermodilution method

CFR: Coronary flow reserve; FFR: Fractional flow reserve; iFR: Instantaneous wave free ratio; HSR: Hyperemic stenosis resistance index; HMR: Hyperemic microvascular resistance index; IMR: Index of microcirculatory resistance.

DISCUSSION

Myocardial ischemia should not be considered to happen exclusively in the presence of critical coronary epicardial stenoses. The physiological significance of intermediate lesions cannot be properly assessed by angiography, and in this case a pressure wire should always be used to decide intervention or deferral. In the absence of significant coronary stenoses, a complete evaluation of the microcirculation and the endothelial function can help identify the fundamental problem, or at the very least reassure the patient and the physician.

When studying a patient with stable angina or acute coronary syndrome, the interventional cardiologist should not be content with an angiography showing non-significant epicardial disease. If there are intermediate lesions (30%-70%), FFR should be performed to rule out ischemic lesions; if the arteries are clearly non-stenotic, or if FFR is normal, we propose that microvascular endothelium-independent (CFR and microvascular resistance), and macro and microvascular endothelium-dependent function should be assessed. This thorough protocol can be performed in a matter of minutes, and with a very low risk[10]. Recent studies[9,10] show that, in most patients with angina who are extensively evaluated, an alteration can be found that explains the symptoms. Figure 4 shows an example from our centre following this protocol in a complex patient. Figure 5 summarizes this diagnostic algorithm.

In other clinical settings, such as stenting, myocardial infarction and heart transplant, vascular function affects clinical outcomes, and can serve as a prognostic marker. Also, coronary physiological parameters can be of interest as surrogate markers of safety and efficacy in clinical trials for new devices, such as drug eluting stents[66,74] and bioabsorbable scaffolds[75]. The interventional cardiologist should be acquainted with the methods used to perform these measurements and their interpretation. Table 2 summarizes the main parameters available to date.

CONCLUSION

Coronary physiology assessment in the catheterization laboratory is essential to help decision making in patients with coronary artery disease, providing functional and prognostic information. Physicians, especially interventional cardiologists should implement its use in daily clinical practise.

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MINIREVIEWS

Adrenal G protein-coupled receptor kinase-2 in regulation of sympathetic nervous system activity in heart failure

Katie A McCrink, Ava Brill, Anastasios Lymperopoulos

Katie A McCrink, Ava Brill, Anastasios Lymperopoulos, Laboratory for the Study of Neurohormonal Control of the Circulation, Department of Pharmaceutical Sciences, Nova Southeastern University College of Pharmacy, Ft. Lauderdale, FL 33328-2018, United States

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Correspondence to: Anastasios Lymperopoulos, PhD, FAHA, Associate Professor of Pharmacology, Laboratory for the Study of Neurohormonal Control of the Circulation, Department of Pharmaceutical Sciences, Nova Southeastern University College of Pharmacy, 3200 S. University Dr., HPD (Terry) Bldg/Room 1338, Ft. Lauderdale, FL 33328-2018, United States. al806@nova.edu
Telephone: +1-954-2621338
Fax: +1-954-2622278

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Abstract

Heart failure (HF), the number one cause of death in the western world, is caused by the insufficient performance of the heart leading to tissue underperfusion in response to an injury or insult. It comprises complex interactions between important neurohormonal mechanisms that try but ultimately fail to sustain cardiac output. The most prominent such mechanism is the sympathetic (adrenergic) nervous system (SNS), whose activity and outflow are greatly elevated in HF. SNS hyperactivity confers significant toxicity to the failing heart and markedly increases HF morbidity and mortality *via* excessive activation of adrenergic receptors, which are G protein-coupled receptors. Thus, ligand binding induces their coupling to heterotrimeric G proteins that transduce intracellular signals. G protein signaling is turned-off by the agonist-bound receptor phosphorylation courtesy of G protein-coupled receptor kinases (GRKs), followed by βarrestin binding, which prevents the GRK-phosphorylated receptor from further interaction with the G proteins and simultaneously leads it inside the cell (receptor sequestration). Recent evidence indicates that adrenal GRK2 and βarrestins can regulate adrenal catecholamine secretion, thereby modulating SNS activity in HF. The present review gives an account of all these studies on adrenal GRKs and βarrestins in HF and discusses the exciting new therapeutic possibilities for chronic HF offered by targeting these proteins pharmacologically.

Key words: G protein-coupled receptor; G protein-coupled receptor kinase; Heart failure; Sympathetic nervous system; Adrenergic receptor; Adrenal medulla

Core tip: The present manuscript is a mini-review describing the current knowledge in the field of adrenal GRKs and βarrestins, both of which are protein families that regulate adrenergic receptor function throughout the cardiovascular system. We specifically discuss the roles of these proteins in the adrenal medulla, as they pertain to regulation of catecholamine secretion and of

sympathetic activity in chronic heart failure (HF). We also outline the exciting new possibilities of targeting these molecules in the adrenal glands for HF therapy.

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INTRODUCTION

The sympathetic (adrenergic) nervous system (SNS) induces in the heart positive chronotropy, inotropy, lusitropy, dromotropy, accompanied by a decrease in venous capacitance, and constriction of resistance and cutaneous vessels[1,2]. All of these effects aim to prepare the body for "fight or flight response" and are mediated by the two catecholamines (CAs) norepinephrine (NE) and epinephrine (Epi)[3,4]. These are synthesized and released *via* the following mechanisms: (1) cardiac sympathetic nerve terminals release NE directly into the heart; (2) the adrenal medulla releases Epi and NE into the circulation; and (3) peripheral, local adrenergic nervous systems[5-7].

The actions of NE and Epi are mediated by the ARs, which are all G protein-coupled receptors (GPCRs) and consist of three α_1AR subtypes, three α_2AR subtypes (α_{2A}, α_{2B}, α_{2C}), and three βAR subtypes[8]. The main role of βARs in the heart is positive inotropy and chronotropy in response to CAs[9]. Agonist activation of GPCRs compels the cognate heterotrimeric G protein to dissociate from guanosine triphosphate and instead bind guanosine diphosphate on its Gα subunit; this results in splitting of the heterotrimer into two active functional components, Gα and $G_{\beta\gamma}$ subunits, both of which mediate signaling[9,10]. With specific regards to the α_2ARs, the α_{2B}AR is expressed in vascular smooth muscle causing vasoconstriction, while centrally located α_2ARs lower sympathetic outflow and systemic blood pressure[11,12]. NE release is controlled by presynaptic α_2ARs[13], since genetic deletion of α_2ARs leads to cardiac hypertrophy and HF, thanks to increased cardiac NE release and adrenal CA secretion[14,15].

Most GPCRs are subject to agonist-promoted desensitization and/or downregulation[16-18]. This process occurs courtesy of the GPCR kinases (GRKs) and the βarrestins[19]. The βarrestins uncouple the receptor from the G proteins, subsequently internalizing it[20]. GRK2 and GRK5 are the prominent GRKs in the heart and in most other tissues, including the adrenals[20,21]. Receptor internalization *via* the βarrestins results in either its resensitization or its degradation (downregulation)[20,21]. The receptor-bound βarrestins can also tranduce their own, G protein-independent intracellular signals[20,21]. Herein, we review the current literature regarding the roles of adrenal GRK2 and βarrestins in regulation of SNS activity in HF, with a focus on the therapeutic targeting of adrenal GRK2 as a sympatholytic strategy in chronic heart failure (HF).

ADRENAL GRK2 AND SNS ACTIVITY IN HF

A salient pathophysiological feature of chronic HF is SNS hyperactivity, reflected by increased levels of circulating Epi and NE[3,4,22]. Although it normally serves as a mechanism to re-adjust the heart from underperforming, it ultimately becomes cardiotoxic, contributing to HF progression, morbidity and mortality[3,4,22]. Adrenal CA secretion is stimulated by nicotinic cholinergic receptors and is refined by presynaptic inhibitory α_2ARs[5,23,24]. α_2ARs, similarly to cardiac βARs, also undergo GRK-dependent desensitization[10]. Of note, increased GRK2 expression and activity occur in the adrenal medulla during HF, which critically influence CA secretion from this source[25]. In particular, as we and others have documented, adrenal GRK2 overexpression is responsible for severe adrenal α_2AR dysfunction in chronic HF, leading to a loss of the sympatho-inhibitory function of these receptors in the adrenal medulla (and possibly also in sympathetic neurons); thus, CA secretion is chronically elevated[25-29]. The importance of the role of adrenal GRK2 in HF is evidenced by that its inhibition leads to a significant reduction in CA circulating levels, restoring not only adrenal, but also cardiac function[25]. In fact, HF rats treated with adrenal-specific βARKct (a GRK2 inhibitory mini-gene[30]) gene delivery show improved cardiac function and cardiac βAR number and signaling[25]. Therefore, an important crosstalk at the level of entire organs seems to exist in chronic HF and adrenal GRK2 is a crucial regulator of the circulating CA levels that affect HF progression. Consequently, adrenal GRK2 targeting to restore α_2AR function and reduce CA secretion from the adrenal medulla may provide a novel sympatholytic strategy for chronic HF treatment[25-29].

Another study demonstrating the advantages of therapeutic targeting of adrenal GRK2 is a study performed in transgenic mice having GRK2 genetically deleted only in cells expressing the phenylethanolamine-N-methyl-transferase enzyme. These mice lack GRK2 in their adrenal medullae[26]. These mice exhibit significantly reduced SNS activity during progression to chronic HF secondary to myocardial infarction (MI), as reflected by their circulating CA levels measured at 4 wk post-MI. In addition, their cardiac contractility, structure/morphology (dilatation), and βAR signaling/function, all show marked improvement at the same time-point (4 wk) post-MI[26]. Thus, prevention of the sympathetic "rush" that attacks the myocardium shortly after an MI thanks to adrenal GRK2 inhibition can help the heart work close to normal and limit its tissue damage, which normally occurs in the period directly following a heart attack. Therefore, adrenal GRK2 inhibition applied as early as possible after an MI may provide significant

survival and quality of life benefits in human HF. Of note, this is exactly the same rationale behind start of β-blocker therapy immediately after the heart attack in MI patients.

Adrenal GRK2 regulates CA secretion also under normal conditions, as adrenal βARKct transduction resulted in lowering of circulating CA levels in normal, otherwise healthy rats, and adrenal GRK2 overexpression increased their CA levels[27]. In addition, exercise training, beneficial for the cardiovascular system as it reduces HF-related SNS overactivation, can also normalize adrenal GRK2 expression and α_2AR function in HF rats[28].

It is also very likely that, in chronic HF, GRK2-mediated α_2AR deregulation also occurs in the cardiac adrenergic terminals, thus contributing to excessive NE release. Thus, global GRK2 blockade will decrease systemic circulating CA's, and perhaps a small molecule GRK2 inhibitor is best-suited for that therapeutic purpose. In that vein, it is interesting to point out that the known antidepressant drug (selective serotonin reuptake inhibitor, SSRI) paroxetine was recently shown to inhibit myocardial GRK2, thereby improving experimental HF in post-MI mice[29]. However, the results of this study must be treated with extreme caution, given that paroxetine exerts a variety of pharmacological effects, which may have contributed to its amelioration of cardiac function post-MI. For instance, it can also inhibit the other major cardiac GRK isoform, GRK5 (albeit to a lesser extent than it inhibits GRK2)[30], and it also activates glycogen synthase kinase (GSK)3-beta[31], which is known to have beneficial actions in cardiac fibrosis, hypertrophy and adverse remodeling[32]. In fact, exactly because it activates pancreatic GSK3β, paroxetine can precipitate insulin resistance/diabetes mellitus[31], a significant adverse effect that can potentially limit the drug's usefulness in HF therapy. Nevertheless, paroxetine may aid in the development of more specific (and potent) pharmacological GRK2 inhibitors by serving as a lead drug compound[30].

In summary, GRK2 inhibition is a novel sympatholytic strategy in HF, curbing CA release from SNS nerve terminals and the adrenal glands. In addition, it can be safely combined with β-blockers, as this combination cuts SNS overactivity and blocks adrenal GRK2 in HF[33]. However, while β-blockers improve inotropy of the failing heart indirectly, by protecting it from the catecholaminergic overstimulation[34,35], adrenal GRK2 inhibition can block also the non-cardiac adverse effects of the SNS (activation of endothelin, renin-angiotensin-aldosterone axis, *etc.*). Additionally, β-blockers acutely lower cardiac contractility and thus, are contraindicated in the acute setting of HF[36]. Adrenal GRK2 blockade, by diminishing global SNS activity in a cardiac-independent manner, may thus be much safer than β-blockers, as a sympatholytic approach, for acute HF. Finally, adrenal GRK2 inhibition would allow for reduction of dose and propensity for adverse effects of β-blocker therapy.

ADRENAL βARRESTINS AND SNS ACTIVITY IN HF

Aldosterone is another elevated hormone in HF and produces various detrimental effects on the failing heart, including adverse cardiac remodeling and HF progression post-MI[37-40]. It can also stimulate sympathetic neurons in the central nervous system to enhance NE release[37-40]. Aldosterone is produced by the adrenocortical zona glomerulosa cells in response to AT_1 receptor activation by angiotensin Ⅱ (Ang Ⅱ)[41,42]. A crucial role for βarrestin1 in mediating AT_1 receptor-induced aldosterone synthesis and secretion in the adrenal cortex has been documented[43]. Specifically, βarrestin1 causes upregulation of steroidogenic acute regulatory protein (StAR), the most critical enzyme in its biosynthesis[43]. Moreover, βarrestin1 does so independently of G proteins[43]. *In vivo*, adrenal βarrestin1 appears to be a major regulator of normal circulating aldosterone levels, since its upregulation, specifically in the adrenal gland, can cause hyperaldosteronism in normal healthy animals[43]. Importantly, in chronic HF, which is also characterized by hyperaldosteronism, adrenal βarrestin1 overexpression/overactivity promotes aldosterone elevation, resulting in accelerated cardiac adverse remodeling and deterioration of heart function[44]. Moreover, the cardio-toxic effects of aldosterone in post-MI HF are prevented by adrenal βarrestin1 inhibition *in vivo*[44] and βarrestin1-knockout mice progressing to HF after experimental MI fail to show any elevation in their circulating aldosterone levels, remaining essentially normal even as late as 4 wk post-MI[45]. CA levels are also significantly reduced in post-MI βarrestin1-knockouts, contributing to the overall better survival and cardiac function of these animals in post-MI HF[45]. Thus, adrenal βarrestin1 is a major driving force behind the cardio-toxic hyperaldosteronism and SNS hyperactivity, both of which accompany and aggaravate chronic HF. Thus, adrenal βarrestin1 inhibition might also be of therapeutic value in post-MI HF therapy. In fact, considering that it also participates in the adrenal GRK2-dependent α_2AR desensitization/downregulation, which chronically elevates CA secretion in HF[25,45], it becomes apparent that adrenal βarrestin1 inhibition could be an attractive therapeutic strategy for countering neurohormonal cardiotoxicity in HF.

CONCLUSION

Preclinical studies on cardiac GRK2 inhibition have established it as a promising therapeutic modality for HF. However, recent studies have brought GRK2 targeting in another organ, the adrenal medulla, to the limelight of potential HF therapies. Adrenal GRK2 inhibition appears to directly lower the neurohormonal (*i.e.*, sympathetic) burden of the failing post-MI heart, without affecting the heart muscle *per se*. As better, safer, and more effective vectors for gene therapy and/

or small molecule inhibitors get developed, the potential for GRK2 inhibition, in both the heart and adrenals, to find its place in the HF therapeutic armamentarium will continue to rise exponentially in the years to come.

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MINIREVIEWS

Challenging aspects of treatment strategies in heart failure with preserved ejection fraction: "Why did recent clinical trials fail?"

Peter Moritz Becher, Nina Fluschnik, Stefan Blankenberg, Dirk Westermann

Peter Moritz Becher, Nina Fluschnik, Stefan Blankenberg, Dirk Westermann, Department of General and Interventional Cardiology, University Heart Center Eppendorf, 20246 Hamburg, Germany

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Correspondence to: Peter Moritz Becher, MD, Department of General and Interventional Cardiology, University Heart Center Hamburg Eppendorf, Martinistraße 52, 20246 Hamburg, Germany. m.becher@uke.de
Telephone: +49-40-741054864
Fax: +49-40-741058862

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Abstract

Heart failure (HF) is the leading cause of hospitalization among older adults and the prevalence is growing with the aging populations in the Western countries. Epidemiologic reports suggest that approximately 50% of patients who have signs or symptoms of HF have preserved left ventricular ejection fraction. This HF type predominantly affects women and the elderly with other co-morbidities, such as diabetes, hypertension, and overt volume status. Most of the current treatment strategies are based on morbidity benefits such as quality of life and reduction of clinical HF symptoms. Treatment of patients with HF with preserved ejection fraction displayed disappointing results from several large randomized controlled trials. The heterogeneity of HF with preserved ejection fraction, understood as complex syndrome, seems to be one of the primary reasons. Here, we present an overview of the current management strategies with available evidence and new therapeutic approach from drugs currently in clinical trials, which target diastolic dysfunction, chronotropic incompetence, and risk factor management. We provide an outline and interpretation of recent clinical trials that failed to improve outcome and survival in patients with HF with preserved ejection fraction.

Key words: Diastolic dysfunction; Preserved ejection fraction; Co-morbidities; Clinical trials

Core tip: Heart failure (HF) has preserved left ventricular ejection fraction (HFpEF) accounts for approximately 50% of all patients diagnosed with HF, with similary poor outcomes. To date, only the prevention of HFpEF by treating the cardiovascular risk factors (coronary artery disease, atrial fibrillation, hypertension, diabetes, and obesity) has been shown to be efficient. This observation suggests that investigators in future trials should specify the indication of hospitalization for HF and may request to verify the details of patients' admissions. We provide an outline and interpretation

of recent clinical trials that failed to improve outcome and survival in patients with HF with preserved ejection fraction.

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INTRODUCTION

Prevalence of heart failure (HF) has been rising in the recent past[1,2]. Epidemiologic reports suggest that approximately 50% of patients who have signs or symptoms of HF have preserved left ventricular ejection fraction (HFpEF)[3-5]. It has been observed that the morbidity and the mortality rates of HFpEF patients are significantly increased when compared to the reference population[3,6]. Moreover, it appears that the all-cause mortality of patients with HFpEF is comparable to patients with HF with reduced ejection fraction (HFrEF).

Patients with HFpEF are older, more likely women, and more often have hypertension[7,8]. Chronic hypertension is the most common cause in addition to age, with suggestion to 60% of patients suffering from HFpEF being hypertensive[7]. Diabetes and obesity also contribute independently to the development of diastolic and vascular dysfunction[9], both being important in the HFpEF pathophysiology. Most of the common treatment of HFpEF is based on morbidity benefits and reduction of clinical HF symptoms. Several co-morbidities are important drivers of the clinical outcome in the HFpEF population. Excluding patients with co-morbidities from clinical trials to enhance the specificity reduces clinical event rate and entails loss of statistical power to detect differences.

Current guidelines recommend the management of treating hypertension, heart rate reduction, volume status, and prevention of myocardial ischemia[10]. However, current intervention strategies available for HFrEF have not been supported by clinical trials for HFpEF[11,12].

Here, we present an overview of the current recommended therapeutic options with available evidence and new therapeutic approaches from drugs currently in clinical trials, which aim at impaired diastolic function, chronotropic incompetence, and risk factor management. We provide an outline and interpretation of previous clinical trials that failed to improve outcome and survival in the HFpEF population.

BETA-BLOCKERS

Study of effects of Nebivolol Intervention on outcomes and Rehospitalisation in Seniors with HF trial (SENIORS).

The mechanism behind β-blockers' therapeutic potential in enhancement diastolic function in patients with HFpEF is believed to be associated with negative chronotropic and inotropic properties in stabilizing heart rate and optimizing left ventricular (LV) relaxation[13].

The SENIORS trial (Study of effects of Nebivolol Intervention on Outcomes and Rehospitalisation in Seniors with HF) enrolled 2128 patients aged greater than 75 years who had either an LVEF less than 35% or a hospitalization for HF in the previous year and randomly assigned them to placebo or nebivolol. In the SENIORS trial 752 patients displayed a preserved LVEF (mean 49.2%).

The SENIORS trial indicated that nebivolol significantly reduced the composite outcome of death and cardiovascular hospitalization. In detail, the SENIORS trial demonstrated a 15% reduction in the relative risk of the composite of all-cause mortality of cardiovascular admission in patients older than 70 years of age with history of congestive HF[14]. The investigators consumed two primary aims distinct from previous trials on β-blockers. First, was to demonstrate the safety and efficacy of nebivolol in elderly HF patients, a group that has been under-represented in previous clinical studies. Secondly, another goal, of this trial was to demonstrate nebivolol's safety and efficiency across a broad range of LVEF, including the HFpEF population.

Conversely, in the SENIORS trial there was no difference in the primary outcome when patients were stratified according to preserved or reduced LVEF using a cut-off of > 35% to define preserved EF[14]. Subsequent analyses suggested no strong interaction between the therapeutic benefit of nebivolol and LVEF above or below 35%, but this does not entirely allay concerns that there might be no benefit in those with an LVEF greater than 45%.

Besides, patients with atrial fibrillation, a common co-morbidity of both HFrEF and HFpEF, do not appear to benefit whether or not LVEF is reduced[15]. In addition, it has to be mentioned that more than half of the patients, included in the SENIORS trial, had LVEF values ranging between 35%-50% and therefore would not be considered to have HFpEF.

However, in a separate analysis of patients with an LVEF cut-off greater than 40%, there was no statistical interaction, suggesting that nebivolol was of comparable benefit in reduced LVEF and preserved LVEF patients. The definition of HFpEF used a low cut-off LVEF of greater than 35% therefore making it difficult to extrapolate these findings to most patients with HFpEF who have a higher LVEF.

Furthermore, the SENIORS echocardiography substudy randomized 112 patients in 29 european centres, of whom 104 were evaluable for the study; 43 with LVEF ⩽ 35% and 61 with an LVEF > 35%[16]. LV end-systolic volume (ESV), LVEF, mitral valve E/A ratio, and E-wave deceleration time were assessed at baseline and after 12 mo.

In the group with LVEF ⩽ 35%, nebivolol reduced

ESV and improved EF; no changes were observed in the E/A ratio or E-wave deceleration time. In LVEF > 35% group, no significant changes in either systolic or diastolic parameters were observed. This absence of detectable differences with standard echocardiography in patients with predominant diastolic dysfunction questions the mechanism of benefit on morbidity and/or mortality in this HF 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.

Swedish HF registry

Lund *et al*[17] from the Karolinska Institute, Stockholm in Sweden, conducted a study to examine whether β-blocker therapy is associated with reduced mortality in patients with HFpEF.

The investigators used data from the Swedish HF Registry, which includes 67 hospitals with inpatient and outpatient units and 95 outpatient primary care clinics in Sweden. The analysis included 41976 patients, 19083 patients with HFpEF[17]. Of these, 8244 were matched 2:1 based on age and β-blocker use, yielding 5496 treated and 2748 untreated patients with HFpEF. Another analysis involved 22893 patients with HFrEF, of whom 6081 were matched, yielding 4054 treated with β-blockers and 2027 untreated patients.

In patients with HFpEF, use of β-blocker therapy was associated with lower all-cause mortality but not with lower combined all-cause mortality or HF hospitalization. In detail, in the matched HFrEF cohort, β-blockers were associated with reduced mortality (HR = 0.89; 95%CI: 0.82-0.97; *P* = 0.005) and also with reduced combined mortality or HF hospitalization (HR = 0.89; 95%CI: 0.84-0.95; *P* = 0.001).

This study provides a rationale for performing large-scale randomized trials with this inexpensive category of drugs.

However, because myocardial ischemia can drive the development of HFpEF, its presence should be detected and treated with anti-ischemic therapies, which still include β-blockers. Patients with evidence of myocardial ischemia could also be considered for revascularization with percutaneous coronary intervention or coronary artery bypass surgery.

However, current guidelines do not recommend the use of β-blockers solely for HFpEF, unless it is used to optimize treatment of comorbidity, such as controlling ventricular rate in atrial fibrillation or tachyarrhythmia, or hypertension.

Since cardiac output is the product of heart rate and stroke volume, patients with HFpEF are often dependent on augmentation of heart rate in order to increase cardiac output.

Negative chronotropic medications are recommended in HFpEF to increase the diastolic filling period, but slowing the heart rate in the absent of tachycardia tends to only prolong diastasis, where transmitral flow is minimal or absence[18]. More importantly, recent studies have repeatedly shown that chronotropic incompetence is highly prevalent and associated with exercise disability in HFpEF[19-21]. Indeed, in the setting of reduced systolic and diastolic reserve, chronotropic reserve may represent the only mechanism to augment cardiac output during exercise, although there is concern that inadequate ability to enhance relaxation with tachycardia may limit stroke volume responses. β-blockers, especially at high doses may aggravate rather than alleviate exercise intolerance.

However, slowing elevated heart rate can prolong LV filling time in abnormally stiff LV and also prolong coronary perfusion. As a result, we recommend the careful use of β-blockade to optimize chronotropic incompetence (induced by atrial fibrillation or tachyarrhythmia) by stabilizing heart rate and optimizing LV relaxation with regard to heart rate profile under basal and exercise conditions in patients with HFpEF. Moreover, additional benefical effects of β-blockers have to be reconsidered. In detail, nebivolol itself would possible confer additional effects due to the NO enhancing action of the drug. This action of nebivolol is exerted *via* a signaling pathway starting from the activation of β3-adrenergic receptors and leading to overexpression of inducible NO synthase. Cardiac NO production by nebivolol could participate in the cardiovascular effects of nebivolol treatment in patients affected by hypertension and HF.

Adequate prospective trial data regarding the effects of β-blockers in HFpEF are not currently available. In this regard it is interesting to know that Pieske *et al* (Charité - Berlin, Germany) are planning an additional large multicenter trial with about 2300 participants with preserved LVEF in order to investigate the effects of β-blockers treatment starting in 2015.

ANGIOTENSIN-CONVERTING ENZYME INHIBITORS AND ANGIOTENSIN RECEPTOR BLOCKERS

Perindopril in elderly people with chronic HF trial

The theoretical benefits of Angiotensin-converting enzyme inhibitors (ACEi) in HFpEF rest on pathophysiological basis that angiotensin II contributes to myocardial hypertrophy and adverse cardiac fibrosis. To date, only one substantial trial of ACEi has been conducted in the HFpEF population, the perindopril in elderly people with chronic HF (PEP-CHF). The PEP-CHF Trial included 850 patients, older than 70 years of age with HFpEF (LVEF > 45%) with echocardiographic evidence of diastolic dysfunction[22]. The primary endpoint of the trial was a composite of all-cause mortality or unplanned HF related hospitalization. A significant reduction in HF hospitalization rate was observed in posthoc analysis of the results at 1 year, when cross over rates to open label ACEi were used. However, early beneficial effects of perindopril treatment

were lost by the end of the trial.

A major limitation of the trial was the high rate of discontinuation at 18 mo (62%), the majority of whom went on open-label ACEi (about 90%). In addition, the event rate in the trial was lower than expected, further reducing the power of the trial. Perindopril appeared favorable at 1-year follow-up when the large majority of patients were on study drug, although these data should not be considered definitive given the post-hoc nature of the analysis. Although the PEP-CHF trial also does not provide conclusive evidence that perindopril is of benefit in this population, the observed favourable trends on hospitalization and days in hospital for HF (early seen beneficial effects), combined with improvements in symptoms and functional capacity provide arguments for its use.

Effects of candesartan in patients with chronic HF and preserved left-ventricular ejection fraction: The CHARM-Preserved trial

In the effects of candesartan in patients with chronic HF and preserved left-ventricular ejection fraction: The CHARM-Preserved trial (CHARM-Preserved) trial, 3023 (mean age 67 years, 40% women) patients were randomly assigned to the angiotensin receptor blocker (ARB) candesartan or placebo and followed 37 mo[23]. Adequate patients were aged greater than 18 years, suffering from HF for more than 4 wk, were in NYHA class II-IV, had a history of hospital admission and had a greater LVEF than 40%. The primary outcome (cardiovascular death or HF admission) was neutral (P = 0.051), but only slightly short of the primary outcome. A possible explanation of this finding could be the rates of study-drug discontinuation due to adverse events or laboratory abnormalities, which were significantly higher in the candesartan group (17.8% *vs* 13.5%, P = 0.001). In detail, candesartan was discontinued in more patients due to hyperkalemia, worsening creatinine levels or hypotension. In an echocardiography substudy of CHARM-Preserved, only 44% had moderate or severe diastolic dysfunction, which conferred a 3-fold increased risk but it is not clear whether these patients obtained a greater benefit from candesartan. Overall, CHARM-Preserved results were related with reduced hospitalization with candesartan[23]. However, the LVEF cut-off value of 40% and a non-defined diastolic function identified the study population as not a true HFpEF population.

Irbesartan in patients with HF and preserved ejection fraction trial

The Irbesartan in patients with HF and preserved ejection fraction trial (I-Preserve), the largest trial in the HFpEF population so far, randomly assigned 4128 patients (mean age 72 years, 60% female) to irbesartan or placebo[24]. The observation period was about 49.5 mo (mean). All included patients were aged greater than 60 years, had symptoms of HF and had a greater LVEF than 40%. The primary outcome (death from any cause or hospitalization for cardiovascular cause) occurred 36% of patients in the irbesartan group and 37% in the placebo treated group[24]. There were no significant differences in the primary endpoints between the two groups. This trial also found no treatment benefit in any group and no significant difference in secondary endpoints such as CV death, HF death, exercise testing, NT-proBNP levels, and quality of life (Table 1).

However, it is essential to mention that in this study a high percentage of patients were already receiving ACEi and spironolactone. The investigators speculated that the treatment of a large proportion of patients with multiple inhibitors of the RAS might have left reduced opportunity for further benefit from the addition of an angiotensin-receptor blocker. Furthermore, it seems to be possible that HFpEF does not appear to involve neurohormonal activation as a critical pathophysiologic mechanism in the same way that HFrEF does.

The rationale for using ACEi and ARBs in patients with HFpEF is blocking the neurohumoral signaling leading to HF progression and poor clinical outcomes. First, the CHARM-Preserved trial showed a significant reduction in hospitalization rate caused by HF, but failed to display a significant reduction in cardiovascular mortality. Moreover, in an echocardiography substudy of CHARM preserved, only 44% had moderate or severe diastolic dysfunction. Second, the I-Preserved trial failed to show a reduction in risk of the composite outcome, cardiovascular hospitalization and all-cause mortality. However, the not insignificant co-medication in this trial could be one reason for the neutral endpoints. Third, the PEP-CHF trial also failed to demonstrate a reduction in composite all-cause mortality and hospitalization caused by HF.

Also because of the neutral results of these three main outcome trials the current guidelines do not recommend the use of ACEi and ARBs for HFpEF. Nevertheless, when hypertension and other co-morbidities like LV hypertrophy and atherosclerotic vascular disease are involved ACEi and ARBs are first-line therapy and should also be given to patients with HFpEF. A possible mechanism for potential benefit of ACEi and ARBs could be afterload reduction and reduced and reduced wall tension, leading to improved diastolic function.

MINERALOCORTICOID RECEPTOR ANTAGONISTS

Randomized controlled aldosterone receptor blockade in diastolic HF trial

Series of RCTs[25,26] have shown that treatment with mineralocorticoid-receptor antagonists (MRAs) improved some properties of cardiac performance in patients suffering from HFpEF. The randomized controlled aldosterone receptor blockade in diastolic HF (ALDO-HF) trial displayed an improvement in ejection fraction,

Table 1 Clinical trials in heart failure with preserved ejection fraction

Acronym (yr)	Drug	Number of patients	Age (mean)	Percentage female (mean, %)	LVEF (mean, %)	Primary outcome	Follow up period
Swedish heart failure registry[17]	Beta-Blocker	8244	78	45	40-49; > 50	ACM, HFH	24 mo
TOPCAT[33]	Aldactone	3445	68.6	52	60.1	CVD-HFH: NS	27 mo
PARAMOUNT[51]	LCZ696	292	70.6	56	57.7	Reductions in NT-proBNP levels	36 wk
RELAX[43]	Sildenafil	216	69	48	60	EC-CS: NS	24 wk
ALDO-DHF[27]	Spironolactone	422	67	52	67	Reduced E/É	12 mo
I-Preserve[24]	Irbesartan	4128	72	60	59.5	D-CVH: NS	49.5 mo
PEP-CHF[22]	Perindopril	850	75	55.5	65	D-HFH: NS	26.2 mo
DIG[26]	Digoxin	6800	63.8	22.7	28.6	ACM: NS; improvements in DFWHF, HFWHF	37 mo
SENIORS[14]	Nebivolol	2128	76.1	38.4	36	Improvements CVD, HFH	21 mo
CHARM-Preserved[23]	Candesartan	3023	67.1	40	54	CVD-HFH: NS	36.6 mo

ALDO-DHF: Aldosterone Receptor Blockade in Diastolic Heart failure; CHARM-Preserved: Effects of candesartan in patients with chronic HF and preserved left-ventricular ejection fraction trial; DIG: The Effect of Digoxin on Mortality and Morbidity in Patients with HF trial; I-Preserve: The irbesartan in HF with preserved systolic function trial; PARADIGM: Angiotensin–Neprilysin Inhibition *vs* Enalapril in HF trial; PARAMOUNT: The angiotensin receptor neprilysin inhibitor LCZ696 in HF with preserved ejection fraction: a phase 2 double-blind randomised controlled trial; PEP-CHF: The perindopril in elderly people with chronic HF trial; RELAX: Phosphodiesterase-5 Inhibition in Diastolic HF: The RELAX Trial Rationale and Design; SENIORS: Randomized trial to determine the effect of nebivolol on mortality and cardiovascular hospital admission in elderly patients with HF trial; TOPCAT: Spironolactone for HF with Preserved Ejection Fraction trial; ACM: All-cause mortality; CS: Clinical status; CVA: Cardiovascular admission; CVD: Cardiovascular death; CVH: Cardiovascular hospilization; DFWHF: Death from worsening HF; EC: Exercise capacity; FHCVE: First hospitalization for a cardiovascular event; HF: Heart failure; HFAR: Hospitalization for any reason; HFH: Heart failure hospitalization; HFWHF: Hospitalization for worsening HF; NS: Not significant.

E/É relation, LV mass and LV end-diastolic volume[27]. However, these findings were not related with an enhancement in exercise capacity.

In the ALDO-HF trial, treatment with MRAs decreases renal function. Therefore, MRAs cannot be recommended based on the mentioned results. Physicians treating patients with MRAs should carefully monitor renal function and potassium levels. Whether the improved left ventricular function observed in the ALDO-HF trial is of clinical significance requires further investigation in larger HFpEF populations.

Treatment of preserved cardiac function HF with an aldosterone antagonist trial

The rationale to use MRAs for HFpEF therapy has been initially generated in experimental studies. These studies suggested that a blockade of the aldosterone-induced signaling may lead to anti-hypertrophic and anti-fibrotic effects[28]. Moreover, clinical trials EPHESUS and EMPHASIS-HF demonstrated significant reductions in risk of death from cardiovascular causes or first hospitalization for HF in patients after myocardial infarction and mild HF symptoms. However, in these trials solely patients with reduced LVEF were included.

MRAs such as spironolactone are highly effective in patients with HF accompanied with reduced LVEF[29-32].

In the treatment of preserved cardiac function HF with an aldosterone antagonist (TOPCAT) trial, patients with at least one symptom of HF were included if those patients had an ejection fraction greater than or equal to 45%[33].

Moreover, increased natriuretic peptide levels in the foregoing 60 d or a hospital admission in the previous year (with management of HF a major component of the care provided) were required, and these eligibility criteria were used for stratification of patients at randomization of this study[33]. Three thousand four hundred and forty-five patients undertook randomization in 6 different countries (United States, Argentina, Brazil, Canada, Russia and Georgia) to spironolactone or placebo.

Regarding a mean follow-up of 3.3 years (mean), the incidence rate of the primary composite outcome of death from cardiovascular causes, cardiac arrest, or hospitalization for HF was 5.9 events per 100 person-years in the spironolactone group and 6.6 events per 100 person-years in the placebo group.

Overall, the TOPCAT trial showed neutral results. There was a significant reduction in the secondary outcome of hospitalization for HF with spironolactone treatment.

Patients randomized to treatment with spironolactone had a fewer admission rate for HF, but an increased risk for renal dysfunction and hyperkalemia[34].

The majority of patients from Russia and Georgia were included in the hospitalization stratum (therefore no increased NT-proBNP was present) and thus were at lower cardiovascular risk, whereas patients from the United States were further balanced between the two mentioned strata. However, a post hoc analysis showed, that spironolactone treatment seemed to benefit patients in the United States but not those patients in Russia or Georgia. In detail, a total of 3445 subjects were recruited over a period of 4 years from 270 clinical centers in the United States (1151), Russia (1066), Georgia (612), Canada (326), Brazil (167) and Argentina (123), and were randomized on 1:1 basis

to either spironolactone (target dose of 30 mg daily) or placebo. Patients with uncontrolled hypertension, those with infiltrative or hypertrophic cardiomyopathy and patients with elevated baseline serum potassium levels (> 5.0 mmol/L) were excluded. The overall event rate was low, with 3-year mortality being 10.2%. This is in sharp contrast with the previously reported annual mortality rates of 22%-29% in large community-based studies[35]. This concern is further intensified by a primary event rate (in the placebo group) of 8.4% in Russia and the Republic of Georgia: A rate which not only is unheard of in HF studies, but also one that is remarkably less than that observed in the "American" arm of the same study (31.8%).

It is remarkable that geographic differences in outcome have been a significant relevance in previous trials involving patients with HF. Possible factors in such geographic variation include differences in the clinical characteristics of the patient population, standards of care and methodological knowledge of clinical trials[34].

To conclude, TOPCAT was a neutral study. Spironolactone failed to reduce the primary outcome compared to placebo in patients with HFpEF. However, it did reduce the rate of HF hospitalizations. A signal of benefit was also seen in patients with elevated natriuretic peptides and in a geographical subset of patients. Based upon these findings, a mixed response from the medical community is expected: Some clinicians will not prescribe spironolactone for HFpEF patients, while others will continue using it especially in patients with elevated natriuretic peptides and/or in those with objective evidence of diastolic dysfunction. Finally, we prescribe spironolactone for HFpEF patients during carefully monitoring of renal function and serum potassium levels given the overall positive data from the Americas in TOPCAT.

DIGITALIS THERAPY

Digitalis investigation group ancillary trial

It has been shown that treatment with digoxin has beneficial effects on hospitalization in patients with HFrEF. Treatment with digoxin reduced the total number of hospitalizations. In the digitalis investigation group ancillary trial 988 patients suffering from chronic HF and ejection fraction greater than 45% were randomized to treatment with digoxin or placebo[36].

After 37 mo (mean follow-up), patients treated with digoxin or placebo had similar rates of the primary composite of hospitalization of HF or cardiovascular death[36]. However, an early benefit in patients with digoxin treatment was lost by the end of follow-up of the trial.

In ambulatory patients with chronic mild to moderate diastolic HF and normal sinus rhythm receiving angiotensin-converting enzyme inhibitor and diuretics, digoxin had no effect on natural history end points such as mortality and all-cause or cardiovascular hospitalizations[36].

To conclude, there is fragile evidence of digoxin in patients with HFpEF. Similar to β-blockers, guidelines do not recommend the use of digoxin solely for HFpEF, unless for treatment of co-morbidities, such as atrial fibrillation or tachyarrhythmia. However, common use of digoxin in the elderly HFpEF population with increased renal dysfunction seems not to be advisable.

INHIBITION OF THE LATE CURRENT OF THE CARDIAC ACTION POTENTIAL (LATE INA)

RAnoLazIne for the treatment of diastolic HF in patients with preserved ejection fraction: the RALI-DHF proof-of-concept study

In a small, randomized (phase Ⅱ) trial 18 patients were included who received ranolazine infusion followed by 2 wk of oral application[37]. It was shown by the investigators that left ventricular end-diastolic pressure and pulmonary capillary wedge pressure were reduced in patient with ranolazine treatment whereas in patients with placebo treatment there were no significant effects seen (clinicaltrails.gov NCT01163734). However, at the end of the trial no significant differences were observed by echocardiography and exercise capacity. In addition, a planned multi-center trial has been abandoned due to low recruitment. Finally, results of two ongoing studies are earliest expected in 2016.

PHOSPHODIESTERASE-5 INHIBITION

Sildenafil, a phosphodiesterase-5 (PDE-5) inhibitor is currently approved for treatment of pulmonary arterial hypertension (PAH)[38-40]. A small clinical trial observed improvements in pulmonary pressure, right ventricular (RV) function and LV relaxation after treatment with sildenafil in patients suffering from HFpEF. In a phase Ⅲ ongoing trial the effect of sildenafil on patients suffering from HFpEF and PAH will be studied[41]. Moreover, sildenafil treatment led to an enhancement of systolic and diastolic LV function in a one-year randomized double-blind study placebo controlled study in patients suffering from stable HF and reduced ejection fraction[42].

PhosphdiesteRasE-5 inhibition to improve clinical status and exercise capacity in diastolic HF trial

Controversial findings have been oberserved from the PhosphdiesteRasE-5 inhibition to improve clinical status and EXercise Capacity in Diastolic Heart Failure (RELAX) trial[43] with HFpEF patients. Here, no significant improvement in diastolic function, exercise capacity and quality of life was observed.

In addition, in a multi-center study 216 patients with HFpEF and increased pulmonary artery pressures did not affect exercise capacity or clinical constitution over a time period of 24 wk[44]. Furthermore, longterm analyses of NT-proBNP and endothelin-1 displayed no significant changes between sildenafil and placebo

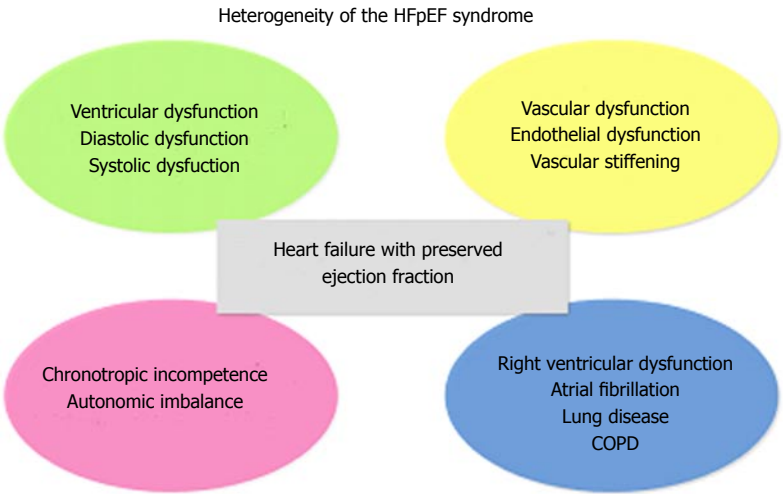

Figure 1 Overview of multiple effectors for the heterogeneity of the heart failure has preserved left ventricular ejection fraction syndrome. COPD: Chronic obstructive pulmonary disease; HFpEF: Heart failure has preserved left ventricular ejection fraction.

treated groups. However, in a one-year single center trial of sildenafil in patients with HFpEF described significant improvements in with sildenafil treated patients when compared to placebo treated patients[41].

The lack of benefit of sildenafil treatment could be because the inclusion and exclusion criteria. In this trial, the included patients did not have pulmonary hypertension and suggested by highly increased NT-proBNP levels had advanced HF; this could explain the less-responding to sildenafil-treatment.

Furthermore, in a small clinical trial including 44 patients suffering from HFpEF (LVEF > 50%) and PAH inhibition by PDE-5 displayed a significant improvement in diastolic dysfunction, pulmonary pressures and right ventricular performance over an oberservation period of 12 mo[41]. Given the results, PDE-5 inhibition for HFpEF without proven increased PAP should not be used.

DEVICE THERAPY

No substantial clinical trials of implantable cardiac defibrillators or cardiac resynchronization therapy exist in the HFpEF population.

A large trial of cardiac resynchronization therapy (CRT) in patients suffering from an LVEF between 36% to 50% has been stopped due to poor outcome[45].

CRT is currently limited to those patients with LVEF < 35%, sinus rhythm, QRS > 150 ms, and left bundle branch block (LBBB) pattern. A retrospective analysis of the predictors of response to CRT has shown that CRT may offer a valuable option for these patients[46,47]. However, this finding has to be proven in a prospective, randomized multicenter trial. To date, CRT should not be used as matter of routine in patients with HFpEF. Furthermore, a current small clinical trial used a cardiovascular simulation to provide insights into the potential effects of an inter-atrial shunt on rest and exercise hemodynamics in patients suffering from HFpEF[48]. The principal finding of this study is that the inter-atrial shunt lowers left atrial (LA) pressure and that this effect is particularly pronounced during the marked increase in LA pressure and increased left-to-right atrial pressure gradient during exercise in this patient population.

However, the marked reduction in LA pressure (and pulmonary capillary pressure) could allow patients to exercise longer, potentially resulting in higher heart rates and higher values of cardiac output.

There exist currently two different devices in clinical development to create a device to make a precisely sized interatrial septal defect that will maintain patency for this purpose. Whether the findings of this theoretical simulation provide insights into patient selection criteria and the expected magnitude of hemodynamic improvement has to be proven in further clinical trials.

Possible optimizations of clinical trials for HFpEF in the future

For future clinical trials in HFpEF better matching of treatments for the precise type of HFpEF seems to be necessary (Figure 1).

However, in retrospect it has been elucidated that the type of therapy tested in previous clinical trials may not be the correct match for the type of HF population included. This line of argument incorporates the ALDO-DHF trial, which included patients with early-stage HFpEF and not manifest volume overload.

Moreover, in the RELAX trial, which enrolled symptomatic HF patients with volume overload but not necessarily those with overt PAH and RV dysfunction. However, the inclusion and exclusion criteria should focus on patients with early HFpEF, in whom exercise intolerance is one of the main indicators and in whom there is objective evidence of exercise-induced increase in LV filling pressures. Excluding patients with co-morbidity to try to increase the specifity of HFpEF may purely make matters worse by excluding those patients at high risk. If co-morbidities drive the clinical course of the patient, then treatment directed only at cardiac function may be ineffective. In addition, diagnosis of HFpEF should not be based solely on clinical criteria and the absence of HFrEF (Figure 2).

Natiuretic peptides provide considerable confidence for improved clinical trial design. HFpEF is a

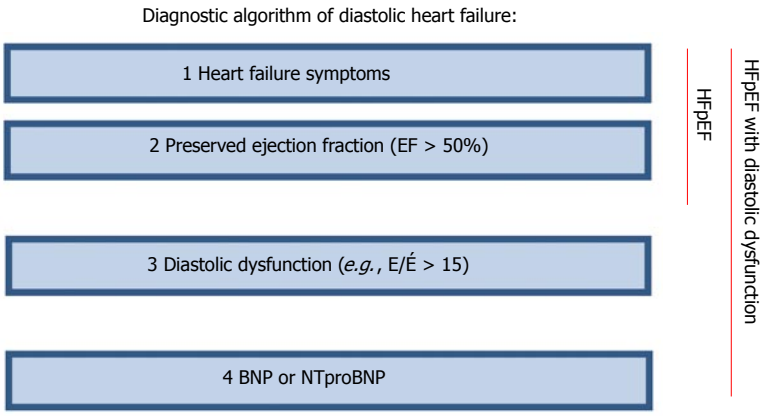

Figure 2 Diagnostic algorithm of diastolic heart failure. BNP: B-type natriuretic peptide; NT-proBNP: N-terminal of the B-type natriuretic peptide; E/É: Pulsed-wave Doppler E wave velocity divided by tissue Doppler E wave velocity; HFpEF: Heart failure has preserved left ventricular ejection fraction.

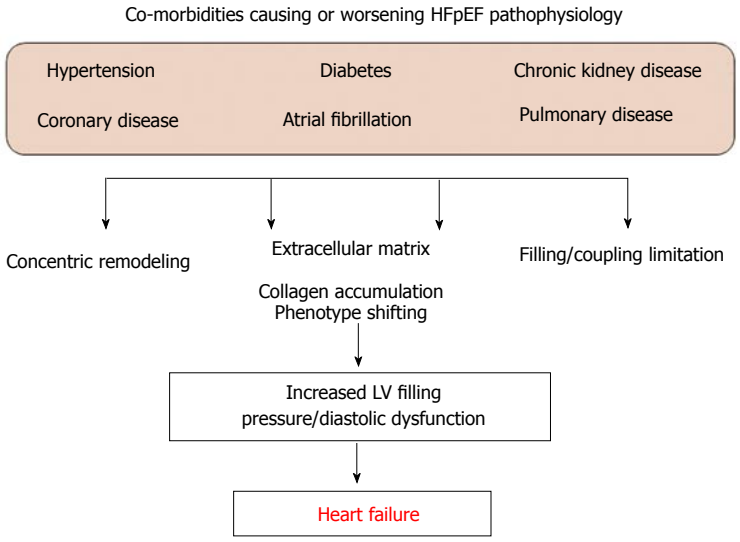

Figure 3 Scheme of co-morbidities causing or worsening heart failure has preserved left ventricular ejection fraction pathophysiology. LV: Left ventricular; HFpEF: Heart failure has preserved left ventricular ejection fraction.

heterogeneous and a complex syndrome and only specific phenotypes may respond to a particular therapeutic intervention (Figure 1).

Sufficient diagnosis and phenotyping seems to be essential. The disappointment in the last clinical trials that have proven so effective in treating HFrEF supports an urgent need for novel drug approaches to HFpEF (Figure 3). Underpowered clinical trials should be avoided and study designs need a focus on more consistent patient populations to control the impact cardiovascular co-morbidities. To conclude, ethnicity, cultural differences, co-medication, cut-off values and local clinical practice might influence results of clinical trials. Additional endpoints that include for example, quality of life evaluation and correct timing for effective therapeutic intervention must be kept in mind when planning expensive multicenter RCTs.

PROMISING NEW THERAPY STRATEGIES

Soluble guanylate cyclase inhibitors

Solid evidence supports augmentation of (cGMP) signaling as a potential therapeutic strategy for HFpEF[49]. Direct soluble guanylate cyclase stimulators target reduced cGMP generation due to insufficient sGC stimulation and represent a promising method for cGMP enhancement.

In the SOCRATES-Preserved trial (soluble guanylate cyclase stimulator in HF patients with PRESERVED EF; clinicaltrial.org NCT01951638) stimulation of the soluble guanylate cyclase by the oral soluble guanylate cyclase stimulator BAY1021189 is currently being investigated over 12 wk in patients with worsening HFpEF.

I_f channel inhibition

The SHIFT trial demonstrated that significant heart rate reduction *via* ivabradine, inhibitor of the I_f channel of the sinoatrial node, led to a significant reduction in hospitalization caused by HF and cardiovascular mortality in the HFrEF population[50]. Interestingly, the effects of ivabradine in HFpEF have been studied in a small recent trial of 61 patients, randomized to placebo or ivabradine (5 mg twice a day). Treatment with ivabradine showed an enhancement in exercise capacity and an improvement in LV filling pressures. In addition, a larger multi-center study enrolling about 400 patients is going to evaluate the properties of ivabradine concerning diastolic function, NT-proBNP levels and exercice capacity (www.clinicaltrialsregister.eu-EUCTR2012-002742-20-DE).

Dual angiotensin receptor blocker-neutral endopeptidase inhibitors

Although studies conducted with ARBs or ACEi alone did not display enhancements in HFpEF patients, pathophysiological evidence support the rationale for targeting the renin angiotensin system (RAS) in this population of patients.

The Prospective comparison of ARNI with ARB on Management of HF with preserved ejectionN fraction (PARAMOUNT) study[51], a phase Ⅱ trial conducted in 308 patients in 13 countries, compared the effects of LCZ696 and the ARB valsartan on the concentrations of natriuretic peptides. The natriuretic peptide investigated in this study, NT-proBNP, is a marker of cardiac wall stress, and levels are increased in patients with HF[51].

The agent LCZ696 in the PARAMOUNT study is the first compound to show both reductions in NT-proBNP and left atrial size (LA) in HFpEF patients, powerful predictors of outcome in HF. The favorable effects of LCZ696 seen in patients with HFpEF in the PARAMOUNT trial are encouraging, and further testing of this agent in this patient population is warranted.

LCZ696 acts by inhibiting both the angiotensin receptor and the enzyme responsible for the breakdown of the natriuretic peptides (neprilysin). LCZ696's dual mechanism of action thus acts to restore the altered neurohormonal balance in HFpEF[52]. These dual effects may be important in the treatment of HFpEF. Moreover, the large outcome trial PARAGON-HF will test the efficacy and safety in HFpEF patients (clinicaltrials.gov NCT01920711).

CONCLUSION

HFpEF accounts for approximately 50% of all patients diagnosed with HF, with similary poor outcomes. To date, only the prevention of HFpEF by treating the cardiovascular risk factors (coronary artery disease, atrial fibrillation, hypertension, diabetes, and obesity) has been shown to be efficient. This observation suggests that investigators in future trials should specify the indication of hospitalization for HF and may request to verify the details of patients' admissions.

However, dual inhibition of the RAS and neprilysin by the agent LCZ696 represents a novel promising therapeutic target for treating patients with HF. LCZ696 in the PARAMOUNT trial is the first agent to show both reductions in NT-proBNP levels and LA size in HFpEF patients, each strong predictors of outcome in HF. The favorable effects of LCZ696 seen in patients with HFpEF in the PARAMOUNT trial are encouraging. Further testing of dual of RAS and neprilysin inhibition in the HFpEF population is warranted.

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Electrical storm: A clinical and electrophysiological overview

Sergio Conti, Salvatore Pala, Viviana Biagioli, Giuseppe Del Giorno, Martina Zucchetti, Eleonora Russo, Vittoria Marino, Antonio Dello Russo, Michela Casella, Francesca Pizzamiglio, Valentina Catto, Claudio Tondo, Corrado Carbucicchio

Sergio Conti, Salvatore Pala, Viviana Biagioli, Giuseppe Del Giorno, Martina Zucchetti, Eleonora Russo, Vittoria Marino, Antonio Dello Russo, Michela Casella, Francesca Pizzamiglio, Valentina Catto, Claudio Tondo, Corrado Carbucicchio, Cardiac Arrhythmia Research Centre, Centro Cardiologico Monzino IRCCS, 20138 Milan, Italy

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Correspondence to: Corrado Carbucicchio, MD, FESC, Cardiac Arrhythmia Research Centre, Centro Cardiologico Monzino IRCCS, Via Carlo Parea 4, 20138 Milan, Italy. corrado.carbucicchio@ccfm.it
Telephone: +39-02-58002616
Fax: +39-02-58002782

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Abstract

Electrical storm (ES) is a clinical condition characterized by three or more ventricular arrhythmia episodes leading to appropriate implantable cardioverter-defibrillator (ICD) therapies in a 24 h period. Mostly, arrhythmias responsible of ES are multiple morphologies of monomorphic ventricular tachycardia (VT), but polymorphic VT and ventricular fibrillation can also result in ES. Clinical presentation is very dramatic in most cases, strictly related to the cardiac disease that may worsen electrical and hemodynamic decompensation. Therefore ES management is challenging in the majority of cases and a high mortality is the rule both in the acute and in the long-term phases. Different underlying cardiomyopathies provide significant clues into the mechanism of ES, which can arise in the setting of structural arrhythmogenic cardiomyopathies or rarely in patients with inherited arrhythmic syndrome, impacting on pharmacological treatment, on ICD programming, and on the opportunity to apply strategies of catheter ablation. This latter has become a pivotal form of treatment due to its high efficacy in modifying the arrhythmogenic substrate and in achieving rhythm stability, aiming at reducing recurrences of ventricular arrhythmia and at improving overall survival. In this review, the most relevant epidemiological and clinical aspects of ES, with regard to the acute and long-term follow-up implications, were evaluated, focusing on these novel therapeutic strategies of treatment.

Key words: Electrical storm; Ventricular tachycardia/fibrillation; Structural heart disease; Antiarrhythmic therapy; Implantable-cardioverter defibrillator; Shock; Catheter ablation

Core tip: Electrical storm is an increasingly common and life-threatening syndrome. The proper management of this arrhythmic emergency is related to a comprehensive assessment of each case. In this review we report all the essential aspects regarding the clinical and

diagnostic evaluation, pharmacological treatment and, with special emphasis, catheter ablation approaches.

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INTRODUCTION

Electrical storm (ES), also referred as arrhythmic storm, refers to a clinical condition characterized by 3 or more arrhythmia episodes of ventricular tachycardia (VT) or ventricular fibrillation (VF) leading to implantable cardioverter-defibrillator (ICD) therapies (Antitachycardia Pacing, ATP, or Direct Current shock, DC-shock), occurring over a single 24 h period[1]. ES represents an arrhythmic emergency that often affects patients at high risk of sudden cardiac death who previously underwent ICD implantation. In this setting ICD correctly interrupts VT/VF episodes; however ventricular arrhythmias, in terms of arrhythmogenic substrate, represent the gradual evolution of the underlying structural heart disease. In this review, we assess the most relevant epidemiological and clinical aspects of ES, with regard to the acute and long-term follow-up implications, focusing on novel therapeutic strategies of treatment.

CLINICAL CHARACTERIZATION

The term ES was introduced in the 1990s to describe an instability condition, highly malignant, characterized by repetitive episodes of ventricular arrhythmias[2]. Nowadays, ES implies several appropriate ICD interventions aimed at terminating the arrhythmic episodes.

ES has been reported in 10%-40% of patients in secondary prevention whereas the incidence of ES is lower (3.5%-4%) in primary prevention[3-16]. However, the correct incidence of ES is uncertain due to several confounding factors such as population considered, type of cardiomyopathy, pharmacological therapy undertaken and other variables. In addition, most of the studies concerning ES incidence were retrospective thus including only the patients who survived the arrhythmic event.

ES mainly affects patients with advanced dilated cardiomyopathy, both ischemic and non-ischemic, representing the gradual evolution of the underlying arrhythmic substrate; however, ES may affect patients with different types of structural heart disease, such as valvular or congenital heart disease, as well as patients without structural heart disease (*i.e.*, Brugada syndrome)[17].

The most significant predictors of ES are severe reduction of left ventricle (LV) function, advanced age and previous VT/VF episodes[8,10,11,13,18,19]. Monomorphic ventricular tachycardia is the most common arrhythmia documented in ES patients. VT episodes, hemodynamically unstable and interrupted with ATP or DC-shock, are the rule, with evidence of multiple VT morphologies[20]. Anyway, clinical presentation of ES is variable[9].

Less commonly, ES has been recorded in patients in whom premature ventricular complexes are the trigger of VT/VF both in acute myocardial infarction and in absence of structural heart disease[16,21]. The latter are often patients with "primitive ventricular fibrillation", in whom the trigger of the arrhythmia has not been documented, presenting with multiple VF episodes after ICD implantation, mostly refractory to pharmacological therapy. Therefore, in this setting, the identification of ES triggers may be of interest in preventing VT/VF episodes, particularly in case of electrolyte disorder[9,14]. The role of adrenergic system in maintaining ES is of special interest as well; in terms of acute event treatment[7,16]. Adrenergic activation seems to play a key role in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia due to the well known arrhythmic sensitivity to adrenergic stimulation; although in the majority of these patients the trigger is unkown[22].

ACUTE PRESENTATION, PROGNOSTIC RELEVANCE

ES is an arrhythmic emergency related to recurrent consecutive episodes of ventricular arrhythmia, with low likelihood of spontaneous termination.

The clinical scenario of ES is the result of a combination of various factors: in patients with structural heart disease affected by chronic heart failure, ES causes worsening of heart failure with high risk of pulmonary edema/cardiogenic shock. These events are much more frequent and severe, less stable the arrhythmic condition and the functional status are. ICD therapies even if allow arrhythmic episodes termination and prevent sudden cardiac death, do not play any role in stabilizing the clinical scenario. Moreover, the continuous intervention of ICD, implies unfavorable hemodynamic effects[3], also resulting in psychological distress, adrenergic hyperactivity, and patient discomfort[22,23].

No reproducible data on acute mortality in ES are available. It should be reminded that acute mortality represents a common cause of death in patients with severe structural heart disease, in most of cases as cardiogenic shock or electromechanical dissociation in the setting of unmanageable arrhythmias[3]. Literature data show that patients with ES present an increased risk of sudden arrhythmic or cardiac death in the mid-term follow up[8-10,16,24]; furthermore, these findings were not confirmed just in few papers[7,13]. The MADIT Ⅱ trial showed that patients with VT episodes interrupted by ICD have a significantly higher risk of sudden and non-sudden cardiac death. Moreover, patients who have survived ventricular arrhythmias have an increased risk

of worsening heart failure and of mortality related to it[25]. Specifically, in the MADIT Ⅱ trial, Sesselberg *et al*[11] have shown that ES is the most important independent predictor of mid-term cardiac death (increased risk of 7-fold), resulting particularly significant in the first 3 mo after ES (increasing risk of 18-fold). The results of SCD-HeFT trial are comparable, in addition, Poole *et al*[26] observed that not only appropriate shocks - directly related with arrhythmic events - but also inappropriate shocks impact on an increased mortality. More specifically the authors reported a significant increase of death in patients with appropriate (HR = 5.68; $P < 0.001$) and inappropriate shocks (HR = 1.98; $P = 0.002$). In particular, multiple shocks were associated with a 8-fold risk of death (HR = 8.23; $P < 0.001$).

These findings support the hypothesis that the recurrence of frequent arrhythmic events (and even more ES) strongly impacts on the evolution of patients' clinical history, particularly by worsening the cardiac function. In this setting multiple shocks could have their own etiopathogenetic role related to repeated myocardial injury.

DIAGNOSIS AND CLINICAL MANAGEMENT

Patients with ES require a diagnostic evaluation of their structural heart disease, the type of arrhythmia and the presence of clinical triggers. The most common triggering factors are acute myocardial ischemia, electrolytic disorders and adverse drug effects. Identification of triggers is a key point: sometimes it allows the suppression of arrhythmias through simple therapeutic interventions, such as in case of hypokalaemia. Acute myocardial ischemia must be accurately identified and excluded through clinical and non-invasive diagnostic parameters. However, in most patients with coronary artery disease and previous history of myocardial infarction presenting with ES, myocardial ischemia is just a secondary effect of the arrhythmias. Myocardial ischemia should therefore be interpreted and consequently treated with the aid of pharmacological and/or interventional therapies in the presence of acute coronary syndrome. In the majority of cases, however, ES represents the evolution of an arrhythmogenic substrate in patients with previous VT/VF episodes.

Therapeutic interventions first depend on the arrhythmic pattern and on the hemodynamic stability of patients. ICD interrogation is the preliminary diagnostic step to evaluate the appropriateness of shock delivery and arrhythmic parameters (heart rate, electrogram analysis, trigger). ICD reprogramming is mandatory in order to both limit ICD shock delivery and attempt VT/VF interruption with antitachycardia pacing[27,28]. The accuracy of the diagnosis of ventricular arrhythmia may only occasionally show interpretative troubles in single-chamber ICD recipients in whom the comparison between basal and arrhythmic electrograms should be carried out carefully privileging reading from multiple recording channels.

ES patients require hospitalization. A continuous ECG and vital signs monitoring must be performed in the Coronary Intensive Care Unit or in a dedicated Emergency Arrhythmia Unit. During the evaluation phase, the possibility to document and characterize different morphologies of VT responsible for the clinical scenario is relevant, also with regard to a possible ablative treatment[20].

Hemodynamic and metabolic evaluations are needed in order to perform urgent interventions through intravenous therapies, such as inotropic agents or hydroelectrolytic infusion.

In the acute setting, prevention of arrhythmic recurrence should be as efficient as possible, by means of: (1) amiodarone is the first choice drug, unless contraindicated (presence of hyperthyroidism, long QT interval)[29]; (2) beta-blocker administration plays an important role because of its antiarrhythmic and antiadrenergic effect. Beta-blockers administration should be limited in patients with labile hemodynamic compensation or severe reduction of LV function; (3) lidocaina and azimilide are second choice drugs, useful in case of contraindications to previous medications[14,30]; (4) verapamile should be used as drug of choice in case of premature ventricular beats originating from His-Purkinje system; and (5) finally, atrial or sequential atrio-(bi)ventricular pacing are useful to avoid bradycardia[31,32].

Sedation is pivotal to stabilize patients with ES, but hemodynamic and/or respiratory instability can limit the use of sedation drugs, such as benzodiazepine. In these cases mechanical ventilation with oro-tracheal intubation are absolutely required in refractory forms of ES. In some cases mechanical ventilation allows safer drugs administration otherwise not tolerated.

ROLE OF CATHETER ABLATION

The role of catheter ablation (CA) in patients with VT is becoming more and more relevant, as a definite treatment of multiple forms of arrhythmias and a complementary intervention in cases of high electrical instability, thus improving prognosis and quality of life in patients with advanced forms of heart disease. This observation creates the rationale to investigate the possibility to apply CA in patients with frequently recurring ventricular arrhythmias and ES[33].

Preliminary reports regarding the role of CA in the treatment of ES are limited to patients with specific clinical characteristics and/or small case series. Silva *et al*[34] reported a success rate of 80% in an ES population with recurrent hemodynamic stable VT; Schreieck *et al*[35] reported acute success in most of cases of a selected population undergoing CA of hemodynamic unstable arrhythmias guided by substrate mapping. Also Bänsch *et al*[16] described CA in patients with acute

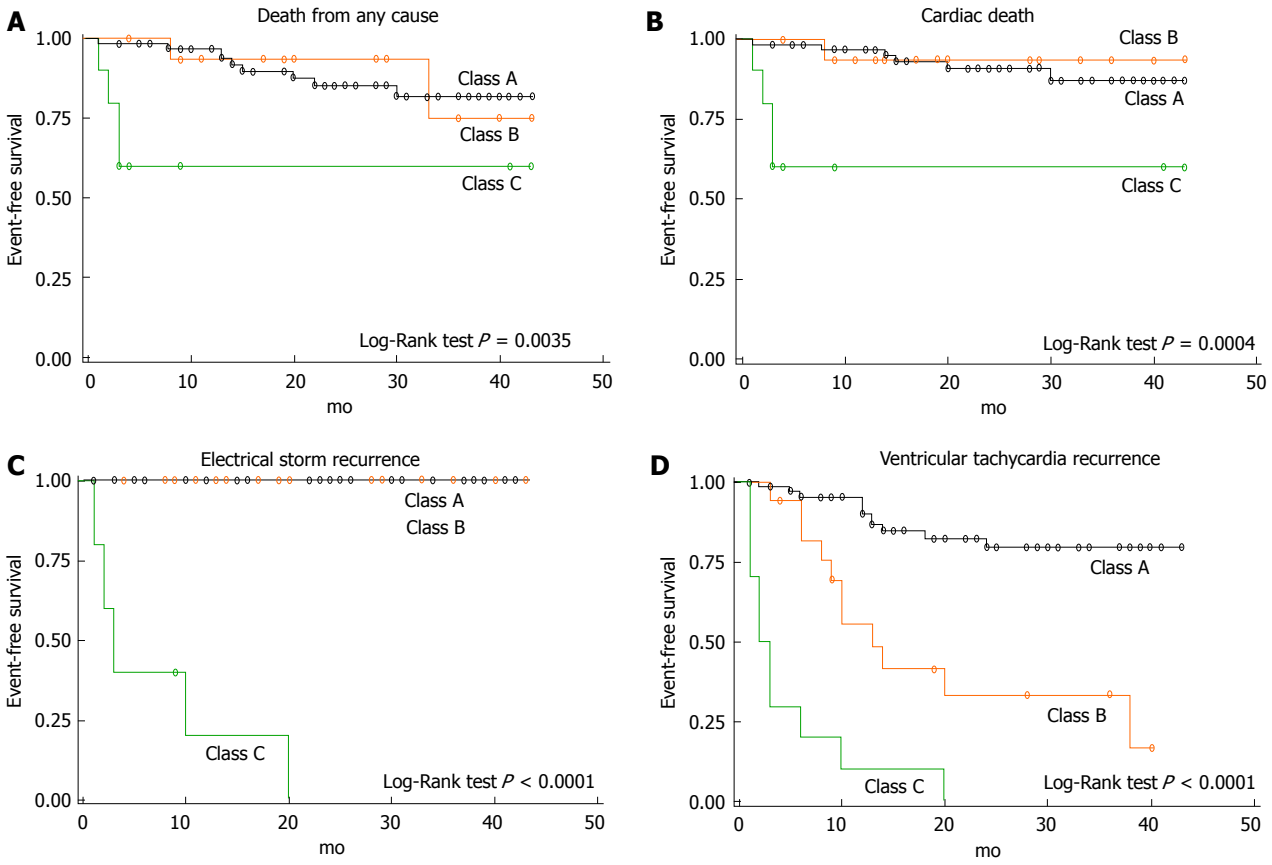

Figure 1 Kaplan-Meier survival analysis after catheter ablation of electrical storm. Class A indicates catheter ablation success, defined as suppression of each ventricular tachycardia (VT) morphology; Class B indicates partial success, defined as suppression of each clinical VT; Class C indicates failure, defined as persistence of one or more clinical VT (reprinted from Carbucicchio *et al*[20]).

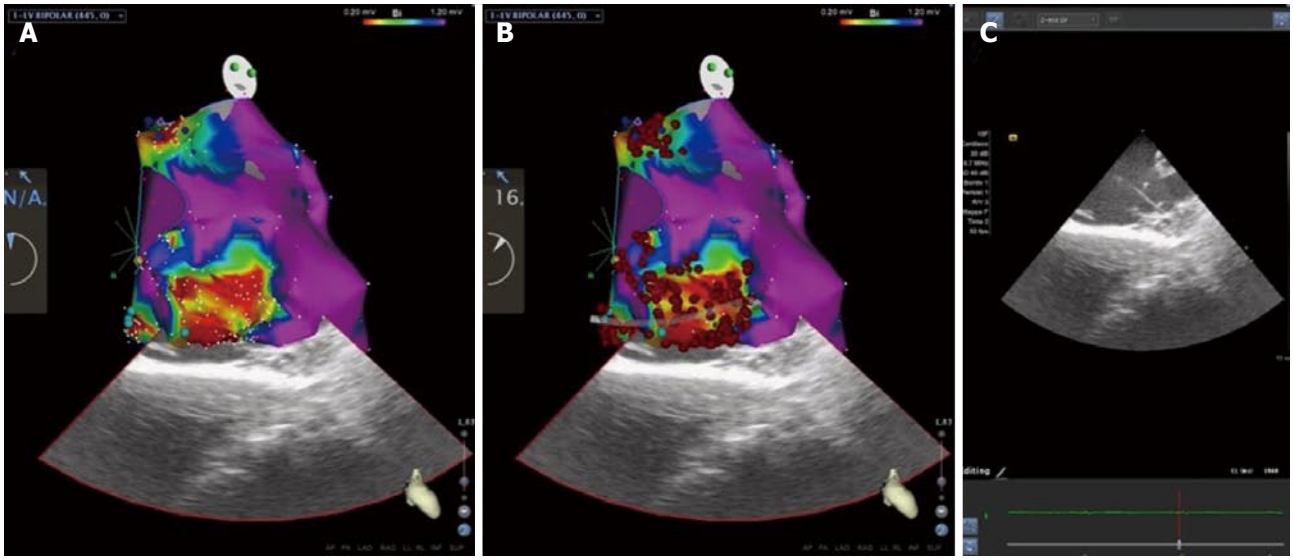

Figure 2 Modern approach of mapping and ablation for the treatment of electrical storm: High-density electroanatomical mapping of the left ventricle using CARTOSOUND contact-force technology (Biosense Webster, Diamond Bar, CA, United States). A: Two distinct peri-valvular scars, in the mitro-aortic continuity and below the mitral annulus, in the setting of idiopathic cardiomyopathy are visualized. Scars are characterized by dense scar (abnormal bipolar electrograms < 0.20 mV), surrounded by a border zone (0.20-1.20 mV) in which late potentials, are tagged (dark and light blue dots); B: Radiofrequency ablation lines are dragged for dechanneling and isolation of all proarhythmic sites; C: Intracardiac echocardiography imaging is aiming at substrate characterization and correct positioning of the catheter during mapping and ablation.

myocardial infarction and ES in whom VF was triggered by premature ventricular contractions, targeted by CA.

The ability to assess the feasibility and effectiveness of CA in a wider ES population arises from more

recent experiences, which better represent the profile of patients with complex, hemodynamically non-tolerated, drug-refractory ventricular arrhythmias, mostly in the setting of structural heart disease with severe impairment of left ventricular function. In this population, Carbucicchio *et al*[20] have described for the first time VT suppression in 90% of patient undergoing one or more CA procedures with or without the use of haemodynamic mechanical support. Moreover, the authors have shown that non-inducibility of VT at the end of the procedure was predictive of no recurrence of ES or VT at 2 years follow-up; accordingly, CA survival was improved in arrhythmia-free patients (Figure 1). This experience once again shows that ES represents a turning point in the natural history of patients with dilated cardiomyopathy and ventricular arrhythmias and that the treatment of arrhythmic burden plays a favourable effect on the clinical history of these patients both in terms of arrhythmic death and acute heart failure. More recent studies have confirmed that CA of ES is effective in reducing mortality in the middle-term follow up[18,36].

Regarding management of patients with ES or with recurrent VT following points must be taken in account: (1) clinical management in this setting is highly demanding. It requires an experienced Intensive Care Unit staff and a multidisciplinary approach that includes anesthesiological and psychological support; (2) advanced CA strategies in these patients are particularly complex (Figure 2). Obviously, the use of electroanatomical mapping (EAM) to guide CA is mandatory, and a substrate-guided approach is commonly more efficient, limiting activation mapping manoeuvres[37]. An epicardial approach should be preferred in all patients with non-ischemic cardiomyopathy to minimize recurrences. In patients with unstable VT or very depressed cardiac function, or in those presenting with cardiogenic shock, hemodynamic mechanical support allows patients stabilization and enhances efficacy and safety of CA, and can be used both during intraprocedurally as in the post-procedural period[38]; and (3) in selected patients, requiring concomitant surgical indications or in whom a percutaneous approach is not feasible, surgical ablation guided by EAM (endo- and/or epicardial) may be taken into account, in an experienced and multidisciplinary setting.

CONCLUSION

ES is an "extreme" ventricular arrhythmia affecting ICD patients with structural heart disease and is a major predictor of cardiac death in the short-term follow-up. Problems related to the treatment of ES patients are complex, depending on the type of patient as well as on the treatment of cardiac emergency, and require high standard facilities and specialized skills.

CA for the treatment of ES is particularly promising and should be considered the elective form of treatment to achieve long-term rhythm stabilization and to prevent heart failure. The possibility to modify the arrhythmic substrate by CA in an early phase, thus preventing critical situations deriving from repetitive ICD interventions, looks promising, but necessitates further corroborations.

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ORIGINAL ARTICLE

Basic Study

Initial clinical experience using the EchoNavigator®-system during structural heart disease interventions

Jan Balzer, Tobias Zeus, Katharina Hellhammer, Verena Veulemans, Silke Eschenhagen, Eva Kehmeier, Christian Meyer, Tienush Rassaf, Malte Kelm

Jan Balzer, Tobias Zeus, Katharina Hellhammer, Verena Veulemans, Silke Eschenhagen, Eva Kehmeier, Christian Meyer, Tienush Rassaf, Malte Kelm, Division of Cardiology, Pneumology and Angiology, Department of Medicine, University Hospital Duesseldorf, 40225 Duesseldorf, Germany

Author contributions: Balzer J and Zeus T designed the concept of the study, prepared the manuscript and figures, performed the interventional procedures and drafted the manuscript; Hellhammer K and Veulemans V performed the clinical and echocardiographic data assessment before and during the intervention; Eschenhagen S and Kehmeier E made critical revisions related to the quality of the acquired data and participated in the creation of figures and images; Meyer C, Rassaf T and Kelm M participated in the performance of the interventional procedures, and in the design and review of the manuscript; all authors read and approved the final manuscript.

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Institutional review board statement: No ethical approval by the local institutional review board was necessary for the additional application of this novel fusion imaging technology. The data analysis was conducted retrospectively without the necessity of providing an ethics committee approval.

Institutional animal care and use committee statement: Animal experiments were not performed for the conduction of this study.

Conflict-of-interest statement: Jan Balzer has received fees for serving as a speaker from Philips Healthcare. Other authors have none conflict of interest to declare.

Data sharing statement: Technical appendix, statistical code, and datasets are available from the corresponding author. Participants gave written informed consent for the performance of each interventional procedure. Written informed consent was not obtained for the additional application of the fusion imaging software. The presented data are anonymized and the risk of identification is low.

Correspondence to: Dr. med. Jan Balzer, Division of Cardiology, Pneumology and Angiology, Department of Medicine, University Hospital Duesseldorf, Moorenstr. 5, 40225 Duesseldorf, Germany. jan.balzer@med.uni-duesseldorf.de
Telephone: +49-211-8118800
Fax: +49-211-8118812

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Abstract

AIM: To present our initial clinical experience using this innovative software solution for guidance of percutaneous structural heart disease interventions.

METHODS: Left atrial appendage, atrial septal defect and paravalvular leak closure, transaortic valve repair and MitraClip® procedures were performed in the catheter laboratory under fluoroscopic and echocardiographic guidance. The two-dimensional and three-dimensional images generated by the transesophageal echocardiography probe were interfaced with the fluoroscopic images in real-time using the EchoNavigator®-system.

RESULTS: The application of the novel image fusion technology was safe and led to a better appreciation of multimodality imaging guidance due to improved visualization of the complex relationship between catheter devices and anatomical structures.

CONCLUSION: The EchoNavigator®-system is a feasible and safe tool for guidance of interventional procedures in structural heart disease. This innovative technology may improve confidence of interventional cardiologists in targeting and positioning interventional devices in order to increase safety, accuracy, and efficacy of percutaneous interventions in the catheter laboratory.

Key words: Fusion imaging; Interventional guidance; Percutaneous interventions; Structural heart disease; Echocardiography

Core tip: Interventions in structural heart disease require adequate echocardiographic and fluoroscopic imaging for safe accomplishment of the procedure. Recently, a novel fusion imaging technology has been introduced, allowing for the first time to merge echocardiographic and fluoroscopic images in the catheter laboratory in real time. As one of the first centers worldwide, we used this innovative technology for guidance of interventions in structural heart disease, demonstrating its potential benefits for guiding complex interventions in structural heart disease.

Balzer J, Zeus T, Hellhammer K, Veulemans V, Eschenhagen S, Kehmeier E, Meyer C, Rassaf T, Kelm M. Initial clinical experience using the EchoNavigator®-system during structural heart disease interventions. *World J Cardiol* 2015; 7(9): 562-570 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i9/562.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i9.562

INTRODUCTION

Adequate peri-procedural image guidance is indispensable for safe accomplishment of cardiovascular interventions[1]. In contrast to coronary interventions where fluoroscopy remains the dominant imaging modality, the evaluation and treatment of structural heart disease requires continuous soft-tissue information that cannot be provided by fluoroscopy alone. Therefore, percutaneous interventions in the catheter laboratory are usually monitored additionally by two-dimensional (2D) and especially three-dimensional (3D) transesophageal echocardiography (TEE)[2]. Both techniques are presented side by side on different screens, necessitating the interventionalist to mentally reconstruct and fuse the presented information. Recently, the EchoNavigator®-system (Philips Healthcare, Best, The Netherlands) has been introduced as a novel software solution, allowing to merge echocardiographic and fluoroscopic images on the same display in real time[3,4]. In this study we aim to present our initial clinical experience with this innovative technology and describe its potential benefits during percutaneous interventions in structural heart disease.

MATERIALS AND METHODS

From January 2014 until July 2014 we used the EchoNavigator® software for guidance of 127 interventions in structural heart disease [3 paravalvular leaks, 11 atrial septal defects (ASDs), 31 transapical transcatheter aortic valve repair (TAVR) procedures, 35 left atrial appendage (LAA) occlusions, and 47 MitraClip® procedures]. Conscious sedation with continuous hemodynamic monitoring was applied in all cases, with the exception of the 31 transapical TAVR procedures, where general anesthesia was applied. After insertion of the procedure specific sheath, patients were sedated, and the TEE probe was inserted by an experienced echocardiographer before initiating the procedure by the interventionalist. For the additional application of this fusion imaging technology no ethical approval was necessary. All patients gave written informed consent for the performance of each interventional procedure.

EchoNavigator®

The technology of the EchoNavigator®-system relies on a real-time co-registration and visualization of 2D/3D TEE and fluoroscopy. The method consists of an image-based TEE probe localization and calibration algorithm. This algorithm automatically finds and tracks the position and the direction of the TEE probe within the fluoroscopic image[5]. After synchronization of TEE and fluoroscopy images, the system automatically tracks and follows the rotation of the C-Arm, based on the angulation of the gantry[6]. The results of this co-registration process are visualized to the interventional cardiologist on a large specific display that can be divided and arranged in up to 4 sections at discretion of the interventionalist. The 4 sections are assigned to different functions, depicting different views and are labeled as follows: (1) Echo: The Echo-view demonstrates online the images from the echo machine that can only be manipulated by the echocardiographer; (2) X-ray: The X-ray-view displays the actual fluoroscopic view depending on the angulation of the gantry. For a precise co-registration of the TEE probe, the probe has to be central in this view, the correctness of the co-registration being illustrated by a green edging of the probe. In case that the co-registration is not correct, *e.g.*, after movement of the TEE probe, the edging of the probe will turn into red; (3) C-Arm: The beam flow of the matrix array transducer is marked as a purple 3D sector in the X-ray-view, presenting the 3D Echo information of this sector in the C-Arm-view. Changes in angulation, rotation or position of the TEE probe are immediately registered

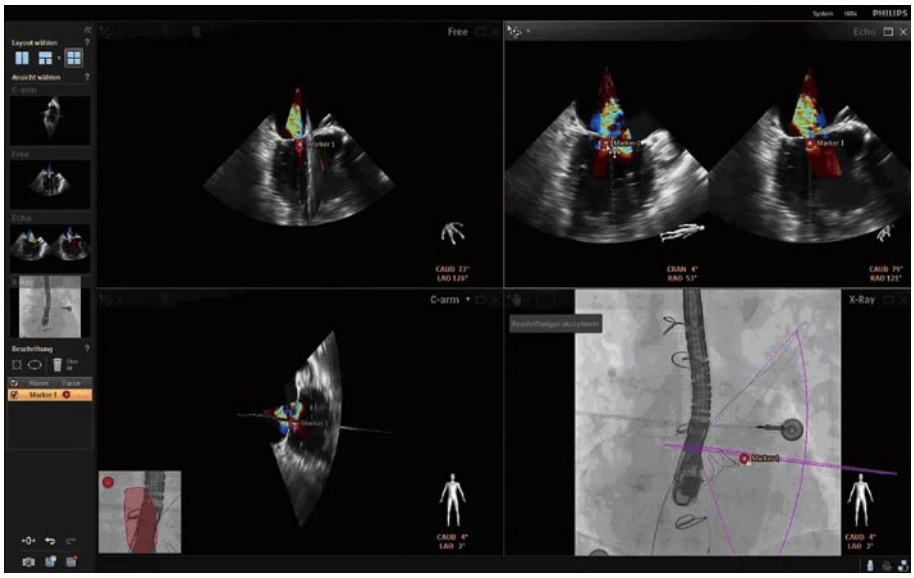

Figure 1 Specific points of interest can be marked in the ultrasound image by the interventionalist that will automatically appear on the fluoroscopic image. In this case of a MitraClip® procedure, the echocardiographer displays the mitral valve with Color Doppler in the X-plane mode in order to define the most regurgitant jet (right upper panel). The interventionalist can then steer particular markers using the table-side control mouse, and place them to define the region of interest for the MitraClip® procedure. After adjusting the marker, it will appear on the fluoroscopic image, representing the exact region of interest, where the MitraClip® should be implanted (right lower panel).

and updated on the fluoroscopic image; and (4) Free: The Free-view also displays 2D and 3D TEE information that can be manipulated by the interventionalist with a sterile covered mouse on the catheter table. The interventionalist can rotate and crop into 3D data sets in any direction.

Specific points of interest can be marked in the ultrasound image by the interventionalist that will automatically appear on the fluoroscopic image (Figure 1). There is also the opportunity to switch between different TEE modalities (2D, 3D, 2D and 3D Color Doppler), and different view settings (2D, X-Plane, 3D Zoom and 3D Full Volume).

RESULTS

Feasibility of the EchoNavigator® during interventions in structural heart disease

All 127 procedures were performed using the EchoNavigator®-system for peri-interventional guidance. The application led to safe accomplishment of all performed procedures without any complications related to the peri-procedural imaging guidance.

Percutaneous edge to edge repair of mitral regurgitation using the MitraClip® system: The unique visualization of the mitral valve apparatus using 3D TEE for planning and performing the procedure allows improved understanding of the morphological and functional changes induced by the MitraClip® system. In this context, a recent study demonstrated that the procedural effects of the MitraClip® system upon the mitral valve apparatus can best be detected using 3D TEE with various offline reconstruction techniques[7]. The peri-interventional evaluation of the mitral valve, including the leaflets, the annulus, and the subvalvular apparatus using 3D TEE is therefore of major importance. On the other hand, the orientation of the guiding system and the dedicated structures of the Clip with its grippers can be much better delineated using fluoroscopy. Therefore, a multimodality approach for guidance of MitraClip® implantations using 2D/3D TEE and fluoroscopy is of essential importance. Our results demonstrate that the translation of specific echocardiographic markers into the X-ray-view can improve the visualization of the complex relationship between catheter devices and anatomical structures during MitraClip® procedures (Figure 2). In this context, the EchoNavigator®-system was especially usefull for the transseptal puncture, that can be performed using fluoroscopy and 2D TEE imaging alone. In special situations, the 3D images improve the visualization of the transseptal puncture side for better definition of the correct height above the mitral valve, necessary for sufficient movement of the delivery guide and the device[8]. The fact, that the designated point of puncture can be marked in either the 2D or the 3D echocardiographic view, is very helpful for placement of the needle in the fluoroscopic image. Severe complications can arise when the Clip perforates the thin wall of the atrial septum, leading to cardiac tamponade[9]. The designation of three echocardiographic orientation points (interatrial septum at puncture site, crista terminalis between pulmonary vein and the LAA, and the center of the mitral valve) into the fuoroscopic image can prevent injury of the left atrium, even when the Clip movements are monitored with X-ray within this virtual triangle. When more than

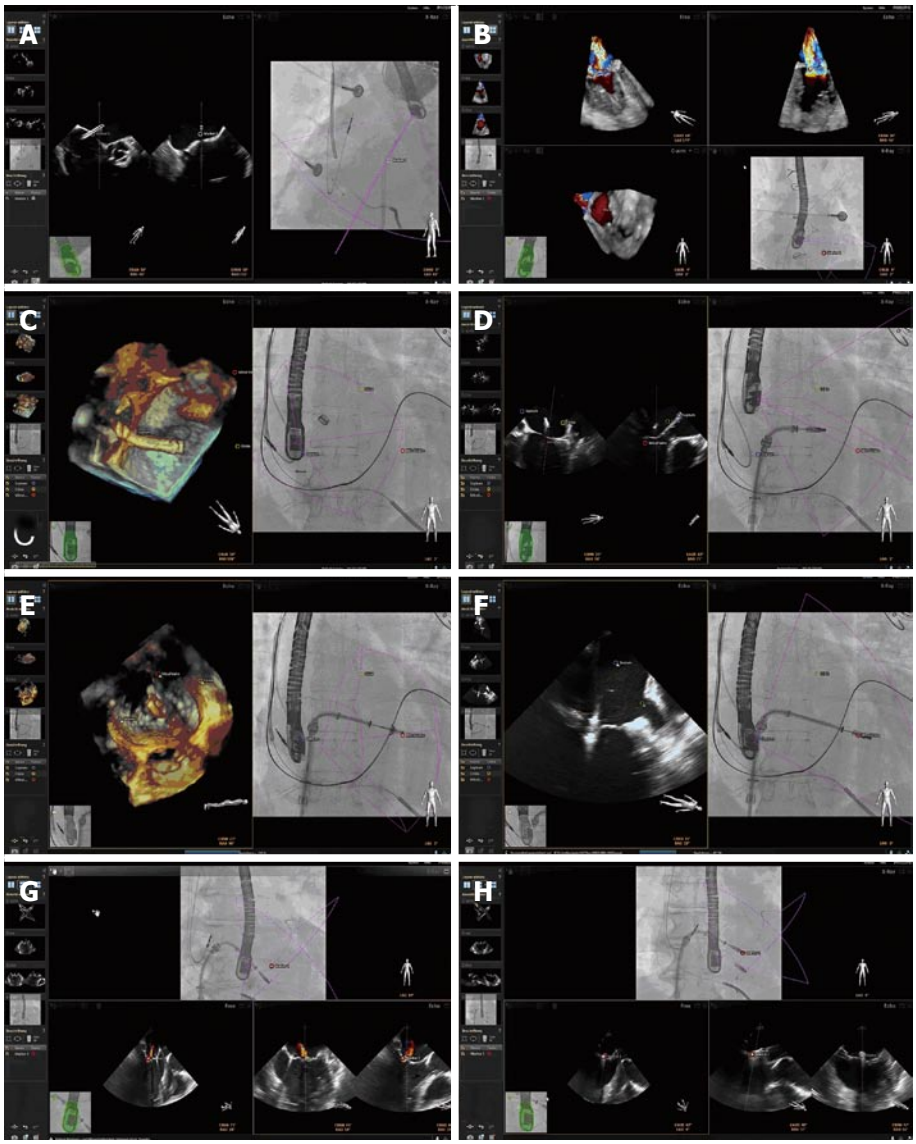

Figure 2 EchoNavigator® during MitraClip® procedures. A demonstrates the Echo-view on the left, depicting an X-plane-view of the interatrial septum. The interventionalist can set markers in this view for exact delineation of the optimal puncture site that will automatically appear in the X-ray-view on the right. A 3D full volume color doppler image is shown in the right upper panel of B. After cropping into this data set, the maximum regurgitant jet was depicted and marked within this image. On the left side of C, a 3D view of the septum with the delivery system and especially the three points of interest (Septum, Crista, and Mitral valve) is demonstrated. The connection between these three points creates a virtual triangle in the X-ray-view on the right side of C, outlining the area at risk outside this triangle in the X-ray-view in D, where contact of the MitraClip® device with its surrounding structures potentially can lead to complications, such as perforation with cardiac tamponade. E demonstrate the process of Clip orientation orthogonally towards the commissure in the 3D view, and the grasping of leaflets in the 2D intercommisural view in F. The X-ray-view in both figures on the right display demonstrates the correct position of the Clip right on top of the red marker, corresponding with the echo images. G demonstrates the residual regurgitant jet on the lateral side of the first Clip. This jet was marked in the echo image and was then used for orientating a second Clip in the X-Ray image, as demonstrated in H. 2D: Two-dimensional; 3D: Three-dimensional.

one Clip needs to be implanted, the exact relation of the Clips to each other can be misjudged due to blooming artefacts of the echocardiographic image[10]. In this situation, fluoroscopy is very helpful to illustrate the spatial relation of both Clips. The EchoNavigator®-system enables the translation of the residual jet from the echocardiographic image into the X-ray image for exact implantation of the second Clip.

LAA occlusion: In non-valvular atrial fibrillation the interventional closure of the LAA has shown to be a successful alternative to oral anticoagulation[11]. The main steps of the procedure are the transseptal crossing of the guiding catheter into the left atrium and the placement of the occluder into the LAA. Basically, the intervention can be performed using fluoroscopy alone, but the advantages of 3D TEE for peri-interventional guidance during the procedure could be clearly demonstrated in recent studies[12]. 2D TEE allows the interventionalist to measure the orifice of the appendage in different 2D cut planes, but 3D TEE additionally allows 3D measurement of the perimeter for exact definition of the landing zone and correct device selection[13]. The EchoNavigator®-system revealed to be very useful for the performance of the transseptal puncture (Figure 3). Furthermore, the exact delineation of the landing zone

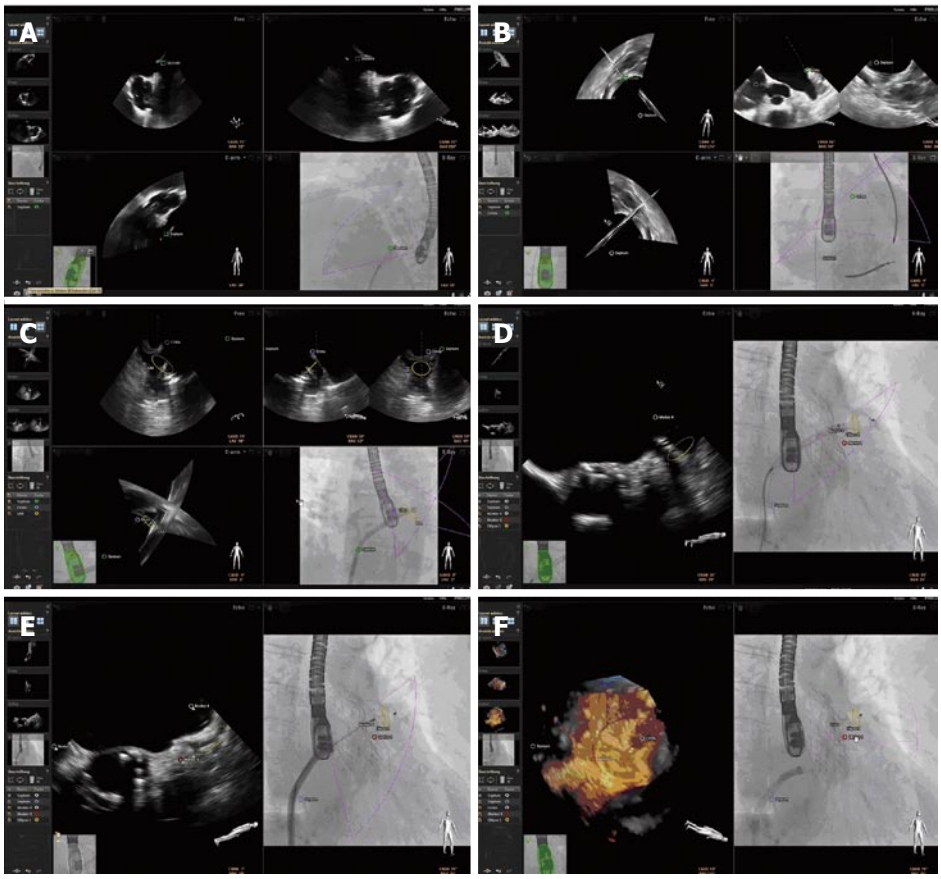

Figure 3 Echonavigator® during left atrial appendage occlusion. A demonstrates how the point of interest for potential transseptal crossing along the interatrial septum is marked in the Echo image. B demonstrates in the X-ray-view, that the wire is located cranial of the Crista terminalis, that initially was assigned in the Echo image. C depicts an ellipsoid ring, representing the landing zone for the occluder device marked yellow in the X-plane view of the 2D Echo image. D displays the deployment of the lobe of the left atrial appendage occluder, detecting that correct device size was chosen. E shows this ellipsoid marker right in between the lobe and the disc of the occluder after deployment of the disc. The red marker represents the location of the circumflex coronary artery. F shows the final result after disconnecting the guiding system from the occcluder. The left panel gives a nice 3D overview of the site of implantation, displaying the difference of the landing zone (yellow circle) and the orifice area of the left atrial appendage.

derived from the echocardiographic images allowed for secure implantation of the device, therewith preventing mismatch and dislocation of the occluder.

ASD occlusion: Interventional techniques for the closure of interatrial communications can be performed using echocardiography as the only imaging modality. In a recent study the feasibility of interventional closure of ASDs without fluoroscopy was demonstrated[14]. TEE provides an imaging technique to guide ASD closures, providing fast and complete information about the underlying pathomorphology, improving spatial orientation, and additionally monitoring online the appropriate position of the device without loss of image quality. Furthermore, the additional information supplied by the 3D images helps to better understand the anatomy and the pathomorphology of the defect during guidance of the intervention, leading to shorter procedure times and less radiation exposure to the patient[15]. Our initial experience with the EchoNavigator®-system in such procedures indicates safe implantation of devices in ASDs. After placement of the echocardiographic marker delineating the ASD into the X-ray-view, the guide wire passage orientated towards this point enormously facilitated the procedure (Figure 4).

TAVR: For safe and precise performance of TAVI procedures, knowledge about the exact anatomy of the aortic root an its surrounding structures obtained by different imaging modalities is indispensable[16]. The use of TEE for peri-interventional guidance is limited, as it often requires general anesthesia and the probe may also partially obstruct the optimal fluoroscopic view. Particularly in patients treated over the transapical approach where general anesthesia is needed anyway, TEE has its place during and after valve implantation. For secure valve implantation, the exact knowledge about the alignment of the hinge points of the three leaflets is crucial[17]. With fluoroscopic imaging alone it is difficult to place the gantry in a position, where the hinge points are in the exact same cut plane. 3D TEE has proven to be very useful for aortic annular sizing and exact delineation of the hinge points during valve sizing and implantation[18]. The EchoNavigator®-system allows transfer of specific 2D and 3D echocardiographic

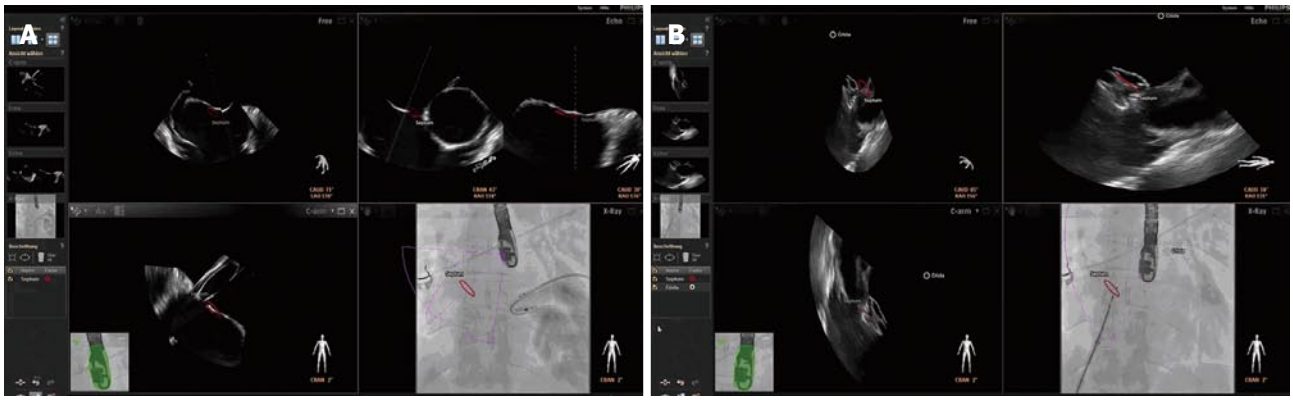

Figure 4 EchoNavigator® during atrial septal defect closure. A: Shows in the Echo-view in the right upper panel an X-plane view of the interatrial septum with a bicaval and a short axis view. The defect is clearly illustrated and marked with a red circle, appearing also in the X-ray-view as the target lesion; B: Demonstrates the occluder device after deployment of both the left and the right atrial disc. Note in the right upper panel that the two discs embrace the red target marker, where the defect was previously located.

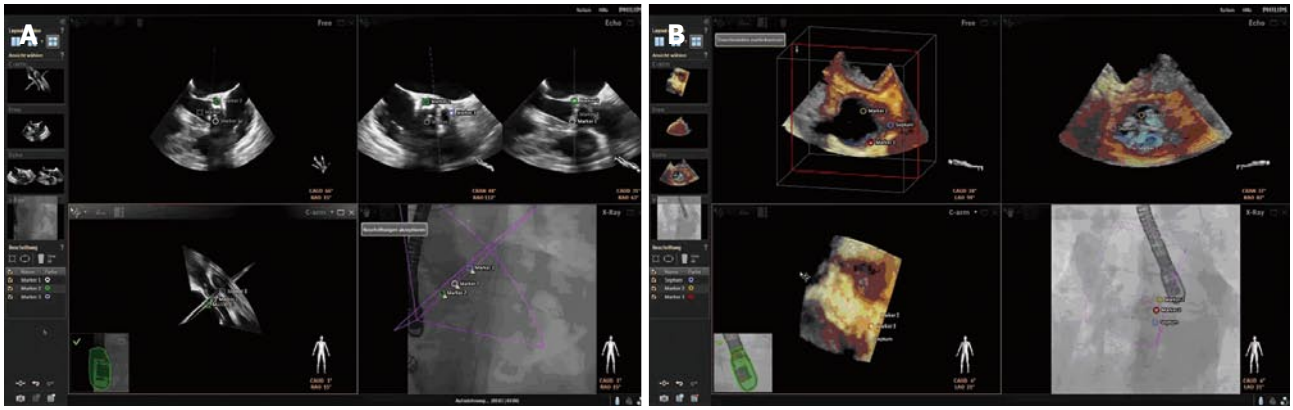

Figure 5 EchoNavigator® during transcatheter aortic valve repair. For precise delineation of the aortic annulus, the hinge points of the three leaflets of the aortic valve were marked in the Echo image, as illustrated in the right upper panel in A. These markers then appeared in the fluoroscopic X-ray-image in the right lower panel. Afterwards, the gantry was moved in order to transfer the three markers in one orthogonal image plane. This was also performed using 3D TEE for a better spatial orientation along the annulus plane of the aortic valve (B).

markers into the fluoroscopic image (Figure 5). This allows the interventionalist to correct the position of the gantry to the point where all three hinge point markers derived from echocardiography create one orthogonal plane.

Percutaneous closure of prosthetic paravalvular leaks: Different multimodality imaging techniques have been proposed for peri-interventional guidance of these procedures, including a combination of fluoroscopy and TEE[19]. The advantage of 3D TEE is the improved spatial resolution of the defect, especially during placement of the guide wire through the defect after transseptal puncture[20]. The interaction between the prosthetic leaflets and the occluder system leading to possible obstruction and malfunction of the prosthetic valve can better be illustrated by 3D TEE. The application of the EchoNavigator®-system is very helpful for percutaneous closure of paravalvular leaks (Figure 6). It allows the interventionalist to focus more upon the fluoroscopic image as it better delineates the catheter and the devices. Especially the precise steering of the guide wire through the defect is facilitated using this innovative technology.

DISCUSSION

The major findings of the present study are: (1) the application of the EchoNavigator®-system is feasible and safe during interventions in structural heart disease; (2) the EchoNavigator®-system allows merged display of echocardiographic (2D, 3D, Color-Doppler, X-Plane Imaging) and fluoroscopic images in real-time, allowing the interventionalist to interact with both imaging modalities simultaneously.

The benefits of fusion imaging technologies have already been described in the recent past, especially for the combination of CT-angiography and fluoroscopy images during transapical TAVR procedures for exact assignment of the correct site for the transapical access[21]. The integration of echocardiographic images into the X-ray image in real-time during percutaneous interventions were hampered so far by the complex nature of the echocardiographic data. The EchoNavigator®-system

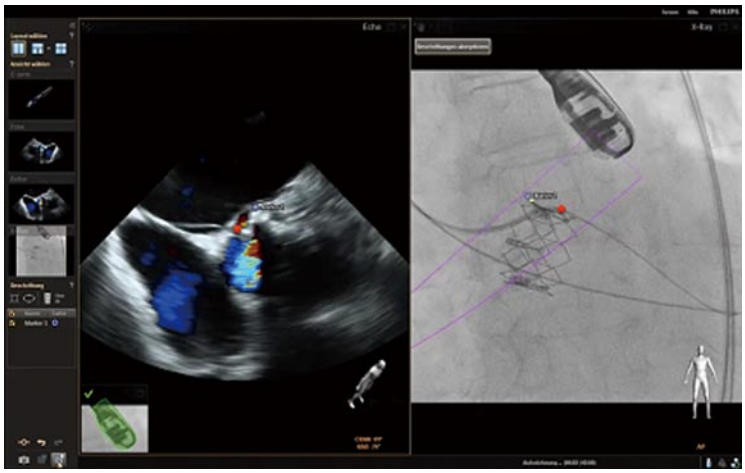

Figure 6 A case of a prosthetic paravalvular leak presenting aortic regurgitation after transcatheter aortic valve repair. The left panel demonstrates the Echo image in a short axis view, presenting the aortic annulus and depicting the regurgitant jet with Color Doppler. Here the marker was placed incorrectly, consequently transfering the marker next to the destination in the fluoroscopic image in the right panel. The red marker demonstrates where the region of interest should have been defined, reflecting the correct position in the fluoroscopic image.

is the first integrative solution to merge the two major important imaging modalities echocardiography and fluoroscopy in real time during interventions in structural heart disease. This technology facilitates the procedures, as echocardiographic soft tissue information is copied into the fluoroscopic image in real-time without time-consuming offline reconstruction. Traditionally, the interventional cardiologist is more familiar with the standard fluoroscopy projections compared with the classical TEE orientations. This new technology therewith fulfills the needs of the traditional interventionalist, who is not always used to the different 2D and 3D image orientation from TEE, by delineating the information from echocardiography into the more familiar fluoroscopic image. Only few studies have described the application of the EchoNavigator® during structural heart disease interventions so far. Sündermann *et al*[4] were the first to document an influence of radiation dose and procedure time under guidance of MitraClip procedures using the EchoNavigator®. They discovered a reduction in radiation and procedure time especially in complex procedures were more than one clip was implanted. This underlines the strength of this technology, simplifying the exact detection of the target point for the implantation of more than one clip next to the already implanted clip. The more complex the intervention becomes during the procedure, the more beneficial is the gain of the additional information given by the supporting software. Recently, González Gómez *et al*[22] described the advantage of the technique for transseptal punctures, according to our own results. Especially in MitraClip procedures the exact height of the puncture is essential for the success of the intervention. Using the EchoNavigator® the puncture site can even be defined before pullback of the needle from the superior caval vein into the right atrium. The precision of this fusion imaging technology is remarkable. Our own experience indicates that the applied markers depicting the target structures correlate very well with the definite location of the catheter devices. Moreover, in cases where the matching of echocardiographic and fluoroscopic data was unsatisfactory, we switched back to the conventional image guidance without the overlay. This approach always warrants an exit strategy in cases where the confidence into the fusion technology is somehow affected, anticipating possible misguidance by the EchoNavigator®-system. Next to the advantages of the EchoNavigator®, there also are some limitations. The technology described in this paper is based on a co-registration process of the TEE probe into the fluoroscopic image, simultaneously transferring the data into the virtual coordinate system of the X-ray display. The specific points of interest that can be marked with the software do not follow the acute movements of the echocardiographic speckles, making the combined image with the marked reference points quite static. The newest release of the EchoNavigator® allows a translation of the entire echocardiographic dataset into the X-ray-image, making the fusion of both modalities even more impressive, as recently published by our group[23].

Limitations

Our manuscript is supposed to give "tips and tricks" while working with the EchoNavigator® technology according to our initial experience in 127 patients. Our study lacks information about the benefit of the technology in the context of reducing the procedure length or the radiation dose. Data demonstrating these effects are currently only available for MitraClip procedures[4]. Prospective randomized multi-center studies with a larger sample size are necessary to demonstrate potential benefits of this promising technology for the patient. At this time, the main benefit of the EchoNavigator®-system is the facilitation of procedures by online fusion of two important imaging modalities, leading to better confidence of the interventionalist into the procedure and producing a better communication between the echocardiographer and the operator.

In conclusion, the EchoNavigator®-system is a feasible and safe tool for guidance of interventional procedures in structural heart disease. This innovative technology may accelerate the learning curve of interventional cardiologists in order to increase safety, accuracy, and efficacy of percutaneous interventions in the catheter laboratory. Further research is necessary

to evaluate the clinical value of this promising new tool, but it is likely that such a visualization technology might have a significant impact on the success and the safety of cardiovascular procedures in the catheter laboratory.

COMMENTS

Background

The number of percutaneous interventional procedures for the treatment of structural heart disease in patients that are ineligible for conventional open heart surgery is increasing permanently. Less invasive techniques allow for save accomplishment of these highly complex interventions in the catheter laboratory. Still, complications can arise during the procedure due to inadequate imaging of the target structures, often with fatal outcome for the patient.

Research frontiers

Side by side imaging of cardiac soft tissue anatomy and catheter devices using echocardiography and fluoroscopy is essential for safe performance of interventional procedures in structural heart disease, though assuming mental fusion and reconstruction of each imaging modality by the interventionalist. Online fusion imaging technologies can determine a faster and better understanding of the complex relationship between anatomical landmarks and catheter devices and have the potential to facilitate the procedure.

Innovations and breakthroughs

In the current study the authors demonstrate for the first time the application of a novel image fusion technology (EchoNavigator®) to guide different types of complex interventions in structural heart disease. To our knowledge, no similar studies presenting such a broad applicability of this hybrid imaging technique have been published so far.

Applications

The study results of the present study suggest that online fusion of echocardiographic soft tissue anatomy and fluoroscopic catheter devices is a breakthrough for precise monitoring of interventions in structural heart disease. The impressive images in this article implicate the value of this innovative technology for upcoming interventional procedures that afford even more an exact delineation of cardiac anatomy for safe performance of complex procedures. Furthermore, the technique does not exclude the standard operating procedure using solely echocardiography and fluoroscopy side-by-side. The generated overlay images rather must be considered accessory with in fact tremendous additional value.

Terminology

The name of the new software "EchoNavigator®" implicates the way it is used for monitoring of interventions in the catheter laboratory. 2D and 3D echo information can be translated into the fluoroscopic image for best navigation of the procedure using a combination of both imaging modalities.

Peer-review

This is an excellent manuscript about the clinical experience using the EchoNavigator®-system. The authors have suggested that the EchoNavigator®-system is a feasible and safe tool for guidance of interventional procedures, such as left atrial appendage, atrial septal defect and paravalvular leak closure, transaortic valve repair and MitraClip® in structural heart disease. This manuscript is nicely structured and very well written.

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ORIGINAL ARTICLE

Observational Study

Single *vs* double antiplatelet therapy in acute coronary syndrome: Predictors of bleeding after coronary artery bypass grafting

Vincenzo Tarzia, Giacomo Bortolussi, Edward Buratto, Carla Paolini, Carlo Dal Lin, Giulio Rizzoli, Tomaso Bottio, Gino Gerosa

Vincenzo Tarzia, Giacomo Bortolussi, Edward Buratto, Giulio Rizzoli, Tomaso Bottio, Gino Gerosa, Division of Cardiac Surgery, Department of Cardiac, Thoracic and Vascular Sciences, University of Padua Medical School, 35128 Padua, Italy

Carla Paolini, Carlo Dal Lin, Division of Cardiology, Department of Cardiac, Thoracic and Vascular Sciences, University of Padua Medical School, 35128 Padua, Italy

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Correspondence to: Vincenzo Tarzia, MD, Division of Cardiac Surgery, Department of Cardiac, Thoracic and Vascular Sciences, University of Padua Medical School, Via Giustiniani, 2, 35128 Padova, Italy. v.tarzia@gmail.com
Telephone: +39-049-8212410
Fax: +39-049-8212409

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Abstract

AIM: To investigate the contribution of anti-platelet therapy and derangements of pre-operative classical coagulation and thromboelastometry parameters to major bleeding post-coronary artery bypass grafting (CABG).

METHODS: Two groups of CABG patients were studied: Group A, treated with aspirin alone (n = 50), and Group B treated with aspirin and clopidogrel (n = 50). Both had similar preoperative, clinical, biologic characteristics and operative management. Classic coagulation parameters and rotational thromboelastometry (ROTEM) profiles were determined preoperatively for both groups and the same heparin treatment was administered. ROTEM profiles (INTEM and EXTEM assays) were analyzed, both for traditional parameters, and thrombin generation potential, expressed by area-under-curve (AUC).

RESULTS: There was no significant difference between

rates of major bleeding between patients treated with aspirin alone, compared with those treated with aspirin and clopidogrel (12% *vs* 16%, $P = 0.77$). In the 14 cases of major bleeding, pre-operative classic coagulation and traditional ROTEM parameters were comparable. Conversely we observed that the AUC in the EXTEM test was significantly lower in bleeders (5030 ± 1115 Ohm*min) than non-bleeders (6568 ± 548 Ohm*min) ($P < 0.0001$).

CONCLUSION: We observed that patients with a low AUC value were at a significantly higher risk of bleeding compared to patients with higher AUC, regardless of antiplatelet treatment. This suggests that thrombin generation potential, irrespective of the degree of platelet inhibition, correlates with surgical bleeding.

Key words: Platelet inhibitors; Thromboelastometry; Bleeding; Acute coronary syndrome; Coronary artery bypass grafting

Core tip: To establish the timing of discontinuation of double antiplatelet therapy before coronary artery bypass grafting (CABG), it is crucial to identify predictors of bleeding. We analysed preoperatively classic parameters and thromboelastometry on 100 patients operated for CABG after presenting with acute coronary syndrome, to investigate the contribution of anti-platelet therapy and derangements of pre-operative coagulation status to major bleeding post-CABG. We observed that patients with a low area-under-curve (AUC) value in EXTEM were at a significantly higher risk of bleeding compared to patients with higher AUC, regardless of anti-platelet treatment. This suggests that thrombin generation potential, irrespective of the degree of platelet inhibition, correlates with surgical bleeding.

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INTRODUCTION

Post-operative bleeding is a major complication following coronary artery bypass grafting (CABG), with excessive bleeding occurring in 10% of patients, causing increased requirements of blood products, re-intervention and mortality[1-3]. Guidelines recommend ceasing clopidogrel therapy 5 d prior to CABG in order to minimise the risk of post-operative haemorrhage[4,5]. However, patients presenting for urgent and emergent CABG are often treated with double antiplatelet therapy, as recommended in major guidelines for the emergency management of acute coronary syndrome (ACS), prior to performing the diagnostic angiogram[6]. Ceasing clopidogrel and waiting the recommended 5 d prior to performing CABG would put these patients at higher risk of adverse coronary events[7]. Furthermore, as newer more potent platelet inhibitors such as ticagrelor and prasugrel become more widely used, patients presenting for CABG will increasingly be treated with these drugs[8-10]. As a result, it would be important to be able to detect patients at risk of excessive bleeding post-CABG, especially among those treated with double anti-platelet therapy. We devised the following study to determine whether bleeding risk could be predicted from the presence of double antiplatelet therapy, classical coagulation parameters or by rotational thrombo-elastometry performed prior to surgery.

MATERIALS AND METHODS

Patient selection

Among 905 patients operated for CABG between January 2006 and December 2008, we selected those presenting with ACS, and prospectively enrolled 50 consecutive patients without pre-operative clopidogrel exposure (Group A = 50 pts) and 50 consecutive patients with preoperative clopidogrel exposure within two days prior to intervention (Group B = 50 pts), who fulfilled inclusion criteria. The decision to stop double-antiplatelet therapy was at the discretion of the treating cardiologist. All patients signed informed consent for this observational study on prospectively collected data.

All patients who, within 2 d of surgery, were either on a daily oral regimen of 75 mg of clopidogrel or received a 300 mg oral loading dose prior to percutaneous coronary intervention (PCI), made up the clopidogrel study group. They were compared with a control-group of patients who had no clopidogrel exposure. Both patient groups received aspirin (100 mg) and low-molecular-weight-heparin (nadroparin calcium) prior to surgery. Patients with a history of previous cardiac surgery, concomitant valvular surgery or preoperative exposure to either warfarin or platelet glycoprotein IIb/IIIa inhibitors were excluded.

Recognized risk factors for perioperative bleeding in cardiac surgery were assessed including advanced age, female gender, low weight and renal insufficiency (Table 1).

Clotting profiles

Baseline haematocrit, platelet counts, prothrombin time (PT) and activated partial thromboplastin time (aPTT) were assessed because of their influence on blood product transfusions.

Thromboelastometry was performed on fresh blood within 2 h of being drawn from patients prior to induction of anaesthesia, with a ROTEM® (Tem International GmbH, München, Germany) coagulation analyzer, according to the standard protocols supplied by the

Table 1 Patient demographic, pre-operative characteristics, operative data

	Group A ASA	Group B ASA + Clopidogrel	*P*
Age	67 ± 9.4	66.3 ± 10.1	0.71 (*T-t*)
Gender (M/F)	42/8	45/5	0.46 (P-χ^2-*t*)
Weight (kg)	75.2 ± 13	76.4 ± 10.3	0.60 (*T-t*)
Hypertension	46/50 (92%)	50/50 (100%)	0.11 (*F-t*)
Dyslipidaemia	45/50 (90%)	45/50 (90%)	1 (*F-t*)
Diabetes	14/50 (28%)	18/50 (36%)	0.39 (P-χ^2-*t*)
COPD	12/50 (24%)	8/50 (16%)	0.45 (*F-t*)
Renal failure	7/50 (14%)	4/50 (8%)	0.33 (P-χ^2-*t*)
History of MI	28/50 (56%)	29/50 (58%)	0.5 (*F-t*)
History of CVA	4/50 (8%)	1/50 (2%)	0.36 (*F-t*)
Urgent surgery	22/50 (44%)	25/50 (50%)	0.34 (*F-t*)
EF%	56 ± 8.5	50 ± 9.5	0.04 (KW)/0.03 (LR)
LVEDV mL/m^2	60.8 ± 10.4	62.6 ± 12.5	0.54 (KW)
No. of anastomoses	2.5 ± 0.8	2.5 ± 0.7	0.99 (P-χ^2-*t*)
No. of grafts	2.5 ± 0.8	2.4 ± 0.7	0.99 (P-χ^2-*t*)

T-t: *T* test; *F-t*: Fisher's exact test; P-χ^2-*t*: Pearson's chi-square test; KW: Kruskal Wallis-test; ASA: Acetylsalycilic acid; COPD: Chronic obstructive pulmonary disease; CVA: Cardio-vascular accident; EF: Ejection fraction; LVEDV: Left ventricular end-diastolic volume.

manufacturer[11].

Prior to analysis, the samples were stored at room temperature. The sample tubes were gently inverted five times to re-suspend any sedimentation before pipetting the blood. Two standard ROTEM® assays named INTEM® and EXTEM® were performed. INTE® and EXTEM® (ellagic acid and tissue factor activation, respectively) represent assays in which the intrinsic and the extrinsic coagulation pathways are triggered, respectively. The ROTEM® method defines various parameters: Clotting time (CT, s) the time from the beginning of the coagulation analysis until an increase in amplitude of 2 mm, which reflects the initiation phase of the clotting process. Clot formation time (CFT, s) the time between an increase in amplitude of the thrombelastogram from 2 to 20 mm. Alpha-angle (°), the tangent to the clotting curve through the 2 mm point. The CFT and alpha-angle reflect measures of the propagation phase of WB clot formation. Maximum clot firmness (MCF, mm) is the maximum amplitude reached in thromboelastogram and correlates with the platelet count and function as well as the concentration of fibrinogen[12]. Area-under-curve (AUC), defined as the area under 1st derivative (*i.e.,* velocity) curve ending at a time point that corresponds to MCF, reflects thrombin generation potential[13].

Intraoperative management

All surgical procedures were performed through a median sternotomy and with cardiopulmonary-bypass (CPB), in a standard fashion. A comparable intraoperative heparin anticoagulation regimen was utilized in all patients; initial heparin dose was calculated using a minimum standard of 400 units/kg with additional dosing administered during the procedure, in order to maintain a target activated-clotting-time (ACT) value greater than 480 s.

Management of bleeding

Post-operative bleeding was carefully managed according to our institution's diagnostic - therapeutic algorithm[14]. PT, aPTT, platelet count, antithrombin III and ROTEM paramaters (INTEM, EXTEM and HEPTEM) are assessed and any abnormalities are corrected with relevant blood products (platelets, fresh frozen plasma, cryoprecipitate), protamine sulphate, fibrinogen or recombinant factor VIIa according to the underlying cause of bleeding.

Ongoing bleeding at a rate of 500 mL/h in the first hour, 400 mL/h in the first 2 h, 300 mL/h in the first 3 h, 200 mL/h in the first 4 h, despite optimisation of coagulation paramaters or the presence of cardiac tamponade are considered indications for re-intervention for bleeding. The amount of post-operative blood loss and the rate and amount of transfused blood products used both intra- and post-operatively were recorded.

Clinical outcomes

Major bleeding was considered to have occurred when $\geqslant$ 3 units of blood, fresh frozen plasma, platelets or surgical revision were required. Chest tube outputs assessed at 12 h were the primary measure of postoperative bleeding. Transfusion quantity was recorded for the three main blood product types (red blood cells, platelets and fresh frozen plasma) during operation and ICU stay. Clinical outcomes specific to CABG recovery included reoperation for bleeding, mortality, acute myocardial infarction, stroke and postoperative atrial fibrillation until discharge. General post-surgical outcomes evaluated were duration of intubation and postoperative length of ICU stay.

Statistical analysis

The prevalence of bleeding-related haematologic, laboratory and clinical risk factors in the two groups was compared by means of contingency tables of categorical variables and the two-sided Fisher exact test. Two sample *t* tests with equal or unequal variances were used to compare continuous variables with reasonably normal distribution of the original or transformed units, otherwise Kruskal-Wallis equality-of-populations rank test was used. Distributional differences were further analyzed with Graphic Box plot comparisons between groups. Multivariable stepwise forward and backward logistic regression of the bleeding event *vs* risk factors was made, with 0.05 significance limits to enter or retain. Confounding factors (age, weight and sex), bleeding related clinical risk factors (hypertension, renal failure, cerebrovascular events and treatment) and the variables enumerated above in the clotting profile paragraph were included. The event probability predicted from the logistic result was compared to the observed empiric probability, calculated on patients' deciles. A correlation analysis of laboratory ROTEM tests

Table 2 Preoperative hematologic and coagulation profile

	Group A ASA	Group B ASA + Clopidogrel	*P* (KW)
Hb (g/dL)	13.44 ± 0.2	13.42 ± 0.2	0.95
Hematocrit (%)	39.9 ± 0.6	39.3 ± 0.5	0.67
Platelet count (10^3/mm^3)	247 ± 8.6	245 ± 0.2	0.83
PT (%)	80.3 ± 1.6	80.5 ± 1.7	0.95
PTT (%)	32.2 ± 0.7	31.8 ± 0.8	0.67
CT-INTEM	153 ± 16	179 ± 15	0.87
CFT-INTEM	57 ± 4	63 ± 7	0.28
Alpha angle-INTEM	81 ± 10	75 ± 8	0.49
MCF-INTEM	65 ± 4	63 ± 5	0.51
AUC-INTEM	6543 ± 520	6320 ± 731	0.65
CT-EXTEM	55 ± 6	58 ± 7	0.52
CFT-EXTEM	96 ± 8	102 ± 9	0.51
Alpha angle-INTEM	71 ± 6	66 ± 5	0.33
MCF-EXTEM	65 ± 6	61 ± 9	0.057
AUC-EXTEM	6529 ± 643	6177 ± 978	0.056

ASA: Acetylsalycilic acid; PT: Prothrombin time; PTT: Partial thromboplastin time; CFT: Clot formation time; MCF: Maximum clot firmness; AUC: Area-under-curve; KW: Kruskal Wallis-test.

Table 3 Postoperative measures of bleeding and blood product transfusions

	Group A ASA	Group B ASA + Clopidogrel	*P*
Major bleeding	6 (12%)	8 (16%)	0.77 (*F-t*)
Re-intervention	1 (2%)	0 (0%)	1.0 (*F-t*)
Chest tube output (12 h)	509 ± 234	539 ± 239	0.41 (KW)
PRBCs (U)	1 ± 1	0.9 ± 0.9	0.87 (KW)
FFP (U)	0.5 ± 2	0.5 ± 1.9	0.99 (KW)
PLTs (U)	0.7 ± 2.7	1 ± 2.5	0.35 (KW)

ASA: Acetylsalycilic acid; FFP: Fresh frozen plasma; PRBCs: Packed red blood cells; PLT: Platelets.

was further performed to identify parameters closely related to the logistic result. All data were manipulated and analyzed using STATA (StataCorp LP, Texas, United States). The statistical review of the study was performed by a biomedical statistician.

RESULTS

Patient characteristics

The baseline characteristics of those with and without preoperative clopidogrel exposure were comparable in age, gender, weight and renal failure (Table 1). There was a significantly higher incidence of ventricular dysfunction (P = 0.02) in the clopidogrel group. The baseline classic coagulation parameters (hematocrit, platelet count, PT, aPTT) and ROTEM parameters (CT, CFT, MCF, alpha angle and AUC of INTEM and EXTEM) were also comparable between the groups (Table 2).

Surgical and clinical outcomes by anti-platelet therapy exposure

No statistically significant differences between the two groups in term of postoperative bleeding, blood product transfusions (Table 3) and clinical outcomes (Table 4) were observed.

Patients on double treatment showed a greater frequency of major bleeding (8 *vs* 6 episodes), greater mean chest tube output at 12 h (539 ± 239 mL *vs* 509 ± 234 mL), and mean number of blood products transfusions, although these differences were not statistically significant. One patient in the single treatment group required surgical re-intervention due to bleeding.

The length of stay in the ICU (days) and of mechanical ventilation (hours) were comparable between the groups. There was 1 death in group A and 0 in group B, 1 myocardial-infarction in group A and 0 in group B, 1 stroke in group A and 0 in group B. Therefore there was a greater number of major adverse events in the group treated with aspirin alone (3 events, 6%; OR = 0.75; P = 0.24) compared with the double treatment group (0 events, 0%).

Table 4 Clinical outcomes

	Group A ASA	Group B ASA + Clopidogrel	*P*
AMI	1 (2%)	0%	0.5 (*F-t*)
IABP	3 (6%)	2 (4%)	0.5 (*F-t*)
Stroke	1 (2%)	0 (0%)	0.5 (*F-t*)
Renal failure	1 (2%)	1 (2%)	1.0 (*F-t*)
AF	12 (24%)	7 (14%)	0.15 (*F-t*)
MV (h)	8 ± 11	6 ± 2	0.22 (KW)
ICU stay (d)	1.8 ± 3.3 (Med 1)	1.4 ± 0.9 (Med 1)	0.77 (KW)
Mortality	1 (2%)	0 (0%)	0.5 (*F-t*)
MACE (death, stroke. AMI)	3 (6%)	0 (0%)	0.24 (*F-t*)

F-t: Fisher's exact test; KW: Kruskal Wallis-test; ASA: Acetylsalycilic acid; AMI: Acute myocardial infarction; AF: Atrial fibrillation; MV: Mechanical ventilation; MACE: Major adverse cardiac events.

Table 5 Logistic multivariate analysis of factors related to bleeding risk

	Coefficient	OR	95%CI	*P*
Age	-0.14	0.87	0.73-1.03	0.11
Weight	-0.35	0.71	0.52-0.97	0.03
Gender (female)	-10.54	0.00003	0.06-3.9	0.04
EF	-0.02	0.98	0.83-1.16	0.81
AUC EXTEM	-0.0099	0.99	0.98-0.99	0.008
Aspirin only	-1.18	0.31	0.02-4.58	0.39

Odds ratios are adjusted for concomitant variables. EF: Ejection fraction; AUC: Area-under-curve.

Preoperative predictors of bleeding risk

From the comparison of the risk-factors for perioperative bleeding (advanced age, female gender, low weight and renal insufficiency), and preoperative haemocoagulatory status between all patients who bled (n = 14) and those who did not, no significant difference was noted.

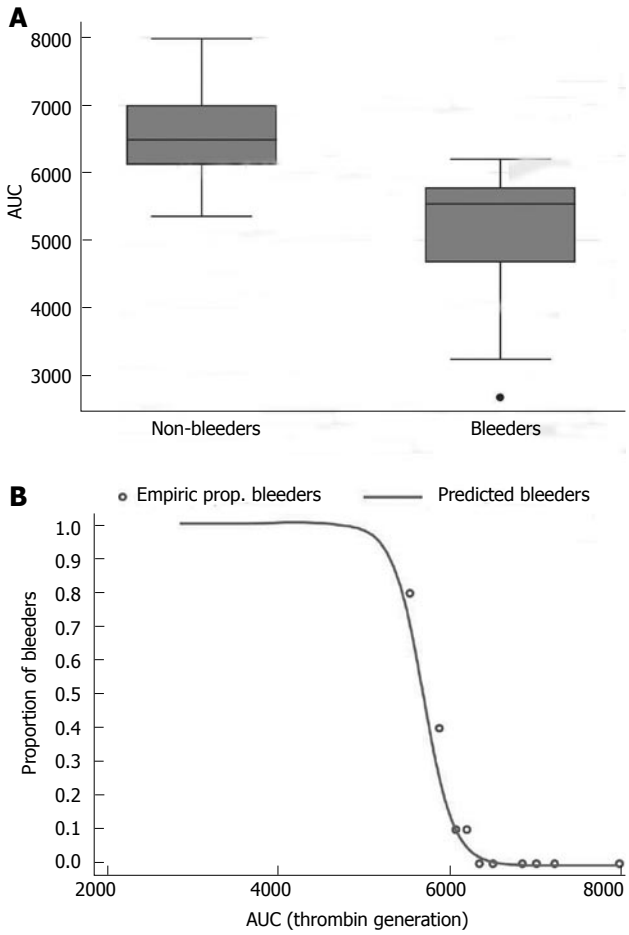

Figure 1 Predictors of bleeding risk. A: AUC-EXTEM in non-bleeders and in bleeders (patients with major bleeding) (P = 0.0001); B: Bleeding risk prediction and AUC-EXTEM: Each circle represents one decile of the patient population, showing the proportion of patients who bled compared with their mean AUC. The curve is a line of best fit, clearly showing a threshold at an AUC of 6000, below which the bleeding risk increases markedly. AUC: Area-under-curve.

After performing a logistic multivariate analysis (details in Table 5), the backward stepwise variable selection showed a significant independent relationship of bleeding risk only with AUC activity (coefficient –0.0099; OR = 0.99; 95%CI: 0.98-0.99; P < 0.001). In fact, patients who bled had lower AUC-value (Thrombin-generation-potential) in the EXTEM test (5030 ± 1115 Ohm*min) than those who did not bleed (6568 ± 548 Ohm*min) (P < 0.0001) (Figure 1A). We observed a good correlation (r = 0.96) between AUC and MCF, a clot quality indicator, that similarly was increased in patients who did not bleed (65.7 ± 5.6 mm) than those who did (51.9 ± 12.5 mm) (P < 0.0001), although these values were still within the normal range (50-72 mm). At this point we identified a threshold value for AUC and MCF which predicted bleeding in our patients, that is 6000 for AUC and 60 for MCF (Figure 1B). In fact, all patients with an AUC and MCF below these cut off values were at increased risk of major bleeding, and the lower these values the greater the risk. Furthermore patients with higher AUC and MCF values were at a lower risk of significant haemorrhagic events.

DISCUSSION

Post-CABG bleeding is a major issue in cardiac surgery and remains difficult to predict. Concerns about bleeding risk have resulted in the recommendation to cease double antiplatelet therapy prior to elective CABG[5]. There are still many patients who present for urgent CABG with double antiplatelet therapy, and increasingly patients will have been exposed to more potent antiplatelet agents such as prasugrel and ticagrelor[8-10]. Hence, we set out to determine if it was possible to predict post-operative bleeding with pre-operative clotting characteristics via analysis of classical coagulation assays (aPTT, PT) as well as a suite of ROTEM analyses. We also compared haemorrhagic and thrombotic complications between single and double treated patients.

Main findings

Our data showed a higher frequency of major bleeding in the double antiplatelet therapy group (16% *vs* 12%), but this was not statistically significant (P = 0.77). However, assuming that it represents a true difference, to attain a P value of 0.05 with a test power of 70% the sample size required is 978 patients on each arm. On the contrary the major adverse events (MACE: Death, stroke and myocardial infarct) were limited to group A (6%) (Table 4), but this difference was not statistically significant (P = 0.24).

Comparison of classic and ROTEM parameters between the 14 "bleeder" patients and the "non-bleeders", showed that differences were limited to AUC and MCF, in the EXTEM test. Patients with lower AUC and MCF values were at a higher risk of suffering major bleeding, regardless of whether they were treated with clopidogrel or not. MCF reveals the quality of the clot and is linked to thrombin generation and the change in AUC observed here is a reflection of the increase in MCF.

Clot generation is a composite process involving both primary and secondary haemostasis, as well as intrinsic and extrinsic pathways. Yet bleeding risk after CABG, the tendency has been to focus on the platelet, probably because of its major role in athero-thrombosis[15,16]. And the extensive experience with the use of antiplatelet agents in minimising further ischemic events. Nevertheless, despite receiving the same standard ACS medical treatment some patients bleed while others do not, and although this may be attributed to the recognized response variability (so called "resistance") observed with clopidogrel or aspirin[15], our results indicate that differences in patients' thrombin generation potential may be an important determinant of bleeding.

We believe that a broader perspective may be necessary: Platelets and platelet-inhibition play very important roles but their complicate interplay with other pieces of the haemostatic process should be taken into account. ROTEM is appropriate for providing such analyses, and unique in allowing evaluation of

every element of thrombosis and clot lysis[17], especially fibrinogen and factor XIII.

Indeed, we found that clot quality is a predictor of post-operative bleeding. We believe that association of MCF and AUC with haemorrhagic risk derives from the role of thrombin, which is fundamental as it affects every step of clot formation: It has a role in activation of fibrinogen to fibrin as the final common pathway from both intrinsic and extrinsic pathways, it activates factor XIII which promotes clot stability by cross linking fibrin polymers within the clot[18] and contributes to platelet activation, acting on at least three diverse platelet receptors[19]. We propose that in post surgical patients a high clot quality prior to surgery, as indicated by higher MCF and AUC values in the EXTEM test, can effectively clot despite the inhibition of platelet activation by aspirin and clopidogrel.

The mechanism by which patients with greater thrombin generation potential are able to form effective clots in the face of double antiplatelet therapy are probably twofold: (1) platelet activation is multifactorial, in addition to adenosine diphosphate (ADP) and thromboxane A2, thrombin also plays a critical role and is the most potent of the platelet activators[15], and patients who are better able to generate thrombin may thus sufficiently activate their platelets despite inhibition of the first two pathways by clopidogrel and aspirin respectively; and (2) patients with a higher MCF and hence higher thrombin generation may be able to affect greater thrombin deposition and stabilisation by factor XIII and hence form a stable clot despite decreased platelet activation and aggregation. In such circumstance the metaphor would be to a brick wall: Despite the presence of fewer effective bricks (platelets) in the presence of clopidogrel therapy, patients with a higher MCF can counterbalance by applying more higher-quality mortar (thrombin), thus producing a stable wall (clot).

Clinical implications

Besides characterizing MCF and AUC as factors influencing post-surgical bleeding, we identified a threshold at which the risk becomes significant: an AUC < 6000, corresponding to an MCF of < 60. This is important in two ways: (1) these values are within the normal range and yet the patients are at high risk of post-operative bleeding; and (2) identification of patients at risk may allow improved management in these patients.

While rotational thromboelastometry has been previously studied in relation to post CPB haemorrhage it has demonstrated mixed results. Published studies generally show that ROTEM parameters obtained intra-operatively or-post operative can predict post-operative excessive blood loss, but not those obtained prior to commencing bypass[20-23]. Our results differ from these as we have seen that ROTEM analysis performed prior to the institution of CPB can predict post-operative bleeding. This difference is difficult to explain, but may be related to the use of different ROTEM parameters, the high proportion of patients treated with clopidogrel in our cohort and differing definitions of major post-operative bleeding amongst these studies.

The capacity to recognize patients at high risk of post-operative bleeding may influence the decision to perform CABG in the first instance and further permit adjustment of surgical procedure and post-operative management to reduce the risk of haemorrhage in patients who are treated with CABG. As an example, patients at a high risk of bleeding may be treated with off pump CABG, avoiding CPB and hence lowering the risk of haemorrhagic events[24]. This is extremely important as CPB results in coagulopathy due to activation and consumption of platelets and coagulation factors, such as thrombin, which results in an increased risk of post-operative bleeding[25]. Other management options would include a more targeted use of blood product derived coagulation factor infusions, as well as the potential development of new pharmacological interventions involving replacement of individual clotting factors, such as recombinant activated factor VII, and factor XIII, prothrombin complexes and fibrinogen.

On the other hand, patients with higher thrombin generation potential may represent a subgroup more prone to develop ischemic rather than hemorrhagic complications (myocardial infarction, recurrent angina, stroke), thus deserving double antiplatelet therapy[26].

Study limitations

While these results are promising for the determination of risk of post-operative bleeding in CABG patients, the study does have several limitations. While AUC and MCF were seen to correlate well with bleeding risk in EXTEM analysis, INTEM analysis did not demonstrate such a relationship. This may result from the use of LWMH in all patients, a factor which affects the intrinsic pathway and hence INTEM analyses. Furthermore, ROTEM, while giving a good overall impression of a patient's haemostatic function, is not the ideal tool for the assessment of platelets, rather a platelet function analyzer would be the gold standard for such analyses[13], which would have allowed better determination of the contribution of antiplatelet response variability to bleeding risk in our cohort[27]. Additionally the sample size in this study was small and the patients were not randomised to the two treatment arms, thus limiting our ability to comment on the differential effect of single and double antiplatelet on postoperative haemorrhage risk. Further studies in groups not treated with LMWH, the use of platelet function analyser and greater sample size and randomization would allow greater understanding of the factors relating to bleeding risk identified in our study. Finally, we cannot generalize our results to newer antiplatelet agents such as prasugrel and ticagrelor.

In conclusion, we have seen that patients with a low AUC value (thrombin-generation-potential) are at a significantly higher risk of bleeding as compared to patients with higher AUC, regardless of whether they were treated with clopidogrel. Hence this study provides

a promising insight into the potential role of ROTEM analyses in the prediction of post-CABG bleeding risk; future research on this topic may contribute to a more effective intra- and post- operative management.

COMMENTS

Background

Post-operative bleeding is a major complication following coronary artery bypass grafting (CABG). Guidelines recommend ceasing clopidogrel therapy 5 d prior to CABG in order to minimise the risk of post-operative haemorrhage. However, patients presenting for urgent and emergent CABG are often treated with double antiplatelet therapy, as recommended in major guidelines for the emergency management of acute coronary syndrome, prior to performing the diagnostic angiogram. Ceasing clopidogrel and waiting the recommended 5 d prior to performing CABG would put these patients at higher risk of adverse coronary events.

Research frontiers

As yet there is no effective way to predict which patients will have significant bleeding after CABG. In fact, it would be important to be able to detect patients at risk of excessive bleeding, especially among those treated with double anti-platelet therapy.

Innovations and breakthroughs

While rotational thromboelastometry has been previously studied in relation to post cardiopulmonary-bypass haemorrhage it has demonstrated mixed results. Published studies generally show that rotational thromboelastometry (ROTEM) parameters obtained intra-operatively or-post operative can predict post-operative excessive blood loss, but not those obtained prior to commencing bypass. The authors devised the following study to determine whether bleeding risk could be predicted from the presence of double antiplatelet therapy, classical coagulation parameters or by rotational thrombo-elastometry performed prior to surgery. The authors found that there was no significant difference between rates of major bleeding between patients treated with aspirin alone, compared with those treated with aspirin and clopidogrel. In the cases of major bleeding, pre-operative classic coagulation and traditional ROTEM parameters were comparable. Conversely the authors observed that the area-under-curve in the EXTEM test was significantly lower in bleeders than non-bleeders, regardless of antiplatelet treatment. This suggests that thrombin generation potential, irrespective of the degree of platelet inhibition, correlates with surgical bleeding. Moreover, the authors were able to define a threshold at which bleeding risk becomes significant.

Applications

The ability to identify patients at high risk of post-operative bleeding is important as it may influence the decision to perform CABG in the first instance and further allow tailoring of surgical technique and post-operative management to reduce the risk of haemorrhage, and achieve a more targeted use of blood products. On the other hand, patients with higher thrombin generation potential may represent a subgroup more prone to develop ischemic rather than hemorrhagic complications, thus deserving double antiplatelet therapy.

Terminology

ROTEM consists in a viscoelastic method for hemostasis testing in whole blood, which can be used to detect clotting disorders and drug effects. Moreover, through appropriate assays, it can provide differential diagnostic information to support decisions in therapy.

Peer-review

This is an interesting manuscript about the predictive factors of bleeding after CABG in patients with previous acute coronary syndrome.

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CASE REPORT

Eggshell calcification of the heart in constrictive pericarditis

Rajesh Vijayvergiya, Ramalingam Vadivelu, Sachin Mahajan, Sandeep S Rana, Manphool Singhal

Rajesh Vijayvergiya, Ramalingam Vadivelu, Department of Cardiology, Advanced Cardiac Centre, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India

Sachin Mahajan, Sandeep S Rana, Department of Cardiac Surgery, Advanced Cardiac Centre, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India

Manphool Singhal, Department of Radiology, Advanced Cardiac Centre, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India

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Correspondence to: Dr. Rajesh Vijayvergiya, MD, DM, FSCAI, FISES, FACC, Professor, Department of Cardiology,Advanced Cardiac Centre, Post Graduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India. rajeshvijay999@hotmail.com
Telephone: +91-172-2756512
Fax: +91-172-2744401

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Abstract

Constrictive pericarditis (CP) is an inflammatory disease of pericardium. Pericardial calcification in X-ray provides a clue for the diagnosis of CP. An extensive "eggshell" type of calcification is rarely seen in CP. We hereby report a case of CP with eggshell calcification of pericardium, encircling whole of the heart. A need for multimodality imaging and hemodynamic assessment followed by surgical pericardiectomy is discussed.

Key words: Contrictive pericarditis; Calcification; Pericardiectomy; Right heart failure

Core tip: Idiopathic contrictive pericarditis (CP) with extensive pericardial calcification is rarely seen. We hereby report a case of CP with extensive "eggshell" calcification of heart, who presented with right heart failure. A need for multimodality imaging and hemodynamic assessment is discussed in the article.

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INTRODUCTION

Constrictive pericarditis (CP) is an inflammatory disease of pericardium. Its etiology includes infection,

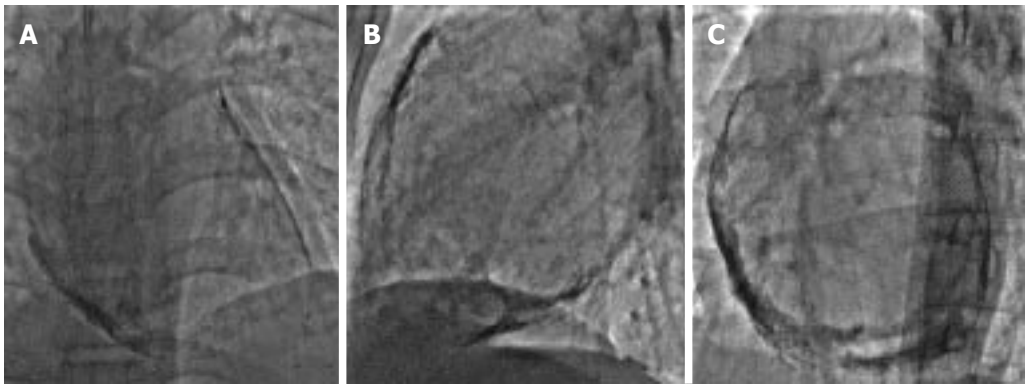

Figure 1 Fluoroscopy images in antero-posterior (A), lateral (B) and left anterior oblique 30º (C) views show circumferential pericardial calcification around the heart.

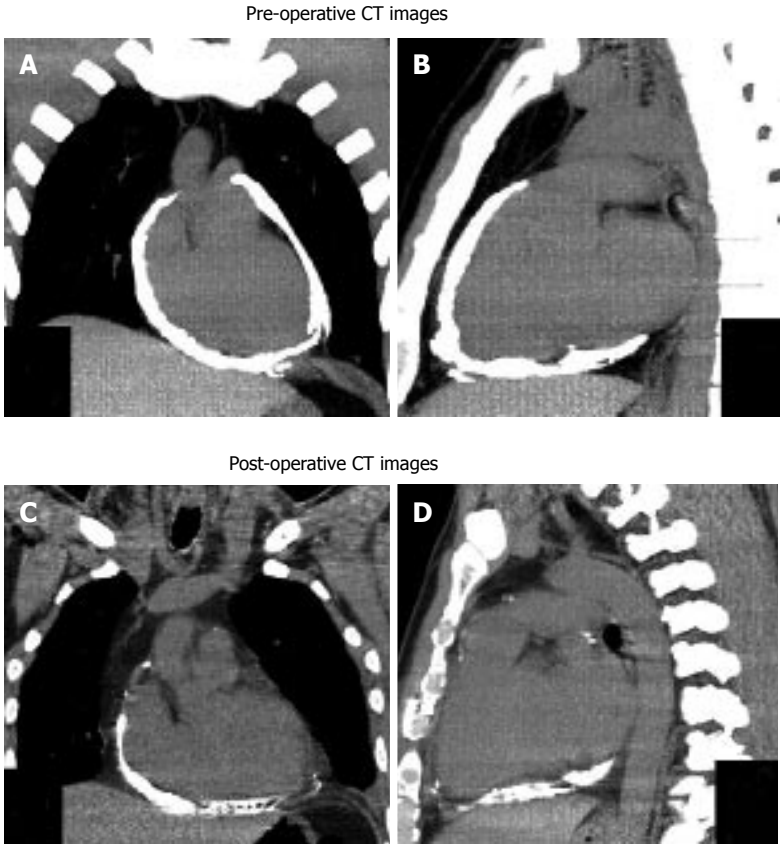

Figure 2 Computed tomography. Non-contrast computed tomography reconstructed images in coronal (A) and sagittal (B) planes show thick, calcified pericardium around the heart. A repeat CT following partial pericardiectomy in coronal (C) and sagittal (D) planes show residual calcified pericardium at right atrial and posterior surface of the heart. Calcified pericardium is absent along the antero-lateral surface of the heart. CT: Computed tomography.

connective tissue disorders, chest trauma, irradiation, post-cardiac surgery and idiopathic-type[1]. Extensive pericardial calcification is rarely seen in CP. We hereby report a case of CP presented with extensive "eggshell" like calcification of whole of pericardium.

CASE REPORT

A 43-year-old male presented with shortness of breath, NYHA class-Ⅲ of 6-mo duration. There was no history of fever, productive cough, joint pain, orthopnea and pedal edema. Clinical examination revealed a jugular venous pulse of 18 cm, prominent X and Y descents. Cardiac auscultation revealed a pericardial knock. Two-dimensional echocardiogram showed 10-mm thick, calcified pericardium; about 25%-variation in mitral diastolic flow velocities with respiration and a dilated inferior vena cava of 24-mm dimension. Fluoroscopy revealed dense circumferential calcification all around the heart (Figure 1). A computed tomography (CT) scan confirmed the circumferential thick pericardial calcification like an eggshell, encircling the heart (Figures 2 and 3). Angiography revealed normal epicardial coronaries without any external compression.

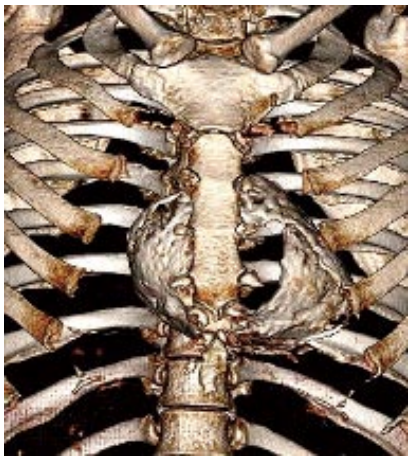

Figure 3 Pre-operative non-contrast computed tomography reconstructed volume rendered image shows pericardial "eggshell" calcification around the heart.

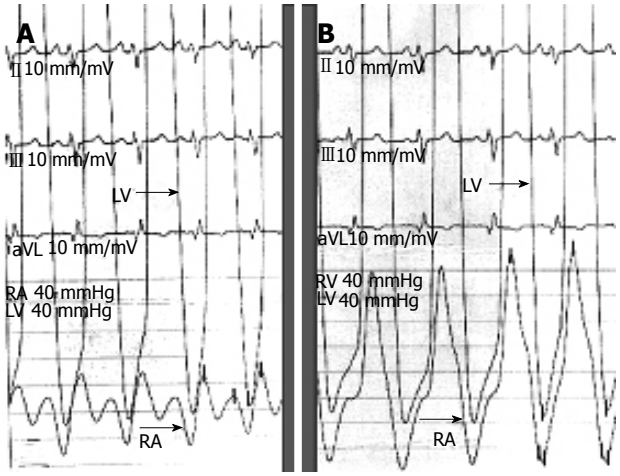

Figure 4 Hemodynamic tracing during catheterization. A: Right atrial (RA) pressure tracing shows prominent X and Y descent; B: Ventricular pressure tracing shows typical "dip-and-plateau configuration" during diastole. RV: Right ventricle; LV: Left ventricle.

Hemodynamic data revealed elevated mean right atrial (RA) pressure of 20 mmHg. Right ventricle (RV) and left ventricle (LV) end-diastolic pressure was 23 and 28 mmHg, respectively. Pulmonary artery systolic and mean pulmonary capillary wedge pressures were 44 and 24 mmHg, respectively. There was near equalization of elevated RA, RV and LV end-diastolic pressures. Right atrial pressure tracing showed prominent X and Y descent (Figure 4A), and ventricular pressure tracing showed typical "dip-and-plateau configuration" (Figure 4B) suggestive of CP. He had surgical pericardiectomy for densely calcified CP. Following median thoracotomy, thickened, calcified, firmly adherent pericardium was resected and excised from anterior and left lateral aspect of the heart (Figure 5). The densely adherent pericardium from surface of right atrium and posterior wall was not resected. Histopathology of pericardium revealed dystrophic calcification without any evidence of granulomatous or giant cell inflammation. Mycobacterial

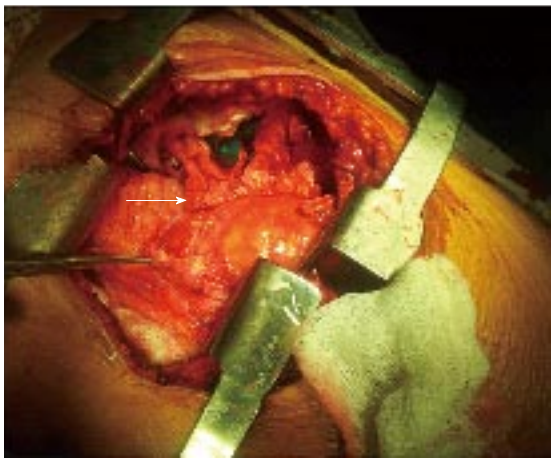

Figure 5 Surgical image shows resected thick and calcified pericardium (white arrow) from anterior surface of heart.

and fungal cultures of the excised pericardium were negative. He had uneventful recovery and was discharged on 7th post-operative day. A post–operative CT scan showed absence of calcified pericardium along antero-lateral surface of the heart (Figures 2C and D). He remained asymptomatic during 1-year follow-up.

DISCUSSION

Constrictive pericarditis is a sequale of chronic inflammation of pericardium. Among the various etiologies, idiopathic type is a common cause for CP[1,2], which was present in index case. The clinical presentation is mostly of right heart failure. Any patient presented with dyspnea, raised jugular venous pulse, hepatic and systemic venous congestion should be evaluated for CP. Pericardial calcification in chest X-ray can suggest CP, however it is present in only 5%-27% of cases[3]. Extensive "eggshell" like calcification is rarely seen in CP[4], as was present in index case. Echocardiography is an initial imaging test for diagnosis of CP[5]. In patients with equivocal echocardiography findings, CT or Cardiac Magnetic Resonance can confirm the diagnosis[5]. The extent of severe calcification and involvement of adjacent structures is clearly defined by CT, as demonstrated in index case. Cardiac catheterization is required for hemodynamic assessment and to rule out coronary compression by calcified thickened pericardium[6]. A multi-modality imaging and hemodynamic assessment is essential for proper evaluation of CP[5]. Surgical pericardiectomy is the definite treatment for CP. Those with calcified and firmly adherent pericardium, median thoracotomy is preferred over antero-lateral thoracotomy[7]. Calcified CP also has higher surgical risk, incomplete pericardial resection and poor hemodynamic outcomes following surgery[7]. However, index case had a successful outcome following partial antero-lateral pericardiectomy *via* median sternotomy approach. In conclusion, we present a case of idiopathic CP with extensive eggshell calcification of

the heart, who had successful surgical pericardiectomy, and had a favourable long term outcome.

COMMENTS

Case characteristics

A 43-years-old male presented with shortness of breath, New York Heart Association class-III of 6-mo duration.

Clinical diagnosis

Clinical examination revealed a raised jugular venous pulse with prominent X and Y descents.

Differential diagnosis

Cardiac auscultation revealed a pericardial knock. Two-dimensional echocardiogram showed 10-mm thick, calcified pericardium with significant trans-mitral diastolic flow velocities variation.

Imaging diagnosis

Fluoroscopy revealed dense circumferential calcification all around the heart. A computed tomography scan confirmed the circumferential thick pericardial calcification like an eggshell, encircling the heart. Cardiac catheterization revealed near equalization of elevated right atrial, right ventricle, and left ventricle end-diastolic pressures and ventricular pressure tracing showed typical "dip-and-plateau" pattern suggestive of constrictive pericarditis.

Pathological diagnosis

He had surgical pericardiectomy for densely calcified CP. Histopathology of pericardium revealed dystrophic calcification without any evidence of granulomatous or giant cell inflammation. An importance of multi-modality imaging with hemodynamic assessment is discussed in management of constrictive pericarditis.

Peer-review

This is an interesting article on calcified constrictive pericarditis. The paper is nicely documented.

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EDITORIAL

Intimal pericytes as the second line of immune defence in atherosclerosis

Ekaterina A Ivanova, Yuri V Bobryshev, Alexander N Orekhov

Ekaterina A Ivanova, Department of Development and Regeneration, Biomedical Sciences Group, KU Leuve, Leuven, Belgium

Yuri V Bobryshev, Faculty of Medicine, School of Medical Sciences, University of New South Wales, Kensington, Sydney NSW 2052, Australia

Yuri V Bobryshev, School of Medicine, University of Western Sydney, Campbelltown NSW 2560, Australia

Alexander N Orekhov, Laboratory of Angiopathology, Institute of General Pathology and Pathophysiology, Russian Academy of Sciences, 125315 Moscow, Russia

Alexander N Orekhov, Institute for Atherosclerosis Research, Skolkovo Innovative Center, 121609 Moscow, Russia

Alexander N Orekhov, Department of Biophysics, Faculty of Biology, Lomonosov Moscow State University, 119991 Moscow, Russia

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Correspondence to: Yuri V Bobryshev, PhD, Faculty of Medicine, School of Medical Sciences, University of New South Wales, High Street, Gate 9, Kensington, Sydney NSW 2052, Australia. y.bobryshev@unsw.edu.au
Telephone: +61-2-93851217
Fax: +61-2-93851217

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Abstract

Inflammation plays an essential role in the development of atherosclerosis. The initiation and growth of atherosclerotic plaques is accompanied by recruitment of inflammatory and precursor cells from the bloodstream and their differentiation towards pro-inflammatory phenotypes. This process is orchestrated by the production of a number of pro-inflammatory cytokines and chemokines. Human arterial intima consists of structurally distinct leaflets, with a proteoglycan-rich layer lying immediately below the endothelial lining. Recent studies reveal the important role of stellate pericyte-like cells (intimal pericytes) populating the proteoglycan-rich layer in the development of atherosclerosis. During the pathologic process, intimal pericytes may participate in the recruitment of inflammatory cells by producing signalling molecules and play a role in the antigen presentation. Intimal pericytes are also involved in lipid accumulation and the formation of foam cells. This review focuses on the role of pericyte-like cells in the development of atherosclerotic lesions.

Key words: Atherosclerosis; Arteries; Intima; Immune-inflammatory processes; Pericyte-like cells

Core tip: Intimal stellate cells, expressing smooth muscle α-actin that co-express antigen 3G5 antigen (known to be specific for pericytes), reside in the intima of human large arteries. There cells have been defined as "pericytes-like satellite cells" or "intimal pericytes". Because of the peculiarities of the distribution of these cells and because of the current lack of our knowledge about the spectrum of the expression of other pericyte-associated markers in the arterial wall, it is reasonable to avoiding at this time to identify smooth muscle α-actin(+)/3G5 antigen(+) stellate-shaped cells as true pericytes. The review highlights the importance of intimal pericytes in atherosclerosis.

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INTRODUCTION

Cardiovascular disorders (CVDs) are currently the main cause of mortality in developed countries, and atherosclerosis is the major contributor to the CVD risk[1]. The disease affects large arteries causing lipid deposition in the arterial wall, local inflammatory process and the formation of plaques. Atherosclerotic plaques progression leads to luminal narrowing of blood vessels, which by itself can result in the ischemia of corresponding organs. However, most dangerous is the acute thrombus formation on the surface of a ruptured atherosclerotic plaque. Thrombosis can lead to rapid and life-threatening events, such as acute coronary syndrome and stroke[2]. Early stages of atherosclerosis development are mostly clinically silent, and the first manifestations of the disease are often fatal. At the same time, subclinical atherosclerosis is a prevalent condition in the modern society, especially among the ageing population. It has been reported recently that up to 5% of women and 12% of men over the age of 80 suffer from asymptomatic atherosclerotic carotid artery stenosis[3]. Other studies demonstrated the high rate of asymptomatic atherosclerosis incidence in young and middle-aged people, with up to 100% of apparently healthy individuals having atherosclerotic lesions at various stages of progression[4,5]. Along with the well-known benefits of life style correction, the development of more specific therapies could help decreasing the risk of atherosclerosis progression and consequent CVD development. It is therefore important to improve our understanding of the pathological mechanisms that trigger atherosclerotic lesion development in the arterial wall.

The intimal barrier of human arterial wall consists of the endothelium and the subendothelial net-like tissue layer formed by pericyte-like cells that have recently been characterized as true pericytes[6,7]. Whereas the endothelium serves as the first line of defence against the development of atherosclerotic lesion, the pericyte-containing subendothelial layer can be regarded as the second defence line essential for normal functioning and protection of the arterial wall. Apart from pericytes, this layer contains other cell types, including smooth muscle cells, macrophages, lymphocytes, mast cells, dendritic cells, and other cell types. Pericyte-containing subendothelial layer is a characteristic component of both arterial and venous intima[6]. Its role in the development of human pathology remained unknown until recently. However, the accumulating evidence demonstrated its importance in many severe vascular disorders, such as saphenous vein graft disease, thrombosis and atherosclerosis. Macrovascular pericytes are capable of rapid proliferation and participate in immune reactions. Moreover, these cells have been identified as a source of coagulation-inducing tissue factor[7]. In this review we will summarize the current knowledge on the structure and function of the sub-endothelial leaflet of human macrovascular intima and its role in the pathogenesis of atherosclerosis.

STRUCTURE OF NORMAL AND ATHEROSCLEROTIC ARTERIAL INTIMA

Aortic intima is the part of the blood vessel wall located between the internal elastic lamina and the lumen with endothelial lining, and consists of two distinct layers (Figure 1)[8,9]. Muscular-elastic layer represents the external part of the intima, and is separated from the internal intimal layer by the limiting membrane. This layer is formed by longitudinally oriented elongated cells and elastic fibres. The internal intimal layer, also known as proteoglycan-rich or connective tissue layer[10,11], contains randomly-oriented connective tissue fibres and morphologically heterogeneous cell population[12]. The intimal layers differ from each other by the composition of glycosaminoglycans and fibres. Muscular-elastic layer is rich in elastin fibres, and proteoglycan-rich layer - in collagen and reticulin[13]. Moreover, collagen composition and structure are different between the two layers: longitudinally oriented collagens Ⅰ and Ⅲ are common in the muscular-elastic layer, while collagens Ⅳ and Ⅴ are detected primarily in the proteoglycan layer, where they form the endothelial basal membrane.

The development of atherosclerotic lesion has been carefully studied by our group on human aorta samples from healthy subjects and atherosclerotic patients[8-13]. Atherosclerotic process is tightly associated with thickening of the arterial intima. Careful analysis of the intima thickness along the arteries affected by atherosclerosis in comparison to normal tissues revealed that muscular-elastic layer remained unaltered in the fatty streak area and became only slightly thicker in the atherosclerotic plaque area[8-13]. By contrast, the proteoglycan layer was 2-fold thicker in the fatty streak and 4-fold - in

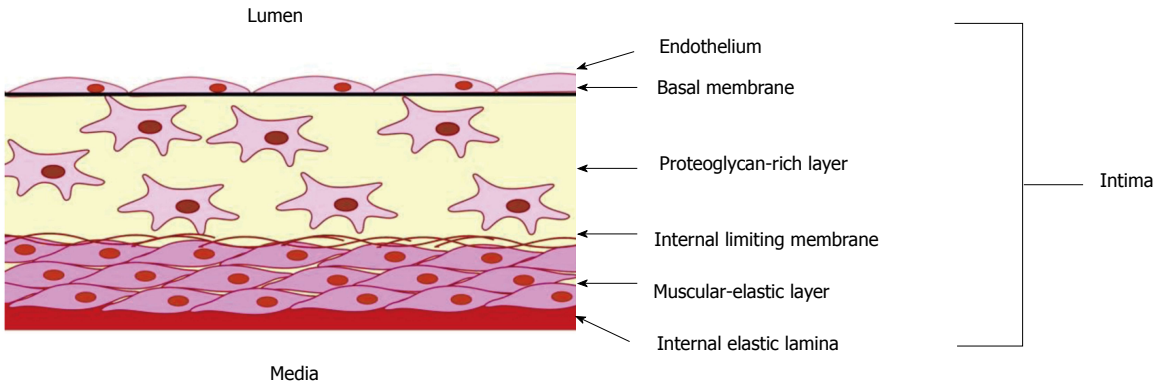

Figure 1 Schema showing the organization of the intima of the arterial wall. The proteoglycan-rich layer which contains a heterogeneous population of cells, including macrovascular pericytes, is located just below the endothelial monolayer. Intimal pericytes form a network of cells interconnected through gap junctions. The muscular-elastic layer, formed by elongated contractile smooth muscular cells, lies below the proteoglycan-rich layer.

the atherosclerotic lesion areas, and was the major contributor of the arterial stenosis. This thickened proteoglycan layer had increased collagen content. Moreover, longitudinal orientation of collagen fibres was altered, and lipid droplets were present between the interstitial collagen fibres[8-13]. Atherosclerotic plaques were characterized by the accumulation of all collagen types, especially in the fibrous cap area. Collagens Ⅳ and Ⅴ formed a thick layer in the endothelial basal membrane area and surrounded the subendothelial cells. At the same time, no prominent alterations of collagen structure and composition were present in the muscular-elastic layer of the intima[8-13].

Changes of lipid content accompany the development of atherosclerotic plaques in the aortic intima. In the normal tissue, extracellular lipid droplets visualised by Oil Red O staining were located along the elastic membrane. In the fatty streaks, both extra- and intracellular droplets were present, mostly in the proximity of the lumen. In atherosclerotic plaques, lipid deposition could be observed in the proteoglycan layer, and total lipid content was 8-fold higher than in the unaffected tissue[6]. Lipid content of muscular-elastic layer was increased to a lesser extent, being 4.4-fold higher than control. Cellular composition of the proteoglycan-rich layer of atherosclerotic intima was also altered in comparison with the healthy tissue[6]. Total cell count assessed by alcohol-alkaline dissociation of the fixed tissue, was 1.5 and 2-fold higher in the fatty streak and atherosclerotic plaque respectively[6].

Taken together, these observations clearly indicate that the subendothelial proteoglycan-rich layer of the arterial intima undergoes the most prominent alterations in course of the atherosclerotic plaque development, including thickening, lipid deposition and the accumulation of collagen fibres with disturbed orientation[6,8-13].

CELLULAR COMPOSITION OF MACROVASCULAR INTIMA

Human macrovascular intima is made of a heterogeneous cell population. Immediately below the endothelial lining and in close contact with it, there is a three-dimensional net formed by pericyte-like cells. Early studies have identified pericytes as mostly microvascular cells that play an important role in angiogenesis and vessel branching and are essential for maintaining the normal structure of the capillary wall and the endothelial barrier function, including the maintenance the blood-brain barrier[14,15]. Pericytes were also demonstrated to contribute to embryonic development of the aorta[16]. It has become evident, however, that pericyte-like cells are also present in the walls of large blood vessels[17]. Apart from vasa vasorum microvessels[18], pericyte-like stellate cells were found in the intima of large arteries[6,7,19-21].

Accurate identification of pericytes, as well as their characterisation in *in vitro* studies, remains challenging, since these cells have flexible phenotype. The expression of numerous pericyte marker proteins is highly dynamic and depends on the surrounding milieu. Moreover, many marker proteins are shared between pericytes and vascular smooth muscle cells (VSMCs)[14]. One of such markers is the α-smooth muscle actin (αSMA), which is normally expressed in VSMCs, but is also common in pericytes. However, its expression in the latter cell type can vary depending on the surrounding milieu[22]. For instance, pericytes from non-contractile capillaries can be αSMA-negative[23]. The expression of αSMA in pericytes can also be regulated by cell maturity[23] or stimulation by endothelin-1[24]. These considerations have to be taken into account while analyzing the cell population of macrovesicular intima. In the intima of large human arteries, the majority of resident cells were found to be αSMA-positive, especially in the muscular-elastic layer, where this population reached two thirds of the total amount of cells[25]. In the proteoglycan layer, the percentage of αSMA-positive cells was much lower. These cells were different from typical VSMCs, were less densely packed and had a characteristic branched morphology, with long processes forming intracellular contacts. Moreover, some αSMA-positive intimal cells expressed marker proteins that were unusual for muscular cells,

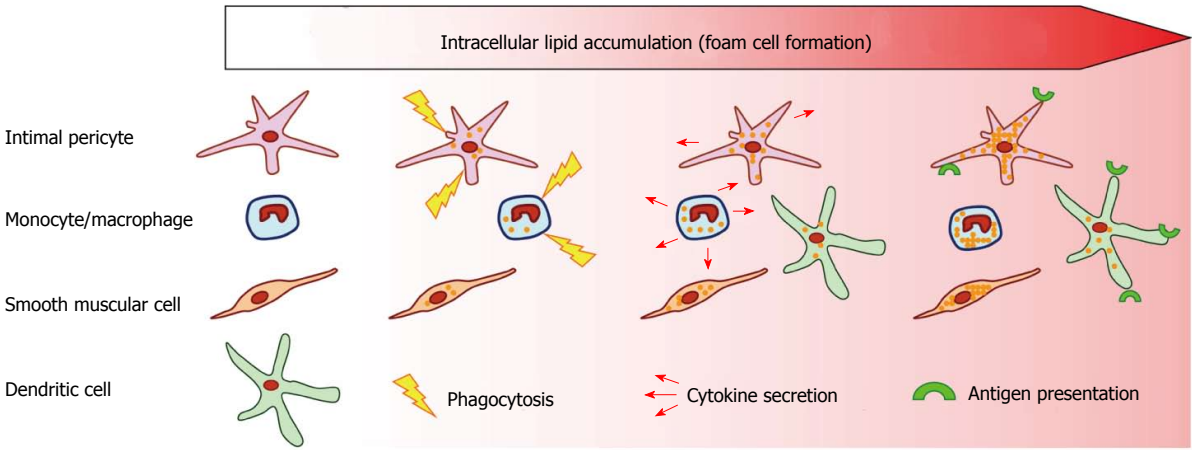

Figure 2 Scheme showing the roles of arterial wall cells in atherogenesis. Several types of arterial wall cells participate in lipid accumulation and foam cell formation. Macrophages and intimal pericytes accumulate lipids through phagocytosis and participate in the local inflammatory process secreting pro-inflammatory cytokines. Dendritic cells, together with macrophages and intimal pericytes express antigen-presenting complexes, further promoting the inflammatory process.

such as CD68, which is considered a macrophage-specific antigen[26]. Interestingly, the proportion of αSMA+CD68+ cells increased in atherosclerotic lesions, as well as in primary cell culture exposed to atherogenic modified low-density lipoprotein (LDL). Apparently, the acquisition of the "macrophage"-specific marker reflects the engagement of cells into phagocytic activity[27,28]. The large percentage of αSMA-negative cells in the arterial intima can partly be explained by the loss of contractile apparatus, since these cells acquire other functions than maintaining the vascular tone, such as metabolic homeostasis and nutrition or participation in the immune response in the blood vessel wall[29].

Therefore, accurate identification of pericytes in the intimal layer should not be based on αSMA expression exclusively, and has to include the analysis of other pericyte markers expression and morphological data. Commonly used pericyte markers include platelet-derived growth factor receptor β, CD146, aminopeptidase A and N (CD13), endoglin, neuron-glial 2, non-muscle myosin, desmin, vimentin and nestin[14,30]. However, most of these markers are shared with VSMCs and/or dependent on pericyte maturity or activation state, and therefore should be used in combination to avoid false-positive results. It has been demonstrated that some antigens expressed in resident intimal cells are not present in VSMCs. Antigen 3G5 is an O-sialoganglioside specific for microvascular pericytes[31]. It was found in some intimal resident cells, but not in the tunica media[26,32]. In humans, 3G5-positive pericytes form the net-like subendothelial tissue layer in normal human arterial intima and account for approximately one third of intimal cells (Figure 2)[26]. Another pericyte antigen detected in the macrovascular intima is 2A7, also known as melanoma chondroitin sulphate proteoglycan[26,33]. The expression of this antigen is typical for activated pericytes in the active angiogenesis phase. The expression of these pericyte marker antigens alters in atherogenesis. As demonstrated on primary cultures of subendothelial cells exposed to atherogenic modified LDL, intracellular lipid deposition results in the reduction of 3G5-positive cell fraction, whereas 2A7-expressing fraction increases[33].

Studies of the primary cell culture obtained from human macrovascular intima helped revealing the mechanisms of pericyte acquisition of characteristic stellate shape. Pericyte arborisation could be induced by increasing the intracellular cAMP concentration[33]. Interestingly, cells originating from the proteoglycan layer were more susceptible of arborisation, with up to 100% of them being capable of acquiring the stellate shape, whereas in population originating from the muscular-elastic layer it could be induced in only 50% of cells. The arborisation was associated with re-distribution of connexion 43 (Cx43), which is responsible for gap junction formation. Stellate cells formed large Cx43-positive sites located at the ends of cellular processes, where intracellular contacts were formed. *In vivo*, pericytes form stable contacts with the endothelial cells, which could also be reproduced in co-culture of the two cell types[7].

Apart from pericytes, other cell types populating the arterial intima have been described. These include cells of hematogenous lineage - macrophages[34-36], lymphocytes[37,38], mast cells[39] and dendritic cells. Cells of hematogenous lineage are located exclusively in the subendothelial proteoglycan layer of intima and are enriched in atherosclerotic plaques, where they can account for up to 20% of total cell population.

ROLE OF PERICYTE-LIKE CELLS (INTIMAL PERICYTES) IN THE PATHOGENESIS OF ATHEROSCLEROSIS

According to current consensus, pericytes are pluripotent cells that can serve as progenitors for other cell types of

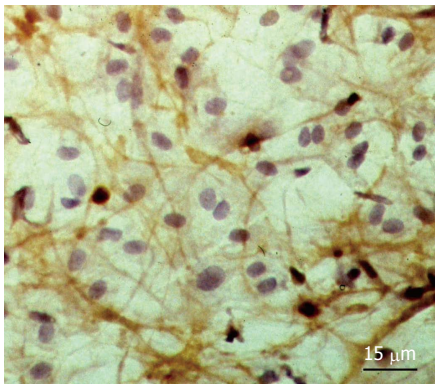

Figure 3 Cellular network formed by 3G5+ cells located under the luminal endothelium in undiseased intima. This cellular network was identified by means of the application of peroxidase-anti-peroxidase immunohistochemical analysis (brown colour of reaction product) in en face preparation of a tissue specimen of the human aorta. Counterstain with haematoxylin.

mesenchymal origin, including smooth muscle cells[40], fibroblasts, osteoblasts[41,42], chondrocytes[40] and adipocytes[43]. Pericytes are actively involved in various conditions associated with impaired microcirculation, such as diabetes, inflammation, wound healing and tumor growth. Converging evidence indicates pericyte implication in the pathogenesis of atherosclerosis. In presence of proinflammatory microenvironment and atherogenic modified LDL typical for early stages of atherosclerosis, intimal pericytes can accumulate lipids and become activated. The development of atherosclerotic plaque is associated with the increase of total cell count and the percentage of pericytes indicative of their involvement into the pathological process[21]. In fatty streaks, these cells actively accumulate lipids, which leads to the increase of cell size, acquisition of irregular shape, loss of Cx43-mediated cell contacts[44] and disturbance of the net-like subendothelial tissue[45]. Pericytes may not only proliferate and store lipids contributing to the plaque growth[46,47], but also promote thrombogenesis being a source of tissue factor[17]. Figure 3 depicts the impact of different arterial cells, including intimal pericytes, in the atherosclerotic process.

Microvascular pericytes are important for atherosclerosis progression, as they participate in neovascularization of the atherosclerotic plaques[48]. The recruitment of pericytes to growing neovessels could potentially be mediated by T-cadherin signalling. T-cadherin is an unusual member of the cadherin family, which is up-regulated in atherosclerosis[49]. It might play a role during LDL-induced pericyte activation through ERK1/2-nuclear factor κB signalling pathway[50]. It has been demonstrated that pericytes could also be recruited to neovessels in atherosclerotic plaques through hepatocyte growth factor signalling triggering c-Met-PI3K/Akt pathway in pericytes[51].

Participation in vascular wall remodelling and calcification is another possible contribution of pericytes to the pathological process in atherosclerosis. Activation of Wnt/β-catenin signalling stimulates chondrogenic differentiation of pericytes[52], which can be enhanced by transforming growth factor-β3[53]. The latter is abundantly produced by macrophages, foam cells and VSMCs in the atherosclerotic plaque[54]. Intimal pericytes were shown to express vascular calcification-associated factor. Moreover, pro-inflammatory environment in the plaque may promote osteogenic differentiation of the resident vascular cells. These observations indicate the potential involvement of pericytes in ectopic vascular calcification associated with atherosclerosis[55,56].

Taken together, the presented observations strongly support the hypothesis that macrovascular pericytes play an important role in all stages of the atherosclerotic lesion development, which makes them an attractive potential target for therapy development[7,57]. Interestingly, pericytes may also take part in the innate immunity reactions, which will be discussed in the next section.

PERICYTE-LIKE CELLS IN INNATE IMMUNITY

The development of atherosclerotic lesion is tightly associated with local inflammatory process. At the initial stages of atherosclerosis, formation of focal intimal thickening is accompanied by the increase of intimal cell count[58-60]. Such increase can be explained by the enhanced proliferation of intimal cells, recruitment of circulating hematologous cells or a combination of these processes. Cell proliferation has been demonstrated to be important for atherosclerotic process[61], although the differences in cell composition depending on different types of large arteries remained unknown. Detailed study of cell count and proliferation was performed on 29 post-mortem specimens of human carotid and coronary arteries with different stages of atherosclerotic plaque development: diffuse intimal thickening (grossly normal tissue), initial lesions, fatty streaks, lipofibrous plaques and fibrous plaques[62]. Proliferating cells in S phase were identified by proliferating cell nuclear antigen (PCNA) staining. The authors report the increased total cell count in atherosclerotic lesions and more frequent macrophages and lymphocytes in comparison with normal tissue. Number of proliferating cells was also increased in atherosclerotic plaques as compared to normal intima. Maximal total cell count and maximal amount of proliferating cells were detected in lipofibrous plaques in both types of arteries. Interestingly, the proliferative index (determined as the percentage of PCNA-positive cells in the total subpopulation) hematogenous cells in atherosclerotic lesions was lower than in the unaffected intima. It is therefore likely that the increase of hematogenous cells in atherosclerotic intima occurs due to immune cell recruitment from the bloodstream and not from the local proliferation[63-65]. By contrast, proliferative index of resident cells was higher in atherosclerotic lesions than in unaffected tissue, reaching its maximum in lipofibrous plaques[62].

It is well documented that modified atherogenic LDL

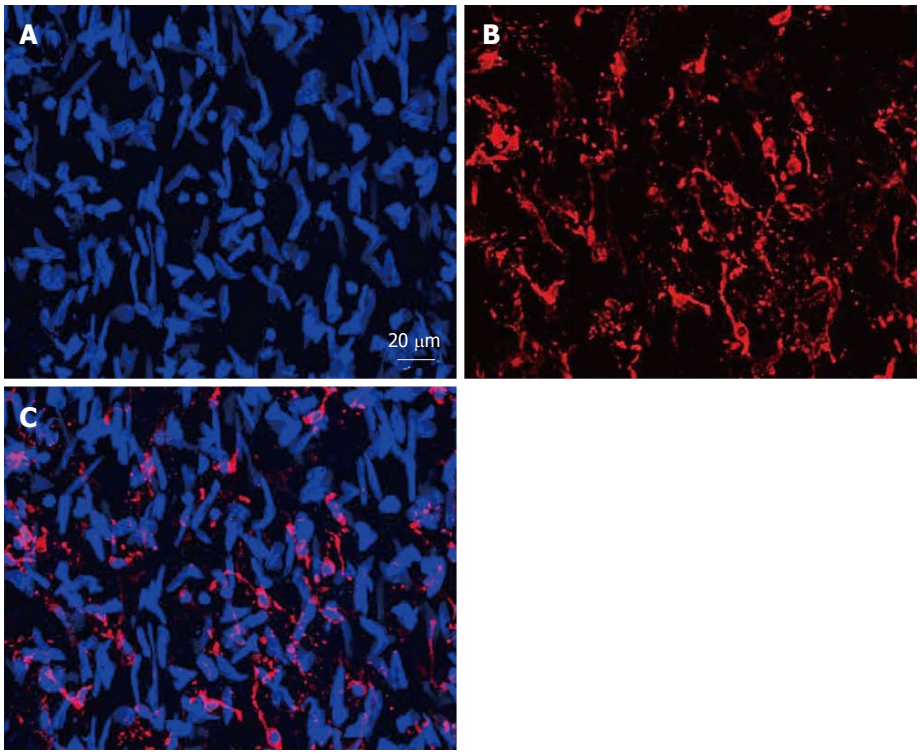

Figure 4 Immunofluorescent images representing the merge of 45 optic "confocal" sections of the intima obtained with interval of 1 μm between consecutive sections (A and B). A: Nuclei were visualized by staining with ethidium bromide; B: Distribution and networks formed by HLA-DR+ cells, detected with the use of anti-HLA-DR antibody conjugated with Alexa 633; C: A merge of the images shown in (A) and (B). Human aorta specimen. Reproduced from Bobryshev *et al*[74], with permission from Elsevier. HLA: Human leukocyte antigen.

not only causes lipid deposition in the arterial intima, but also initiates pro-inflammatory conditions, stimulating both adaptive and innate immunity[66,67]. During the atherosclerotic plaque formation, inflammatory cells are migrating into the lesion zone, where they release proinflammatory cytokines and chemokines, inducing proliferation of the resident intimal cells[65,68]. This model may explain the observed divergence of inflammatory and resident cells proliferative index dynamics in atherosclerotic lesions. Study of immune-inflammatory processes in diffuse intimal thickening demonstrated the link between lipid deposition in the arterial wall and the immune-inflammatory cell content[69]. Lipid deposition in the proteoglycan-rich layer of intima positively correlated with the expression of major histocompatibility complex class Ⅱ (MHC Ⅱ) molecule HLA-DR[70] and the amount of immune-inflammatory cells in that area. These findings demonstrate that lipid accumulation at the early stages of atherosclerotic lesion development is accompanied by local immune activation. HLA-DR is a marker of antigen presenting cells (APCs)[70,71] that include macrophages, B cells and dendritic cells. The latter were described as professional APCs, as they play the most prominent role in antigen presentation and initiation of the immune response. Apart from these cell types of hematogenous origin, some non-hematogenous cells can serve as APCs. For instance, epithelial cells of the thymus are involved in antigen presentation[71]. Human endothelial cells are capable of T cell activation and basally express both MHCI and MHC Ⅱ, although the expression is less prominent in larger arteries[72]. Moreover, fibroblasts and endothelial cells can become activated to express MHC Ⅱ under certain conditions both *in vitro* and *in vivo*[70,73].

Immunofluorescent analysis of HLA-DR expression in the aortic intima revealed a large population of interconnected HLA-DR-positive cells that formed a net-like tissue (Figure 4)[74]. In some segments of the intima up to 15% of total cell population was expressing HLA-DR[74]. Morphologically, these cells could be identified as pericytes, with their typical net-forming intercellular contacts.

These results indicate that antigen presentation in the arterial wall can be performed by a variety of intimal cell types, including macrovascular pericytes. Importantly, microscopic study has revealed some HLA-DR-positive cells that also contained apo-B in the perinuclear space, possibly representing the early events of lipid accumulation in the intimal cells[74]. It is therefore possible that pericytes play a double role in atherogenesis, participating both in lipid accumulation and in local immune reaction.

Immunohistochemistry demonstrated the expression of tumor necrosis factor (TNF)-α and CCL18 cytokines in both normal and atherosclerotic human aorta. These two cytokines were expressed by distinct populations of cells, with CCL18 mostly located in the subendothelial layer, and TNF-α - in deeper layers of the unaffected intima. The expression pattern was altered in atherosclerotic plaques, with both TNF-α and CCL18 upregulated compared to grossly normal arterial intima. Apart from macrophages, other cytokine-producing cells types were detected, including stellate pericyte-like cells.

The immune functions of pericytes remain an attractive topic for future investigation[75]. Microvascular placental pericytes were demonstrated to interact with $CD4^+$ T cells in co-culture. Pericytes stimulated the expression of CD25 and CD69 in resting allogenic $CD4^+$

T cells, indicative of MHC recognition. This, however, did not lead to the induction of cytokine production and proliferation of T cells[76]. The possibility that macrovascular pericytes have immune-modulating properties remains to be explored. The three-dimensional network of subendothelial cells participating in immune reactions may play important protective functions in the blood vessel wall. As demonstrated on the biopsy material from arteries, unaffected by atherosclerosis or with different stages of atherosclerotic lesion development, changes in this network occur early in the pathological process.

OTHER CELL TYPES PARTICIPATING IN IMMUNE REACTIONS IN THE SUBENDOTHELIAL INTIMA

Cells of hematogenous origin actively participate in immune reactions in the blood vessel wall. The development of atherosclerotic lesion is accompanied by infiltration and population of the lesion site by lymphocytes and macrophages. In human atherosclerotic lesions, the population of T cells consists mostly of the effector memory T cells. Substantial part of them are $CD4^+$ T helper cells[77]. $CD8^+$ cytotoxic T lymphocytes are also present in atherosclerotic lesions. T cells are especially numerous in so called vulnerable plaques that are susceptible to rupture and thrombosis initiation[78]. B cells have also been demonstrated to participate in atherogenesis[79,80]. However, these cells are mostly found in the adventitia of the affected vessels, and not in the atherosclerotic plaques[77,81].

Pro-inflammatory stimuli in blood vessels affected by atherosclerosis attract circulating monocytes that rapidly migrate to the atherosclerotic lesion site and differentiate where, predominantly to macrophages. This polarization is driven by stimulation of specific receptors, including C-C chemokine receptor type 2 and CX3C chemokine receptor 1[82,83]. Both of these chemokines are implicated in the atherosclerotic plaque progression[84]. Macrophages play a prominent role in the inflammatory process associated with the initiation and progression of atherosclerotic lesions. Macrophages are involved in the uptake of atherogenic modified LDL and form typical foam cells. Toll-like receptor (TLR) signalling in macrophages plays important role in lipid accumulation through regulation of scavenger receptor expression and suppression of cholesterol efflux[85,86]. Macrophages also produce a number of inflammatory cytokines and contribute to vascular remodelling.

In atherosclerotic lesions, two distinct macrophage subpopulations can be found: M1 (classical) and M2 (alternative). Pro-inflammatory M1 phenotype can be induced in response to bacterial infection and pro-inflammatory cytokines, such as interferon (IFN)-γ and TNF-α[87]. They produce a spectrum of cytokines and chemokines stimulating the inflammatory response, as well as nitric oxide and reactive oxygen species. M2 macrophages have anti-inflammatory properties, producing interleukin (IL)-10. In addition, M2 subpopulation of macrophages is heterogeneous: M2a macrophages are induced in response to IL-4 and IL-13 and participate in tissue remodelling, M2b cells are involved in immunoregulation, and M2c, induced by IL-10 and transforming growth factor-β, are involved in clearance of apoptotic cells[88,89]. All three subtypes of M2 macrophages can be found in human atherosclerotic lesions. Human-specific M4 macrophages can be induced by platelet-derived chemokine CXCL4 and are characterized by resistance to excessive lipid uptake. This makes them susceptible to rapid transformation into foam cells and indicates their potential role in atherosclerotic lesion development[90]. Specific populations of macrophages can be found in hemorrhagic atherosclerotic plaques. In humans, they represent M(Hb), HA-mac and Mhem subsets. HA-mac and M(Hb) macrophages are involved in hemoglobine clearance[91]. All three subpopulations possess anti-inflammatory properties and can play a protective role in atherosclerosis.

Dendritic cells are also frequent in the subendothelial layer of intima, just below the liminal endothelium, where they contribute up to 10% of total cell population[74,92,93]. In the subendothelial space, dendritic cells form cellular networks by means of their long interconnected processes[74,92,93]. Dendritic cells play an important role in the immune response, producing a spectrum of cytokines and surface co-stimulatory molecules. They can be differentiated from monocytes at the periphery through stimulation with granulocyte-macrophage colony-stimulating factor (GM-CSF) and TLR4 ligands[94]. These monocyte-derived dendritic cells are involved in antigen presentation and cross-presentation[95]. It has been demonstrated that monocyte-derived DCs from patients with atherosclerosis had increased sensitivity to GM-CSF and IL-4 as compared to healthy subjects[96]. Pro-inflammatory microenvironment of the atherosclerotic plaque accounts for preferential differentiation of monocytes and dendritic cell precursors towards pro-inflammatory dendritic cells that play an important role in the inflammatory process and stimulate differentiation of the naïve T cells to Th1 and Th17 phenotypes[97]. Dendritic cells also produce pro-inflammatory IFN-α and a number of chemokines that induce the migration of inflammatory cells to the lesion site[98]. In summary, pro-inflammatory dendritic cells play a prominent role in the development of atherosclerotic plaque. Populations of atheroprotective tolerogenic and anti-inflammatory dendritic cells could also be found in atherosclerotic plaques. In Apo-E-deficient mice, tolerogenic dendritic cells generated in response to immunogenic bacterial peptides were demonstrated to decrease inflammation and contribute to the plaque decrease through induction of IL-10-producing Tregs[99]. It is possible that employing the

pathways of tolerogenic dendritic cell induction can be beneficial for treatment of atherosclerosis.

CONCLUSION

The subendothelial layer of human arterial intima contains a cellular network made of pluripotent pericytes that form multiple intracellular contacts through gap junctions. Together with the professional immune cells, such as macrophages and dendritic cells, pericytes may execute functions, typical for innate immune cell types: participate in LDL trapping by phagocytosis, produce pro- and anti-inflammatory cytokines and other factors and present antigens (Figure 2). Whereas the aortic endothelium plays a role as the first line of defence in atherosclerosis development, the subendothelial pericytes can be regarded as the second defence line and should be considered for the development therapeutic approaches for atherosclerosis treatment.

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FRONTIER

Cost-effectiveness modelling of percutaneous coronary interventions in stable coronary artery disease

Ariel Beresniak, Thibaut Caruba, Brigitte Sabatier, Yves Juillière, Olivier Dubourg, Nicolas Danchin

Ariel Beresniak, Data Mining International, Research and Development, CH-1215 Geneva, Switzerland

Ariel Beresniak, Paris-Descartes University, LIRAES, 75006 Paris, France

Thibaut Caruba, Brigitte Sabatier, Department of Pharmacy, Hôpital Européen Georges Pompidou, 75015 Paris, France

Yves Juillière, Department of Cardiology, Institut Lorrain du Coeur et des Vaisseaux, 54500 Vandœuvre-lès-Nancy, France

Olivier Dubourg, Department of Cardiology, Hôpital Ambroise Paré, 92100 Boulogne, France

Olivier Dubourg, UFR des Sciences de la Santé Simone Veil, Université Versailles Saint Quentin en Yvelines, 78000 Versailles, France

Nicolas Danchin, Division of Coronary Artery Disease and Intensive Cardiac Care, Hôpital Européen Georges Pompidou, 75015 Paris, France

Author contributions: Beresniak A developed the cost-effectiveness model; Caruba T and Sabatier B carried out the literature review and performed the cost study; Juillière Y, Dubourg O and Danchin N contributed to design the structure of the model and validated the model assumptions; all the authors contributed to review the manuscript.

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Correspondence to: Ariel Beresniak, MD, MPH, PhD, Data Mining International, Research and Development, Route de l'Aéroport, 29-31, CP221, CH-1215 Geneva, Switzerland. aberesniak@datamining-international.com
Telephone: +41-22-7993400
Fax: +41-22-7883850

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Abstract

The objective of this study is to develop a cost-effectiveness model comparing drug eluting stents (DES) *vs* bare metal stent (BMS) in patients suffering of stable coronary artery disease. Using a 2-years time horizon, two simulation models have been developed: BMS first line strategy and DES first line strategy. Direct medical costs were estimated considering ambulatory and hospital costs. The effectiveness endpoint was defined as treatment success, which is the absence of major adverse cardiac events. Probabilistic sensitivity analyses were carried out using 10000 Monte-Carlo simulations. DES appeared slightly more efficacious over 2 years (60% of success) when compared to BMS (58% of success). Total costs over 2 years were estimated at 9303 € for the DES and at 8926 € for bare metal stent. Hence, corresponding mean cost-effectiveness ratios showed slightly lower costs ($P < 0.05$) per success for the BMS strategy (15520 €/success), as compared to the DES strategy (15588 €/success). Incremental cost-effectiveness ratio is 18850 € for one additional percent of success. The sequential strategy including BMS as the first option appears to be slightly less efficacious but more cost-effective compared to the strategy including DES as first option. Future modelling approaches should confirm these results as further comparative data in

stable coronary artery disease and long-term evidence become available.

Key words: Cost-effectiveness; Percutaneous coronary; Coronary artery disease; Drug eluting stent

Core tip: The objective of this study is to develop a robust cost-effectiveness model comparing drug eluting stents (DES) *vs* bare metal stent (BMS) in patients suffering of stable coronary artery disease. DES appeared slightly more efficacious over 2 years (60% of success) when compared to BMS (58% of success). Mean cost-effectiveness ratios showed slightly lower costs per success for the BMS strategy (15520 €/success), as compared to the DES strategy (15588 €/success). The sequential strategy including BMS as the first option appears to be less efficacious but more cost-effective compared to the strategy including DES as first option.

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INTRODUCTION

Coronary heart disease is an important disorder in Western industrialized societies, with regard to both the epidemiologic and economic burden of illness[1]. Stable angina (SA) is a clinical syndrome subset of the acute coronary artery disease (CAD), which is a major cause of emergency medical care in developed countries. The prognosis of SA is highly variable and depends on the initial treatment strategy which could be invasive (surgical procedure) or conservative (medical management). Angiographic and angioscopic studies suggest that CAD often results from the disruption of an atherosclerotic plaque and a subsequent cascade of pathological processes that decrease coronary blood flow. A modern therapeutic strategy consists of coronary interventions and the implantation of drug-eluting vascular stents. The idea that devices could be placed inside the arteries to maintain the blood flow came to a reality in 1986 when the first stents were successfully implanted in coronary arteries[2,3]. The technology evolved rapidly even if the incidence of in-stent restenosis was between 20% and 30%[4]. Then different generations of Drug Eluting Stents (DES) from heparin coated Palmaz-Schatz stents[5] to chemotherapeutic releasing agent or copolymer coating have been proposed to lower the incidence of restenosis. Because of their high efficacy and good safety profile, DES is reported to be used in 45% to 80% of all percutaneous coronary interventions[6,7]. However, clinical evidence of medical devices is not really supported by robust randomized control clinical trials such as for pharmaceutical agents. Furthermore, cost-effectiveness of such strategies is rarely fully documented and based on numerous assumptions, making difficult the full evaluation of such strategies. Recent studies have continued to show improved procedural and clinical outcomes with DES both in the setting of acute coronary syndromes and stable coronary artery disease[8]. A recent meta-analysis published by Palmerini *et al*[9] analyzed twenty-two trials involving a total of 12453 patients and established that at one year DES were associated with lower rates of cardiac death or myocardial infarction and stent thrombosis than bare metal stents (BMS). Peterson *et al*[10] studied the medical costs and outcomes of coronary stenting *vs* simple balloon angioplasty and estimated that the mean in-hospital cost for stent patients was $3268 higher than for those receiving coronary angioplasty ($14802 *vs* $11534, $P < 0.001$). However, stent patients were less likely to be re-hospitalized (22% *vs* 34%, $P = 0.002$) or to undergo repeat revascularization (9% *vs* 26%, $P = 0.001$) than coronary angioplasty patients within six months of the procedure. A South Korean cost-minimisation model established that DES resulted in higher costs than Bare metal stent by 985 Euros per patient[11]. However, it is possible that some selection bias influenced the results of such studies based on descriptive clinical data sources. A United States study published by Amin *et al*[12] specifically focused on DES indications in current practices and concluded that the use of DES in the United Sates would vary widely among physicians, with only a modest correlation to patients' risk of restenosis. Thus less DES used among patients with low risk of restenosis would have the potential for significant cost savings for the United States health care system while minimally increasing restenosis events. As large controlled clinical trials are very difficult to implement in this indication, a modelling approach would allow to generate robust comparative results and assess the value for money[13]. The objective of this study is to develop a cost-effectiveness model comparing DES *vs* BMS in patients suffering of stable coronary artery disease according to the French health system.

LITERATURE STUDY

Given the scarcity of head-to-head clinical trials, there is a need to use decision analytic models to assess and compare expected costs and effectiveness of percutaneous coronary interventions (PCI) strategies, using published evidence and cost estimates. Hence, evaluating the cost-effectiveness of a therapeutic sequence may identify the most clinically suitable population for a new strategy, and the most effective

Table 1 Charge table (i€)

Resource utilization	Mini	Mean	Max	Tariff
GP visit				22
Specialist visit (cardiologist)				23
Lab tests without markers of myocardial damage				21
Lab tests with markers of myocardial damage				39
ECG at rest				14
Stress test ECG				77
Stress test echocardiography				165
Myocardial scintigraphy				409
Coronary angiography				1954
Standard combination therapy (1 yr): aspirin + statin + ACE inhibitors	364		743	
GTN (1 yr)	39.4		73.2	
β-Blocker (1 yr)	52.9		210.9	
Calcium Inhibitors (1 yr)	87.5		311	
Hospitalization stay for PCI with BMS (1.6 stent)	4074		4573	
Hospitalization stay for PCI with DES (1.6 stent)	5284		5704	
Hospitalization stay for CABG surgery		14065		
Hospitalization stay for a clinical failure		2096		

DES: Drug eluting stents; BMS: Bare metal stent; PCI: Percutaneous coronary interventions; CABG: Coronary artery bypass graft; ACE: Angiotensin-converting-enzyme; ECG: Electrocardiogram.

and cost-effective treatment sequence. A model is a mathematic formula linking different variables to generate results relevant to a given environment based on local medical practices. A cost-effectiveness model is classically composed of a framework structure populated with costing and effectiveness data. Best modelling practices suggest that data populating a model should be based on relevant costs and existing published clinical data at the time of model development[14]. The model assumptions should also be validated by expert clinicians according to their current medical practice in a given country. Specific to stable coronary artery disease management, results generated by such modelling approach provide unique information on the expected effectiveness, overall costs and cost-effectiveness of different PCI strategies to assist medical decision-making as well as resource allocation decisions.

Resource utilization

French direct medical costs were derived from a standard costing approach performed with a panel of three expert clinicians highly experienced in CAD management. Direct medical costs were estimated per 12 mo considering ambulatory costs (GP visits, cardiologist visit, laboratory tests, imaging, drugs) and hospital costs (percutaneous intervention, coronary artery bypass graft, coronary angiography alone, hospitalization due to complications), while initial diagnostic costs were not considered. Unit costs of interventions were derived from Diagnosis Related Groups list and from the national payer perspective for ambulatory costs. Item costs were collected from minimum and maximum costs. Costs are reported in Euros (2012), and the charge table is reported in Table 1.

Resource utilizations were assessed from existing clinical guidelines. In the absence of guidelines, expert opinions were used from the clinician co-authors (YJ, OD and ND). Guidelines used were: "diagnosis and management of patients with stable ischemic heart disease" (American Heart Association 2012), "acts and services of long-term disease coronary heart disease" (Haute Autorité de Santé 2012) and "guidelines on the management of stable angina pectoris" (European Society of Cardiology 2006)[15-17]. Treatment costs include a combination of drugs prescribed to every patient (low-dose aspirin, statin and angiotensin-converting enzyme inhibitor) and specific costs of the selected treatment. When the options are "PCI with BMS" or "PCI with DES", specific costs are associated with hospitalisation costs required for the angioplasty, the number and type of the implanted stents, and the costs of clopidogrel prescription to prevent intrastent thrombosis. Costs for the hospitalisation are determined using the French official hospital information system according to the specific diagnosis-related groups (DRGs) named "PCI without myocardial infarction". DRG costs include treatments and all examinations (invasive or non-invasive cardiac investigations, biological analyses, *etc.*). Total costs of stents are calculated according to the average number of stent implanted per patient: 1.6 per patien[7]. The therapeutic option "drugs" includes the costs of basic drugs (see above) plus either β-blockers, calcium antagonists or long acting nitrates.

Costs of follow up after a clinical success include costs of ambulatory medicals visits, lab and medical imaging. The number of medical visits during follow-up has been estimated to 3 per year for GPs and 1 for specialist (cardiologist). Medical imaging includes one echocardiography and one myocardial scintigraphy. Lab tests (2 per year) include fasting lipid profile, including total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides, fasting glucose, full blood count including haemoglobin and white cell count, and serum creatininemia. Cost of follow up after a clinical failure are similar to costs after a clinical success plus one specific hospitalisation for management of the clinical failure. As no specific DRG exists for clinical failure, the following DRGs were considered: "myocardial infarction", "angina", "chest pain", "coronary atherosclerosis" and "arrhythmias and cardiac conduction disturbances". When a coronary angiography is not followed by an intervention, but by a drug prescription, the costs of the DRG "intravascular diagnostic procedure" have been used (mean costs of 5 related DRG).

Effectiveness

One relevant effectiveness endpoint aligned with stable coronary artery disease treatment goals has been used

Table 2 Effectiveness data of drug eluting stents model

	Minimum	Maximum	Ref.
Probability of success of a CABG surgery following a coronary angiography after a failure of a DES	90%	-	Sheiban *et al*[18]
Probability of success of pharmaceutical treatment after a failure of a DES	85%	90%	Sheiban *et al*[18]
Probability of success after a first DES	82.2%	97.5%	Simsek *et al*[19] Bakhai *et al*[20] Meredith *et al*[21] Morice *et al*[22] Toutouzas *et al*[23] Weisz *et al*[24] Serruys *et al*[25] Yan *et al*[26]
Probability of success after 1 yr of surveillance following a first DES	86.3%	98.7%	Simsek *et al*[19] Meredith *et al*[21] Park *et al*[27]
Probability of undergoing a second PCI following a coronary angiography after a failure of a DES	50%	60%	Malenka *et al*[28]
Probability of undergoing a surgery following a coronary angiography after a failure of a bare metal stent	30%	40%	Malenka *et al*[28]
Probability of undergoing a DES after a failure of a first DES	60%	70%	Sheiban *et al*[18]
Probability of success of a DES after a failure of a first DES	74.8%	90%	Steinberg *et al*[29] Ge *et al*[30] Lee *et al*[31]
Probability of success of a CBA after a failure of a first DES	60%	77%	Park *et al*[32]

DES: Drug eluting stents; PCI: Percutaneous coronary interventions; CABG: Coronary artery bypass graft; CBA: Coronary balloon angioplasty.

and expressed as treatment "success rate". The success endpoint was defined as the absence of a MACE (major adverse cardiac events), that is to mean the absence of death or non fatal myocardial infarction or the need of a subsequent revascularization. Effectiveness estimates of PCI were derived directly from published clinical trials at the time of model development.

Assuming comparable patient populations, probabilities of patients in success at each simulated 12-mo time point have been estimated from an extensive literature review. If different values are presented in different publications, expert opinions were used to validate the use of a range between the minimum value and the maximum value observed in the literature. When no relevant values have been reported in the literature, a range of potential values is estimated based on the clinical experience of the expert panel. Then 10000 Monte-Carlo simulations were carried out to screen every possible value according to a uniform distribution shape. Effectiveness values are presented in Table 2 for the DES strategy and Table 3 for the BMS strategy.

Model structure

Using a 2-year time horizon, two PCI simulation models have been developed: BMS first line strategy (Figure 1A) and DES first line strategy (Figure 1B). The first step of the BMS model is the coronary angiography, followed by bare metal stent. After one year, patient could be in clinical situation of success or failure. In case of success, the surveillance without new treatment will occur during the second year. In case of failure at the end of the first year, a new coronary angiography will lead to either a PCI (either bare metal stent or DES), a Coronary Artery Bypass surgery (CABG), or a pharmaceutical treatment. The first step of the DES model is the coronary angiography, followed by DES. In case of success, a simple surveillance is proposed as in the first model. In case of failure, a new coronary angiography will lead to either a PCI (new DES or balloon alone without any stent), a CABG, or a pharmaceutical treatment.

The models were specifically programmed in D-script language (Vanguard Studio 5.2). To manage uncertainty, and as per best practice in economic modeling, 10000 Monte-Carlo simulations generated mean values and standard deviations of the sub-model outputs: costs, effectiveness, and average cost-effectiveness over 2 years. Monte Carlo simulations consist of a class of computational algorithms that rely on repeated random sampling to compute their results. This approach, also called "probabilistic sensitivity analysis", allows screening all possible values of a given parameter according to a defined distribution shape and to recalculate the results. For the purpose of this study, uniform distributions have been programmed between minimum-maximum ranges of values. Therefore, the models were able to construct distributions of results which are presented with their standard deviations (SD). Statistical tests (two groups mean tests with known variances deducted from cost-effectiveness SD) were performed to calculate potential significant differences between cost-effectiveness ratios of treatment strategies.

COST-EFFECTIVENESS

Using stable coronary artery disease management medical costs, cost of interventions and pharmaceutical therapies and published effectiveness data, the models

Table 3 Effectiveness data of bare metal stent model

	Minimum	Maximum	Ref.
Probability of success after a first bare metal stent	70.9%	86.4%	Simsek *et al*[19] Daemen *et al*[33] Bakhai *et al*[20] Morice *et al*[22] Weisz *et al*[24]
Probability of success after 1 yr of surveillance following a first bare metal stent	85%	96%	Simsek *et al*[19]
Probability of undergoing a second PCI following a coronary angiography after a failure of a bare metal stent	80%	-	Malenka *et al*[28]
Probability of undergoing a surgery following a coronary angiography after a failure of a bare metal stent	10%	15%	Malenka *et al*[28]
Probability of undergoing a DES after a failure of a bare metal stent	50%	-	Konstance *et al*[34]
Probability of success of a DES after a failure of a bare metal stent	78%	89%	Steinberg *et al*[29]
Probability of success of a second bare metal stent after a failure of a first bare metal stent	58%	67.3%	Singh *et al*[35]
Probability of success of a CABG surgery following a coronary angiography after a failure of a bare metal stent	90%	-	Malenka *et al*[28] Konstance *et al*[34]
Probability of success of pharmaceutical treatment after a failure of a bare metal stent	85%	90%	Sheiban *et al*[18]

DES: Drug eluting stents; PCI: Percutaneous coronary interventions; CABG: Coronary artery bypass graft.

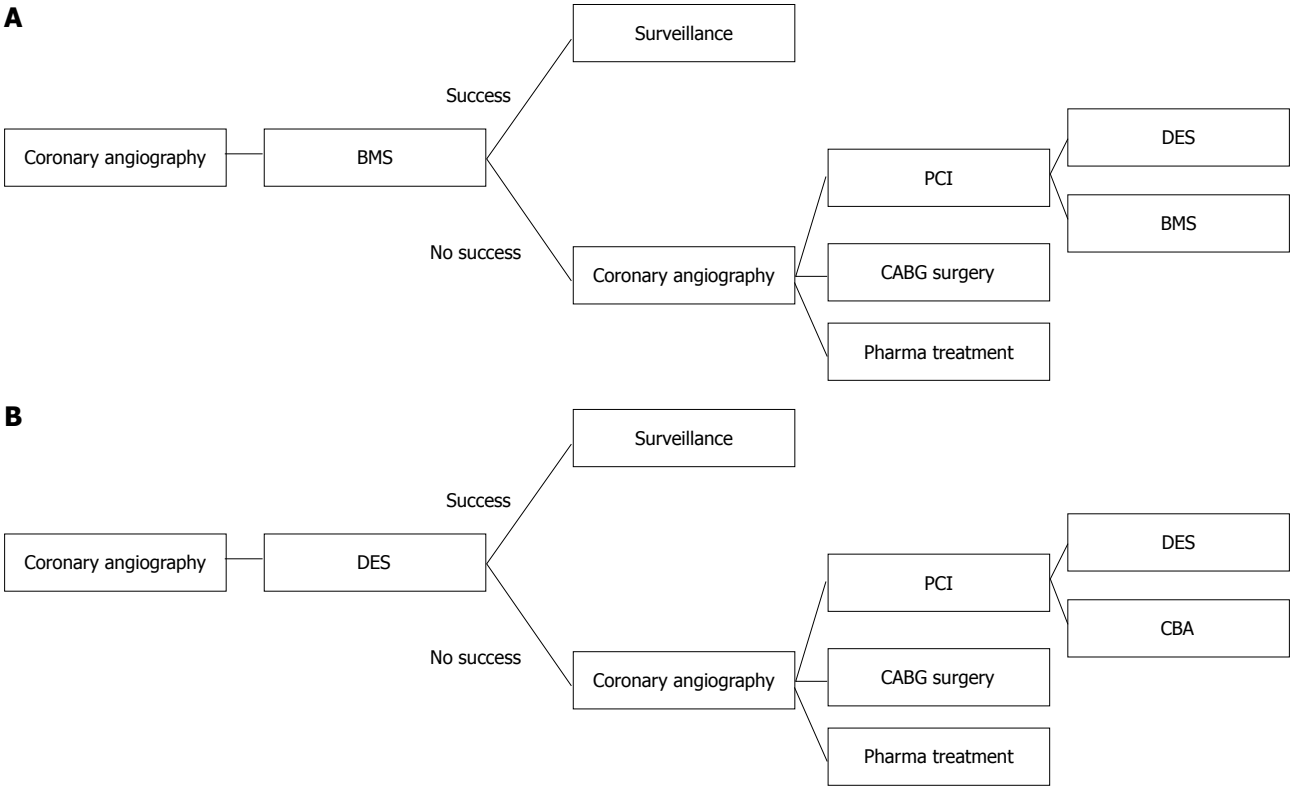

Figure 1 General architecture of the bare metal stent (A) and drug eluting stents (B) sequential model. DES: Drug eluting stents; BMS: Bare metal stent; PCI: Percutaneous coronary interventions; CABG: Coronary artery bypass graft; CBA: Coronary balloon angioplasty.

generated the overall treatment costs over 2 years, the probability of success, and the cost-effectiveness expressed in cost per success.

Strategy DES appeared slightly more efficacious (Figures 2 and 3) over 2 years (60% of success, SD 0.03) when compared to the strategy BMS (58% of success, SD 0.02). Total costs over 2 years were estimated at 9303 € (SD 3415) for the strategy DES and at 8926 € (SD 3778) for the strategy bare metal stent. Hence, corresponding mean cost-effectiveness ratios showed slightly lower costs per success for the bare metal stent strategy (15520 €/success, SD 6634), as compared to the strategy DES (15588 €/success, SD 5787). Incremental Cost-Effectiveness Ratio is 18850 € for one additional percent of success.

DISCUSSION

The results of this cost-effectiveness model based on published clinical evidence suggest that in patients in stable coronary artery disease, the sequential strategy including bare metal stent as the first PCI option

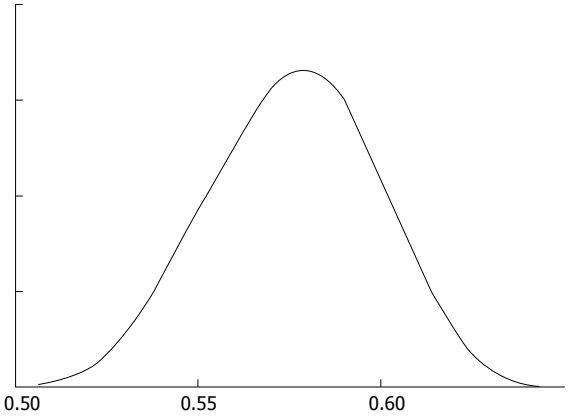

Figure 2 Cost distribution shape of the bare metal stent strategy (X axis: % success rate; Y axis: Occurrence probability).

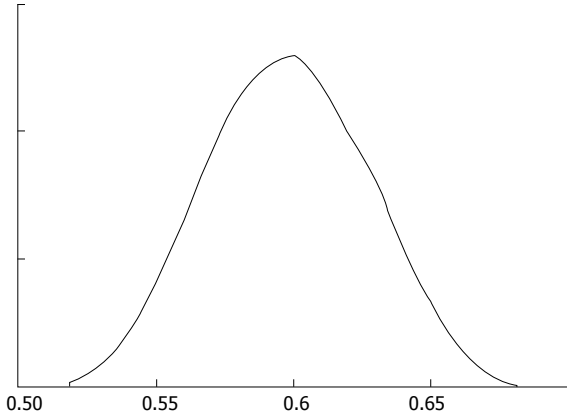

Figure 3 Cost distribution shape of the drug eluting stents strategy (X axis: % success rate; Y axis: Occurrence probability).

appears to be slightly less efficacious but more cost-effective compared to the strategy including DES as the first option. Two factors contribute to the better cost-effectiveness of the bare metal stent strategy. First, the results are driven by the efficacy reported in clinical trials. Secondly, the slightly higher success rates of the DES strategy do not offset the difference in costs between the two strategies. One of the most important issues in the creation of valid medico-economic models is the use of clinical effectiveness endpoints that are clinically meaningful and consistent across different settings. Selecting objective and consistent clinical outcomes allow defining clinical effectiveness of a given treatment more accurately and to compare across different treatment strategies for a specific patient population. Given that the goal of percutaneous coronary interventions is to reach a therapeutic success, this clinical endpoint appeared to be the most relevant effectiveness criteria for the purpose of conducting this cost-effectiveness analysis. Using a dichotomous approach of achieving success or no-success also appeared more clinically meaningful. Such cut-off points also avoid the need of using continuous scales with cardinal metrics (same origin and regular degrees) to compute effectiveness endpoints. Not only the proposed dichotomous approach success/no success is clinically meaningful, but it also requires fewer assumptions than using other outcomes,

The model assumes a 12-mo treatment period prior to allowing a potential switch to the next potential intervention in case of treatment failure. This assumption is based on the fact that most clinical trials report effectiveness data at 12 mo time points. These assumptions could be further discussed but they appeared to be consistent with medical practices in France, as validated with the expert panel. Furthermore, the time horizon of the model is limited to 2 years in order to reflect the data available at the time of model development. Hence, no long term effectiveness assumptions were made, as is it often the case in published "lifetime" cost-effectiveness models in stable coronary artery disease. Wisløff *et al*[36] carried out a cost-effectiveness model comparing DES *vs* BMS and concluded that DES was more cost-effective over a life time horizon using life years saved as an effectiveness endpoint. However, this model is based on life-time horizon projections speculating well beyond clinical trials evidence and diluting costs over years.

Co-morbidity such as diabetes is a risk factor of re-stenose after stent implantation, particularly for BMS as confirmed by the meta-analysis of Bangalore *et al*[37] carried out on 42 controlled randomized clinical studies. However, no specific calculations have been carried out for patients suffering of co-morbidities such as diabetes. Data inputs come from studies where proportion of diabetes patients were similar than European population, as described in the Euro Heart Survey[38].

This approach does not capture potential Quality of Life (QOL) improvement, as we would recommend that such evidence be considered separately, to its full merit. Furthermore, it is not the purpose of one clinical indicator to capture all the dimensions of life, so QOL dimensions should be collected separately using appropriate validated instruments. Many published "cost-utility" models (often presented under the label of "cost-effectiveness" models) consider the use of Quality Adjusted Life Years (QALY) as "effectiveness" criteria in order to take into account both the Quality of Life and the survival perspectives[39]. Not only the QALY approach is not specifically recommended in France (and several other countries such as USA and Germany) for methodological issues, but such approach reveals to be inconsistent in Stable coronary artery disease[40]. This is because the results are directly dependent on how the utility scores have been derived, explaining the possibility of data manipulation and why these studies often lead to divergent results[41,42]. The advantage of cost-effectiveness models using clinical effectiveness outcomes (such as Success probabilities) from published clinical trials is that the effectiveness criteria are not further transformed into

utilities. Hence, classic cost-effectiveness assessments (cost/clinical outcome achieved or per medical event avoided) generate more transparent and consistent results. Also, this cost-effectiveness analysis does not use a societal perspective but the perspective of the public payer in France. In such case, the results do not take into account the reported favourable impact of PCI on indirect costs. As indirect costs related to CAD are substantial and are estimated to be 2-3 times as high as direct costs, the results of this economic evaluation are likely to be understated. Finally, a frequent concern about cost-effectiveness models is that most publications seem to support the product of the study sponsor, suggesting a potential publication bias such as for publications of clinical trials. As they are used to inform and optimize resource allocation decisions, cost-effectiveness models are country specific and should always define the assumptions and conditions underlying the results where a therapeutic strategy is found to be cost-effective[14], which should also be in line with medical practices. Any model assuming very hypothetical clinical practices or theoretical outcomes should be considered with caution, as for any scientific studies.

CONCLUSION

This sequential cost-effectiveness model proposes a new approach to assess complex strategies based on clinical evidence based data and avoids any extrapolation over time, which could be subject to criticism. The model outcome expressed in costs per clinical success appears to be a clinically meaningful endpoint, allowing to compare various strategies. The sequential strategy including BMS as the first option appears to be slightly less efficacious but more cost-effective compared to the strategy including DES as first option in the frame of the French health system. Future modelling approaches should confirm these results as further comparative data in stable coronary artery disease and long-term evidence become available, but also to assess the value of innovative strategies such as biodegradable coronary stents.

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REVIEW

"Obesity paradox" in coronary artery disease

Ibrahim Akin, Christoph A Nienaber

Ibrahim Akin, Department of Cardiology, Universitätsmedizin Mannheim, 68167 Mannheim, Germany

Christoph A Nienaber, Cardiology and Aortic Centre, the Royal Brompton Hospital and Harefield Trust, London SW3 6NP, United Kingdom

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Correspondence to: Dr. Christoph A Nienaber, Professor, Cardiology and Aortic Centre, the Royal Brompton Hospital and Harefield Trust, Sydney Street, London SW3 6NP, United Kingdom. c.nienaber@rbht.nhs.uk
Telephone: +44-0207-3528121
Fax: +44-0207-3518634

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Abstract

Obesity used to be among the more neglected public health problems, but has unfolded as a growing medical and socioeconomic burden of epidemic proportions. Morbid obesity is linked to traditional cardiovascular risk factors like, hypertension, hyperlipidemia and diabetes, and suspected to incur increased morbidity and mortality in the Western and even third world populations. This patient cohort is also at greater risk to develop coronary artery disease. Recent population-based registries revealed that 43% and 24% of all cases of coronary revascularization were carried out in overweight and obese patients, respectively. However, despite evidence of a positive correlation between obesity and increased cardiovascular morbidity, some authors have described a better clinical outcome in overweight and obese patients, a phenomenon they coined "obesity paradoxon". Thus, there is an ongoing debate in light of conflicting data and the possibility of confounding bias causing misconception and challenging the "obesity paradox". In this review article we present the current evidence and throughly discuss the validity of the "obesity paradoxon" in a variety of clinical settings.

Key words: Coronary stent; Obesity paradox; Mortality; Body mass index

Core tip: Obesity is one of the leading health problems within the last years and is associated with several cardiovascular risk factors resulting in increased prevalence of coronary artery disease as well as atherosclerotic disease in head vessels and peripheral artery system. Despite these positive correlations there are reports describing a protective effect in patients undergoing coronary revascularization. This review will enlight the potential causes and bias regarding this "obesity paradoxon" in several clinical setting and will present the latest data.

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INTRODUCTION

Epidemiologic data on obesity, traditionally defined

as a body mass index (BMI) > 30 kg/m^2, range in the Western population with a prevalence of up to 36.5%[1]. Prevalence in United States is up to 70% that is much higher than 40 years ago (25%)[2]. Obesity and associated disorders like arterial hypertension, dyslipidemia, diabetes mellitus and sleep apnoea syndrome are linked to increased morbidity and mortality[3,4]. Common recommendation is weight loss to modify cardiovascular risk as an attempt for primary and secondary prevention of cardiovascular disease in overweight and obese patients[5-7]. Obesity is associated with increased atherosclerotic diseases, especially coronary artery disease by reduced insulin sensitivity, enhanced free fatty acid turnover, increased basal sympathetic tone, a hypercoagulable state, and finally by promotion of systemic inflammation[8,9]. Population-based data revealed that 43% and 24% of all coronary revascularization were carried out in overweight and obese patients, respectively[10]. Clinical outcome speculations should indicate that these patient cohorts would be associated with worse outcomes as compared to normal weight patients. Nevertheless, despite proven evidence of a causative association between morbid obesity and increased cardiovascular morbidity, previous studies have described the phenomenon of "obesity paradoxon", reporting a protective effect of obesity with regards to postoperative morbidity and mortality in patients receiving a surgical of interventional revascularization[11]. The observation of better clinical outcome is not only restricted to coronary revascularization but also in other clinical settings like acute myocardial infarction and heart failure[12,13]. With this review article we would like to give an overview and summary on the evidence of an "obesity paradoxon" in coronary artery disease.

STABLE CORONARY ARTERY DISEASE

Correlation of BMI with clinical endpoints in the setting of interventional coronary revascularization was first reported in 1996 from a single-center experience in patients (n = 3571) receiving balloon angioplasty[14]. In-hospital outcomes revealed higher rates of mortality (2.8% *vs* 0.9% *vs* 3.7%; P < 0.001) in normal weight and obese patients as compared to overweight patients. Similar differences were seen in need for blood transfusion (11.9% *vs* 7.4% *vs* 8.4%; P = 0.003) and rise in creatinine > 1 mg/dL (3.6% *vs* 1.8% *vs* 1.8%; P = 0.018); whereas rates for myocardial infarction were not different (3.5% *vs* 3.4% *vs* 4.7%; P = ns). A total of 3634 patients from the multicenter BARI registry undergoing elective revascularization [2108 by interventional procedure (PCI) and 1526 by surgery (CABG)] between 1988 and 1991 were evaluated for BMI at study entry[15]. Body mass index (BMI) was associated with increased risk of a major in-hospital event just in the PCI arm. However, at five year follow-up there was a correlation between mortality and BMI only in the CABG arm. While these results from the BARI trial suggested an inverse correlation of BMI with in-hospital outcome after PCI and no such difference with long-term follow-up, Gruberg *et al*[11] revealed an inverse correlation during 12-mo follow-up in 9633 patients that had been evaluated between 1994 and 1999 for mortality (10.6% *vs* 5.7% *vs* 4.9%; P < 0.0001); conversely rates of myocardial infarction (7.4% *vs* 7.0% *vs* 6.7%; P = 0.66) and target vessel revascularization (20.2% *vs* 22.0% *vs* 22.4%; P = 0.16) were not different.

Postprocedural clinical events like arterial hypotension, pulmonary congestion, impairment of renal function, as well as bleeding events, access site complications and mortality rates were more frequently seen in underweight patients than in overweight or obese ones. Different from previous all-comers trials, the Scottish Coronary Revascularisation Register included only patients (n = 4880) receiving elective PCI between 1997 and 2006 without a history of previous known coronary artery disease. During five years of follow up BMI between 27 and 30 kg/m^2 was correlated with lower all-cause mortality as compared to other weight groups. Introduction of a blanking time (< 30 d) to exclude periprocedural events and an adjustment to different baseline data did not impact on outcomes of their study[16]. These results could be confirmed in the APPROACH registry in 31021 patients treated medically (n = 7801), by PCI (n = 7017) or by CABG (n = 15601)[17]. In the first group, mortality rates were lower in overweight and obese patients as compared to normal ones. Similar findings were found in both the CABG and PCI group. Interestingly, the use of bare-metal stents (BMS), or any metaanalysis of these single trials revealed an inverse relationship between BMI and clinical outcome after stenting[18]. In contrast to the above-mentioned results from the balloon angioplasty and BMS era, some other studies from that time do not support the "obesity paradoxon" in patients with both, BMS or DES. Poston *et al*[19] revealed in 1631 patients that normal weight patients were older than the non-normal weight ones at the time of hospital admission[18]. During one year follow-up mortality and risk for repeat procedures was not different between groups. In the TAXUS trials, 1307 patients were stratified according to BMI and use of stent type (BMS *vs* DES)[20]; restenosis rates with use of BMS were higher in obese and overweight patients as compared to normal weight ones (29.2% *vs* 30.5% *vs* 9.3%, respectively; P = 0.01). Although rates for major cardiac events were also significant different in favour of normal weight patients, clinical event rates were not different in patients receiving DES. These findings were underlined by the results of the German DES.DE registry[21]. At 98 sites in Germany 5806 patients receiving DES in an all-comers design were included and followed over 12 mo. Similar to previous trials baseline comorbidity index was higher in obese patients as compared to overweight and normal weight patient are in-hospital events were similar in all three

Table 1 Overview of literature addressing the "obesity paradox" in patients suffering from stable coronary artery disease undergoing coronary angiography and/or revascularization

Ref.	Year	*n*	Follow-up (mo)	Mortality	Myocardial infarction	Target vessel revascularization	Renal insufficiency	Vascular complications
Ellis *et al*[14]	1996	3571	12	+	-	-	+	+
Gurm *et al*[15]	2002	3634	60	+	NA	NA	NA	-
Gruberg *et al*[11]	2002	9633	12	+	-	-	+	-
Poston *et al*[19]	2004	1631	12	-	NA	-	NA	NA
Nikolsky *et al*[20]	2005	1301	12	-	-	-	NA	NA
Romero-Corral *et al*[18]	2006	250152	45	+	NA	NA	NA	NA
Oreopoulos *et al*[17]	2009	31021	46	+	NA	NA	NA	NA
Hastie *et al*[16]	2010	4880	60	+	NA	NA	NA	NA
Akin *et al*[21]	2012	5806	12	-	-	-	-	-

NA: Not available.

groups. One-year follow-up revealed no differences in rates of death (3.3% *vs* 2.4% *vs* 2.4%; $P = 0.17$), myocardial infarction (2.8% *vs* 2.3% *vs* 2.3%; $P = 0.45$), target vessel revascularization (10.9% *vs* 11.7% *vs* 11.6%; $P = 0.56$) and major bleeding (2.5% *vs* 2.1% *vs* 2.8%; $P = 0.53$) between normal weight, overweight and obese patients, respectively (Table 1).

ACUTE CORONARY SYNDROME

In contrast to stable coronary artery disease an acute myocardial infarction is characterized by a proinflammatory state with different forms of hemodynamic, rhythmogenic and hemostatic disturbances. The phenomenon of an "obesity paradoxon" has also been evaluated in this patient population; yet, data on a potential link between BMI and clinical events in patients with acute myocardial infarction are scarce and inhomogenous in the literature. Data from the PREMIER and TRIUMPH registries including 6359 patients with acute coronary syndrome were taken to look for any relationship between BMI with survival rate[22]. Similar to patients with stable coronary artery disease there was an inverse relationship between BMI and rate of mortality (9.2% *vs* 6.1% *vs* 4.7%; $P < 0.001$) without any interactions of demographic data like age and sex. Similar findings were revealed in the KAMIR registry involving 3824 patients with ST-elevated myocardial infarction[23]. Baseline characteristics were characterized by the fact that normal weight patients were older, had more impairment of left ventricular ejection fraction, and a higher comorbidity index. Nevertheless, normal weight in this scenario was associated with higher mortality. In contrast of these trials several other trials showed no inverse relationship between BMI and clinical outcome[24,25]. Our group analyzed 890 patients suffering from ST-elevated myocardial infarction including patients with cardiogenic shock and followed them up to 12 mo; clinical events were not significantly different between all three weight groups, again challenging an obesity paradox. These findings were also seen in patients suffering from cardiogenic shock[26] (Table 2).

RATIONALE FOR THE "OBESITY PARADOX"

Self-reported obesity increased by 37% from 13.6% to 18.6% among men aged 35-49 since 1970. Simultaneously, epidemiology of other cardiovascular risk factors like arterial hypertension and diabetes increased[27,28]. However, mortality attributed to coronary events declined during the last 40 years mainly due to decreased cholesterol levels and damaging smoking habits with greatest reduction seen in overweight and obese patients, and to some degree as a result of more frequent revascularization[29-31]. Nevertheless, overweight and obesity, as part of the metabolic syndrome, are still linked to other cardiovascular risk factors, endothelial dysfunction, and inflammation and are often associated with an increased risk of suffering from atherosclerosis.

The key question to answer is how to explain better survival rates from coronary events despite increasing rates of obesity in light of above mentioned correlation. There is an ongoing debate whether the phenomenon of "obesity paradoxon" is real in the space of coronary artery disease[10-26].

Close examination of the current literature revealed that some published and most often retrospective data just claimed a U-shaped nonsignificant trend towards lower survival among underweight patients, compared with normal or mildly overweight patients; this however might predominantly result from a technical bias, that cannot be completely corrected by statistical means.

Detailed analyses reveal that up to 2% of patients who were underweight were likely to suffer from comorbid conditions, including malignancies, heart failure, malnutrition, multiorgan dysfunction, and happened to be significantly older than the normal and obese patients[10,11,15]. There is clear evidence that elderly and frail patients have worse clinical outcomes after any coronary event regardless of reperfusion or reperfusion strategy[32,33]. It is important to recognise that increasing age with its concomitant comobidity index results in weight change[34,35]. Chronic diseases might lead to gradual weight-loss, which is not taken

Table 2 Overview of literature addressing the "obesity paradox" in patients suffering from acute coronary syndrome including cardiogenic shock undergoing coronary revascularization

Ref.	Year	*n*	Follow-up (mo)	Mortality	Myocardial infarction	Target vessel revascularization	Renal insufficiency	Vascular complications
Kosuge *et al*[25]	2008	3076	Hospital	-	NA	NA	NA	NA
Kang *et al*[23]	2010	3824	12	+	-	-	NA	NA
Camprubi *et al*[24]	2012	824	Hospital	-	NA	NA	NA	NA
Bucholz *et al*[22]	2012	6359	12	+	NA	NA	NA	NA
Li *et al*[51]	2013	1429	12	-	-	-	NA	NA
Shehab *et al*[52]	2014	4379	1	-	-	-	NA	NA
Akin *et al*[26]	2015	890	12	-	-	-	-	-

NA: Not available.

into consideration in presented trials. Along these lines, another important confounding observation was the fact that obese patients tended to be diagnosed and treated at an earlier stage than lean patients[36]. Furthermore, the "obesity paradoxon" is clearly challenged by a recent survey on > 130000 patients, revealing that adherence to guidelines was better with higher BMI with regards to standard medication such as aspirin, b-blockers, acetylcholinesterase inhibitor and angiotensin Ⅱ receptor blockers, as well as lipid lowering drugs and also an increased likelihood of receiving invasive diagnostic and treatment[15,18,21]. Furthermore overweight and obese patients present in a much more stable status with lack of hemodynamic compromise, lower Killip class, and less impairment of ventricular function. Novel theories to explain the "obesity paradoxon" after PCI have included the suggestion that obese patients have "larger vessels" and outcome after PCI is worse in patients with smaller vessel[37,38]. Antithrombotic medications usually given as standard dosage and not weight adjusted, may be too high in normal weight and underweight patients according to their BMI, resulting in more bleeding events which are associated with higher mortality rate[39]. Similarly, sheath-to-artery size ratio is different in BMI groups resulting in different rates of vascular complications[15]. All these differences in periprocedural events can contribute to improved survival among overweight patients[11,40]. The fact that BMI alone was the only measure of obesity is containly a limiting factor. There is no information in several trials regarding the distribution of obesity that might be very important, as there is a worse outcome in patients with central obesity[41]. Moreover additional information regarding waist circumference, waist-to-hip ratio and weight changes are missing in several trials describing the "obesity paradoxon"[42-45]. Additionally, all trials supporting the "obesity paradoxon" suffer from the inherent limitation of an observational retrospective registry. Potentially confounding variables such as physical inactivity, unintended weight loss and even socioeconomic factors were not analyzed, and may be the source to further bias, not to mention the short follow-up of these registries. Eventually any potential relation between obesity and in-hospital and short-term survival may be lost the longer patients are followed. Therefore with extended follow-up a cumulative detrimental effect of obesity may manifest over time as increased late mortality[46,47].

It is sensible to ask what is left to support the so-called "obesity paradoxon", or is it just a paradoxical concept? Protagonists claim that adipose tissue is being recognized as an endocrine organ[48] that produces soluble tissue necrosis factor receptor with its protective effect[49]. Morbidly obese patients (BMI > 40 kg/m^2) certainly have higher adjusted rates of post-PCI mortality that might be due to higher levels of prothrombotic factors as well as elevated levels of plasminogen activator inhibitor- Ⅰ[50]. On aggregate, while early studies may have suggested an inverse relationship between being underweight with outcomes in heart failure, which led to the assumption of a "obesity paradoxon", the analysis of the published evidence denies any such "obesity paradoxon" in the context of coronary artery disease and modern coronary interventions. In fact there is no plausible concept to turn away from the classic relationship between risk factors, confounding variables and prognostic outcomes. The limitations of such association studies are not only the lack of a pathophysiologic underpinnings, but moreover the mere association with descriptive notions and the unknown impact of confounding variables. With respect to the neutralizing results from the German DES.DE Registry[21], the perception of obesity as a protective condition of outcomes after PCI is shattered and the provocative construct of an "obesity paradoxon" evaporates, as such hypothesis was never really substantiated in the clinical setting of coronary artery disease and PCI. Finally, as it turns out, associative studies with little to no statistical evidence lended support to invent a "obesity paradox" which was never supported by biological evidence and seems now shattered by new interpertation of recent clinical data. Any concept will eventually survive only if supported by plausible physiology. In the context of coronary artery disease and PCI there is hardly any plausible explanation and certainly no clinical data to justify an "obesity paradoxon".

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REVIEW

Lipoprotein-associated phospholipase A2 prognostic role in atherosclerotic complications

Giuseppe Maiolino, Valeria Bisogni, Giacomo Rossitto, Gian Paolo Rossi

Giuseppe Maiolino, Valeria Bisogni, Giacomo Rossitto, Gian Paolo Rossi, Department of Medicine - DIMED, Hypertension Clinic, University of Padua, 35128 Padova, Italy

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Correspondence to: Gian Paolo Rossi, MD, FACC, FAHA, Professor, Department of Medicine - DIMED, Hypertension Clinic, University of Padua, Via Giustiniani, 2, 35128 Padova, Italy. gianpaolo.rossi@unipd.it
Telephone: +39-049-8212279
Fax: +39-049-8217873

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Abstract

Atherosclerosis manifests itself clinically at advanced stages when plaques undergo hemorrhage and/or rupture with superimposed thrombosis, thus abruptly stopping blood supply. Identification of markers of plaque destabilization at a pre-clinical stage is, therefore, a major goal of cardiovascular research. Promising results along this line were provided by studies investigating the lipoprotein-associated phospholipase A2 (Lp-PLA2), a member of phospholipase A2 proteins family that plays a key role in the metabolism of pro-inflammatory phospholipids, as oxidized low-density lipoproteins, and in the generation of pro-atherogenic metabolites, including lysophosphatidylcholine and oxidized free fatty acids. We herein review the experimental and clinical studies supporting use of Lp-PLA2 activity for predicting cardiovascular events. To his end we considered not only Lp-PLA2 activity and mass, but also *Lp-PLA2* gene variations and their association with incident coronary artery disease, stroke, and cardiovascular mortality. Based on these evidences the major scientific societies have included in their guidelines the measurement of Lp-PLA2 activity among the biomarkers that are useful in risk stratification of adult asymptomatic patients at intermediate cardiovascular risk. The results of two recently published major clinical trials with the Lp-PLA2 inhibitor darapladib, which seem to challenge the pathogenic role of Lp-PLA2, will also be discussed.

Key words: Lipoprotein-associated phospholipase A2; Atherosclerosis; Coronary artery disease; Myocardial infarction; Prognosis

Core tip: Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a promising new marker of atherosclerotic plaque destabilization, which plays a key role in the metabolism of pro-inflammatory phospholipids and in the generation of pro-atherogenic metabolites. This review focuses on the experimental and clinical studies supporting use of Lp-PLA2 for predicting cardiovascular events considering not only Lp-PLA2 activity and mass, but also *Lp-PLA2* gene variations. Based on current evidences the major scientific

societies have included Lp-PLA2 activity measurement in their guidelines among the biomarkers that are useful in risk stratification of adult asymptomatic patients at intermediate cardiovascular risk.

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INTRODUCTION

Exposure of endothelial cells to damaging stimuli, as smoking, arterial hypertension, diabetes mellitus, dyslipidemia can induce qualitative changes that are collectively defined as "endothelial activation", and are currently postulated regarded as one of the earliest events in atherogenesis[1]. An "activated" endothelium expresses adhesion molecules and chemotactic substances, increases its permeability to macromolecules with ensuing variation of the sub-endothelial extracellular matrix composition. As a result, low-density lipoproteins (LDLs), particularly those that are smaller and denser and therefore more pro-atherogenic, penetrate the vessel wall and remain trapped in the sub-intimal space, where they undergo oxidative changes. Oxidized LDLs induce recruitment of monocytes by vascular cells and promote their differentiation into macrophages[2]. The latter internalize oxidized LDLs and become foam cells[3], the distinctive feature of the atherosclerotic lesions.

Atherosclerosis manifests itself clinically either when the arterial vessel stenosis prevents the increase of blood flow and oxygen supply during augmented demand (*e.g.*, exercise or digestion) causing the onset of pain (angina pectoris, abdominis or claudication intermittens, depending on the segments involved), or when an unstable plaque undergoes hemorrhage and/or rupture with superimposed thrombosis.

Several studies showed that athero-thrombosis, which is responsible for acute ischemic events, does not correlate strictly with the degree of atherosclerotic plaque narrowing[4,5], but rather with the plaque features, and, more specifically, with the extent of inflammation, thinning of the fibrous cap, and expression of inflammatory cytokines and metalloproteinases that degrade the fibrous cap[6,7]. This explains why the atherosclerotic disease might manifest clinically with acute catastrophic events even in patients with apparently mild lesions.

Identification of circulating markers that can be useful to improve the prediction of cardiovascular events is akin the current frontiers of Cardiology. C-reactive protein and cholesterol levels, despite being among the most studied biomarkers[8,9], bear a rather small predictive value: for example, in the Framingham Heart Study most of the patients who developed ischemic heart disease during twenty-six years follow-up had "normal" total cholesterol levels comparable to those not developing any cardiovascular disease[10].

In the Get with the Guidelines study database[11], which included 231896 patients admitted to 541 United States hospitals with a diagnosis of acute coronary syndrome, 136905 (59%) subjects had the lipid levels determined at admission and 21.1% of them were treated with cholesterol-lowering drugs. The average lipid profile was: LDL cholesterol 104.9 mg/dL (2.17 mmol/L), high-density lipoprotein (HDL) cholesterol 39.7 mg/dL (1.03 mmol/L), and triglycerides 161 mg/dL (1.82 mmol/L). According to this study about half of the patients admitted to the hospital with an acute coronary syndrome had LDL cholesterol levels in the normal range (Figure 1)[11]. These data provide compelling evidence for the urgent need to perform clinical and laboratory research to identify new biomarkers of imminent plaque destabilization. Along this line, encouraging results were provided by studies investigating the lipoprotein-associated phospholipase A2 (Lp-PLA2), a member of phospholipase A2 proteins family that plays a crucial role in the metabolism of pro-inflammatory phospholipids, such as oxidized LDLs, and in the generation of pro-atherogenic metabolites, such as lysophosphatidylcholine and oxidized free fatty acids (Figure 2).

ROLE OF LP-PLA2 IN ATHEROSCLEROSIS

Lp-PLA2 is a calcium-independent lipase mainly produced by monocytes and macrophages[12], which catalyze the hydrolysis of the sn-2 acyl chain of the phospholipid substrate[13] on the surface of LDLs[14], releasing lysophosphatidylcholine and oxidized fatty acids. The latter are well-established triggers of the inflammatory cascade[14-16], *via* stimulation of endothelial cells expression of adhesion molecules and cytokines, induction of chemotaxis of monocytes and leucocytes, and promotion of their entry in the sub-intimal space of the arterial walls.

The accumulation of lysophosphatidylcholine and oxidized fatty acids in the sub-intimal space contributes to the development of the plaque lipid "core". Moreover, these substrates once taken up by macrophages promote their conversion into foam cells[17]. In addition, lysophosphatidylcholine induces the production of reactive oxygen species, such as superoxide, by activating the endothelial nicotinamide adenine dinucleotide phosphate oxidase and by inducing the endothelial nitric oxide synthase (eNOS) "uncoupling"[18,19]. Through the latter mechanism the enzyme becomes a superoxide and peroxynitrite producer, thus contributing to atherogenesis and plaque destabilization, as corroborated by the increased cardiovascular mortality found in coronary artery disease patients carrying an *eNOS* gene polymorphism that implies enhanced eNOS

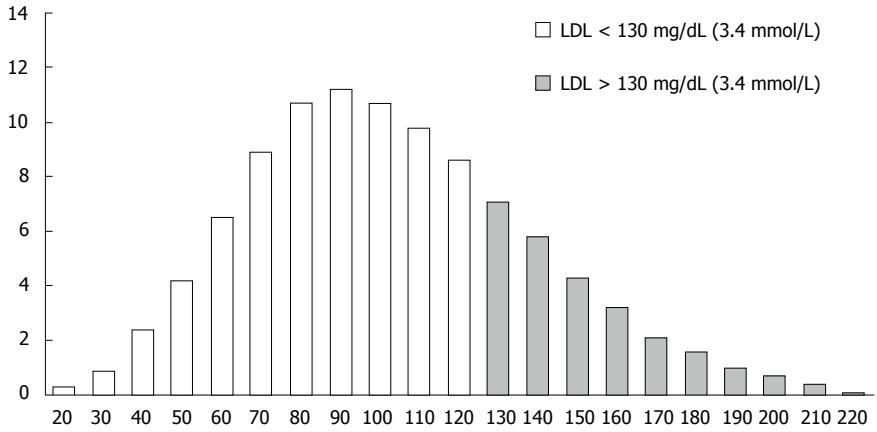
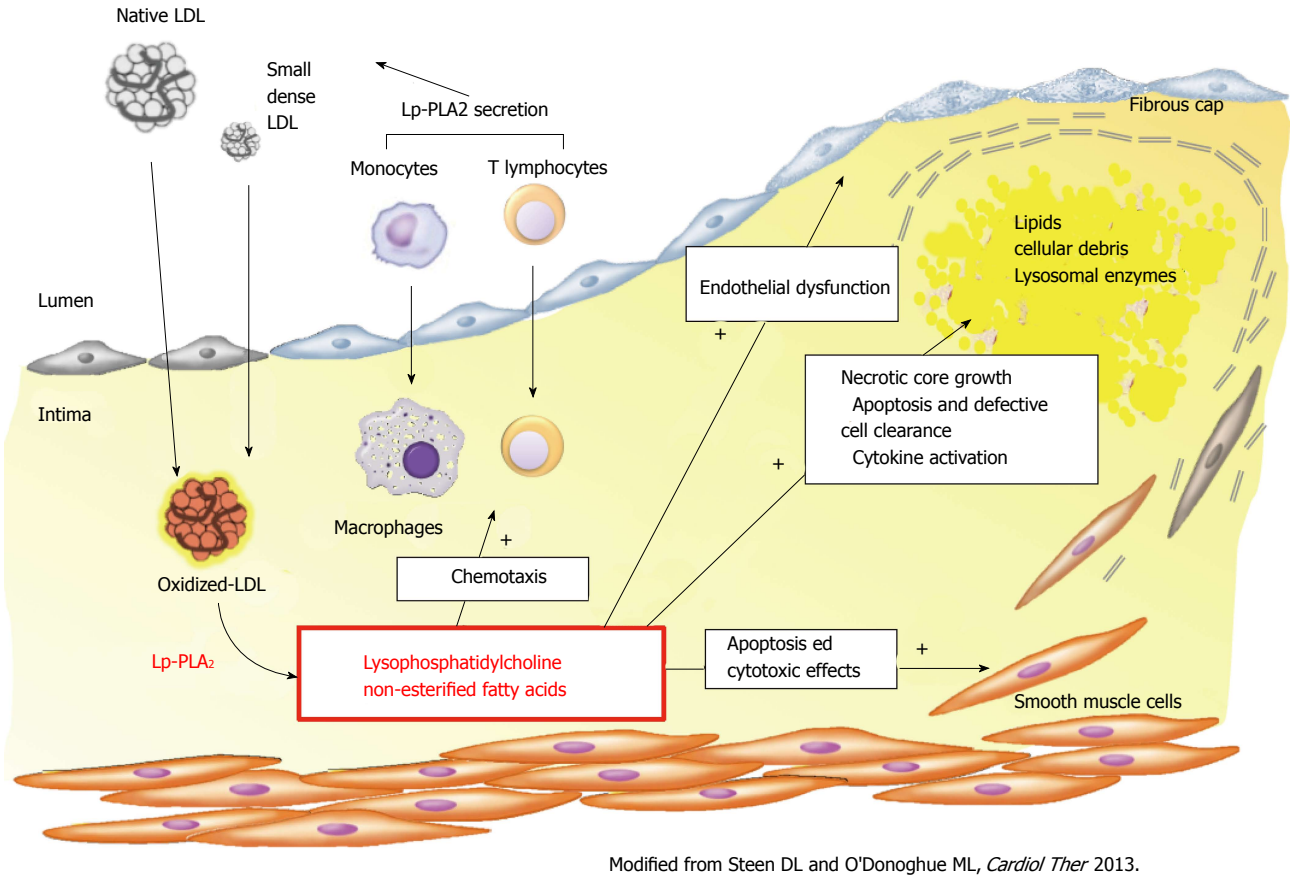


Figure 1 Low-density lipoprotein cholesterol levels on admission in patients with acute coronary syndrome[74]. LDL: Low-density lipoprotein.



Modified from Steen DL and O'Donoghue ML, *Cardiol Ther* 2013.

Figure 2 Pathogenic role of lipoprotein-associated phospholipase A2 in atherosclerosis development. LDL: Low-density lipoprotein; Lp-PLA2: Lipoprotein-associated phospholipase A2.

expression and reactive oxygen species generation[20].

In summary, experimental evidences indicate that due to its pro-inflammatory and pro-oxidative effects Lp-PLA2 plays a key role in the pathogenesis of atherosclerosis.

LP-PLA2 SECRETION AND CIRCULATION IN THE BLOOD-STREAM

Hematopoietic (monocytes, macrophages, lymphocytes, mastocytes, and platelets) and hepatic cells (Kupffer's cells) produce Lp-PLA2; however, its synthesis and release in the circulation occur with monocytes maturation into macrophages[21] alongside activation by inflammatory mediators[22].

In the bloodstream Lp-PLA$_2$ circulates by two-thirds bound to the LDLs and by one third to HDLs[23,24]. It is, however, worth highlighting that with normal LDL-levels the total plasma Lp-PLA2 activity associated with HDL accounts for only 4.9% of the total enzyme activity[25].

Plasma ultracentrifugation leads to partial separation

of Lp-PLA2 from the lipoproteins, thus indicating that there is a dissociable and a non-dissociable form of the enzyme[26]. The transition between them might be one of the mechanisms regulating the activity of Lp-PLA2 *in vivo*. The association with HDL and LDL is controlled by post-translational chemical modifications: Glycosylation of specific residues decreases the association of Lp-PLA2 and lipoproteins, even though these changes do not seem to influence the enzyme secretion by the cells[27].

As regards the relationship of Lp-PLA2 with apolipoproteins, B100 plays a key role in the association of Lp-PLA2 with LDLs, especially its carboxyl terminus, which interacts with the Lp-PLA2 residues Tyr-205, Trp-115, and Leu-116 and, to lesser extent, with the Met-117[28]. In spite of the fact that Lp-PLA2 preferentially associates with the most dense and electronegative LDLs fractions, even among the latters only 1% of the particles contain Lp-PLA2[29-31]. As mentioned, only one third of Lp-PLA$_2$ circulates in plasma with HDLs. Multiple amino-acid residues, as well as the carbohydrate content of the enzyme, appear to play a crucial role for its association with HDL apolipoprotein A-I[27,32].

Finally, when plasma lipoprotein(a) concentrations are $\geqslant$ 30 mg/dL detectable amounts of Lp-PLA2 are associated with this lipoprotein. A major role for its attachment is played by apolipoprotein B-100[33].

PLASMA LP-PLA2 DETERMINATION

Originally specific tests were developed to determine the Lp-PLA2 plasma concentration (mass) and enzymatic activity. The plasma mass assays were thereafter abandoned due to lack of significant advantages and lower accuracy in patients' risk stratification than enzymatic activity assays. The assessment of Lp-PLA2 activity exploits enzymatic substrates, such as 2 Tio-PAF, whose degradation releases free thiol groups, which are detectable by spectrophotometric reading.

GENETIC DETERMINANTS OF LP-PLA2 ACTIVITY

The prognostic relevance of Lp-PLA2 measurement raised the question whether the enzyme levels and activity are genetically determined ("nature") or influenced by environmental factors ("nurture"). According to one study and a recent meta-analysis Caucasians carry higher Lp-PLA2 activity levels than Hispanics and African-Americans, suggesting that Lp-PLA2 is genetically influenced[34,35]. Moreover, Lp-PLA2 activity was reported to be 10% lower in females compared to males, possibly due to higher estrogen levels in the former, which down-regulate Lp-PLA2 activity and decrease LDL-cholesterol[34,35]. A conclusive demonstration of hereditability was provided by twins' studies. In fact, genetically identical monozygotic twins showed differences in their plasma levels of Lp-PLA2 much smaller than dizygotic twins, who share only half of their genes, thus showing that about 62% of the variance of Lp-PLA2 activity levels is under genetic control[36].

The *Lp-PLA2* gene (*PLA2G7*) is located in chromosome 6p21.2 to 12 and entails 12 exons. Its cDNA was cloned in 1995[37] and comprises an open reading frame codifying a precursor of 441 amino acids that is cleaved into a 45.4 kDa mature protein[38]. The *PLA2G7* gene is characterized by non-synonymous polymorphisms that could cause reduction or loss of the enzymatic activity.

The first evidence of the functional relevance of these mutations dates back to the identification of five Japanese families with absent circulating Lp-PLA2, an autosomal recessive trait[39] linked to a Val279Phe polymorphism on the exon 9[40]. This variant causes the absence of Lp-PLA2 enzymatic activity because of an amino acid change in proximity of Ser-273 and Asp-296 that is responsible of folding and, thus, functioning of the mature protein.

The Val279Phe polymorphism was associated to atherosclerosis[41,42], stroke[43], and dilated cardiomyopathy[44]. However, these early evidences, which have been produced when Lp-PLA2 was believed to be anti-atherogenic, were not confirmed by subsequent studies that, in fact, showed just opposite results[45]. Thus, it remains unclear whether the lack of Lp-PLA2 activity is pro- or anti-atherogenic and if carriers of this genetic variant, who are exclusively Asian, could have inherited compensatory mechanisms that change unpredictably the final clinical phenotype.

Other polymorphisms were thereafter identified in Caucasians[46,47]: Arg92His (exon 4), Ile198Thr (exon 7), Ala379Val (exon 11). In particular, the Ile198Thr variation is located near the Tyr205 residue, a binding site for LDLs, in a position that might decrease the affinity for the substrate, thus explaining the observed reduction of enzymatic activity[46]. Another polymorphism, the Ala379Val, is located near the residue belonging to the catalytic triad of lipase (Hys-351), suggesting that it could influence the enzymatic activity[48].

Ala379Val and Arg92His variants have been associated with coronary artery disease (CAD)[49], but only the former correlated with the severity of atherosclerosis in a Taiwanese population[50] and to acute myocardial infarction in two case-control studies[49,50]. In other studies this association was neither confirmed[51] nor denied[52,53]. Two recent meta-analyses[54,55], which included more than 10000 patients of European ancestry, failed to demonstrate any association between the *PLA2G7* gene polymorphisms and CAD risk. These studies, as well as the meta-analyses that included them, were biased and affected by confounding factors, in that: (1) only a minority of studies used a prospective cohort study design, which is more reliable compared to case-control studies; and (2) the adjustment for potential confounders by multivariate analysis was not consistently performed. Therefore, likely their results

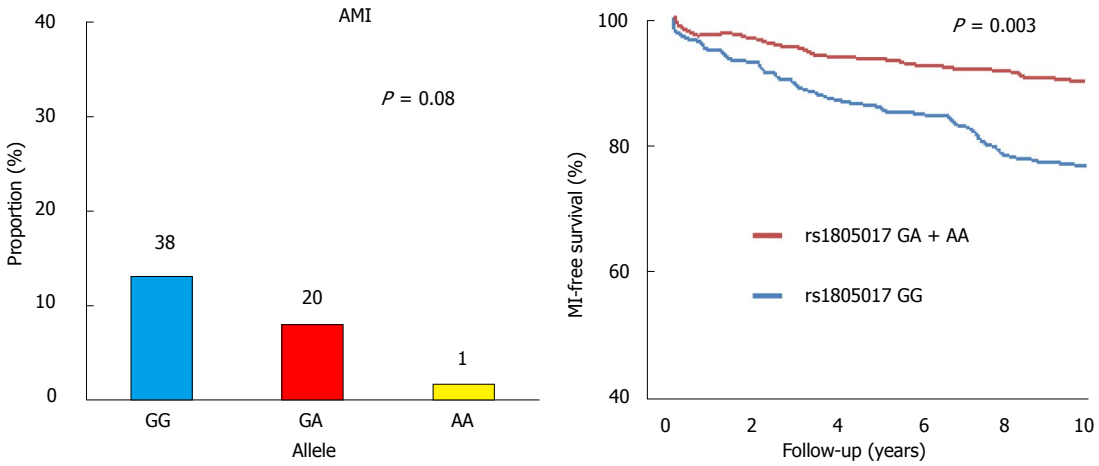


Figure 3 Increased number of acute myocardial infarction depending on the variant gene Arg92His. A: The patients with variant gene GG (Arg92) have a greater number of infarcts compared to the other two variants; B: The Kaplan-Meyer curve shows a lower survival free from acute myocardial infarction in patients with variant GG (Arg92). AMI: Acute myocardial infarction.

could not be considered conclusive even despite the large number of patients analyzed.

A recently published prospective cohort study performed with an appropriate prospective cohort design and an utmost care to the role of potential confounders, showed that Arg92His is associated to both high levels of Lp-PLA2 activity, and a 1.75-fold increase of relative risk of acute myocardial infarction (Figure 3)[56]. Hence, it would appear that genetic predisposition to high Lp-PLA2 activity translates into increased susceptibility to acute coronary events.

LP-PLA2 ACTIVITY AND CARDIOVASCULAR DISEASE

The first study showing an association between elevated Lp-PLA2 plasma levels and cardiovascular events was the West of Scotland Coronary Prevention Study (WOSCOPS)[57]. Other studies thereafter confirmed Lp-PLA2 to be a predictor of cardiovascular events in different cohorts[58-66], but the large Women Health Study[67], which enrolled a healthy female population, found just an opposite association. In apparently healthy populations, three trials demonstrated the Lp-PLA2 prognostic role. In the ARIC study, which enrolled a large cohort of healthy subjects of both genders, those with low LDL cholesterol (< 130 mg/dL) and high Lp-PLA2 levels had an increased relative risk of ischemic heart disease [HR 2.08, 95% confidence interval (CI): 1.20-3.62] compared to those with low levels of Lp-PLA2[68]. The JUPITER trial also showed that patients with high Lp-PLA2 activity (fourth quartile) had a more than two-fold increased relative risk (HR 2.15, 95%CI: 1.13-4.08) of developing cardiovascular events than those with low activity (first quartile)[69]. Finally, the Bruneck study also reported that the population in the third tertile of Lp-PLA2 activity had a higher relative risk of incident cardiovascular events (HR 2.2, 95%CI: 1.1-4.8) compared to those in the first tertile[70].

Lp-PLA2 activity might predict the occurrence of events also in patients at high cardiovascular risk: in the MDCS study, which enrolled healthy subjects, those with metabolic syndrome and high Lp-PLA2 activity had a 1.97 (95%CI: 1.34-2.90) relative risk of cardiovascular events[71]. The combined analysis of two studies, HPFS and NHS, including patients with diabetes mellitus, showed that those with a high Lp-PLA2 activity had a 1.75 (95%CI: 1.05-2.92) relative risk of cardiovascular mortality and AMI[72].

The ability of Lp-PLA2 to predict cardiovascular events was also confirmed in subjects with cardiovascular disease. The VA-HIT study, which included patients with ischemic heart disease, the increase of Lp-PLA2 activity levels was associated with a higher relative risk of cardiovascular events (HR 1.17, 95%CI: 1.04-1.32) and death (HR 1.23, 95%CI: 1.01-1.50)[73]. Similar results were obtained in the LIPID trial that entailed subjects with history of acute coronary syndrome in whom Lp-PLA2 activity was associated to a higher risk of cardiovascular mortality (HR 1.32, 95%CI: 1.00-1.75)[74]. Another study that included 1051 patients affected by CAD showed that Lp-PLA2 activity predicted the risk of cardiovascular events (HR 2.40, 95%CI: 1.35-4.29)[63]. Finally, in a large cohort of subjects with CAD of the GENICA Study, we demonstrated that a high Lp-PLA2 activity level predicted both cardiovascular mortality (HR 1.01, 95%CI: 1.00-1.02) and acute myocardial infarction (HR 1.01, 95%CI: 1.00-1.02) (Figure 4)[75].

Circulating Lp-PLA2 activity levels could be an index of systemic inflammation as suggested by the finding of a direct link between Lp-PLA2 enzymatic activity and activation of lympho-monocytic cells[76]. These data were confirmed by studies on CAD patients (Rotterdam Study and Ludwigshafen Risk and Cardiovascular Health Study) that demonstrated an association

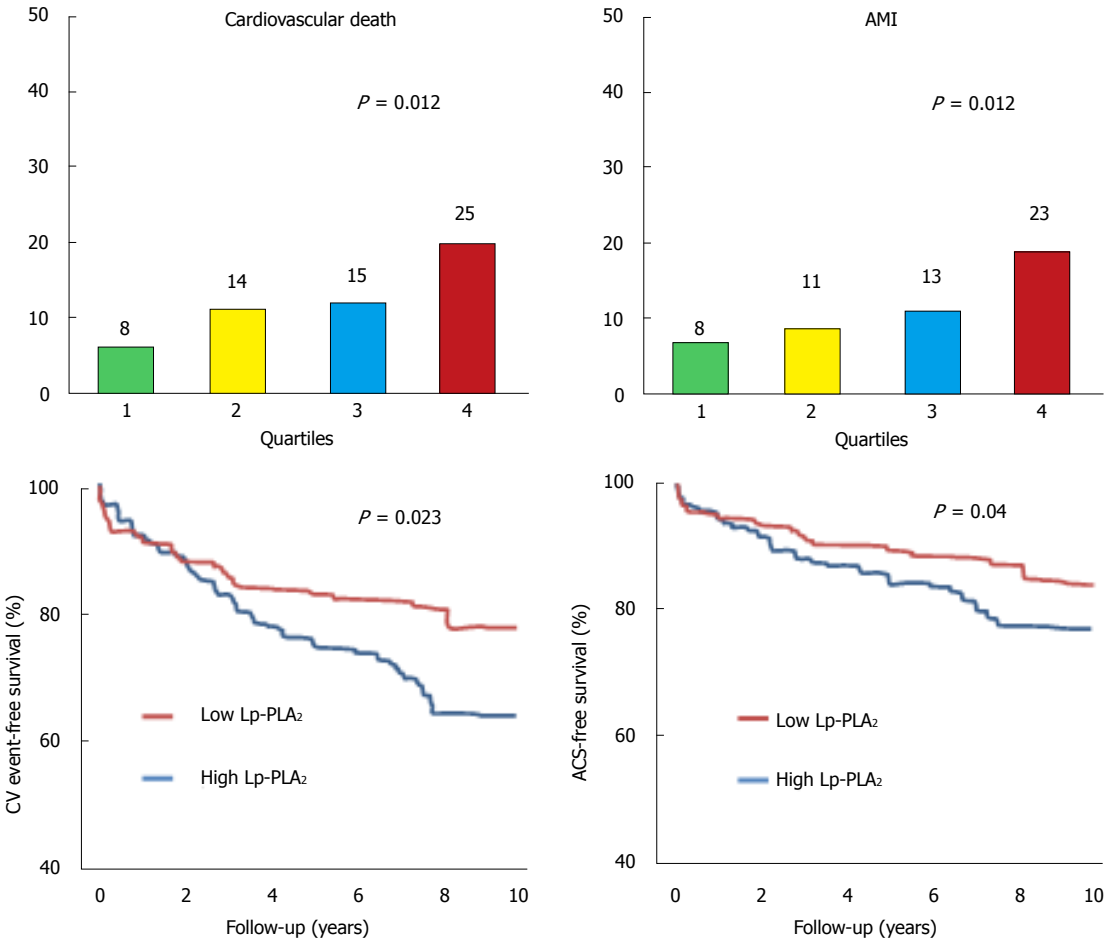


Figure 4 The Kaplan-Meyer curves underline a greater survival free from cardiovascular events (death, acute myocardial infarction) in patients with lower lipoprotein-associated phospholipase A2 activity. ACS: Acute coronary syndrome; CV: Cardiovascular.

between Lp-PLA2 enzymatic activity and CAD risk[77,78].

A meta-analysis that included all prospective studies conducted on Lp-PLA2 including a total of 79036 patients showed a relationship between Lp-PLA2 activity and mass and incidence of CAD, stroke, and cardiovascular mortality[79].

LP-PLA2 AND GUIDELINES

Based on these evidences, the guidelines of four major international societies, including the European Society of Cardiology, the American College of Cardiology, the American Heart Association, and the American Society of Endocrinology, included the Lp-PLA2 activity measurement among the biomarkers that are useful for risk stratification of asymptomatic adult patients. The use of this marker is particularly advantageous in patients at moderate cardiovascular risk (> 2 risk factors) and in those at high-risk in whom an increase of Lp-PLA2 activity levels should guide the lipid-lowering treatment to reach a target LDL-cholesterol lower than, respectively, 130 mg/dL (< 3.3 mmol/L) or 100 mg/dL (< 2.5 mmol/L) in primary prevention[80] (Figure 5).

THERAPEUTIC STRATEGY TO REDUCE LP-PLA2 LEVELS

As the majority of plasma Lp-PLA2 is linked to LDLs, a therapeutic strategy aimed at decreasing LDL cholesterol levels could be expected to reduce Lp-PLA2 activity. Accordingly, several cholesterol-lowering treatments, such as statins[66,74,81,82], fibrates[81,83], ezetimibe[81], and omega-3 fatty acids[84], were found to reduce plasma Lp-PLA2 activity. However, it remained unclear whether the reduction of Lp-PLA2 activity with a lipid-lowering treatment translates into a lower mortality and cardiovascular event rate, and if these benefits could be explained by the reduction of plasma lipids, of Lp-PLA2 activity levels, or of both. This hypothesis has been tested in the LIPID (Long-term Intervention with Pravastatin in Ischemic Disease) study, a double blind multicenter trial that randomized to placebo or pravastatin 9014 patients with CAD[74]. The levels of many biomarkers, such as cholesterol fractions and Lp-PLA2 activity were determined at "baseline" and after one year of treatment. The study showed that after one-year follow-up, the statins group

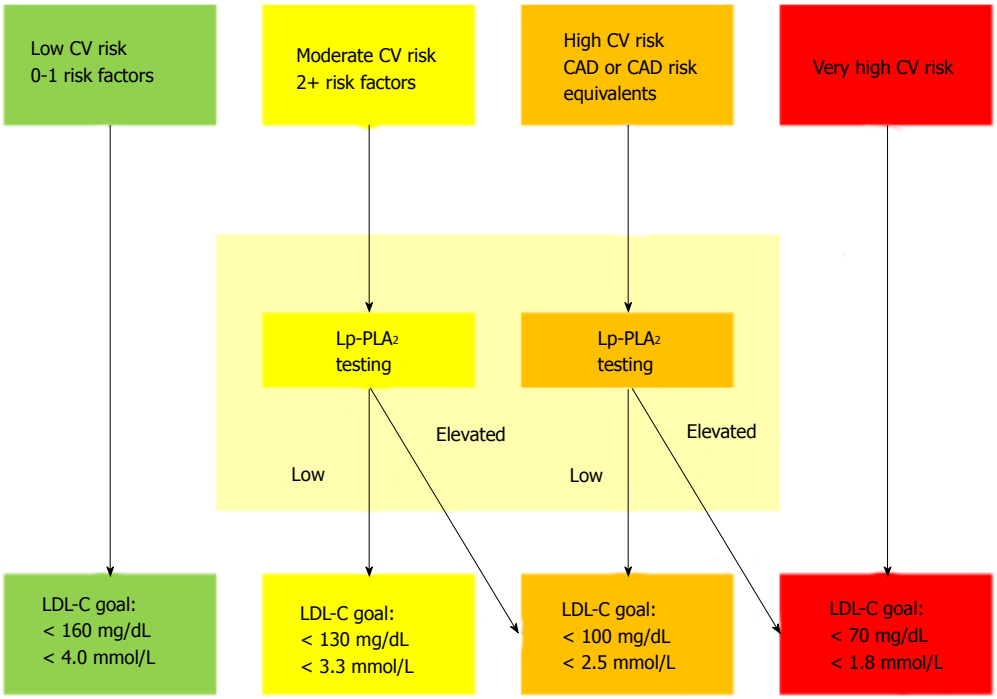


Figure 5 Relevance of measuring of lipoprotein-associated phospholipase A2 activity for risk stratification in adult patients with moderate cardiovascular risk (> 2 risk factors) or higher[80]. Lp-PLA2: Lipoprotein-associated phospholipase A2; LDL: Low-density lipoprotein; CAD: Coronary artery disease; CV: Cardiovascular.

had a reduction Lp-PLA2 activity (50 nmol/min per milliliter, $P < 0.001$) compared to both baseline values and to the placebo group. Similarly to previous studies, the "baseline" values of Lp-PLA2 activity predicted the risk of cardiovascular events, including CAD mortality and acute myocardial infarction, and total mortality; after adjustment at multivariate analysis, the baseline values of Lp-PLA2 activity predicted only CAD mortality. The key finding was that after one year of treatment low Lp-PLA2 levels predicted less major CAD events (HR 0.65, 95%CI: 0.50-0.86, $P = 0.002$), less major cardiovascular events (cardiovascular death, less non fatal acute myocardial infarction or stroke, HR 0.70, 95%CI: 0.55-0.89, $P = 0.003$), and less cumulative cardiovascular events (major cardiovascular events, unstable angina, revascularization, HR 0.70, 95%CI: 0.59-0.83; $P < 0.001$), comparing the first with the fourth quartile of Lp-PLA2 levels. These prognostic value persisted unaltered after adjustment for twenty-three risk factors at enrolling, which led the authors to conclude that the reduction of Lp-PLA2 during treatment with statin was as predictive, or even more predictive, than the decrease of LDL cholesterol[74]: about 59% of the beneficial effects of pravastatin were explained by a decrease of Lp-PLA2 values. This study could not, however, verify whether the reduction of circulating Lp-PLA2 was associated with a decrease of the enzyme activity into the atherosclerotic plaque, a data that, if confirmed, could explain the observed decrease of events.

RANDOMIZED CONTROLLED CLINICAL TRIALS TESTING THE EFFICACY OF LP-PLA2 INHIBITORS

This piece of information was, however, obtained, in diabetic and dyslipidemic pigs: darapladib, a Lp-PLA2 inhibitor, reduced the lysophosphatidylcholine levels in coronary artery plaques and decreased macrophage infiltration and necrotic "core" in the plaques[85]. Moreover, in the IBIS-2 study in humans, darapladib decreased the Lp-PLA2 activity and the necrotic "core" in coronary plaques[85]. Two randomized trials were conducted to test whether pharmacological inhibition of Lp-PLA2 with darapladib reduces cardiovascular events in stable and unstable CAD and were recently published, the STABILITY[86] and the SOLID-TIMI 52[87].

The STABILITY trial randomized 15828 patients with stable CAD to receive darapladib or placebo for a median period of 3.7 years with a composite primary end-point of cardiovascular death, myocardial infarction, or stroke[86]. The trial fell short of proving its primary end-point and could not demonstrate any beneficial effect of darapladib on each individual component of the primary end-point, or on the overall mortality. However, it demonstrated a beneficial effect of Lp-PLA2 inhibition in that darapladib reduced the rate of major coronary events and total coronary events.

The SOLID-TIMI 52 trial enrolled 13026 patients with an acute coronary syndrome in the last 30 d

before randomization to darapladib or placebo for a median period of 2.5 years with a composite primary end-point of cardiovascular death, myocardial infarction or urgent coronary artery revascularization[87]. The results did not demonstrate any beneficial effect of darapladib on any of the either primary or secondary endpoints.

These disappointing results might seem to challenge the pathogenic role of Lp-PLA2 in atherosclerosis and plaque destabilization. However, considering the wealth of data demonstrating that Lp-PLA2 predicts cardiovascular events, one could argue that the plasma activity of Lp-PLA2 is only a prognostic marker and does not play a causative role. Another possibility is that the trials, due to the high rate of drug discontinuation (20% in the STABILITY trial and 17% in the SOLID TIMI 52), ultimately lacked the statistical power to challenge the hypothesis that darapladib is efficacious in reducing cardiovascular events. If this was the case the possibility that another, possibly better tolerated, antagonist could improve outcomes needs to be tested. Furthermore, the selection criteria did not include a threshold Lp-PLA$_2$ level, whereas it is well known that the risk of cardiovascular events is dependent on the Lp-PLA2 levels. Moreover, the drug adherence assessment used, *e.g.*, pill count, is well known to underestimate the true therapeutic compliance[88]. The absence of any report of the Lp-PLA2 levels reached after darapladib administration, testing the therapy efficacy and the patients' compliance, is a missing crucial piece of information in these trials.

At present, from the available data it is possible to speculate that darapladib is not an efficacious therapy. Considering the extensive proof of the Lp-PLA2 prognostic value and the crucial information missing in the completed trials, it is possible to hypothesize that Lp-PLA2 is a marker of disease and does not have a pathogenic role, or that another more efficacious way to inhibit Lp-PLA2 activity by means of new drugs should be investigated.

CONCLUSION

In summary, compelling evidence indicate that high Lp-PLA2 activity levels predict an increased risk of cardiovascular events in the general population, as well as in patients with metabolic syndrome, diabetes, and coronary heart disease[63,68-75]. Many cholesterol-lowering medications besides decreasing LDL-cholesterol lower circulating Lp-PLA2 levels. Moreover, the Lp-PLA2 levels achieved with pravastatin treatment is a marker of cardiovascular risk and coronary events, even better than the LDL cholesterol level[74]. The available evidences support the usefulness of the measurement of plasma Lp-PLA2 activity in the clinical practice to stratify the cardiovascular risk, especially in patients at intermediate or high risk. In these subjects Lp-PLA2 activity levels should prompt the physician to pursue two aims: (1) a more aggressive LDL-cholesterol treatment; and (2) the normalization of Lp-PLA2 levels (Figure 5). For this reason, the scientific societies guidelines introduced the measurement of Lp-PLA2 as a marker of risk in these categories of patients.

The role of Lp-PLA2 as a therapeutic target has been disproven by two large randomized clinical trials thus far. However, due to their intrinsic limitations, it remains unclear if these results depended on the Lp-PLA2 being only a marker of cardiovascular events devoid of a pathogenic role, or on the lack of efficacy of the drug tested in these trials. Further studies are needed to resolve this dilemma.

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REVIEW

Cardioprotection by remote ischemic conditioning: Mechanisms and clinical evidences

Alberto Aimo, Chiara Borrelli, Alberto Giannoni, Luigi Emilio Pastormerlo, Andrea Barison, Gianluca Mirizzi, Michele Emdin, Claudio Passino

Alberto Aimo, Chiara Borrelli, Michele Emdin, Claudio Passino, Institute of Life Sciences, Scuola Superiore Sant'Anna, 56126 Pisa, Italy

Alberto Giannoni, Luigi Emilio Pastormerlo, Andrea Barison, Gianluca Mirizzi, Michele Emdin, Claudio Passino, Division of Cardiology and Cardiovascular Medicine, Fondazione Toscana Gabriele Monasterio, 56124 Pisa, Italy

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Correspondence to: Claudio Passino, MD, Division of Cardiology and Cardiovascular Medicine, Fondazione Toscana Gabriele Monasterio, via Giuseppe Moruzzi 1, 56124 Pisa, Italy. passino@ftgm.it
Telephone: +39-050-3152191
Fax: +39-050-3152109

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Abstract

In remote ischemic conditioning (RIC), several cycles of ischemia and reperfusion render distant organ and tissues more resistant to the ischemia-reperfusion injury. The intermittent ischemia can be applied before the ischemic insult in the target site (remote ischemic preconditioning), during the ischemic insult (remote ischemic perconditioning) or at the onset of reperfusion (remote ischemic postconditioning). The mechanisms of RIC have not been completely defined yet; however, these mechanisms must be represented by the release of humoral mediators and/or the activation of a neural reflex. RIC has been discovered in the heart, and has been arising great enthusiasm in the cardiovascular field. Its efficacy has been evaluated in many clinical trials, which provided controversial results. Our incomplete comprehension of the mechanisms underlying the RIC could be impairing the design of clinical trials and the interpretation of their results. In the present review we summarize current knowledge about RIC pathophysiology and the data about its cardioprotective efficacy.

Key words: Remote ischemic conditioning; Ischemic heart disease; Percutaneous coronary intervention; Cardiac surgery; Atherosclerosis

Core tip: Remote ischemic conditioning (RIC) is a safe, non-invasive, and inexpensive technique that has the potential to protect the heart against the ischemia-reperfusion injury. Its cardioprotective efficacy is currently being evaluated, and diverging results are emerging. It is thus worth resuming current understanding of RIC pathophysiology and clinical efficacy.

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INTRODUCTION

The myocardium can tolerate brief periods (up to 15 min) of severe and even total ischemia. Such ischemic episodes occur in the settings of angina, coronary vasospasm, and balloon angioplasty, and are not associated with concomitant myocyte cell death. With increasing duration and severity of ischemia, greater myocardial damage, and the predisposition to further damage during reperfusion develop. The combined deleterious effects of coronary occlusion and revascularization configure the "ischemia-reperfusion (IR) injury"[1].

The counterintuitive idea to apply several brief episodes of IR cycles to protect the myocardium against IR injury was firstly advanced in 1986, when Murry *et al*[2] reported that the infarcted area following a 40-min coronary occlusion was reduced if preceded by four 5-min IR cycles. This phenomenon was called "ischemic preconditioning". Its clinical application is hindered by the unpredictable timing of acute myocardial infarction (AMI), and by the necessity to intervene on coronary vessels[3]. However, several IR cycles were found to confer cardioprotection even when applied at the onset of coronary revascularization (ischemic postconditioning)[4,5], both in animals and in human patients undergoing primary percutaneous coronary intervention (PCI).

In 1993, it was demonstrated in anesthetized dogs that 4 episodes of 5-min ischemia and reperfusion in the left circumflex coronary territory, followed by a 1-h occlusion of the left anterior descending coronary artery, significantly reduced the infarct size[6]. The "remote ischemic conditioning (RIC)" paradigm has been progressively extended[7]. At present, RIC is defined as the phenomenon by which brief episodes of ischemia and reperfusion in one vascular bed, tissue, or organ render distant sites resistant to the ischemia-reperfusion injury[7,8]. The IR cycles are effective when applied before myocardial ischemia (remote ischemic preconditioning), during coronary occlusion (remote ischemic perconditioning), and during cardiac revascularization (remote ischemic post-conditioning)[7-12].

The mechanisms conferring protection at distance have not been completely defined[7], yet their characterization would be relevant to achieve a full comprehension of the phenomenon, and to exploit its full potential in clinical practice. In fact, understanding whether humoral mediators, neural mechanisms, or their combination mediate remote ischemic conditioning would be crucial to determine the optimal number of IR cycles, the better site and timing of their application, to select the patients according to age, comorbidities, and medical treatment, and to optimize the overall therapeutic management of the patient.

In the first part of the present review, we analyze current knowledge of the mechanisms underlying RIC, comparing humoral and reflex-mediated mechanisms. In the second half of this work, we attempt a critical analysis of the available literature concerning the cardioprotective potential of RIC in different settings.

THE "HUMORAL HYPOTHESIS" OF RIC, AND POTENTIAL CIRCULATING MEDIATORS

The "humoral hypothesis" has been formulated in the setting of remote ischemic preconditioning (RIPC). It postulates that the IR cycles in a distant site cause the local accumulation of mediators which are then released into the bloodstream and finally reach the heart[7] (Figure 1).

Several data from animal models support this hypothesis. In particular, it has been demonstrated that the effluent from preconditioned hearts could transfer the protection to naïve recipients[13-15]; this protection seems to be mediated by small hydrophobic proteins whose molecular weight ranges between 3.5 and 15-30 kDa[16].

Since these humoral mediators must be effective in remote sites after dilution into the bloodstream, their release from the peripheral tissue has to be massive[16]. The identification of humoral mediators should therefore be relatively easy to perform in animals or humans undergoing a RIPC protocol[16]. Indeed, proteomic approaches have been attempted in both animals and humans, still they have yielded controversial results[17-19].

Among the proteins potentially involved, there are kallistatin, apolipoprotein A-I, and stromal-derived factor 1α (SDF-1α)[16].

Kallistatin is a serine protease which reduces inflammation, apoptosis, and oxidative stress in endothelial cells[20]. It has been recently characterized as a protective factor against renal ischemia-reperfusion injury in mice[21], and has been found to be increased in the plasma of healthy humans undergoing a RIPC protocol[16]. However, its role as a humoral mediator of RIPC has not been properly evaluated yet.

Apolipoprotein A-I has anti-inflammatory properties, which could prove useful in the protection against ischemia-reperfusion injury[18]. In humans, its circulating levels have been found to be either increased[18] or decreased[17,22] after a RIPC protocol; therefore, its exact role is still debated.

Finally, SDF-1α has been proposed to be an important, and possibly the main, mediator of RIPC[23]. In a study on rats, a 50% plasma increment was detected in rats subjected to a RIPC protocol compared to control animals (890 ± 70 pg/mL *vs* 590 ± 50 pg/mL; n = 8, $P < 0.01$)[23]. Nevertheless, the administration of a selective inhibitor of SDF-1α did not completely abrogate the reduction in infarct size following RIPC[24], suggesting the existence of other mechanisms of cardioprotection[24].

Several potential other mediators have been identified: among them, there are microRNAs (miRNAs),

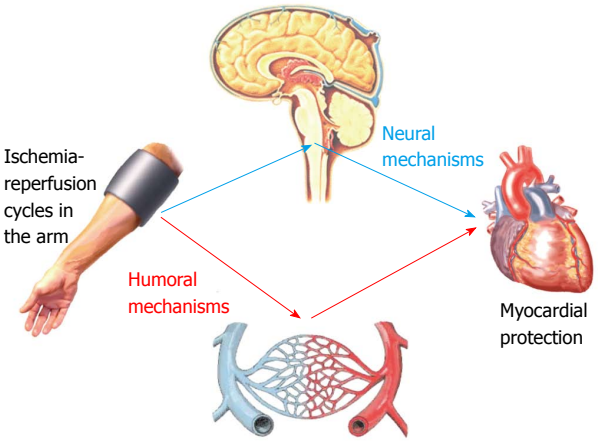

Figure 1 Mechanisms of remote ischemic conditioning. The cardioprotective effects of several ischemia/reperfusion cycles applied in a distant site (most commonly the upper limb) have been ascribed to the activation of humoral and/or neural pathways. The pathogenesis of remote ischemic conditioning is incompletely known, however it is possi-ble that both humoral and neural mechanisms underlie this response.

bradykinin, adenosine, and nitric oxide (NO).

miRNAs have been involved in both muscle ischemia[25] and protection against myocardial IR injury[26]. The circulating levels of miR-144 have been found to increase by 1.6 folds in healthy human subjects undergoing a RIPC protocol, even though the exact mechanism of its action is still unknown[27].

Bradykinin is released by damaged tissues and can activate afferent fibers (see below), possibly contributing to a cardioprotective effect known as "remote preconditioning of trauma"[28]. A release of bradykinin from ischemic tissues into the bloodstream has been reported[29]; the involvement of bradykinin in the RIPC response has been postulated, but the data from an animal study were inconclusive[30].

Finally, both adenosine and NO have been extensively studied in the setting of ischemic preconditioning[31], and have been considered potential mediators of RIPC as well[7,32], although their extremely short half-life makes unlikely that they could exert a significant cardioprotective effect.

To summarize, it is quite established the existence of humoral mechanisms underlying RIPC, but the nature of the mediator(s) is currently unclear. Potential humoral mediators should be assessed with respect to their potential mechanism of action, the increase in their circulating levels following a conditioning protocol, and their half-life; in fact, all these parameters should be compatible with a cardioprotective role. Further studies are required to define the existence and the role of humoral mechanisms underlying RIPC, as well as the other forms of remote ischemic conditioning.

NEURALLY-MEDIATED CARDIOPROTECTION

Neural control of the heart

The autonomic nervous system consists of an afferent pathway, integrating centers located into the central nervous system, and two efferent limbs, the sympathetic and the parasympathetic nervous systems[33]. In the heart, sensory innervation is provided by afferent neurons located into the nodose and dorsal root ganglia, and projecting to brainstem areas controlling the activity of both sympathetic and parasympathetic nuclei[33]. Sympathetic efferent fibers innervate the sinoatrial and atrioventricular nodes, the atria, and the ventricles[33]. Parasympathetic efferent fibers are traditionally believed to control exclusively the nodal tissue and the atria[33]. Nevertheless, the presence of cholinergic innervations have been detected in both ventricles, and it has been demonstrated that vagal activation decreases the force of ventricular contraction irrespective of its effect on heart rate, in both animals and humans[33].

The "neural hypothesis" of RIPC, and potential role of the sympathetic system

The "neural hypothesis" of remote ischemic preconditioning (RIPC) postulates that ischemia-reperfusion (IR) cycles in peripheral sites might activate a neural reflex resulting in myocardial protection against a subsequent myocardial insult[34] (Figure 1).

In animals, cycles of occlusion and reopening of the renal artery, mesenteric artery, or femoral artery resulted in significant cardioprotection; in all these cases, the resection of the afferent fibers projecting to the ischemized territories abolished the cardioprotective effect[35-37]. In rats, IR cycles in the mesenteric artery conferred a cardioprotection similar in entity to that provided by ischemic preconditioning (*i.e.*, IR cycles of the coronary vessel before sustained occlusion); the systemic administration of hexametonium, a blocker of both sympathetic and parasympathetic ganglia, abolished this effect[38].

Conflicting results have been provided by Kingma *et al*[39], who reported that, in dogs anesthetized with isoflurane, a RIPC protocol conferred robust myocardial protection against a subsequent ischemic injury even during autonomic blockade or surgical denervation of the heart. It should be noted that in this study the animals were anesthetized with isoflurane[39], which is *per se* a powerful preconditioning agent (see below).

The activation of neural afferents during RIPC has been ascribed to local accumulation of mediators such as calcitonin-gene related peptide (CGRP), adenosine, and bradykinin[40]. Interestingly, the accumulation of adenosine[41,42], bradykinin[43], and other mediators[44,45] in the exercising muscle has been implied as a determinant of the metaboreflex[46], which is a neural mechanism coupling sympathetic tone to exercise requirements[47,48]. It could then be speculated that the IR cycles of a conditioning protocol cause metaboreflex activation. The subsequent increase in sympathetic outflow could confer myocardial protection through the activation of β1 and/or β2 adrenergic receptors; this phenomenon has been discovered in animal hearts perfused with β-agonists, and has been named

"β-adrenergic preconditioning"[49-51].

Evidences of vagal activation following RIPC

As discussed above, a theoretical framework could be provided for sympathetic outflow as the final mediator of RIPC. Nevertheless, a growing body of evidences points to RIPC as a vagal reflex.

The possibility to precondition the heart by the infusion of acetylcholine (ACh) was demonstrated in 1993 by Yao *et al*[52] The preconditioning potential of ACh has been confirmed by several other studies[53-55]. It has been demonstrated that cardioprotection by RIPC is suppressed by spinal cord section, bilateral vagotomy or the systemic administration of the muscarinic receptor antagonist atropine, while vagal stimulation closely recapitulates the effects of RIPC[56,57].

Using a viral transfer gene approach in rats, Mastitskaya *et al*[58] confirmed that an intact parasympathetic outflow is crucial for myocardial protection by RIPC. The neurons in the dorsal motor nucleus of the vagus nerve were selectively silenced, thus abolishing the cardioprotective effect of a RIPC protocol[58]. The selective activation of the same neurons closely recapitulated the cardioprotective effect of RIPC; this response was suppressed by atropine[58]. Again in rats, Basalay *et al*[59] reported that IR cycles in the limb conferred cardioprotection when applied 25 min prior to myocardial ischemia. The authors then found that the cardioprotective effect was abolished by the denervation of the peripheral ischemic organ or bilateral vagotomy[59].

Autonomic function in RIPC: What happens in humans?

To our knowledge, only two studies have evaluated the consequences of a RIPC protocol on the autonomic function in humans. In 2005, Loukogeorgakis *et al*[60] evaluated the possibility to protect against endothelial ischemia-reperfusion injury by RIPC. A cuff inflation to 200 mmHg for 20 min in the non-dominant arm was used as the ischemic insult; the subsequent endothelial damage was denoted by reduced flow-mediated dilation (FMD)[60]. When arm ischemia was preceded by a RIPC protocol in the dominant arm, the ischemic insult in the other arm caused no significant reduction in FMD compared to baseline, suggesting endothelial protection by RIPC[60]. Such response was abolished by the autonomic ganglion blocker trimetaphan[60]. These results suggested an autonomic activation underlying the endothelial protection by RIPC; however, being trimetaphan an aspecific autonomic blocker, it was not possible to ascertain if either the sympathetic or the parasympathetic system accounted for the protection by RIPC[60].

Parasympathetic activation was detected as the underlying mechanism by Enko *et al*[61] in 2011. After 3 cycles of 5 min ischemia and 5 min reperfusion in the left arm, a significant dilation of the right brachial artery was observed; in the power spectral analysis of heart rate, the high frequency domain displayed a simultaneous increase, denoting increased parasympathetic outflow[61].

Remote ischemic perconditioning and postconditioning: does vagal activation play a role?

The first demonstration of remote ischemic perconditioning was provided in 2007: in pigs, four 5-min cycles of lower limb ischemia during a 40-min left anterior descending coronary artery occlusion caused a significant reduction in infarct size, improved indexes of systolic and diastolic function, and less arrhythmic events during the reperfusion phase[11]. With respect to remote ischemic postconditioning, a cardioprotective effect of IR cycles at the beginning of reperfusion was demonstrated for the first time in 2005[12], and subsequently corroborated by other animal studies[62,63].

To our knowledge, only Basalay *et al*[59] assessed the pathophysiology of remote ischemic perconditioning and postconditioning. These authors reported that deafferenting the site of IR cycles or cutting both vagus nerves abolished the preconditioning and perconditioning responses in rats, but did not alter the postconditioning effect[59]. These results suggest that remote ischemic perconditioning relies on neural mechanisms, while remote ischemic postconditioning is mediated by humoral mediators[59].

Further studies are required to assess this hypothesis. However, it should be noted that neural mechanisms are more qualified than humoral mechanisms to protect the ischemic myocardium in the setting of remote ischemic perconditioning, at least when the coronary flow is completely blocked. In the same setting, an activation of the parasympathetic system would probably be more effective than a sympathetic response.

Excessive concentrations of catecholamines have been detected in the ischemic area during an acute myocardial infarction[64]. Increased cardiac sympathetic outflow is due to pain, anxiety, and a fall of cardiac output or arterial blood pressure; a further release of catecholamines is promoted by the ischemic damage of nerve endings[64]. As a result, extracellular norepinephrine reaches up to 100-1000 times its normal plasma concentrations within 30 min of coronary occlusion[64]. Far from being protective, local concentrations of this magnitude are capable of producing myocardial necrosis even in nonischemic myocardium, and might promote malignant arrhythmias[64]. This mechanism accounts for the positive effects of early administration of β-blockers during acute myocardial infarction[65,66], and probably excludes increased sympathetic outflow as the final mediator of cardioprotection by remote ischemic perconditioning.

PROTECTING THE HEART IN THE SETTING OF PCI

Stable coronary artery disease (SCAD) is associated with impaired quality of life, reduced physical endurance,

recurrent hospitalizations and outpatient visits[67]. Revascularization by either elective PCI or CABG can relieve symptoms, reduce the use of anti-ischemic drugs, improve exercise capacity and quality of life, compared to medical therapy alone[67]. The efficacy of elective PCI in addition to medical therapy in patients with SCAD has been demonstrated in a large number of randomized controlled trials, meta-analyses, and large-scale registries[67].

Albeit elective PCI is becoming increasingly safe, balloon inflation during PCI often causes transient ischemia[68]. Myocardial injury with necrosis may derive from recognizable peri-procedural events such as coronary dissection, occlusion of a major coronary artery or a side-branch, disruption of collateral flow, slow flow or no-reflow, distal embolization, and microvascular plugging; alternatively, the ischemic insult can have no detectable cause[68]. Myocardial ischemia is attested by a rise and fall of cardiac biomarkers after the procedure, with values rising five or more folds over the 99th percentile being indicative of peri-procedural myocardial infarction (PMI)[68].

Four recent meta-analyses have demonstrated that RIPC is effective in reducing PMIs in patients undergoing elective PCI[69-72]. For example, in the meta-analysis by Zografos *et al*[71], PMI occurred in 40.3% of patients in the RIPC group and in 51.3% of patients in the control group (odds ratio 0.57).

Several trials have assessed the long-term outcomes after elective PCI. An improvement in prognosis was not found by Prasad *et al*[73] over 1 year follow-up. By contrast, in the Cardiac Remote Ischemic Preconditioning in Coronary Stenting study, a significant reduction of major adverse cardiac and cerebral events (MACCE; a composite of all-cause mortality, myocardial infarction, readmission for heart failure, and ischemic stroke or transient ischemic attack) was found at 6 mo[74]. A recent follow-up study evaluating the same cohort demonstrated that the MACCE rate at 6 years remained lower in the RIPC group[75].

The significant heterogeneity of the study protocols could be hindering a careful assessment of RIPC efficacy in the setting of elective PCI. For example, the studies assessed in the meta-analysis by Zografos *et al*[71] differed with regard to the RIPC procedure (number of IR cycles, duration of the IR periods, site of application of the IR cycles), the percentage of patients with multivessel disease, and the positivity or the negativity of cardiac troponin I (cTnI) before PCI[71]. By contrast, in all the studies evaluated in this meta-analysis the IR cycles were performed immediately before elective PCI[74-80], so that a different time span between the IR cycles and the angioplasty procedure cannot be regarded as a potential confounding factor.

On the whole, a protective role for RIPC in the setting of elective PCI is emerging, even though its efficacy seems to be lower than in primary PCI. Nevertheless, only one long-term follow-up study has been published so far[75]; the prognostic role of RIPC should therefore receive extensive evaluation, as well as the optimal RIPC protocol to achieve effective cardioprotection.

Remote ischemic perconditioning refers to the application of ischemia-reperfusion cycles in a distant site shortly before revascularization. The first evidences of a protective role of remote ischemic perconditioning in human patients was provided in 2010 by Bøtker *et al*[81], who assessed 333 patients with suspected first ST-elevation myocardial infarction. The primary endpoint was myocardial salvage index (MSI), quantified as the proportion of the area at risk preserved by the treatment, 30 d after primary PCI. MSI was significant higher in the conditioning group than in controls; the protective effect of remote ischemic perconditioning seemed to be strongest in patients with more severe infarctions, *i.e*., presenting with occluded vessels or infarcts in the left anterior descending artery[81].

The long-term outcome of remote ischemic perconditioning was assessed in the same study population[82]. A significant reduction of MACCE and all cause mortality was observed in the conditioning group over a median follow-up of 3.8 years. There was also a trend toward reduced myocardial reinfarction, readmission for heart failure, and ischemic stroke or transient ischemic attack[82].

In 2013, remote ischemic postconditioning was assessed on 232 patients undergoing elective PCI[83]. In the conditioning group, the patients underwent three 5-min cycles of cuff inflation in the nondominant arm just after the end of the angioplasty. No significant difference was found between the conditioning group and the control group in terms of peak troponin I levels, PMI rate, recurrence of myocardial ischemia[83]. In another study, the incidence of PMIs was similar between all groups, and no difference was remarked with respect to the creatine kinase (CK) levels or the incidence of acute kidney failure[84]. In other studies, significant reductions in the incidence of acute kidney failure were observed; the prevention of acute kidney failure is currently regarded as the most promising perspective for the application of remote ischemic postconditioning during PCI[85,86].

For the details of the studies cited in the present paragraph, see Table 1.

REMOTE ISCHEMIC PRECONDITIONING BEFORE ELECTIVE CARDIAC SURGERY

Elective coronary artery bypass surgery (CABG) stands as an alternative to elective PCI for the management of SCAD[87]. The safety and efficacy of both techniques are similar, as well as the incidence of PMIs[87].

In 2007, a randomized controlled study enrolled 57 adult patients undergoing elective CABG surgery[88]. In the RIPC group, three IR cycles were performed

Table 1 Clinical studies on remote ischemic conditioning in percutaneous coronary intervention

Ref.	Patients *n* (CTRLS/RIPC)	ST or LT outcome	Conditioning protocol			Primary endpoint	Results		
			I/R cycles	Cuff pressure	Limb		RIPC	CTRLS	*P*
Remote ischemic preconditioning									
Prasad *et al*[73], 2013	48/47	ST	3 × 3′	200 mmHg	Upper	Post-PCI myonecrosis (cTnT ⩾ 0.03 ng/dL)	40%	47%	0.42
Ahmed *et al*[76], 2013	72/77	ST	3 × 5′	200 mmHg	Upper	cTnT 16 h post-PCI	0.02 ng/mL	0.047 ng/mL	0.047
Ghaemian *et al*[77], 2010	40/40	ST	2 × 5′	Above systolic	Lower	TnT 24 h post-PCI	12.50%	40%	0.01
Hoole *et al*[74], 2009	117/125	ST	3 × 5′	200 mmHg	Upper	cTnI 24 h post-PCI	0.06 ng/mL	0.16 ng/mL	0.04
Luo e *et al*[78], 2013	104/101	ST	3 × 5′	200 mmHg	Upper	hs-cTnI 16 h post-PCI	0.11 ng/mL	0.21 ng/mL	< 0.01
Xu *et al*[79], 2014	98/102	ST	3 × 5′	200 mmHg	Upper	hs-cTnI 16 h post-PCI	0.29 ng/mL	0.38 ng/mL	0.256
Davies *et al*[75], 2013	117/125	LT	3 × 5′	200 mmHg	Upper	MACCE (6 yr follow up)	23	36	0.039
Bøtker *et al*[81], 2010	167/166	ST	4 × 5′	200 mmHg	Upper	MSI after 30 d	0.75	0.55	0.033
Sloth *et al*[82], 2014	167/166	LT	4 × 5′	200 mmHg	Upper	MACCE rates (5 yr follow-up)	25.60%	13.50%	0.018
Carrasco-Chinchilla *et al*[83], 2013	114/118	ST	3 × 5′	200 mmHg	Upper	TnI 24 h post-PCI	0.476 ng/mL	0.478 ng/mL	0.378

cTnI: Cardiac troponin I; cTnT: Cardiac Tro-ponin T; CTRLS: Controls; hs-cTnT: High sensitivity cardiac troponin T; I/R: Ische-mia/reperfusion; MACCE: Major adverse cardiac and cerebral events; RIPC: Remote ischemic preconditioning; ST: Short term; LT: Long term.

after the induction of anesthesia, resulting in a 43% reduction in the 72 h area under the curve (AUC) of cTnT compared with the control group[88]. Other randomized trials confirmed a cardioprotective role of RIPC, in terms of reduced cTnT[89], cTnI[90], and CK isoenzyme MB[91] levels. By contrast, several studies failed to detect significant differences among the RIPC group and the control group[92-95]; the use of volatile anesthetics with preconditioning potential (isoflurane, enflurane, sevoflurane) possibly accounts for discrepant results[96-98].

In a meta-analysis, D'Ascenzo *et al*[99] reported a significant reduction in cTnI and cTnT levels in the RIPC group after elective CABG surgery. Such difference persisted after excluding the trials with potentially confounding factors (among them, the use of isoflurane)[99]. It has been suggested that the cardioprotective effect of RIPC could be masked by the administration of volatile anesthetics and blunted by the perioperative administration of β-blockers[99,100]. Indeed, previous studies on animals or isolated human atrial trabeculae had demonstrated that β-blockers could attenuate ischemic preconditioning-induced cardioprotection[100,101], perhaps since even the activation of β-adrenergic receptor is protective against ischemia-reperfusion injury (β-adrenergic preconditioning; see paragraph The "neural hypothesis" of RIPC, and potential role of the sympathetic system).

Two studies evaluating the long-term efficacy of RIPC provided diverging results. Lucchinetti *et al*[94] did not find any difference at 6 mo in terms of deaths or revascularizations, whereas Thielmann *et al*[102] found significantly lower mortality rates in the RIPC group than in controls over a mean 1.54 year follow-up.

With respect to the settings of elective valve replacement surgery and congenital cardiac surgery, a recent metanalysis detected a significant cardioprotective role for RIPC[103]. Nevertheless, the small number of studies, and the high heterogeneity among them[103] might undermine the reliability of these conclusions. Another recent meta-analysis considered cumulatively CABG, valve replacement surgery, and congenital cardiac surgery, and detected a significant reduction in the post-operative cTnI levels among the patients undergoing RIPC[104]. No subgroup analysis was performed, and the heterogeneity among studies assessing non-CABG surgery was marked[104]. A third meta-analysis took into consideration the studies evaluating RIPC efficacy in adult patients undergoing "major elective or emergency cardiac or vascular surgery"[105]. In such a broad and mixed setting, no significant efficacy of RIPC was detected with regard to several outcomes: perioperative death, myocardial infarction, new-onset cardiac arrhythmias requiring treatment, cerebrovascular accidents, renal failure requiring renal replacement therapy, mesenteric

Table 2 Clinical studies on remote ischemic preconditioning before elective coronary artery bypass graft surgery

Ref.	Patients *n* (CTRLS/ RIPC)	RIPC protocol			Primary endpoint	Results			Notes	
		I/R cycles	Cuff pressure	Limb		RIPC	CTRLS	*P*	β-blockers	Isoflurane, desflurane
Short terms results										
Hausenloy *et al*[88], 2007	30/27	3 × 5 min	200 mmHg	Upper	cTnT at				+	+
					6 h	0.31 mcg/L	0.59 mcg/L	0.039		
					12 h	0.37mcg/L	0.69 mcg/L	0.002		
					24 h	0.30 mcg/L	0.52 mcg/L	0.003		
					48 h	0.30 mcg/L	0.52 mcg/L	0.036		
					72 h	0.25 mcg/L	0.48 mcg/L	0.111		
Wagner *et al*[90], 2010	35/33	3 × 5 min	40 mmHg above systolic pressure	Upper	cTnI at 8 h	2.54 mcg/L	2.90 mcg/L	0.043	-	-
Ali *et al*[91], 2010	50/50	3 × 5 min	200 mmHg	Upper	CK-MB at					
					8 h	27.22 IU/L	30.24 IU/L	0.026	+	NS
					16 h	33.3 IU/L	37.2 IU/L	0.021		
					24 h	22.74 IU/L	25.22 IU/L	0.052		
					48 h	17.20 IU/L	19.72 IU/L	0.003		
Lomivorotov *et al*[93], 2012	40/40	3 × 5 min	200 mmHg	Upper	48 h cTnI AUC	54.4 ng/mL	53.3 ng/mL	> 0.05	+	+
Thielmann *et al*[102], 2013	167/162	3 × 5 min	200 mmHg	Upper	72 h cTnI AUC	266 ng/mL	321 ng/mL	0.022	+	+
Rahman *et al*[95], 2010	55	3 × 5 min	200 mmHg	Upper	48 h cTnT AUC	30 ng/mL	28 ng/mL	0.721	+	+
Lucchinetti *et al*[94], 2012	28/27	4 × 5 min	400 mmHg	Lower	72 h hs-cTnT AUC	11708 pg/mL	9574 pg/mL	0.33	+	+
Long term results										
Thielmann *et al*[102], 2013	167/162	3 × 5 min	200 mmHg	Upper	ACE at 1.54 yr	3	11	0.046	+	+
					death MACCE	8	23	0.005		
Lucchinetti *et al*[94], 2012	28/27	4 × 5 min	400 mmHg	Lower	ACE at 6 mo death	0	1	1.00	+	+
					rehospitalization	3	3	1.00		

ACE: Adverse cardiovascular events; AUC: Area under the curve; cTnI: Cardiac troponin I; cTnT: Cardiac Troponin T; CTRLS: Controls; hs-cTnT: High sensitivity cardiac troponin T; I/R: Ischemia/reperfusion; MACCE: Major adverse cardiac and cerebral events; NS: Not specified; RIPC: Remote ischemic preconditioning.

ischemia, length of hospital stay and intensive care unit stay[105].

The few studies evaluating the effects of RIPC in the sole setting of valve replacement yielded conflicting results. For example, Wu *et al*[106] did not find a significant effect of a standard RIPC protocol on cTnI release after mitral valve replacement surgery, whereas Xie *et al*[107] reported a significant reduction of the 72 h cTnI-AUC in patients undergoing mitral valve, aortic valve or tricuspid valve surgery.

On the whole, RIPC seems to exert a protective role against PMIs caused by elective CABG surgery, while its long term effects are still uncertain. Furthermore, no definite statement can be made about the RIPC efficacy in other forms of elective cardiac surgery, namely valve replacement surgery and congenital cardiac surgery (Table 2). Finally, volatile anesthetics and β-blockers are emerging as potential confounding factors, although the mechanisms are still unclear.

CONCLUSION

Remote ischemic conditioning (RIC) has been proposed as a "non-invasive, simple, safe, and cheap"[108] strategy to protect the heart against ischemic insults. A great research effort has been performed in order to verify the existence of a myocardial protection by RIC, and to evaluate the extent of such protection. Nevertheless, clinical studies have provided conflicting results. A deeper comprehension of the mechanisms underlying RIC is advisable in order to correctly assess the cardioprotective potential of RIC, and to guide future clinical research.

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REVIEW

Cardiovascular disease in human immunodeficiency virus infected patients: A true or perceived risk?

Shima Shahbaz, Marcella Manicardi, Giovanni Guaraldi, Paolo Raggi

Shima Shahbaz, Paolo Raggi, Mazankowski Alberta Heart Institute, University of Alberta, Edmonton T6G 2B7, Alberta, Canada

Marcella Manicardi, Cardiology Department, Azienda Ospedaliero-Universitaria Policlinico di Modena, University of Modena and Reggio Emilia, 41124 Modena, Italy

Marcella Manicardi, Giovanni Guaraldi, Metabolic Clinic, Infectious and Tropical Disease Unit, Department of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, 41124 Modena, Italy

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Correspondence to: Paolo Raggi, MD, Mazankowski Alberta Heart Institute, University of Alberta, 8440-112 Street, Suite 4A7.050, Edmonton T6G 2B7, Alberta, Canada. raggi@ualberta.ca
Telephone: +1-780-4074575
Fax: +1-780-4077834

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Abstract

After the successful introduction of highly active antiretroviral agents the survival of patients infected with the human immunodeficiency virus (HIV) in developed countries has increased substantially. This has allowed the surfacing of several chronic diseases among which cardiovascular disease (CVD) is prominent. The pathogenesis of CVD in HIV is complex and involves a combination of traditional and HIV related factors. An accurate assessment of risk of CVD in these patients is still elusive and as a consequence the most appropriate preventive and therapeutic interventions remain controversial.

Key words: Human immunodeficiency virus infection; Atherosclerosis; Cardiovascular risk; Antiretroviral therapy; Dyslipidemia; Hypertension; Smoking; Cardiovascular death

Core tip: Infection with the human immunodeficiency virus (HIV) was initially universally lethal but with the introduction of highly active antiretroviral therapies (HAART) the life span of HIV infected patients has drastically increased. Along with the lengthening of life span chronic diseases such as non-acquired immuno-deficiency syndrome related cancers and cardiovascular diseases surfaced. Currently cardiovascular disease is the primary cause of death among HIV infected patients in industrialized countries and its pathogenesis is very complex. A combination of direct virion injury, chronic low-grade inflammation, adverse cardiometabolic effects of HAART and high burden of traditional risk factors

contribute to this new epidemic.

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INTRODUCTION

In the era of highly active antiretroviral therapy (HAART), the prognosis for human immunodeficiency positive (HIV+) patients in developed countries has dramatically improved[1,2]. As a consequence HIV infected patients live longer[3] and the medical care for this population is becoming more focused on the management of non-acquired immunodeficiency syndrome (AIDS) related morbidities, including cardiovascular diseases (CVDs)[4].

Although CVD is a leading cause of mortality and morbidity in HIV+ patients[5,6], there remains some controversy as to whether the disease is accelerated (promoted by traditional and non-traditional risk factors for atherosclerosis) or accentuated (greater prevalence of traditional cardiovascular risk factors) in these patients[7,8]. The prevention and treatment may vary considerably according to which mechanism is the prevailing one. In this review, we address the epidemiology of CVD in HIV+ patients, we discuss the impact of traditional and HIV-related risk factors; we review risk assessment for CVD in HIV and provide a brief overview of future therapeutic approaches to prevention of CVD in patients infected with HIV.

BURDEN OF CVD IN HIV

Epidemiology of CVD in HIV (Table 1)

A large body of evidence supports the notion that the burden of both clinical and sub-clinical CVD is increased in HIV infected patients. Investigators in the Veterans Aging Cohort Study Virtual Cohort (VACS-VC) analyzed data collected in 82459 veterans followed for an average of 5.9 years. The 27350 veterans infected with HIV had a significantly higher risk of myocardial infarction (AMI) compared with uninfected veterans (HR, 1.48; 95%CI: 1.27-1.72)[6]. The highest AMI risk was recorded among patients with HIV-RNA levels of at least 500 copies/mL and CD4 cell (CD4+) count less than 200 cell/mL; the risk remained elevated among patients who achieved HIV-RNA levels less than 500 copies/mL over time, suggesting that HAART may have contributed to some of the AMI risk[6]. The increased risk of CVD was noted both in HIV infected men and women[9]. When the VACS-VC participants were categorized according to the presence or absence of standard risk factors (diabetes mellitus, current smoking, total cholesterol, blood pressure, statins and antihypertensive medications use), HIV-infected veterans without major CVD risk factors had a 2-fold greater risk of AMI compared with uninfected veterans and the risk increased rapidly with each additional risk factor added[10]. In a cohort of 3851 HIV-infected patients examined at two Boston health care facilities the rate of AMI was significantly higher than in 1044589 controls after adjustment for age, sex, race, hypertension, and dyslipidemia (RR, 1.75; 95%CI: 1.51-2.02; $P < 0.0001$). Importantly, race appeared to have a different influence on the rate of AMI, with risk being higher in African-Americans and in Hispanics compared to Caucasians[11]. Although the prevalence of hypertension, diabetes mellitus and dyslipidemia was higher in HIV patients, this study further suggested that traditional risk factors cannot fully account for the increased risk of CVD in HIV[11]. Other studies performed outside of the US confirmed an increased risk of CVD in infected individuals[12,13].

The evidence of an increased risk of CVD in HIV extends to studies of subclinical atherosclerosis. Hsue *et al*[14] measured carotid artery intima-media thickness (IMT), an independent predictor of AMI and stroke[15,16], in 148 HIV+ patients and 63 age and sex matched controls. They reported that the mean carotid IMT of HIV+ patients was significantly greater and progressed faster in HIV+ patients than in controls. Of note, HIV infection was a predictor of carotid IMT independent of all other risk factors such as age, sex, smoking, HTN, lipid abnormalities and diabetes mellitus; a nadir CD4 count < 200 cells/mL was associated with IMT progression. However, Currier *et al*[17] failed to show any association between HIV infection and rate of carotid IMT progression.

Coronary computed tomography with and without intravascular iodinated contrast provides information about coronary artery calcium and non-calcified plaques both measures of subclinical atherosclerosis. Post *et al*[18] showed that HIV-infected men had a greater prevalence of coronary artery plaques compared to uninfected men and more extensive non-calcified plaques. On the contrary, the extent of coronary calcification was similar in the two groups.

In contrast with the above reported increased prevalence of CVD, Klein *et al*[19] recently reported a decline in incidence of AMI and CVD in HIV-patients. They reviewed data collected among the members of Kaiser Permanente Southern California and Northern California health plans between 1996 and 2011 (24768 HIV-infected patients and 257600 controls). The unadjusted relative risk of AMI for HIV+ patients decreased from 2.0 in 1996-1999 to 1.2 in 2010-2011, and the adjusted RR declined from 1.8 in 1996-1999 to 1.0 in 2010-2011 (Table 2). The decreased incidence of AMI in HIV+ patients may be due to the use of more lipid-friendly HAART medications, earlier initiation of HAART, resulting in lower incidence of severe immunodeficiency, and better control of CVD risk factors. The latter notion was supported by the finding that the

Table 1 Epidemiological studies evaluating the impact of human immunodeficiency virus on cardiovascular disease

Ref.	Size	Follow-up	Findings
Freiberg *et al*[6]	82459 27350 HIV+ 55109 HIV-	5.9 yr	Increased risk of MI among HIV+ patients VACS-VS study[6] (HR: 1.48; 95%CI: 1.27-1.72)
Womack *et al*[9] VACS-VS study	2187 women 32% HIV+	6 yr	Increased risk of CVD in HIV+ women compared to uninfected women (HR: 2.8; 95%CI: 1.7-4.6)
Paisible *et al*[10] VACS-VS study	81322 33% HIV+	5.9 yr	HIV+ veterans without major CVD risk factors had a 2-fold increased risk of MI compared with HIV- veterans without CVD risk factors (HR: 2.0; 95%CI: 1.0- 3.9)
Triant *et al*[11]	3851 HIV+ 1044589 HIV-	8 yr	Increased risk of MI among HIV+ patients (RR: 1.75; $P < 0.0001$)
Silverberg *et al*[12]	22081 HIV+ 23069 HIV-	13 yr	Higher risk of MI among HIV+ patients with a low recent or nadir CD4 cells (< 200) compared with HIV- subjects (RR, 1.76; 95%CI: 1.31-2.37 for low recent CD4 RR, 1.74; 95%CI: 1.47-2.06 for low nadir CD4)
Lang *et al*[13] FHDH-ANRS CO4	74958 HIV+	6 yr	The risk of MI was higher in both HIV+ men and women compared with the general population Standardized mortality ratio: 1.4 (95%CI: 1.3-1.6) for HIV+ men and 2.7 (95%CI: 1.8 -3.9) for HIV+ women compared with the general population
Hsue *et al*[14]	148 HIV+ 63 HIV-	1 yr	Higher baseline carotid IMT of HIV+ patients ($P = 0.0001$) and faster progression ($P = 0.002$)
Currier *et al*[17]	133 subjects in 45 triads[1]	144 wk	HIV infection and PI use did not contribute to the rate of carotid IMT progression. The median paired difference in IMT change between the PI and non-PI subjects was not statistically significant ($P = 0.19$). When the HIV+ groups were combined and compared with the HIV-negative group, the difference in progression was also not significant ($P = 0.71$)
Post *et al*[18]	618HIV+ 383HIV- men cross-sectional study		HIV-infected men had a greater prevalence of CAC [PR: 1.21 (95%CI: 1.08); $P = 0.001$] and any plaque [PR: 1.14 (CI: 1.05- 1.24); $P = 0.001$], including non-calcified [PR: 1.28 (CI: 1.13-1.45); $P < 0.001$) and mixed [PR: 1.35 (CI: 1.10-1.65); $P = 0.004$) plaque, than uninfected men
Klein *et al*[19] VACS-VS study	95687 31% HIV+	15 yr	Decline in adjusted MI rate ratio for HIV status over time, reaching 1 (95%CI: 7-1.4) in 2010-2011

[1]Each triad consisted of one subject from each of the following categories: (1) HIV+ with continuous PI therapy; (2) HIV+ without prior PI use; (3) HIV- subject. HIV: Human immunodeficiency virus; IMT: Intima-media thickness; MI: Myocardial infarction; PR: Prevalence ratio; CVD: Cardiovascular disease.

Table 2 Crude and adjusted rate ratios (95%CI) of myocardial infarction comparing human immunodeficiency virus infected and uninfected patients during a 13-year time span in the California Kaiser Permanente health system

Year	1996-2011	1996-1999	2000-2003	2004-2007	2008-2009	2010-2011
Crude	1.6 (1.5, 1.8)	2.0 (1.5, 2.8)	2.0(1.6, 2.5)	1.5 (1.2, 1.9)	1.5 (1.1-2.0)	1.2 (0.9-1.6)
Adjusted	1.4 (1.2, 1.6)	1.8 (1.3, 2.6)	1.7(1.4, 2.1)	1.3 (1.0, 1.6)	1.3 (0.9, 1.7)	1.0 (0.7, 1.4)

Modified from Klein *et al*[19].

Framingham risk scores were lower in HIV+ patients in more recent years compared to the late 90's[19].

In summary, a considerable body of literature shows a greater incidence of clinical CVD and a greater prevalence of subclinical atherosclerosis in HIV infected patients compared to the general population, supporting the notion that HIV is an independent risk factor for CVD. Whether the recently reported trend reversal is due to a greater awareness and more effective implementation of preventive measures in HIV+ patients remains to be demonstrated in different geographical areas and health delivery settings.

Etiopathogenesis of atherosclerosis in HIV

Infection related factors: The increased CVD risk in HIV may be dependent on a direct role of the HIV virus and on the immunological dysregulations caused by chronic HIV infection (Figures 1 and 2); data from observational studies provide evidence that both of these elements are involved[6,10,11].

The importance of continuous suppression of viral replication was investigated in the strategies for management of antiretroviral therapy (SMART) study[20]. The investigators compared the risk of all-cause death and cardiovascular, renal or hepatic complications in 2720 HIV+ patients receiving intermittent antiretroviral therapy and 2752 HIV+ patients receiving continuous antiretroviral therapy[20]. After a mean follow-up of 16 mo, the risk of death from any cause and for major cardiovascular, renal and hepatic diseases was significantly higher in patients who received intermittent compared to those who received continuous antiretroviral therapy (HR for all-cause death: 2.6; 95%CI: 1.9-3.7; $P < 0.001$, HR for major CVD, renal and hepatic disease: 1.7; 95%CI: 1.1-2.5; $P = 0.009$). The authors suggested that the increased CV risk may be

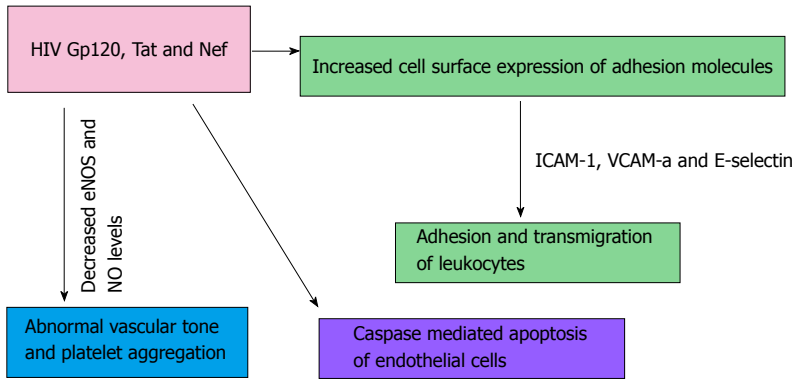

Figure 1 Putative mechanisms by which the human immunodeficiency virus virion increases the risk of atherosclerosis. The virus induces expression of adhesion molecules for leukocytes, reduce the secretion of nitric oxide with reduced vasodilation and induce endothelial cells apoptosis. ICAM: Intercellular adhesion molecule; VCAM: Vascular cell adhesion molecule; eNOS: Endothelial nitric oxide synthase; NO: Nitric oxide; HIV: Human immunodeficiency virus.

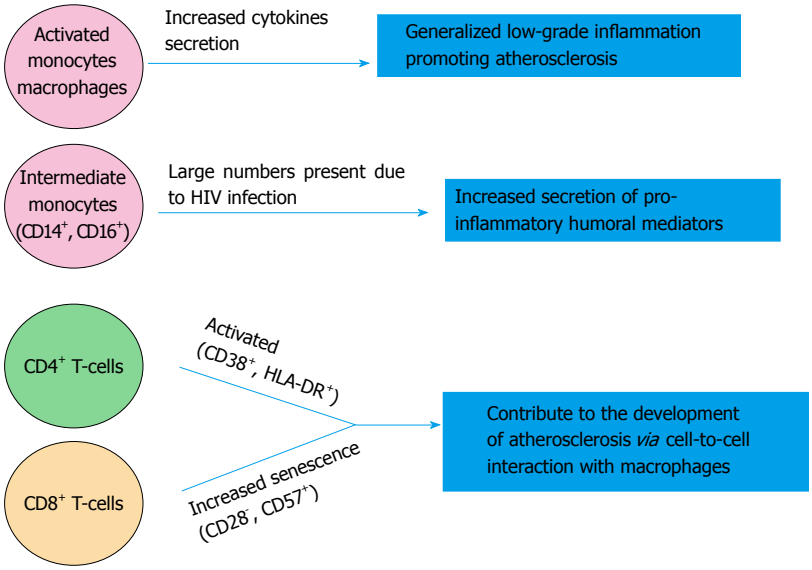

Figure 2 The human immunodeficiency virus promotes a state of low-grade chronic inflammation that increases the risk of atherosclerosis through the activation of lymphocytes, monocytes and macrophages. HIV: Human immunodeficiency virus; HLA: Human leukocyte antigen.

the consequence of alternating low and high CD4 cell numbers and viral loads experienced by the patients while receiving intermittent antiretroviral therapy, hence the importance of early and vigorous control of HIV replication and immune dysfunction[20].

In a cohort study of 22081 HIV+ and 230069 HIV- adult patients Silverberg *et al*[12] showed that the risk of AMI was 44% higher in HIV+ subjects compared with HIV- controls after adjustment for traditional risk factors. HIV+ patients with a nadir CD4 cell count < 200/mcl had a greater risk of AMI compared to controls[12]. However, the AMI rate of HIV+ subjects with a recent or nadir CD4 cell count ⩾ 500 /mcl and that of HIV- subjects was the same. In a case-control study of 289 HIV+ patients and 884 HIV-infected controls[21], a viral RNA level > 50 copies/mL, a low CD4 nadir and a high current CD8 count (> 1150/mm^3) were significantly associated with an increased risk of AMI. The ratio of nadir CD4/current CD8 count was the best predictor of an event[21].

In summary it would appear that a tight control of the HIV reproduction and maintenance of a good CD4 count may be protective against the risk of CV events. However, there exists conflicting evidence as to the actual association between CD4 cell count and the risk of AMI[22,23], as well as the HIV RNA levels and CV risk. Some investigators reported a direct association[21,22], while others were not able to identify one[24-26]; therefore this aspect of the CV pathogenicity of HIV needs to be clarified further.

The HIV can penetrate into endothelial cells utilizing CD4 receptors, galactosyl-ceramide receptors or chemokine receptors pathway[27-29], and different components of the HIV may have a role in the pathogenesis of CVD. Gp120 is a glycoprotein exposed on the surface of the HIV envelope and can also be found in the circulation from viral turn over[30,31]. It mediates HIV-1 entry into human cells by interacting with the HIV receptor for CD4 and co-receptors CXCR4 or CCR5[32]. Jiang *et al*[33] showed that Gp120 and tumor necrosis-alpha (TNF-α) synergistically decrease endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) levels in both porcine and human coronary artery endothelial cells. NO is an important factor in the regulation of vascular tone and inhibition of platelet adhesion and aggregation[34]. HIV infection is a chronic inflammatory state, characterized by elevated serum levels of factors such as TNF-α and TNF-β, interferon gamma (IFN-γ) and monocyte chemo-attractant protein-1 (MCP-1)[32,35,36] and Gp120 can magnify the pro-atherosclerotic effects of these mediators. Gp120 can also induce apoptosis by interacting with CXCR4,

a chemokine receptor[37,38], which is also expressed on vascular endothelium[39,40].

The trans-activator of transcription (Tat) protein is a regulatory protein that enhances the efficiency of viral transcription[41]. In a study on the role of Tat on the expression of adhesion molecules in human endothelial cells, Tat was shown to induce the expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin[42]. In the early phases of atherosclerosis, leukocytes adhere on the surface of endothelial cells and subsequently transmigrate between vascular endothelial cells into the intima layer of the vessel wall. While E-selectin is involved in the initial rolling of leukocytes on the endothelial cells[40], ICAM-1 and VCAM-1 induce firm adhesion and transmigration of leukocytes across the vascular endothelium[43]. High levels of soluble ICAM-1, VCAM-1 and E-selectin are associated with and increased risk of AMI in healthy men and women[44-46]. The levels of ICAM-1, VCAM-1 and E-selectin are elevated in HIV+ patients and there is a correlation between ICAM-1 concentration and the progression of HIV disease as well as the reduction of CD4 count[47,48]. Similarly to Gp120, Tat can also decrease endothelium dependent vasorelaxation and eNOS secretion in porcine coronary arteries[49].

Negative factor (Nef) protein is an HIV regulatory protein with an important role in cell apoptosis and enhancement of viral infectivity[50]. Nef blocks the ATP-binding cassette transporter A1 (ABCA1) pathway, leading to impaired cholesterol efflux from HIV infected macrophages to HDL particles[51]. As a result, HIV-infected macrophages accumulate lipids turning into foam cells, a step that may contribute to atherosclerosis formation[51]. Like Gp120 and Tat protein, Nef decreases endothelium-dependent vasorelaxation in porcine pulmonary arteries and reduces eNOS expression in both porcine pulmonary artery and human pulmonary artery endothelial cells[52]. In the same model Nef increased the levels of reactive oxygen species (ROS)[52], with an attendant decrease in NO bioavailability[53]. In endothelial cells, Nef can induce monocyte chemoattractant protein-1 (MCP-1) expression and apoptosis through NF-κB signaling and ROS-dependent mechanisms, respectively[54].

In addition to the humoral effects described above, cellular immune activation may play a role in the increased incidence of CVD in infected patients[35]. Monocytes are readily infected by HIV[55]; they adhere to the endothelial surface and eventually penetrate in the subendothelial space and intima. Monocytes, especially intermediate monocytes expressing $CD14^{++}$ and $CD16^{+}$, are prone to a greater pro-inflammatory activity once infected with HIV[56]. Furthermore, Hearps *et al*[57] showed that infected monocytes and macrophages of HIV+ patients have a reduced phagocytic activity and demonstrate telomere shortening a marker of premature ageing. High levels of monocyte activation markers, such as soluble CD163, CD14 and MCP-1 have been associated with subclinical coronary artery atherosclerosis, after adjustment for traditional CVD risk factors, in a large cohort of HIV-infected men[58]. T-lymphocytes are also activated in HIV infection[59]. In the Women Interagency HIV study, Kaplan *et al*[59] showed that HIV infection was associated with significantly elevated levels of activated [$CD38^{+}$ human leukocyte antigen (HLA)-DR^{+}] peripheral $CD4^{+}$ and $CD8^{+}$ cells and $CD8^{+}$ senescent cells ($CD28^{-}CD57^{+}$). The trend was reduced but not totally reversed after effective viral suppression with HAART. After adjustment for multiple confounders, $CD4^{+}$ and $CD8^{+}$ cell activation and $CD8^{+}$ senescent cells were associated with subclinical carotid artery lesions detected by 2D ultrasound[59].

Traditional risk factors in HIV: Traditional risk factors are more prevalent in HIV infected patients and likely represent a major driver for CVD in HIV.

(1) Cigarette smoking: The prevalence of cigarette smoking in HIV infected patients has been reported to be higher than in the general population. Analyzing data from 4217 infected (who participated in the Medical Monitoring Project) and 27731 non infected adults (who participated in the National Health Interview Survey in 2009), Mdodo *et al*[60] reported that 42.4% of HIV patients (95%CI: 39.7%-45.1%) were current cigarette smokers, while 20.3% (CI: 18.6%-22.1%) were former smokers, and 37.3% (CI: 34.9%-39.6%) had never smoked. Compared with the US adult population, in which an estimated 20.6% of adults smoked cigarettes in 2009, adults with HIV were nearly twice as likely to smoke [adjusted prevalence difference, 17.0% (CI: 14.0%-20.1%)], but were less likely to quit smoking (quit ratio, 32.4% *vs* 51.7%). A higher prevalence of smoking was also described in the SMART trial[61] and in the D:A:D study[62], where the rates of smoking were 40.5% and 51.5% respectively in HIV infected patients. Social and psychological factors such as ethnicity, lower educational level, poverty, illicit drug use, depression are likely contributing to the tobacco epidemic in HIV[60]. The noxious effects of smoking may be enhanced in HIV patients. Recently Helleberg *et al*[63] reported a greater number of life-years lost due to smoking in HIV infected patients compared to smoking controls [12.3 years (95%CI: 11.5-13.0) *vs* 3.6 years (95%CI: 3.1-4.0), respectively].

(2) Diabetes mellitus: Using data from the Multicenter AIDS Cohort Study (MACS), Brown *et al*[64] reported that the incidence of diabetes mellitus in HIV-infected men with HAART exposure was four fold higher than that of HIV-seronegative men. Subsequently De Wit *et al*[65] reported an incidence rate of 5.72 per 1000 patient follow-up year (95%CI: 5.31-6.13). The incidence of diabetes increased with cumulative exposure to HAART, and the association remained significant after adjustment for confounding factors. Besides the possible influence of HAART, some investigators reported a higher incidence

of insulin resistance and diabetes mellitus in patients co-infected with hepatitis C virus (HCV). The impact of the directly active agents used in the therapy of HCV to reduce the incidence of diabetes mellitus is currently unknown[66,67].

(3) Dyslipidemia: The dyslipidemia that develops during HAART is characterized by an increase in total and LDL cholesterol, and triglycerides levels. The effect varies according to the different HAART classes and within each class with different drugs. While this drug-induced toxicity was very common with the older antiretroviral drugs, it has become much less problematic with second generation nucleoside reverse transcriptase inhibitors (NRTI), (namely Rilpivirine) and with integrase inhibitors (Dolutegravir, Elvitegravir and Raltegravir). The HIV is a metabolically active virus capable to alter reverse cholesterol transport *via* modification of the HDL particles functionality and/or impairment of cellular cholesterol efflux. As discussed above, *in vitro* experiments have demonstrated that the HIV-related Nef protein can impair cellular cholesterol efflux through down-regulation of ABCA1[68,69]. ABCA1 plays a crucial role in stimulating cholesterol export from macrophages. Recently Lo *et al*[68] showed that, in the acute phase of HIV infection, HAART can restore the HDL-mediated cholesterol efflux capacity primarily through the suppression of viremia, providing additional evidence that prompt HAART initiation can potentially reduce atherosclerotic risk.

(4) Systemic hypertension: Among HIV infected patients the prevalence and incidence of systemic hypertension ranges from 20%-40% in high-income countries[70-72] to 11%-28% in low and middle income countries[73,74]. These trends may reflect the distribution of risk in the general populations of the same regions of the world. As a result, it is currently unclear whether hypertension is more prevalent in HIV infected patients than the general population.

Several studies failed to show a correlation between blood pressure levels and CD4 cell count and between viral load and hypertension. There are sparse and conflicting data on the role of antiretroviral therapy in the pathogenesis of hypertension[75,76], and the association remains inconclusive.

Impact of antiretroviral therapy on CVD: The first cases of myocardial infarction in HIV-infected patients receiving protease inhibitor were described in the late 1990s; since then several epidemiological studies have examined the association between HIV infection, HAART and the risk of CVD. In 2003 the investigators of the D:A:D study (Data Collection on Adverse Events of Anti-HIV Drugs) reported for the first time an increased incidence of myocardial infarction with longer exposure to combination antiretroviral therapy [adjusted risk rate per year of exposure, 1.26 (95%CI: 1.12-1.41); $P < 0.001$]. Patients with no exposure to therapy had a lower incidence of myocardial infarction than any of the treated groups[24]. Several years later the same investigators showed that cumulative exposure to some of the protease inhibitors (Indinavir, Lopinavir-Ritonavir) was associated with an increased risk of myocardial infarction (relative rate per year, 1.12 and 1.13, respectively) after adjustment for the impact of these drugs on lipid metabolism[77]. This was the first report that suggested that HAART might be responsible for an increased incidence of cardiovascular events independent of their lipid effects. The case of Abacavir remains paradigmatic in this setting. Several observational cohorts and a few randomized clinical trials reported an association between current, but not cumulative exposure of Abacavir and myocardial infarction. Abacavir, as a guanosine analogue, inhibits soluble guanylyl cyclase leading to enhanced platelet adhesion and, ultimately, to increased risk of myocardial infarction[78].

Numerous other studies implicated Abacavir and protease inhibitors in the development of cardiovascular events, however none of the reports provided conclusive evidence. The adverse effects of these drugs may be particularly harmful in patients with pre-existing high cardiovascular risk, and they should therefore be avoided in those patients.

In spite of early observations reporting an increased risk of CVD in patients receiving HAART, particularly protease inhibitors, more recent evidence suggests a safer cardio-metabolic profile of some categories of HAART[20,79]. Current strategies to decrease the risk of CVD in HIV infected patients include early initiation of HAART regimens known to be associated with the fewest metabolic adverse effects and careful management of traditional CV risk factors during HAART treatment.

SCREENING ALGORITHMS FOR CARDIOVASCULAR RISK STRATIFICATION IN HIV

Cardiovascular risk assessment in HIV infected patients has traditionally been based on recommendations drafted for the general population. In 2010 Friis-Møller *et al*[80] reported on the performance of a new model (derived from the D.A.D. cohort) that included exposure to HAART along with traditional risk factors. Although the new model estimated outcomes more accurately than the Framingham Risk Score (FRS) in the HIV population, it has not been widely adopted.

A new risk prediction algorithm for the general population [atherosclerotic cardiovascular disease (ASCVD)] was introduced in 2013 by the American College of Cardiology/American Heart Association[81]. In preliminary analyses it appeared that the ASCVD might overestimate CVD risk in the general population[82]. However the situation is probably the opposite in HIV disease. In a cohort of 2270 HIV infected patients, Regan *et al*[83] recently reported that the ASCVD algorithm classified a larger proportion of HIV patients as high-

risk compared to the FRS (25% *vs* 10%). However, comparing the 5-year observed-*vs*-predicted event rates, both models underestimated the actual CVD risk in HIV. Similarly, Thompson-Paul *et al*[84] compared ASCVD[81], FRS[85], the European algorithm known as SCORE[86] and the DAD score[80] to predict events in 2392 ambulatory HIV+ patients. After a median follow-up of 6.5 years they recorded 204 events; all models underestimated the actual risk of events. The FRS, ASCVD, and DAD equations showed moderate discrimination (C-statistic range, 0.68 to 0.72), while SCORE showed poor discrimination (C-statistic = 0.59).

Imaging of subclinical atherosclerosis has been suggested as a method to improve risk prediction and implementation of preventive therapies in the general population with variable success. Zanni *et al*[87] described a disproportion in HIV infected individuals between presence of subclinical atherosclerosis and statins recommendation. One third of 108 HIV infected patients without known CVD who underwent computed tomography angiography hosted coronary artery plaques with features of high vulnerability. Although a larger number of patients would require statins according to the new ASCVD algorithm compared to the FRS recommendations (26% *vs* 10%), the majority (74%) of HIV infected patients with subclinical coronary atherosclerosis did not have an indication to receive treatment even with the new algorithm.

Coronary artery calcium (CAC) has been proven to be incremental to risk factors for the prediction of events in the general population[88,89], but to date it has not been shown to be as useful in HIV infected patients. In a metanalysis Hulten *et al*[90] described a similar prevalence of CAC between HIV-positive and uninfected patients (OR: 0.95, P = 0.851). In the MACS[91], among 615 HIV infected men and 332 controls, the adjusted odds ratio for CAC was 1.35 (95%CI: 0.7-2.61). No outcome study has yet been published demonstrating the utility of CAC as an incremental prognostic factor in HIV.

Inflammation plays a central role in the patho-physiology of atherosclerosis and 18-fluorede-oxyglucose positron emission tomography can identify activated macrophages infiltrating the arterial wall. Subramanian *et al*[92] compared 27 HIV positive patients receiving HAART without prior CVD, with two non-HIV control groups: one group was matched for age, sex and FRS (non-HIV, FRS-matched controls), while the second one was sex-matched but had known atherosclerotic CVD (non-HIV, atherosclerotic controls). Inflammation in the aortic wall (measured as target to background signal ratio, TBR) was similar between HIV infected patients and non-HIV atherosclerotic controls (2.23; 95%CI: 2.07-2.40 *vs* 2.13; 95%CI: 2.03-2.23; P = 0.29), but was higher than in the non-HIV FRS-matched controls (2.23; 95%CI: 2.07-2.40 *vs* 1.89; 95%CI: 1.80-1.97; P = 0.001).

In HIV infected patients the aortic TBR was associated with the serum concentration of soluble CD163, a novel marker of activated macrophages (P = 0.04)[93], but not with C-reactive protein (P = 0.65) or D-dimer (P = 0.08) levels.

More recently, Tawakol *et al*[94] performed 18-FDG-PET imaging in 41 HIV+ patients on stable HAART regimen without prior CVD, but with coronary plaques on coronary CT angiography. A higher tracer uptake was correlated with the presence of low-attenuation plaques (P = 0.02) and positive remodeling (P = 0.04), both features of plaque instability.

Despite these encouraging results, no algorithm to date has incorporated imaging information in risk models for HIV patients. Therefore risk assessment remains reliant on the use of traditional risk factors despite their demonstrated limitations, although there is hope that imaging may add useful information in the future (Figure 3).

MANAGEMENT OF HIV INFECTED PATIENTS TO PREVENT CVD

In view of the information discussed so far it appears true that the CV risk of HIV infected patients is greater than that of the general population; a combination of higher prevalence of traditional risk factors and HIV specific factors likely predispose patients to such increased risk. Four questions emerge regarding risk reduction management in HIV patients: (1) should HIV infected patients be treated more aggressively than the general population for traditional risk factors? (2) As a corollary of the former, should a lower risk threshold be used to initiate treatment? (3) What HAART should be chosen to minimize CV risk in HIV? and (4) Finally, should therapy for HIV infected patients be guided by imaging and/or non-imaging biomarkers?

To date no study has addressed the impact of more aggressive therapy of traditional risk factors in HIV patients to reduce the attendant CV risk. While it would appear that there is a trend toward a relative reduction of CV morbidity and mortality in the past 10-15 years[95], it is unclear whether this is due to greater physicians' awareness and increased preventive efforts in HIV patients or other as yet unknown factors. The current recommendations for risk assessment and risk reduction in HIV patients mimic those of the general population. However, as discussed above, in spite of a slightly better performance of newer versus older risk assessment algorithms, the majority of patients at risk are not identified as high-risk and are therefore not receiving potentially life saving therapies. The newest algorithm (ASCVD) lowered the threshold for identification of high-risk patients to 7.5% from 20% as recommended in the ATP-Ⅲ guidelines[96], but it continues to under perform in HIV patients. In fact, despite the greater proportion of patients at risk identified by the ASCVD, as many as 40% of the patients suffering an acute cardiovascular event would not have met the requirement for prescription

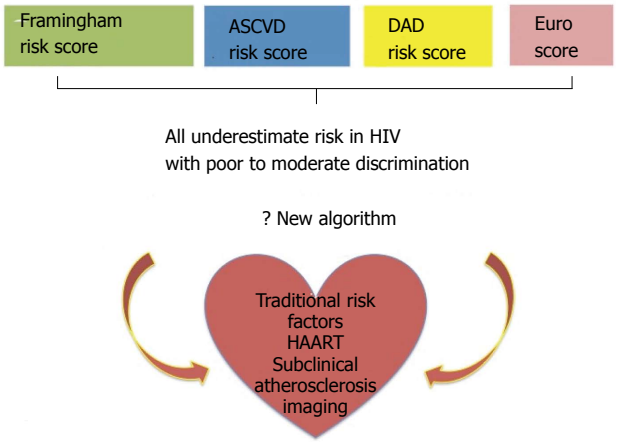

Figure 3 All algorithms currently used to estimate risk of cardiovascular disease in the general population underestimate the actual risk of human immunodeficiency virus positive patients. Although unproven, it is likely that a combination of traditional risk factors, risk linked with some anti-retroviral agents and data on subclinical atherosclerosis collected *via* imaging, may improve risk prediction in the future. ASCVD: Atherosclerotic cardiovascular disease; HAART: Highly active antiretroviral therapies; HIV: Human immunodeficiency virus.

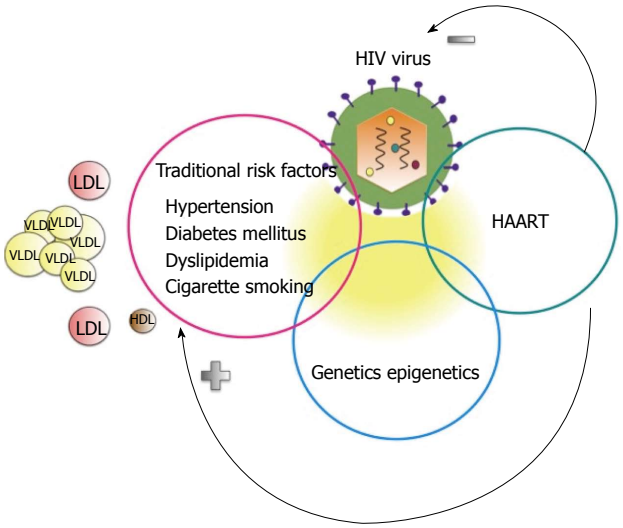

Figure 4 The pathophysiology of human immunodeficiency virus associated atherosclerosis is very complex; a high prevalence of traditional risk factors, direct effects of the human immunodeficiency virus virion and side effects of some the antiretroviral agents, along with yet unknown genetic and epigenetic factors predispose these patients to a high incidence of cardiovascular disease. The impact of anti-retroviral therapy is particularly difficult to estimate since suppression of viral replication may have an anti-atherosclerotic activity (curved arrow with negative sign) while side effect of some antiretroviral drugs may promote atherosclerosis (curved arrow with positive sign). HAART: Highly active antiretroviral therapies; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein; HIV: Human immunodeficiency virus.

of statins prior to the event according to two recent studies[83,97]. Hence, a mere lowering of the threshold for initiation of preventive therapies may be insufficient to effectively lower risk of events in HIV patients. In view of the difficulties highlighted above, a new study was launched[98] that aims to show that early statin treatment in asymptomatic HIV infected patients will delay the development of inflamed atherosclerotic plaques and reduce cardiovascular events (death, myocardial infarction, angina, stroke and revascularizations).

The choice of HAART and timing of therapy initiation likely carry a significant weight in risk reduction, as some of these drugs appear to have both direct and indirect cardiovascular toxicity while others may lower CV risk. Since the initial observation from the SMART study[20] reporting a reduced incidence of CV events in patients reaching a stable viral suppression, newer antiretroviral agents have been introduced with improved cardiometabolic toxicity. However, there are currently no long-term studies to demonstrate the safety and effectiveness of new anti-retroviral agents such as integrase inhibitors, although the safety profile of drugs such as atanazavir (a protease inhibitor) and tenofovir (member of the NRTI family) has been established. To address the paucity of prospective follow-up data, the D.A.D. registry has been tasked with the collection of safety data on drugs with a minimum of 30000 person-year follow-up[99].

Surrogate markers of atherosclerosis and serological biomarkers may provide some insight into the effectiveness of a novel therapeutic agent. In a preliminary communication, Stein *et al*[79] reported the effect of various antiretroviral combinations on the progression of the intima-medial thickness (IMT) of the carotid artery bulb in 234 HIV+ patients followed for a maximum of 144 mo from randomization. Atanazavir (protease inhibitor) was associated with less IMT progression than darunavir (integrase inhibitor), but the progression was similar for atanazavir and raltegravir (first generation integrase inhibitor) despite the better lipid profile of patients receiving raltegravir. Hence, it would appear that only part of the pro-atherogenic effect of HAART may be dependent upon lipid metabolism alterations. Additionally, in a recent publication the authors reported that various HAART regimen did not differ as far as expression of markers of inflammation and immune activation, confirming the notion that the anti- or pro-atherogenic effects of HAART cannot be gauged by their effect on serological biomarkers at the current state of research[100]. The lack of effect on biomarkers and a not markedly different effect on measures of atherosclerosis suggests that despite a reduction of viral load with HAART, there are residual immunological perturbations and reservoirs of viral replication that may induce atherosclerosis progression. Timing of HAART initiation has recently received renewed interest with the publication of the INSIGHT-START trial results[101]. In this trial 4685 HIV infected patients were randomized to receive HAART (mainly based on tenofovir, emtricitabine and efavirenz) with a $CD4^+$ cell count > 500/cc (median $CD4^+$ at initiation 651/cc) or only once the $CD4^+$ cell count dropped below 350/cc. The primary end point was a composite of death from all causes, and any serious AIDS related and non-AIDS related events (mostly cardiovascular). After a mean follow-up of 3 years the patient group

that received early HAART demonstrated a significantly reduced event rate (HR: 0.43; CI: 0.30-0.62, $P < 0.001$) compared to the delayed treatment group. These results fuelled an intense debate as to the pros- and cons of early HAART initiation, an approach that needs to be carefully considered in view of the potential side effects of some HAART discussed above.

CONCLUSION

The etio-pathogenesis of CVD in HIV is very complex, with contributions from the retrovirus and the attendant immunologic perturbations, HAART, highly prevalent traditional risk factors and genetics (Figure 4). Therefore a multifaceted approach will be necessary to effectively prevent its development and progression. Since atherosclerosis in HIV patients is characterized by immune activation in association with highly inflamed, non-calcified, potentially vulnerable plaques, these may become the targets of choice in future clinical trials to test the effectiveness of therapy.

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MINIREVIEWS

Concepts of hypoxic NO signaling in remote ischemic preconditioning

Matthias Totzeck, Ulrike Hendgen-Cotta, Tienush Rassaf

Matthias Totzeck, Ulrike Hendgen-Cotta, Tienush Rassaf, Department of Cardiology, West German Heart and Vascular Center, University of Essen Medical School, 45157 Essen, Germany

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Correspondence to: Dr. Tienush Rassaf, MD, Professor, Department of Cardiology, West German Heart and Vascular Center, University of Essen Medical School, Hufelandstrasse 55, 45157 Essen, Germany. rassaf@gmx.de
Telephone: +49-201-7234801
Fax: +49-201-7235401

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Abstract

Acute coronary syndromes remain a leading single cause of death worldwide. Therapeutic strategies to treat cardiomyocyte threatening ischemia/reperfusion injury are urgently needed. Remote ischemic preconditioning (rIPC) applied by brief ischemic episodes to heart-distant organs has been tested in several clinical studies, and the major body of evidence points to beneficial effects of rIPC for patients. The underlying signaling, however, remains incompletely understood. This relates particularly to the mechanism by which the protective signal is transferred from the remote site to the target organ. Many pathways have been forwarded but none can explain the protective effects completely. In light of recent experimental studies, we here outline the current knowledge relating to the generation of the protective signal in the remote organ, the signal transfer to the target organ and the transduction of the transferred signal into cardioprotection. The majority of studies favors a humoral factor that activates cardiomyocyte downstream signaling - receptor-dependent and independently. Cellular targets include deleterious calcium (Ca^{2+}) signaling, reactive oxygen species, mitochondrial function and structure, and cellular apoptosis and necrosis. Following an outline of the existing evidence, we will furthermore characterize the existing knowledge and discuss future perspectives with particular emphasis on the interaction between the recently discovered hypoxic nitrite-nitric oxide signaling in rIPC. This refers to the protective role of nitrite, which can be activated endogenously using rIPC and which then contributes to cardioprotection by rIPC.

Key words: Remote ischemic preconditioning; Ischemia/reperfusion injury; Nitrite; S-nitrosation; Mitochondria

Core tip: Therapeutic strategies to treat ischemia/reperfusion injury are urgently needed. Remote ischemic preconditioning (rIPC) appears to exert beneficial effects for patients. The underlying signaling remains incompletely understood. Following an outline of the existing evidence, we will characterize the existing knowledge and discuss future perspectives with particular emphasis on the interaction between the recently discovered hypoxic nitrite-

nitric oxide signaling in rIPC.

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INTRODUCTION

Current guidelines of both European and United States cardiac societies emphasize one primary goal for patients with acute myocardial infarctions - the timely and successful reperfusion[1]. This concept among many additional pharmaceutical treatments has significantly reduced mortality and morbidity in patients suffering from acute coronary syndromes. Experimental evidence, however, has largely implicated that rapid restoration of coronary perfusion, in turn, may paradoxically harm cardiomyocytes[2,3]. This phenomenon has been named ischemia/reperfusion (I/R) injury. The underlying signaling is complex and yet incompletely understood. Current concepts for the modulation of myocardial I/R injury have particularly emphasized three major contributors to the final I/R injury: reactive oxygen species (ROS), Ca^{2+}, and mitochondria. Particularly the latter are now not only regarded as targets during I/R but also as mediators of cellular injury and death. For a detailed description of the signaling in myocardial I/R injury the reader is kindly referred to the recently published excellent review sources[2-7]. Experimental and initial clinical studies implicate that all three signaling components of I/R injury may serve as potential targets in attempt to reduce I/R injury. Although early reperfusion therapy has led to a marked decrease in mortality following acute myocardial infarction, application of such cardioprotective strategy might further reduce the burden of cardiovascular disease.

Ischemic conditioning regimens are among the most effective cardioprotective approaches. These techniques are capable of reducing the final infarct size by up to approximately 30%-60%[8-10]. Brief episodes of sublethal I/R applied to the index organ [ischemic preconditioning (IPC)] - or a remote organ (rIPC) - before, during or after a main ischemic event initiate a powerful cellular signaling cascade rendering the cardiomyocyte capable of protecting itself. The majority of relevant clinical phase Ⅱ trials have produced favorable results for ischemic conditioning techniques. However, a definitive implementation into the clinical routine is still warranted. The underlying signaling in the scope of rIPC remains incompletely understood. Here we first outline the events leading to myocardial I/R injury, followed by an introduction to ischemic conditioning. Finally, we will put a major focus on the recently discovered nitrite-nitrogen oxides (NO) signaling, which targets mitochondria during I/R and protects the heart from lethal injury.

MYOCARDIAL I/R INJURY

Reperfusion therapy either by invasive coronary intervention, bypass surgery or pharmacological lysis causes a rapid increase in cellular oxygen levels[2]. One key pathological consequence of this is an insufficient mitochondrial respiration in consequence of an incomplete electron transport over mitochondrial membranes. This can cause an excess formation of ROS[11]. While ROS at physiological concentrations contribute to the general cellular homeostasis, higher levels may initiate a deleterious signaling. This contributes to an increase in cell death by necrosis or apoptosis[12].

The reperfusion phase is not only characterized by increased ROS levels, but also by a reduction in the bioavailability of NO. NO is a gaseous signaling molecule that regulates a wide variety of cardiomyocyte functions including scavenging of radicals, cardiac immune response, improvement of blood flow and left ventricular function[13-17]. By consequence, these functions are impaired during reperfusion. In addition, interactions between the ROS system and NO signaling may further contribute to the final myocardial I/R injury. This relates to the increase formation of peroxynitrite (an oxidant) in the reperfusion period[18], which in turn may contribute to nitrosative stress-related cell injury to membranes[19,20]. The exact role of peroxynitrite and whether peroxynitrite signaling can be effectively modulated in patients remains to be determined.

The second major contributor to cardiomyocyte dysfunction in I/R is a deteriorated Ca^{2+} signaling[21-24]. Elevated intracellular Ca^{2+} levels can not only trigger arrhythmias, and cardiomyocyte hypercontracture[25], they may also activate Ca^{2+}-dependent signaling pathways. This pertains especially to Ca^{2+}-dependent proteases named calpains, which then cleave cellular elements involved, *e.g.*, in apoptosis, mitochondrial respiration or mitochondrial turnover[26-28]. Hypercontracture and necrosis are not limited to one cell but can also be distributed from cell to cell either by direct contracture or by cell-to-cell progression through intercellular gap junctions[29]. Off note, high calcium levels may also directly target and disrupt cell membranes[20]. While both ROS and intracellular Ca^{2+} elevation mark the initial events of myocardial I/R injury, the central target of reperfusion-associated impaired signaling is the mitochondrion.

MITOCHONDRIA IN I/R INJURY

Mitochondrial injury in I/R comprises at least three relevant entities: (1) Destruction of mitochondrial membrane integrity by permeabilization; (2) deficits in mitochondrial structural dynamism, the recently termed mitochondrial fusion and fission and, finally; and (3) the deterioration of mitochondrial respiration[30].

The exact events leading to mitochondrion-driven cell death by mechanisms of necrosis, apoptosis, or autophagy are incompletely understood. Dysregulated Ca^{2+} signals and ROS concentrations, as found in early reperfusion, provide excellent circumstances to deteriorate mitochondrial integrity[31].

Apoptosis causes cell shrinkage, cellular fragmentation, and finally phagocytosis. The general characteristics of necrosis include cellular swelling and rupture, and a marked depletion of energy resources. Newer studies argue that apoptosis as well as necrosis are regulated by a complex signaling machinery with overlapping processes[32]. The critical step for apoptosis is the permeabilization of the mitochondrial outer membrane pore (MOMP). This occurs by activation of pro-apoptotic BH3 proteins, *e.g.*, Bax[31]. Subsequently, a release of apoptogenic factors is initiated, *e.g.*, cytochrome c or apoptosis-inducing factor (AIF)[33,34] By contrast, the key characteristic of necrotic cell death is the permeabilization of the inner mitochondrial membrane causing the formation of a yet incompletely identified mitochondrial permeability transition pore (mPTP).

Mitochondrial morphology and structural dynamism is regulated by fission and fusion[35]. Interestingly, Bax - previously introduced as major contributor to cell death - is also involved in mitochondrial fusion and fission. As a consequence, Bax supplementation deteriorates mitochondrial structural dynamism in conjunction with a much-increased I/R injury. Taken together both regulation of mitochondria-driven cell death as well as mitochondrial structure is complex but many overlapping yet incompletely defined processes exists that contribute to cardiomyocyte damage in I/R injury.

In case the initial phase of reperfusion is survived the preservation and restoration of mitochondrial function becomes a major goal. Mitochondria comprise one third of the cell mass of cardiomyocytes. Recent evidence suggests that at least three subpupulations exist: Subsarcolemal (SSM), interfibrillary (IFM) and perinuclear (PNM) mitochondria. Their relative contribution to the function of cardiac cells remains to be completely elucidated[30,32]. Mitochondria generate a vast amount of adenosine triphosphate per day[36] and the electron transfer through the complexes of the mitochondrial respiratory chain is under control of a delicate regulatory machinery. I/R may cause a major functional disturbance with a subsequent incomplete respiration and a burst in the generation of ROS[37]. Many cardioprotective strategies have attempted to reduce these excessive ROS levels. Newer approaches, *e.g.*, rIPC, have focused on regulatory posttranslational modifications of respiratory chain elements and particularly complex I, which we will outline in the following.

THE CONCEPT OF RIPC

rIPC is among the most effective techniques in rendering the myocardium capable of protecting itself against I/R injury[38]. rIPC-associated protection is initiated *via* short non-deleterious phases of I/R prior to an index ischemia. The maneuver is applied to an organ or tissue at distance to the one undergoing the main ischemic event[38,39]. Clinical evidence from recent phase Ⅱ trials favors a potential translation into clinical routine[40-42]. The underlying rIPC-related signal mechanism remains under intense debate. Generally, the signal transduction machinery initiated by the rIPC stimulus involves a trigger, the transfer of the trigger to the target organ and a distinct cardiomyocyte signaling leaving the cardiomyocyte protected from I/R[13]. As triggering pathways both humoral/blood borne factors[43-45] and neuronal transmission[46] have been proposed. However, the cardioprotection can be transferred when transfusing blood from rIPC animals to unconditioned littermates[43,45,47]. This argues in favor of important contribution of a humoral factor. This does not preclude a role for the nervous system to be involved in the modulation of the rIPC response[48]. However, an intact nervous system is particularly important for the remote and not the target organ[48]. Off note, the signaling is at least to certain degrees species-specific with some of the pathways that are active in murine studies being irrelevant in large animal models and humans[48,49].

TRANSFER OF PROTECTION *VIA* HUMORAL FACTORS

It is now generally accepted that rIPC initiates a complex interplay between several mediators not only at the remote site, but also in the circulation as well as in the target cell. Over the past two decades many components have been forwarded, and it is presently unlikely that one single mediator is responsible for the complete protection associated with rIPC. Among the factors which have been evaluated in experimental studies are adenosine, bradykinin, opioids, interleukins, stromal cell-derived factor, hypoxia-inducible factor 1α and members of the so-called RISK pathway. These studies have in part revealed conflicting results especially when comparing experimental results with those from trials in patients[6].

We and other groups have recently investigated a potential involvement of hypoxic NO signaling in the course of rIPC. Using a mouse model of warm liver I/R, it was demonstrated that ablation of endothelial NO synthase (eNOS) abrogates the protective effects seen with rIPC on microscopic liver damage[50]. eNOS generates NO which can then modulate cardiovascular functions either at the place of synthesis or at a distance when transported as nitrite or nitroso species (nitrosated proteins)[51]. Changes in shear stress, *e.g.*, due to an increase in blood flow as seen in hyperemia after short phases of ischemia, are the strongest physiological stimulus of eNOS activity, which is mirrored in higher circulating NO metabolites[13]. This led to the hypothesis that nitrite-NO signaling is involved in the pathways

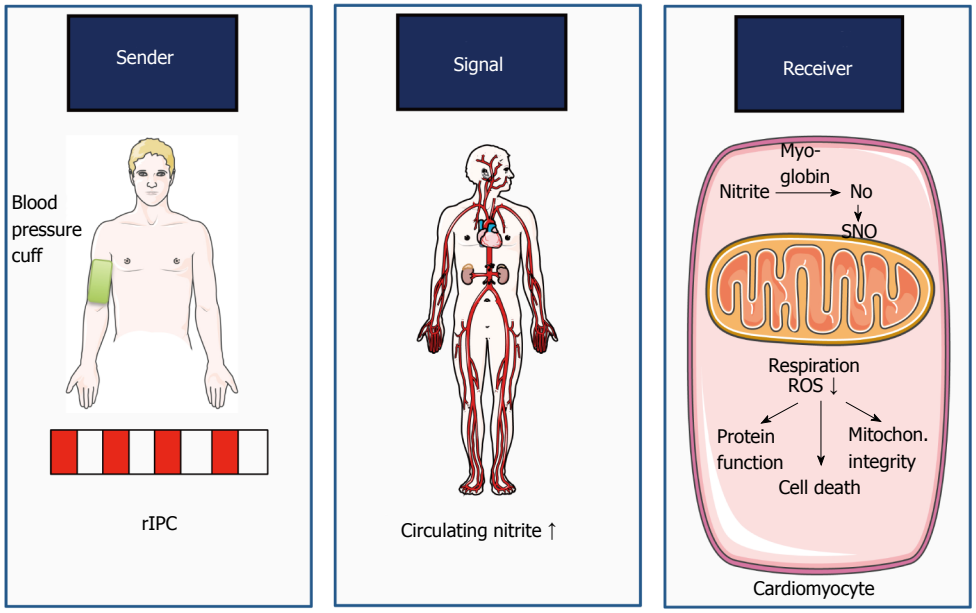

Figure 1 Nitrite/nitrogen oxides related signaling in remote ischemic preconditioning - proposed mechanism. The nitrite/NO signaling involves three components. The sender of the signal is shown in the left panel. In this case the upper extremity receiving rIPC *via* repetitive in-/deflations of a blood pressure cuff. During this maneuver, the classical L-arginine-NOS-nitrogen oxides (NO) pathway is activated. This includes the activation of eNOS, which generates NO from l-arginine while using several cofactors. Off note, the eNOS system is under tight control of several systems, *e.g.*, the calcium-calmodulin pathway. The middle panel represents the signal transmitted from the sender (remote organ) to the receiver (cardiomyocytes), as nitrite is transmitted from the site of generation *via* the circulation to the heart. In the receiving target organ, this signal is translated into cardioprotection *via* myoglobin-dependent nitrite reduction and S-nitrosation of mitochondrial complex I with a protective regulation of mitochondrial functions and reduced reactive oxygen species (ROS). The generation of NO in the last panel activates the alternative pathway for NO generation, which reduces nitrite to NO *via* hemeglobins, *e.g.*, myoglobin in the heart or hemoglobin in the circulation. The reduction of ROS in turn may help reduce cellular stress as indicated. This relates to an improvement of protein function, cell death and mitochondrial integrity[8]. rIPC: Remote ischemic preconditioning; NOS: NO synthase.

leading to cardioprotection from the rIPC stimulus.

THE ROLE OF NITRITE/NO SIGNALING IN REMOTE ISCHEMIC PRECONDITIONING

NO is a signaling molecule with a wide variety of functions in the cardiovascular system. Generally, NO protects the heart from I/R injury except for excessive levels during inflammation. However, in the course of I/R the canonical NO generation pathway *via* the classical L-arginine-NOS-NO signaling pathway becomes impaired due to a lack of molecular oxygen[52]. Nitrite, formerly regarded a mere NO oxidation product, may serve as an alternative source under these conditions[14,53]. The majority of the bodily nitrite provision derives from the oxidation of NO, which is enzymatically formed by one of at least three NO synthase (NOS) isoforms[54]. The remainder of nitrite in the circulation and tissues has been related to nutritional sources[55]. The half-life of nitrite is quite long in contrast to NO - approximately 35-60 min depending on the models and compartments used to evaluate nitrite distribution[56]. We have previously shown that the endogenous levels of nitrite can be modified by intravenous infusion of nitrite and certain diets[14,57,58]. An adequate nitrite production along with sufficient endogenous levels has implications for numerous cardiovascular functions. We have shown that trained athletes with higher nitrite levels performed superior in exercise tests as compared to those with lower concentrations[59].

In hypoxia or ischemia, nitrite may be reduced to bioactive NO, thus providing an alternative pathway to the enzymatic formation of NO, which is inactive under these circumstances. Nitrite activation may occur through reaction with hemoglobins such as myoglobin[60]. With decreasing oxygen gradients, cardiac myoglobin changes its function from an oxygen storage and NO scavenger to an NO producer by reducing nitrite to bioactive NO[13,15,37,60,61]. This can significantly reduce myocardial I/R injury[37].

The downstream mechanism following a formation of NO from nitrite largely involves mitochondria. In an experimental approach using exogenous nitrite during myocardial I/R injury we identified an S-nitrosation modification of mitochondrial complex Ⅰ. This, in turn, regulates myocardial energetics *via* the modulation of mitochondrial respiration and formation of mitochondria derived ROS. S-nitrosation of complex Ⅰ is furthermore associated with an adaption of myocardial functions to a reduced O_2 supply. This mechanism has been recognized as hibernation. Finally, nitrite reduction to NO and the downstream signaling cascade contributes to a decrease in myocardial necrosis and apoptosis[16,37,60,62].

In our recent study using rIPC as protective regimen we revealed that this maneuver also involves a protective nitrite/NO signaling with similarities to the

one described above for exogenous nitrite[13]. Figure 1 summarizes the general concept. This involves a sender, the transfer of the signal to the target organ, and a receiver - the cardiomyocyte. We propose the sender to be the endothelium of the extremity treated with rIPC. Repetitive phases of in- and deflations of the blood-pressure cuff cause high blood flow and a subsequently increased shear stress. This results in shear-stress dependent eNOS activation and NO formation, which converts to nitrite - the stable oxidation product.

This circulating nitrite is transmitted as signal of cardioprotection to the heart. The stability of nitrite and its half time argues in favor of nitrite[56]. In transfer experiments, the effects were preserved when infusing conditioned human plasma to isolated mouse hearts. Nitrite scavenging in conditioned human plasma abolished the transmission of cardioprotection to the conditioning-naïve Langendorff hearts.

The receiver of protection is the cardiomyocyte. In the myocardium, cardiomyocyte myoglobin reduces nitrite to NO during I/R with subsequent S-nitrosation modifications of mitochondrial complex I finally leading to cardioprotection. Presently, it is not known whether other signaling molecules regulating for instance apoptosis, necrosis and mitochondrial structure, *e.g.*, the BH3 proteins, are also affected by S-nitrosation modifications by rIPC.

Our recent findings were further evidenced by studies by a group who demonstrated that ischemic conditioning applied locally leads to a wide variety of proteins being S-nitrosated[63,64]. However, this appears to be a selective process in which SSM mitochondria are favored. This furthermore required connexin-43[64] to be active. It is tempting to speculate that particularly the subgroup of SSM are largely responsible for cardioprotection during I/R injury. Taken together, current evidence from experimental studies currently implicates a substantial contribution of nitrite/NO to cardioprotection by ischemic conditioning. This involves a specific modulation of mitochondria a targeted S-nitrosation and a reduction in the formation of ROS as well a distinct signaling *via* connexins.

CONCLUSION

In summary, rIPC has been demonstrated to protect the myocardium from I/R injury. Several clinical trials have demonstrated beneficial results for patients. Arguably, the underlying signaling is delicate involving many signaling processes. This pertains to humoral factors, blood cells, neurohumoral mediators and presumably a complex interplay. The data from ours and several other groups implicate a role for hypoxic NO signaling in the course of rIPC[13,50,64,65]. This requires particularly an S-nitrosation modification of mitochondrial elements. Naturally, many previously forwarded mechanisms will work along-side the proposed one based on NO[6]. Further studies will be required to elucidate the complex interplay involved in signal generation, signal transfer and final cardioprotection in the target organ. It will be of particular interest to evaluate the decisive signal transduction pathway in patients treated with rIPC. This is naturally a limitation of the current studies in the field of nitrite-NO signaling in rIPC[13,50,64,66-68], which involve healthy mice without significant co-morbidities and relevant medications.

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MINIREVIEWS

Potential of dietary nitrate in angiogenesis

Christos Rammos, Peter Luedike, Ulrike Hendgen-Cotta, Tienush Rassaf

Christos Rammos, Peter Luedike, Ulrike Hendgen-Cotta, Tienush Rassaf, Department of Cardiology, Westgerman Heart and Vascular Center, University Hospital Essen, 45122 Essen, Germany

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Correspondence to: Tienush Rassaf, MD, Department of Cardiology, Westgerman Heart and Vascular Center, University Hospital Essen, Hufelandstraße 55, 45122 Essen, Germany. tienush.rassaf@uk-essen.de
Telephone: +49-201-7234801
Fax: +49-201-7235618

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Abstract

Endothelial dysfunction with impaired bioavailability of nitric oxide (NO) is the hallmark in the development of cardiovascular disease. Endothelial dysfunction leads to atherosclerosis, characterized by chronic inflammation of the arterial wall and stepwise narrowing of the vessel lumen. Atherosclerosis causes deprivation of adequate tissue blood flow with compromised oxygen supply. To overcome this undersupply, remodeling of the vascular network is necessary to reconstitute and sustain tissue viability. This physiological response is often not sufficient and therapeutic angiogenesis remains an unmet medical need in critical limb ischemia or coronary artery disease. Feasible approaches to promote blood vessel formation are sparse. Administration of pro-angiogenic factors, gene therapy, or targeting of microRNAs has not yet entered the daily practice. Nitric oxide is an important mediator of angiogenesis that becomes limited under ischemic conditions and the maintenance of NO availability might constitute an attractive therapeutic target. Until recently it was unknown how the organism provides NO under ischemia. In recent years it could be demonstrated that NO can be formed independently of its enzymatic synthesis in the endothelium by reduction of inorganic nitrite under hypoxic conditions. Circulating nitrite derives from oxidation of NO or reduction of inorganic nitrate by commensal bacteria in the oral cavity. Intriguingly, nitrate is a common constituent of our everyday diet and particularly high concentrations are found in leafy green vegetables such as spinach, lettuce, or beetroot. Evidence suggests that dietary nitrate supplementation increases the regenerative capacity of ischemic tissue and that this effect may offer an attractive nutrition-based strategy to improve ischemia-induced revascularization. We here summarize and discuss the regenerative capacity of dietary nitrate on the vascular system.

Key words: Dietary; Nitrate; Vasculature; Regeneration; Hind limb

Core tip: Nitrate is a common constituent of our everyday diet and particularly high concentrations are found in leafy green vegetables such as spinach, lettuce,

or beetroot. Evidence suggests that dietary nitrate supplementation increases the regenerative capacity of ischemic tissue and that this effect may offer an attractive nutrition-based strategy to improve ischemia-induced revascularization. We here summarize and discuss the regenerative capacity of dietary nitrate on the vascular system.

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BREAKDOWN OF NITRIC OXIDE AVAILABILITY IS THE HALLMARK OF CARDIOVASCULAR DISEASE

Nitric oxide (NO) is biosynthesized endogenously from the amino acid L-Arginine (L-Arg) and oxygen by various NO synthases (NOS) which are termed either according to their distribution within the body or allowing for the order where they first purified and cloned. NOS produce NO by catalyzing a five-electron oxidation of guanidino nitrogen of L-Arg that requires binding of five cofactors. These cofactors are: flavin adenine dinucleotide, flavin mononucleotide, heme iron, tetrahydrobiopterin, and calcium-calmodulin[1]. If any of these co-factors becomes limited, NO production from NOS is restricted and NOS produce superoxide (O_2^-) instead. This mechanism has been termed "NOS uncoupling"[2]. Consequently, a physiological oxygen concentration as well as sufficient substrate supply is necessary for a proper NOS function. NO is involved in a wide variety of regulatory mechanisms of the cardiovascular system, including vascular tone (as a major mediator of endothelium dependent vasodilatation), vascular structure (inhibition of smooth muscle cell proliferation), and cell-cell interactions in blood vessels (inhibition of platelet adhesion and aggregation and inhibition of monocyte adhesion). Risk factors like hypercholesterolemia, hypertension, diabetes mellitus or cigarette smoking lead to the inability of the endothelium to produce NO[3-5]. A decrease of endothelial NO formation due to insufficient oxygen and cofactor supply or inactivation by reactive oxygen species is the hallmark of endothelial dysfunction. Importantly, this is the key element and a facilitative factor in the development of atherosclerosis. Lack of NO in turn promotes aggregation and invasion of inflammatory cells in the vessel wall and aggravates sclerosis of arteries. Thus, a vicious cycle takes place that results in progressive deprivation of blood supply with hypoxia of tissues and organs. Growth of new vessels result as an adaptive mechanism in response to tissue hypoxia or ischemic injury, called angiogenesis. The physiological repair response, however, is often not sufficient and therapeutic angiogenesis remains an unmet medical need.

ROLE OF NO IN ANGIOGENESIS

Angiogenesis is strongly stimulated in response to tissue hypoxia or ischemic injury and requires several key processes, including dissolution of matrix, endothelial cell proliferation and migration, and organization into tubes followed by lumen formation. One of the most potent angiogenic growth factors is represented by the vascular endothelial growth factor (VEGF) that induces proliferation, migration, survival and permeability of endothelial cells[6,7]. VEGF upregulates the expression of endothelial NO synthase (eNOS) and stimulates the release of endothelium-derived NO what is believed to play a critical role in the angiogenic action of this factor[8]. In line with these findings, it could be demonstrated that eNOS gene delivery promotes angiogenesis in animal models of ischemia[9]. On the contrary, the angiogenic response following hind limb ischemia in mice is impaired in eNOS- deficient mice and this cannot be reversed by VEGF substitution[10]. From these findings, NO appears to be a downstream mediator of VEGF-induced endothelial cell proliferation and migration and is suggested to even regulate VEGF expression[11]. However, it should be noted that a major limitation of these investigations is the use of NO donors. The drawback of this approach is that the influence of the released NO might be masked by the NO-independent actions of donating compounds or their derivatives. Likewise, animal models using eNOS overexpression to determine whether the effect of NO on VEGF synthesis could be achieved in ischemic limb neglect that eNOS is dysfunctional in ischemic tissues[12].

NO GENERATION WITHOUT NOS: THE NITRATE-NITRITE-NO PATHWAY

Since the classical NO-pathway is not functional during ischemia, NOS independent mechanisms must exist to maintain NO homeostasis under hypoxic conditions. The reduction of nitrite, the oxidation product of NO, by several "nitrite-reductases" under hypoxia was identified to be such an alternative pathway (Figure 1 and Table 1)[13-17]. These nitrite reductases operate along the physiological and pathological oxygen gradient and allow a graded nitrite reduction to NO according to the circulating and metabolic need. The reduction of nitrite to NO reflects a major mechanism by which the NO homeostasis is maintained independent of NOS. New insights evidence that nitrate and nitrite metabolism occurs in blood and tissues to recycle NO and other bioactive nitrogen oxides[18,19]. Commensal bacteria in the crypts of the tongue own a nitrate reductase enzyme that is utilized for energy metabolism in the absence of oxygen[20,21]. It was known that nitrate is

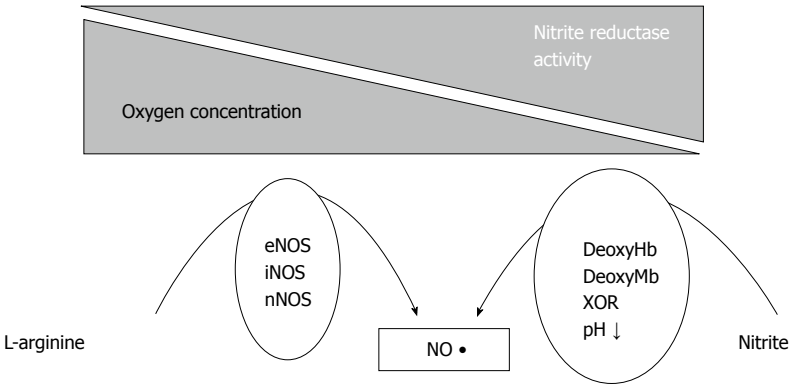

Figure 1 Non-enzymatic nitric oxide formation. One-electron reduction of nitrite (NO_2^-) to NO by ferrous heme proteins like hemoglobin in the blood or myoglobin in the heart can occur under conditions of low oxygen (O_2); the nitrite-reductase activity of these proteins contributes to NOS independent NO formation. eNOS: Endothelial NO synthase; iNOS: Inducible NO synthase; nNOS: Neuronal NO synthase.

Table 1 Nitric oxide generation pathways

Nitrate-nitrite-NO pathway	
NO synthases	
	Endothelial NO synthase
	Neuronal NO synthase
	Inducible NO synthase
Nitrate reductases	
	Xanthin oxidoreductase
	Mitochondrial respiratory chain enzymes
	Cytrochrome P-450
	Acidic reduction
	Myoglobin
	Neuroglobin
	Hemoglobin

NO: Nitric oxide.

taken up by the salivary glands and concentrated in the saliva. However, the reason for this active process could not be explained until the finding that nitrate serves as substrate for the nitrate reductase enzyme of bacteria in the mouth. These bacteria reduce both plasma extracted nitrate as well as dietary nitrate to form nitrite resulting in salivary nitrite levels that are 1000-fold higher than those found in human plasma[22]. When nitrite-rich saliva meets the acidic gastric juice after swallowing, nitrite is protonated to form nitrous acid (HNO_2), which then decomposes to NO. This acidic disproportionation takes part in the human defense against pathogens entering *via* the alimentary tract. Furthermore it could provide protection against ulcers from drugs or stress[23-25]. Beside the intragastric formation of NO it has been demonstrated that ingested nitrite reaches the systemic circulation, thus making it systemically available[22]. Nitrite in turn can be reduced *in vivo via* numerous pathways to form bioactive NO. These include the reduction *via* deoxygenated myoglobin within the heart muscle, deoxygenated hemoglobin, intracellular xanthin oxidoreductase, enzymes of the mitochondrial respiratory chain, cytochrome P-450 and even *via* the NOS[13,15,17,26-29]. Thus, several mechanisms exist by which NO is generated in the body, including the NOS enzymes or the non-enzymatically acidic reduction of nitrite. Nitrite mediates hypoxic vasodilation, enhances blood flow and matches oxygen supply to increased metabolic demands under hypoxic conditions[30]. Moreover, application of exogenous nitrite bears the potential to reduce myocardial damage after myocardial ischemia and reperfusion injury[26,31]. In addition, dietary approaches using nitrate to elevate circulating nitrite levels are emerging as a potential treatment regimen for high blood pressure[32]. Considering the upcoming evidence that nitrite and nitrate mediate cytoprotective effects in human physiology and especially under pathophysiological conditions, it is not unlikely that dietary nitrate and nitrite may positively affect human health and disease. Recognizing that NO is the most important molecule in regulating blood pressure and maintaining vascular homeostasis, food sources rich in NO compounds may provide beneficial effects primarily to the heart and vessels. Although there are clear reports on certain foods and diets that have shown a benefit in terms of preventing cancer and cardiovascular disease, the specific nature of the active constituents responsible for the cardioprotective effects of certain foods is still unknown. Viable candidates are fibers, minerals or antioxidants. High intake of fruits and vegetables is indeed associated with reduced risk for coronary artery disease and apoplectic stroke and the strongest protection against coronary heart disease was seen with high intake of green leafy vegetables[33]. Dietary intakes of nitrate-rich vegetables lowers blood pressure in subjects with borderline hypertension to the same extend as mono-therapy with a standard antihypertensive drug[34,35]. Likewise, blood pressure lowering effects could be demonstrated for dietary nitrate and ingestion of beetroot juice respectively[32,36]. We could recently demonstrate that dietary supplementation with inorganic nitrate improves prognostic relevant outcome measures that have been shown to predict cardiovascular events, namely endothelial dysfunction, vascular stiffness and systolic blood pressure in the elderly with moderately increased cardiovascular risk[37]. Improvements in blood pressure following the nitrate rich diet were associated with reductions of pro-inflammatory cytokines, which points to the potential anti-inflammatory actions of the nitrate-nitrite-NO pathway[38,39]. The hypothesis that dietary nitrate might provide cardiovascular benefit is further encouraged by animal models of myocardial infarction,

where dietary supplementation of these anions provided beneficial effects on I/R injury[40]. Interestingly, a diet rich in vegetables, such as the Mediterranean and the traditional Japanese diets, contains more nitrate than the recommended acceptable daily intake by the World Health Organization[41]. Even a portion of spinach consumed in one serving of salad can exceed the acceptable daily intake for nitrate[22]. Taken together, the current evidence supports the conclusion of the European Food Safety Authority that benefits of vegetable and fruit consumption outweigh any perceived risk of developing cancer from the consumption of nitrate and nitrite in these foods. The outlined above data from observational epidemiologic and human clinical studies support the hypothesis that nitrates and nitrites of plant origin play essential physiologic roles in supporting cardiovascular health.

REGENERATIVE CAPACITY OF DIETARY NITRATE ON VASCULATURE

Increased oxygen radicals (ROS) and subsequent reduced NO bioavailability impair vascular growth and remodeling, which highlights important targets for therapeutic angiogenesis in ischaemic myocardial infarction, peripheral vascular disease, as well as stroke. Significant advances have been made with respect to quantitative analytical methods to detect specific ROS and NO species. This must be employed in future studies to enhance the understanding of the relationship between ROS levels and stimulation of vascular remodeling in conjunction with NO bioavailability. Moreover, studies indicate impaired eNOS function in diabetes and atherosclerosis as a result of increased vascular generation of ROS and reduced NO bioavailability[42,43]. Despite endeavors to promote blood vessel formation by administration of pro-angiogenic factors, gene therapy or targeting of microRNAs, clinically applicable strategies have not been developed yet or are still in a preclinical phase[44-47]. In this context, dietary nitrate becomes an attractive candidate in the field. A nitrate rich diet can be achieved *via* consumption of leefy green vegetables, as spinach or lettuce, or by consumption of beet-root. However, for comparative reasons, most studies have used nitrate in the pure chemical form of sodium nitrate or potassium nitrate. Mechanistically, nitrate and nitrite can be viewed as stable storage pools for NO-like bioactivity. To further determine the mechanisms of a healthy diet through dietary nitrate on the vasculature we investigated the age-related changes that occur at a molecular level and determined the age-related vascular transcriptome altered by dietary nitrate[48,49]. Intriguingly, a chronic nitrate supplementation was shown to act as a modifier of gene expression, highlighting the plethora of putative mechanisms, which dietary nitrate influences[49]. Our results highlight the potential of a dietary approach counteracting the compromised cardiovascular system. Further investigations in ischemic tissues applying the hind limb model in mice highlighted the potential of dietary nitrate in angiogenesis[50]. A cytoprotective role of nitrite in the setting of myocardial, liver, kidney, and brain ischemia-reperfusion (I/R) injury has previously been demonstrated and continuous pharmacological intervention with nitrite injections increases vascular density in hind limb models[51-55]. We could show that dietary nitrate supplementation strongly augments perfusion recovery in chronic hind limb ischemia *in vivo via* a significant increase in capillary density. This improvement was associated with an increase in circulating nitrite concentrations, an elevated mobilization of $CD34^+/Flk\text{-}1^+$ cells and migration of bone marrow-derived $CD31^+/CD45^-$ cells into ischemic tissue[50]. The mobilization of circulating angiogenic cells following dietary nitrate supplementation was recently supported by a phase I clinical study[56]. A further effect of the dietary nitrate supplementation to drinking water was an attenuated apoptosis in myoblasts in chronic hind limb ischemia. Intriguingly, disruption of the nitrate-NO pathway by chronic eradication of the oral bacteria completely abolished beneficial effects of dietary nitrate supplementation and likewise effectively suppressed circulating nitrite levels, which were observed after intake of nitrate-rich food or dietary nitrate supplementation in drinking water. In line with these findings, the results of this study further point to a distinct contribution of dietary nitrate supplementation on tissue viability. Dietary nitrate ameliorated the remarkable capacity of adult skeletal muscles to regenerate myofibers after damage. This rapid repair process is mainly carried out by satellite cells (SCs) with contribution of NO[57,58]. Quiescent SCs become active and proliferate upon injury and display the regenerative capacity of the muscle. Committed daughter cells, the myoblasts, continue to proliferate followed by definite differentiation as initialized by a coordinated cellular signaling[59]. The precise pathways that are influenced by the nitrate-nitrite-NO pathway are under intensive investigations and not fully understood yet.

In summary dietary nitrate supplementation increases the regenerative capacity of ischemic tissue and may offer an attractive nutrition-based strategy to improve ischemia-induced revascularization.

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MINIREVIEWS

Is there a rationale for short cardioplegia re-dosing intervals?

Yves D Durandy

Yves D Durandy, Department of Intensive Care and Perfusion, Centre Chirurgical Marie Lannelongue, F-92350 Le Plessis, Robinson, France

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Correspondence to: Yves D Durandy, MD, Consultant, Department of Intensive Care and Perfusion, Centre Chirurgical Marie Lannelongue, Avenue de la Résistance, F-92350 Le Plessis, Robinson, France. yves.durandy@gmail.com
Telephone: +33-661-697416

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Abstract

While cardioplegia has been used on millions of patients during the last decades, the debate over the best technique is still going on. Cardioplegia is not only meant to provide a non-contracting heart and a field without blood, thus avoiding the risk of gas emboli, but also used for myocardial protection. Its electromechanical effect is easily confirmed through direct vision of the heart and continuous electrocardiogram monitoring, but there is no consensus on the best way to assess the quality of myocardial protection. The optimal approach is thus far from clear and the considerable amount of literature on the subject fails to provide a definite answer. Cardioplegia composition (crystalloid *vs* blood, with or without various substrate enhancement), temperature and site(s) of injection have been extensively researched. While less frequently studied, re-dosing interval is also an important factor. A common and intuitive idea is that shorter re-dosing intervals lead to improved myocardial protection. A vast majority of surgeons use re-dosing intervals of 20-30 min, or even less, during coronary artery bypass graft and multidose cardioplegia has been the "gold standard" for decades. However, one-shot cardioplegia is becoming more commonly used and is likely to be a valuable alternative. Some surgeons prefer the comfort of single-shot cardioplegia while others feel more confident with shorter re-dosing intervals. There is no guarantee that a single strategy can be safely applied to all patients, irrespective of their age, comorbidities or cardiopathy. The goal of this review is to discuss the rationale for short re-dosing intervals.

Key words: Myocardial protection; Del Nido cardioplegia; Continuous cardioplegia; Intermittent cardioplegia; Single-shot cardioplegia; Multidose cardioplegia; Crystalloid cardioplegia; Blood cardioplegia; Custodiol®; Histidine-ketoglutarate-tryptophan

Core tip: During myocardial ischemia, cardioplegia is the preferred method of myocardial protection. However, decades after its implementation, there is still no consensus on the optimal re-dosing interval. Shorter re-dosing (15-30 min) has been preferred to longer intervals (45-60 min), but the choice of one approach over another relies more on the surgeon's preference than on clear advantages. As the interest for one-shot

cardioplegia has been increasing recently, we intend to discuss the rationale, if any, for short cardioplegia re-dosing interval.

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INTRODUCTION

The successful curative treatment of certain congenital or acquired heart diseases requires a method allowing direct vision of the open heart. For decades, cardioplegia has been the preferred and best method to provide a non-contracting heart and a field without blood. However, despite its use on millions of patients, the debate over the ideal cardioplegic technique is still going on. Strictly speaking, cardioplegia means paralysis of the heart: the visual appearance of standstill heart and continuous monitoring of the electrocardiogram allow for an easy control. But cardioplegia is also the major component of myocardial protection. There is no consensus on the best tools for myocardial protection assessment. First, there is no way to routinely perform real-time myocardial protection evaluation. Second, myocardial protection is clinically assessed post-operatively *via* a number of indirect factors such as troponin I/T or creatine kinase MB levels, ischemic electric signs on EKG or myocardial infarction, stroke, atrial fibrillation, myocardial function on echo, low cardiac output state, inotropic support, intra-aortic balloon pump, extracorporeal membrane oxygenation, as well as time to extubation and length of stay in intensive care unit[1-6]. These indirect factors are affected by several causes including surgery, anesthesia, intensive care, and, in the case of complex cardiopathies, resultant physiology following surgical palliation or cure. It is impossible to determine the role played by each cause. Myocardial protection is a concept without clear and specific clinical signs.

Experimental works on cardioplegia aiming to assess myocardial protection use time-consuming, invasive and often expensive approaches such as continuous monitoring of intra-myocardial pH, myocardial lactate production, or myocardial biopsy for ATP dosage, which are unrealistic in routine clinical practice[7-9]. However, there is a huge amount of publications on cardioplegia trying to find the best technique for cardiac arrest and myocardial protection. The composition of the cardioplegic solution (blood *vs* crystalloid, with various substrate enhancement), the injection site(s) (antegrade, retrograde or both), the temperature (cold, tepid or warm) and the re-dosing intervals were the main factors explored. The large number of combinations of these factors makes studies difficult to compare. Furthermore, the type of cardiac repair interferes with re-dosing intervals. During coronary artery surgery, re-dosing is usually done after each distal anastomosis, *i.e.*, every 10 ± 5 min, but 60-min re-dosing intervals have also been described[10]. During valvular or complex procedures, re-dosing intervals are variable from one surgeon to another[2,11-22].

Shorter re-dosing intervals could be a more critical factor than total aortic cross-clamp time in terms of myocardial protection, which would imply that shorter intervals lead to improved protection[23]. This intuitive observation was supported by experimental and clinical works[24-26]. Unexpectedly though, other works suggested that single-shot cardioplegia was better or at least equivalent to multidose cardioplegia[27-30].

We have learned from heart transplantation that myocardial ischemia may be tolerated for several hours[31]. The upper limit for donor heart ischemic time, which was 4 h in the early days of heart transplant surgery, was progressively increased to 6-8 h[32]. Successful heart transplantation with a donor heart ischemic time of 13 h was published, but in this clinical case, the follow-up was limited to three months[33]. Cold blood cardioplegia[34], histidine-tryptophan-ketoglutarate (HTK) solution or Custodiol®, a solution developed for organ preservation in transplantation[35,36], and Del Nido cardioplegia were all proposed for single-shot cardioplegia[37,38].

The debate over the best re-dosing interval is not new but while multidose cardioplegia has been the "gold standard" for decades, one-shot cardioplegia is becoming more commonly used and is likely to be a valuable alternative. This is particularly true for minimally invasive vascular surgery[39,40]. However, the use of single-shot cardioplegia relies more on the surgeons' preference than on true advantages in terms of immediate outcomes[41].

The goal of this work is to discuss the rationale for short re-dosing intervals.

FROM CONTINUOUS BLOOD CARDIOPLEGIA TO SINGLE-SHOT CARDIOPLEGIA *VIA* INTERMITTENT CARDIOPLEGIA

From a theoretical point of view, a continuous injection of normothermic oxygenated blood containing the arresting agent is the best way to perform cardiac arrest and optimal myocardial protection. Gott *et al*[42] proposed this technique of aerobic arrest in 1957, but it was long to establish itself. In 1989, a clinical case was published on retrograde continuous blood cardioplegic warm infusion *via* the coronary sinus during mitral surgery. After a cross-clamp time of 393 min, the patient was easily weaned from bypass without intra-aortic balloon pump or inotropic support. It is noteworthy that the cardiac output was higher

immediately after bypass than before surgery[43]. The same team used the same technique of aerobic arrest in 308 consecutive procedures with warm surgery. Twenty-two of these patients needing an aortic cross-clamp time greater or equal to 3 h had excellent results[44]. The technic never became really popular for several reasons. First, it is technically more demanding and blind insertion of the cannula into the coronary sinus may be uneasy. Second, drawbacks were described, such as hyperkalemia, coronary sinus damage, catheter misplacement, migration or dislodgment in the right atrium[45-47]. Third, a major concern was the distribution of cardioplegia to the right hypertrophic ventricle or when the right coronary vein is next to the coronary sinus[48,49]. Last, for coronary artery bypass graft, a micro blower delivering compressed air is often needed for completion of distal anastomosis[50].

To overcome constraints related to retrograde continuous cardioplegia, intermittent, antegrade warm blood cardioplegia was proposed as an alternative. From November 5, 1990 to December 31, 1992, a study was conducted at three adult cardiac surgical centers of the University of Toronto on 720 patients operated on for aortocoronary. Warm blood cardioplegia was interrupted during 5-15 min for enhancing visualization during distal anastomosis. The longest ischemic time, equivalent to the longest time off cardioplegia (LTOC, in minutes per patient) and the total duration of ischemic time as a proportion of the aortic cross-clamp time were collected. The quality of myocardial protection was assessed on post-operative mortality at 30 d (or in-hospital deaths for patients with a length of stay > 30 d), myocardial infarction by enzyme criteria and low-output syndrome. The authors postulated that short periods of normothermic ischemia should be well tolerated if followed by adequate cardioplegic reinfusion. Their study aimed to evaluate the relation between intermittency of cardioplegia and cardiac events.

The results suggested that the longest ischemic time was more important than the cumulative ischemic time and that prolonged LTOC > 13 min was a risk factor for adverse outcomes[23]. For a vast majority of surgeons, thirteen minutes is a reasonable time for distal anastomosis construction. In summary, intermittent, antegrade warm blood cardioplegia is a valuable alternative to antegrade cold blood cardioplegia when the time off cardioplegia remains under 13 min. In a clinical scenario, this time off cardioplegia is more realistic than the maximal cardioplegia halted time of 5 min proposed by Menasché *et al*[51] in 1992. In 1995, a simpler technique was described, allowing a re-dosing interval of 15 min. Two groups of 250 patients undergoing elective coronary artery bypass grafting were compared using either intermittent warm blood cardioplegia or intermittent cold blood cardioplegia. For the surgeon, the two types of cardioplegia were similarly demanding. After an initial injection in the aortic root, re-dosing was done after each distal anastomosis or after 15 min. This maximal ischemic time was chosen to be long enough for a difficult distal anastomosis. The outcome was superior in the warm group, with significantly less cardiac-related deaths and a dramatic decrease in morbidity. There was lactate washout in both groups 1 min after aortic cross-clamping, but lactate production was still present 20 min after reperfusion in the cold group, while there was evidence of normal lactate extraction in the warm group, suggesting a rapid restoration of a normal metabolism. In summary, in this study, intermittent warm cardioplegia was superior to intermittent cold blood cardioplegia, with lower morbidity, mortality and decreased length of stay in ICU and hospital[52]. The re-dosing interval was later increased to 20-30 min without complication[53,54]. Other authors confirmed the good tolerance of 30-min warm ischemia time in 1996 and 2000[55,56]. In 2009, a comparison between intermittent warm blood cardioplegia and single-shot warm blood cardioplegia was published. The study was done from January 2001 to December 2006 and included 4014 patients: 1708 had single-shot cardioplegia and 2306 had intermittent warm blood cardioplegia with a 20-min re-dosing interval. There was statistical insignificance for mortality, intra or postoperative intra-aortic balloon pump, postoperative inotropics or postoperative arrhythmia. Single-shot had a favorable effect on post-operative myocardial infarction and an unfavorable effect on intraoperative inotropics and postoperative dialysis. Authors found that the first shot of warm cardioplegia may safely exceed 20 min and, in case of short cross-clamping (35 to 40 min), cover the whole cross-clamping time without increased risk[57]. However, there is probably an individual threshold for tolerance due to pre-, intra- and post-operative factors.

Interestingly, the same evolution in re-dosing interval was described in pediatric cardiac surgery. We introduced this technique in 2002 with a cardioplegia protocol identical to the one described in adults in 1995: warm oxygenated blood was diverted from the arterial line *via* a roller pump and St Thomas' solution was added downstream the pump with an electrical syringe. The blood to arresting agent ratio was 60:1, therefore the hydric balance of cardioplegia was negligible and it was named microplegia[58]. The re-dosing interval was 15 min and a nomogram was developed for volume and duration of the first injection and re-injection[59].

Another group implemented the same approach of intermittent warm blood microplegia, except for the re-dosing interval (10 min). They also suggested, on a group of arterial switch, that intermittent warm blood microplegia was a valid alternative to intermittent cold blood cardioplegia[60].

Following our initial experience on 1400 pediatric patients, we demonstrated that the technique was safe for long aortic cross-clamp time on 38 patients with a cross-clamp time > 90 min[61]. Using the same protocol for cardioplegia with a 15-min re-dosing interval, a group from Brussels used cardiac biopsies to demonstrate a significant increase in myocardial ATP

stores during the first cardioplegic ischemic time and a return to initial values after coronary reperfusion. This reproducible method was considered safe, with low morbidity and mortality and a similar quality of cardiac repair[62].

The technique was gradually implemented in several European units and more than 10000 cases with re-dosing intervals varying from 10 to 25 min were published in 2010[63]. As in adult surgery, intervals gradually increased between 2001 and 2013, from 10 to 35-40 min, without any adverse effect[64,65]. However, the major issue remains: how long is too long? It is likely that re-dosing intervals cannot be indefinitely increased without inducing adverse cardiac events.

The merits of single-dose *vs* multidose cardioplegia in the infant heart were described in animal experiments several decades ago[27-30], but there was a certain lack of enthusiasm for its clinical use. However, in 1988, a clinical study was published, comparing two groups of arterial switch operated on with single-dose or multidose cold blood cardioplegia with a 15-min re-dosing interval. The incidence of mortality and ST-T changes was significantly higher in the multidose group. The conclusion was that single-dose is as good as, or better than, multidose cardioplegia[34]. In 1989, another group confirmed the efficiency of single-shot cold crystalloid cardioplegia in arterial switch procedure[66].

There is increasing interest in HTK or Custodiol® single-dose cardioplegia. In 1998, Sakata suggested that single high-volume HTK provided a more adequate myocardial protection for mitral surgery than multidose cold blood cardioplegia[67]. In 2001, the benefit of single-dose HTK cardioplegia over multidose cold crystalloid cardioplegia was also suggested in a clinical work comparing two groups of 15 patients. The incidence of arrhythmia and inotropic support decreased significantly in the HTK group and so did the ICU length of stay[68]. The feasibility and safety of single-shot HTK cardioplegia was suggested in adult and pediatric surgery[35,36,69,70]. A new modified HTK solution named Custodiol-N, likely to enhance the organ protective potential of the previous solution, was tested on animals during a 60-min hypothermic cardiac arrest[71,72].

Del Nido cardioplegia has been used for two decades at the Boston Children's Hospital, generally in a single-dose fashion[73]. Its use was expanded to adult surgery, triggering a growing interest in this solution[74]. Recent works in adults and pediatrics suggest that it is a safe and valuable alternative to conventional multidose cardioplegia[37].

WHERE ARE WE? WHERE DO WE GO FROM HERE?

A review of the literature published prior to 1975 concluded that a 20-min ischemic period at 32℃ could be tolerated by the heart without the need for inotropic support, while the anoxic safe period was extended to 30 min when temperature was lowered to 16℃-20℃[75]. Forty years later, despite ample evidence that the ischemia time can be safely increased, even during warm surgery, a vast majority of surgeons use re-dosing intervals of 20-30 min, or even less, during coronary artery bypass graft. Some surgeons prefer the comfort of single-shot cardioplegia while others feel more confident with shorter intervals. How can we explain the myocardial tolerance to anoxia? Is it due to the composition of the arresting solution, the temperature of cardioplegia, or both?

We have seen that for cold and warm blood cardioplegia, short-term outcomes are equivalent with identical re-dosing intervals, just as they are identical for cold blood and cold crystalloid cardioplegia. The temperature is likely to have little effect, if any, but studies focused on aortic cross-clamp times < 90 min. We have also seen that different cardioplegic solutions can be safely used for hypothermic single-shot cardioplegia[34-38]. The composition did not seem to be critical, or, at least, different solutions can be used for single-shot cardioplegia and comparisons between these solutions are missing.

It is probably a fool's errand to look for a universal gold standard. The best cardioplegia with optimal re-dosing interval is likely to vary with different patients having different pathologies, and different aortic cross-clamp time. Dr J Vaage's stated: "If you had a clamp time of < 60 min, you could actually use whatever cardioplegia or myocardial protection you wanted, you could always get to the shore, so to say"[57]. It is probably true, but we are still looking for the optimal cardioplegia, for simple and complex cardiopathies. The goal is not just to get to the shore, but also to use the best, simplest, fastest and cheapest way to deliver optimal results to our patients. Furthermore, we intend to prevent cardiac events not just during the initial outcome - the one that allows getting to the shore - but also during mid- and long-term outcomes[76]. Myocardial fibrosis could be a late side effect of cardioplegia[77]. This is more challenging and less extensively studied.

CONCLUSION

This review does not solve the issue on the rationale for short-term re-dosing interval. However, facts are facts, and many works suggest or demonstrate that short-term re-dosing intervals are not critical for every patient. There is probably no rationale to use the same re-dosing interval for all patients needing aortic cross-clamping for surgical cardiac repair. Despite the lack of consensus on cardioplegia composition, temperature, way of administration and re-dosing interval, the outcomes of adult and pediatric cardiac surgery are continuously improving.

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MINIREVIEWS

Role of platelet-rich plasma in ischemic heart disease: An update on the latest evidence

Eleftherios Spartalis, Periklis Tomos, Demetrios Moris, Antonios Athanasiou, Charalampos Markakis, Michael D Spartalis, Theodore Troupis, Dimitrios Dimitroulis, Despina Perrea

Eleftherios Spartalis, Periklis Tomos, Demetrios Moris, Antonios Athanasiou, Charalampos Markakis, Dimitrios Dimitroulis, Second Department of Propedeutic Surgery, Athens University Medical School, "Laiko" Hospital, 11527 Athens, Greece

Michael D Spartalis, Department of Cardiology, Onassis Cardiac Surgery Center, 17674 Athens, Greece

Theodore Troupis, Department of Anatomy, Medical School, University of Athens, 11527 Athens, Greece

Despina Perrea, Laboratory of Experimental Surgery and Surgical Research "N.S. Christeas", Medical School, University of Athens, 11527 Athens, Greece

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Correspondence to: Eleftherios Spartalis, MD, MSc, PhD, Second Department of Propedeutic Surgery, Athens University Medical School, "Laiko" Hospital, Vasilissis Sofias 49, 11527 Athens, Greece. eleftherios.spartalis@gmail.com
Telephone: +30-069-74714078
Fax: +30-210-7456972

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Abstract

Myocardial infarction is the most common cause of congestive heart failure. Novel strategies such as directly reprogramming cardiac fibroblasts into cardiomyocytes are an exciting area of investigation for repair of injured myocardial tissue. The ultimate goal is to rebuild functional myocardium by transplanting exogenous stem cells or by activating native stem cells to induce endogenous repair. Cell-based myocardial restoration, however, has not penetrated broad clinical practice yet. Platelet-rich plasma, an autologous fractionation of whole blood containing high concentrations of growth factors, has been shown to safely and effectively enhance healing and angiogenesis primarily by reparative cell signaling. In this review, we collected all recent advances in novel therapies as well as experimental evidence demonstrating the role of platelet-rich plasma in ischemic heart disease, focusing on aspects that might be important for future successful clinical application.

Key words: Platelet-rich plasma; Ischemic heart disease; Myocardial infarction; Myocardial regeneration; Cardiac repair

Core tip: Tissue regeneration requires precise coordination among endothelial, epithelial and mesenchymal morphogenesis. Growth factor-induced angiogenesis plays a key role in recovery from ischemic disease and organ regeneration. Recent studies show that stem-cells and PRP together have opened new horizons in the myocardial infarction treatment.

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INTRODUCTION

Coronary infarction is the most frequent cause of death globally[1]. The loss of cells during ischemia and resultant fibrosis are the main reasons for cardiac failure[2].

As cardiac muscle has very little potential to create new cells, methods of heart regeneration have been studied further as repair modalities for failing myocardium after acute coronary infarction or chronic ischemia[3].

In this article, we investigate current advances and demonstrate approaches such as the upcoming challenges of platelet-rich plasma (PRP) application as well as opportunities to develop its role.

We have gathered all experimental and clinical studies in which PRP was used as a therapy post-MI, and have focused on aspects that might be important for future successful clinical application. The PubMed database was searched for articles using the terms "platelet-rich plasma" and keywords "ischemic heart disease", or "myocardial infarction", or "coronary disease".

NOVEL REGENERATIVE THERAPIES

The majority of patients survive a myocardial infarction (MI). Their outcome, however, is negatively influenced by several events, such as loss of viable cardiomyocytes due to a post-MI inflammatory response, eventually resulting in heart failure and/or death. Regenerating the human heart is a challenge that has engaged researchers around the globe almost a century. Although the human cardiac muscle has not been regenerated yet, decades of experimental progress have guided us onto a promising path[4].

Stem cell approach has become a promising tool for cardiac regeneration[5]. The main target is to repair functional myocardial tissue by implanting exogenous or by activating native stem cells.

Cardiac stem progenitor cells (CS/PCs) are one kind of adult stem cell with the ability to differentiate into heart lineages. Induced pluripotent stem cells (iPSCs) may differentiate into the needed cells in order to repair injured myocardium. These two types of stem cells play a key role in cardiac regeneration. Two main delivery modes of stem cells (percutaneous intramyocardial or intracoronary) are used today for patients with recent acute MI or ischemic cardiomyopathy[6]. Other delivery routes, such as intravenous *via* coronary sinus or peripheral veins and surgical have also been used with less success[6].

While further studies intent to increase the efficacy of current approaches, experimental protocols using new methods such as exploiting paracrine effect and tissue engineering could enhance repair of injured human heart.

Various chemical methods, including both microRNA and anti-microRNA approaches, proteins and modified peptides demonstrate serious potential[7].

Takahashi *et al*[8] investigated pluripotent stem cells (iPSCs) revealing in a new horizon of cellular reprogramming in organ regeneration. It has been shown that iPSCs can be differentiated efficiently into multiple cell types that may be used in the future for regenerative strategies[9].

Finally, transmyocardial laser revascularization (TMR) is a controversial therapeutic technique that relieves angina but can't create a significant effect on heart function[10]. It improves the clinical status without confronting the underlying atherosclerotic disease. Therefore, TMR offers palliation and not cure[10].

ROLE OF PRP

What is PRP

Autologous PRP is an increased amount of platelets in a small portion of plasma[11]. This is why the term PRP is preferred to plasma-rich growth factors (PRGFs), platelet concentrate or platelet-rich gel. PRP is a source of autologous growth factors, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epithelial growth factor and transforming growth factor beta 1 (TGF-β1), that is secreted by platelets in order to trigger the healing cascade[11,12]. Structurally similar to the natural fibrin clot, it can be used as scaffold for cells infiltration and assembly of vascular networks.

One of the most crucial questions regarding methodology, refers to the ideal mechanisms of intramyocardial delivery of PRP. Surgical (epicardial) application is performed into ischemic areas with a thin needle, allowing for multiple injections within and especially around the infarct area. The other less invasive interventional delivery route is the transendocardial catheter injection.

PRP and neovascularization

Neovascularization plays a significant role post-ischemia regeneration and organ repair.

It has been reported that the mixture of angiogenic factors in an certain percentage is crucial for the creation of functional blood vessels[13,14]. Angiogenesis-induced vessels, not only deliver nutrients and oxygen but also provide instructive regulatory signals to surrounding tissue affecting organ regeneration[15,16].

Neovascularization involves multiple complex events such as the maturation and enlargement of size of the preexisting small vessels through vascular remodeling

(arteriogenesis), sprouting of pre-existing resident endothelial cells (angiogenesis) and the recruitment of bone marrow derived endothelial progenitor cells (vasculogenesis)[17].

Angiogenesis involves both microvascular and macrovascular mechanisms. At the microvascular level, neovascularization is the genesis of capillaries, which, however, regress after pause of basic fibroblast growth factor (bFGF) triggering if pericytes are not gathered efficiently. Therefore, the stabilization of newly formed capillary networks by pericytes, known to be recruited by PDGF-BB, is crucial for therapeutic angiogenesis[18]. The enhancement of blood vessel maturation is one of the main modalities implemented to treat such patients.

According to the above-mentioned issues, a limited portion of plasma enriched in platelets, is an attracting attention as a safe and cost-effective source of various growth factors[19]. PRP, by containing these various cytokines, plays an important role in repairing damaged tissue[20]. As we have already discussed, little is known about the mechanism of PRP-related regeneration of damaged tissue. Successful reperfusion of ischemic tissue depends not only on stimulation of angiogenesis but also on arteriogenic activity. Different growth factors in PRP have different roles in angiogenesis and restoration of blood flow following ischemia[21]. It has also been shown that PRP effectively restores blood flow by significant increase of the number of capillaries (angiogenesis) as well as mature vessels (arteriogenesis) in the murine hind limb ischemia, which was confirmed by double staining with endothelial marker and pericytes marker respectively[22].

The VEGF, TGF-β and PDGF-BB, have a significant effect as pro-angiogenic stimulators. Evidence shows that PDGF-BB has a potential as arteriogenic factor, promoting differentiation of endothelial cells[17,23]. VEGF is known to trigger post-ischemia neovascularization[24], and TGF-β enhances cell mitosis[25]. Other reports, however, demonstrated that many growth factors, such as TGF-β and PDGF-BB, inhibit the angiogenic ability of bFGF[26]. These studies evaluated the angiogenic impact using combined solutions of growth factors. Within growth factors, PDGF-BB is the one that allows blood vessels to grow functionally[27,28]. According to this fact, multiple releases of prostaglandin F2 alpha metabolite and bFGF will improve the maturation of blood vessels. It is also demonstrated that the mixed release of VEGF and PDGF promoted the maturation of newly created blood vessels against VEGF release alone[29].

VEGF is the principle stimulatory factor of angiogenesis after ischemia[30]. However, VEGF enhances the creation of unstable capillaries[31]. It promotes mural cell accumulation, presumably through the release of PDGF-BB. It also causes endothelial cell proliferation and migration, resulting in capillary sprouting or angiogenesis. Lastly, it recruits hematopoietic stem cells to ischemic site from bone marrow *via* circulation.

Basic FGF and PDGF are chemoattractants to smooth muscle cells. Those are also causes of growth of smooth muscle cell as well as enlargement of vessel (formation of mature vessels or arteriogenesis). These stem cells produce a capillary plexus and eventually form mature vessels. All of these together cause formation of new vessels for vascular supply in ischemic limbs. So, combined administration of different growth factors may lead to potentially therapeutic angiogenesis[31,32]. PDGFR-beta are needed for vascular stabilization by gathering of mesenchymal progenitors. Absence of PDGF leads to fragile neovasculature[33], indicating that PDGF-BB has potent arteriogenic effect after ischemia.

Insulin-like growth factor-1 (IGF-1) triggers angiogenesis and myogenesis, the pro-angiogenic impact, however, seems to be less efficient than that of other angiogenic factors[34]. Finally stromal cell-derived factor 1 (SDF-1) has direct or indirect (*via* certain secondary cytokines) effects on endogenous angiogenesis[35]. There is also cross talk between VEGF and bFGF; bFGF and PDGF-BB to induce post-ischemia angiogenesis[36]. Finally, inhibition of Ang1-Tie2 signaling suppresses angiogenic ability of the PRP *in vivo* and PRP-induced angiogenesis *in vitro*.

Experimental evidence

Inspite of a large amount of evidence on PRP's usefulness, limited work has been conducted using PRP in myocardium.

Gallo *et al*[37] evaluated histological and morphological impact of the injection of PRP in ischemic sheep myocardium. Noteworthy was the formation of new blood vessels in hematoxylin-eosin-stained sections and factor Ⅷ in PRGF-treated myocardia. According to this report, implantation of platelet growth factors in previously infarcted sheep hearts promoted neovascularization.

Hargrave *et al*[38] utilized the technique of nanosecond pulsed electric fields (nsPEF) in order to determine the efficiency of a protocol involving the *in vivo* treatment of the ischemic and reperfused myocardial cells in culture with PRP in rabbits. The left ventricle had faster contraction/relaxation rate and the size of the infarct was diminished in PRP-treated hearts compared to saline-treated. Mitochondrial depolarization and reactive oxygen species (ROS) production were reduced in PRP-treated cells. These facts show that PRP contributes in cardiac protection by stabilizing the mitochondria and reducing ROS generation of the ischemic-reperfused heart.

Mishra *et al*[39] used permanent ligation, in order to find out whether PRP, enhances cardiac function in an ischemia-reperfusion model as measured by left ventricular ejection fraction (LVEF).

Compared with phosphate-buffered saline (PBS) controls, PRP-treated animals had a higher LVEF after ischemia, while PRP-treated animals who underwent ischemia-reperfusion had higher LVEF after ischemia. Histology revealed increased granulation in the control group *vs* the PRP group. In the same time, magnetic resonance imaging (MRI) revealed a positive impact

Table 1 Summary of the effects of platelet-rich plasma in ischemic heart disease

Ref.	Type of study	Animal model	Delivery method	Effect
Gallo *et al*[37]	Experimental	Sheep	Implantation	Increased formation of new vessels
Hargrave *et al*[38]	Experimental	Rabbit	Intramyocardial injection	Reduced reactive oxygen species generation Stabilized the mitochondria of the ischemic/reperfused heart
Mishra *et al*[39]	Experimental	Murine (Mouse)	Intramyocardial injection	Higher left ventricular ejection fraction after ischemia
Vu *et al*[40]	Experimental	Porcine	Intramyocardial injection	Attenuated adverse cardiac remodeling
Yu *et al*[41]	Experimental	Murine (Rat)	Intramyocardial injection	Decreased infarct size Increased ventricular wall thickness Improved cardiac function and reperfusion
Li *et al*[42]	Experimental	Murine (Rat)	Intramyocardial injection	Limitation of ventricular expansion, Attenuated myocardial hypertrophy in the noninfarct region Facilitated angiogenesis and arteriogenesis in the infarct.
Sun *et al*[43]	Experimental	Murine (Rat)	Intramyocardial injection	Improved LV performance
Wehberg *et al*[10]	Clinical	-	Intramyocardial injection	More efficacious at relieving angina Improved myocardial function

of PRP on left ventricular function in both ligation and ischemia/reperfusion murine model.

Vu *et al*[40] attempted a translational, large-scale restorative but minimally invasive approach in a porcine model, aiming at both structurally stabilizing the LV wall and improving function following ischemic injury.

In this study, a combination of PRP, anti-oxidant and anti-inflammatory factors with intramyocardial injection of hydrogel had the potential to structurally and functionally enhance the injured heart muscle while attenuating adverse cardiac remodeling after acute myocardial infarction.

Yu *et al*[41] conducted a study in order to investigate the impact of direct myocardial injection of PRP on cardiac function, ventricular remodeling and myocardial perfusion in rats. EF was significantly higher and myocardial perfusion significantly improved in the PRP group. Histological examination also confirmed that PRP treatment can decrease infarct size, increase ventricular wall thickness and improve cardiac function.

Li *et al*[42] demonstrated that a platelet-mediated paracrine effect may accelerate the healing process after myocardial infarction in rats. According to this experimental protocol, implantation of thrombin-activated PRP into the ischemic myocardium lead in enhancement of ventricular remodeling and accelerated repair, as shown through the limitation of ventricular expansion, facilitation of neovascularization, arteriogenesis in the infarct and attenuation of myocardial hypertrophy in the noninfarct part.

Sun *et al*[43] reported that adipose-derived mesenchymal stem cells (ADMSC) in a platelet-rich fibrin (PRF) scaffold were superior to direct ADMSC injection in enhancing LV function and diminishing LV remodeling in a post-MI animal model.

PRP and TMR

TMR induces a reconfiguration of the microcirculation, with blood shunted from epicardial to endocardial areas. Current literatures propose a synergistic effect among TMR and exogenously delivered growth factors.

Wehberg *et al*[10] assessed the impact of PRP intramyocardial injection combined with TMR. Angina relief was similar in both groups (TMR-alone and TMR + PRP); the TMR + PRP group, however, had a decreased average angina score and more were angina free compared to the TMR-alone group. EF improved significantly in the TMR + PRP group compared to the TMR-alone group. This study suggested that intramyocardial injection of PRP and TMR may be more effective at treating angina and enhancing heart function than TMR alone.

All above-mentioned studies are summarized in Table 1.

CONCLUSION

While stem-cell therapies and cellular reprogramming hold promise, the use of PRP emerges as an additional modality for repairing cardiac muscle.

Development of tissues is based on accurate coordination among epithelial, mesenchymal and endothelial morphogenesis. Furthermore, growth factor-induced angiogenesis is significant in organ regeneration after ischemia. Recent tissue engineering researches suggest that cells and PRP-derived growth factors together into biomaterials have opened new horizons in the myocardial infarction treatment.

PRP should be investigated for its potential regenerative properties and its use as a therapeutic modality for ischemic myocardium.

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ORIGINAL ARTICLE

Basic Study

Enhanced caveolin-1 expression in smooth muscle cells: Possible prelude to neointima formation

Jing Huang, John H Wolk, Michael H Gewitz, James E Loyd, James West, Eric D Austin, Rajamma Mathew

Jing Huang, Michael H Gewitz, Rajamma Mathew, Department of Pediatrics, Maria Fareri Children's Hospital, New York Medical College, Valhalla, NY 10595, United States

John H Wolk, Department of Pathology, New York Medical College, Valhalla, NY 10595, United States

James E Loyd, James West, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232, United States

Eric D Austin, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN 37232, United States

Rajamma Mathew, Department of Physiology, New York Medical College, Valhalla, NY 10595, United States

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Correspondence to: Rajamma Mathew, MD, Department of Physiology, New York Medical College, Rm # A11, Basic Science Building, Valhalla, NY 10595, United States. rajamma_mathew@nymc.edu
Telephone: +1-914-5944750

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Abstract

AIM: To study the genesis of neointima formation in pulmonary hypertension (PH), we investigated the role of caveolin-1 and related proteins.

METHODS: Male Sprague Dawley rats were given monocrotaline (M, 40 mg/kg) or subjected to hypobaric hypoxia (H) to induce PH. Another group was given M and subjected to H to accelerate the disease process (M + H). Right ventricular systolic pressure, right ventricular hypertrophy, lung histology for medial hypertrophy and the presence of neointimal lesions were examined at 2 and 4 wk. The expression of caveolin-1 and its regulatory protein peroxisome proliferator-activated receptor (PPAR) γ, caveolin-2, proliferative and anti-apoptotic factors (PY-STAT3, p-Erk, Bcl-xL), endothelial nitric oxide synthase (eNOS) and heat shock protein (HSP) 90 in the lungs were analyzed, and the results from M + H group were compared with the controls, M and H groups. Double immunofluorescence technique was used to identify the localization of caveolin-1 in

pulmonary arteries in rat lungs and in human PH lung tissue.

RESULTS: In the M + H group, PH was more severe compared with M or H group. In the 4 wk M+H group, several arteries with reduced caveolin-1 expression in endothelial layer coupled with an increased expression in smooth muscle cells (SMC), exhibited neointimal lesions. Neointima was present only in the arteries exhibiting enhanced caveolin-1 expression in SMC. Lung tissue obtained from patients with PH also revealed neointimal lesions only in the arteries exhibiting endothelial caveolin-1 loss accompanied by an increased caveolin-1 expression in SMC. Reduction in eNOS and HSP90 expression was present in the M groups (2 and 4 wk), but not in the M + H groups. In both M groups and in the M + H group at 2 wk, endothelial caveolin-1 loss was accompanied by an increase in PPARγ expression. In the M + H group at 4 wk, increase in caveolin-1 expression was accompanied by a reduction in the PPARγ expression. In the H group, there was neither a loss of endothelial caveolin-1, eNOS or HSP90, nor an increase in SMC caveolin-1 expression; or any alteration in PPARγ expression. Proliferative pathways were activated in all experimental groups.

CONCLUSION: Enhanced caveolin-1 expression in SMC follows extensive endothelial caveolin-1 loss with subsequent neointima formation. Increased caveolin-1 expression in SMC, thus, may be a prelude to neointima formation.

Key words: Endothelial cells; Neointima; Pulmonary hypertension; Smooth muscle cells

Core tip: Neointima in pulmonary hypertension (PH) is associated with poor prognosis. Caveolin-1, a cell membrane protein has a critical role in PH. We investigated the association of caveolin-1 and neointima formation in monocrotaline (MCT) + hypoxia-treated rats, and in human PH lung sections. The progressive caveolin-1 reduction in endothelial cells is followed by an increased caveolin-1 expression in smooth muscle cells (SMC). In human PH as well as in the MCT + hypoxia model, neointima was observed only in the arteries exhibiting an increased caveolin-1 expression in SMC. Thus, the increased caveolin-1 expression in SMC may in part, facilitate neointima formation.

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INTRODUCTION

Pulmonary hypertension (PH) is a rare, but a progressive disease with a high morbidity and mortality rate. Although considerable progress has been made in the field; the pathogenesis of PH, however, is not yet fully understood, which makes the design of preventive and curative treatment a daunting challenge. The advances in therapeutic modalities have improved the life expectancy as well as the quality of life; the pulmonary vascular remodeling, however, remains progressive[1]. A number of diverse diseases can develop PH, and several PH-associated gene mutations are known to significantly increase the risk of familial PH[2,3]. Irrespective of the underlying disease, severe PH is typically characterized by endothelial dysfunction, impaired vasodilatation, increased vasoconstriction, cell proliferation, medial wall thickening, PH and right ventricular hypertrophy (RVH)[4]. The development of neointima and plexiform lesions in pulmonary arteries associate with poor outcomes although whether or not they are causative of disease or result from an abnormal hemodynamic milieu remains unclear in the human PH[5].

In the monocrotaline (MCT) model, endothelial caveolin-1 loss and the activation of proliferative and anti-apoptotic pathways are observed before PH becomes evident. Concurrent loss of several endothelial cell (EC) membrane proteins including PECAM-1, soluble guanylate cyclase and Tie2 is suggestive of an extensive EC membrane damage. At 2 wk post-MCT, PH and RVH are observed, accompanied by a further disruption of EC as indicated by the loss of cytosolic proteins such as heat shock protein (HSP) 90, Akt and IκB-α[6-8]. Importantly, preventive measures restore endothelial caveolin-1 resulting in the inhibition of proliferative pathways and attenuation of PH[9,10]. Caveolin-1 is a major scaffolding protein of caveolae (50-100 nm), a subset of lipid rafts in the plasma membrane of a number of different cell types including EC and smooth muscle cells (SMC). It plays a pivotal role in maintaining vascular homeostasis. It directly interacts with transducing molecules within caveolae and stabilizes them in an inactive form. It regulates cell proliferation, apoptosis, cell differentiation, cell cycle, and also eNOS function[11-13].

The presence of pulmonary arterial hypertension (PAH) in patients with *CAV-1* mutation associated with reduced endothelial caveolin-1 expression, further supports a critical role of caveolin-1 in the lung vasculature[14,15]. Importantly, the loss of endothelial caveolin-1 and vWF accompanied by an increased caveolin-1 expression in SMC has recently been reported in children and adults with PAH associated with drug toxicity, congenital heart disease and idiopathic PAH (IPAH)[16-18]. Furthermore, pulmonary arterial SMC from the patients with IPAH revealed increased capacitative Ca^{2+} entry and DNA synthesis; both could be attenuated by silencing caveolin-1[18]. Thus, caveolin-1 switches from being an anti-proliferative to a pro-proliferative factor. Interestingly, the dual role of caveolin-1 is a known phenomenon in cancer[19].

Studies with rat models of PH using "VEGF receptor blocker (Sugen) + hypoxia"[20], MCT + pneumonectomy[21] and MCT + hypoxia[22] have shown severe PH with neointima and plexiform lesions, closely mimicking human PH. In these models, underlying EC damage is an important initial phase. We hypothesized that the extensive EC damage and/or loss might be a prerequisite for the increased caveolin-1 expression in SMC and subsequent development of neointima. To test this hypothesis, we treated rats with MCT and exposed them to hypobaric hypoxia (MCT + hypoxia) to accelerate the disease process. Hemodynamic data, lung histopathology, the expression of caveolin-1, and proliferative and anti-apoptotic factors, endothelial nitric oxide synthase (eNOS) and HSP90 proteins were examined. We evaluated the expression of caveolin-2 because it co-localizes with caveolin-1[23], and the expression of peroxisome proliferator-activated receptor (PPAR) γ, because it regulates caveolin-1 expression[24,25], and its loss is implicated in the pathogenesis of PH[26,27]. In addition, we examined caveolin-1 expression in the lung tissue from patients with IPAH and heritable PAH (HPAH).

MATERIALS AND METHODS

Male Sprague-Dawley rats (150-175 g, Charles River Wilmington, MA) were maintained at 22℃ on a 12 h light and dark cycle in the Animal Facility. They were allowed to acclimatize for 5 d, with free access to laboratory chow and water. The Protocols were approved by the Institutional Animal Care and Use Committee at New York Medical College (IACUC # 4-1-0113), and conform to the guiding principles for the use and care of laboratory animals of the American Physiological Society, and the National Institutes of Health. Rats were divided into 4 groups: Gr1, Control rats maintained in room air; Gr2, rats received MCT (40 mg/kg, sc), and kept in room air; Gr3, rats subjected to hypobaric hypoxia (atmospheric pressure 380 mmHg); and Gr4, rats received MCT 40 mg/kg and were subjected to hypobaric hypoxia starting on day 1. The hypoxia chamber was opened twice per week for 15 min to weigh the rats, replenish food and water, and to provide clean bedding similar to the other rats in room air. At the end of 2 and 4 wk, these rats were studied.

Human lung tissue was obtained from PAH patients at the time of post-mortem autopsy or lung transplantation; control tissue was obtained from healthy subjects who died due to traumatic injuries. Vanderbilt Pulmonary Hypertension Research Cohort study participants were recruited *via* the Vanderbilt Pulmonary Hypertension Center. The Vanderbilt University Medical Center Institutional Review Board approved all study protocols (IRB #9401). All participants, or their surrogate custodians as appropriate, gave informed written consent to participate in genetic and clinical studies. PAH was defined either by autopsy results showing plexogenic pulmonary arteriopathy in the absence of other causes such as congenital heart disease, or by clinical and cardiac catheterization criteria. These criteria included a mean pulmonary artery pressure ⩾ 25 mmHg with a pulmonary capillary wedge or left atrial pressure ⩽ 15 mmHg, and exclusion of other causes of PH in accordance with accepted international standards of diagnostic criteria[2]. HPAH was considered the type of PAH if a subject met one or both of the following criteria: (1) family history of two or more subjects with confirmed PAH according to international standards of diagnostic criteria; or (2) detection of a mutation in a PAH-specific gene, such as *BMPR2*. The majority of lung tissue specimens available for this study from PAH patients were from subjects deceased prior to the discovery of the *BMPR2* gene and other genes that could be considered PAH-specific genes which are mutated in association with HPAH. Included in this study were 7 patients: 3 with IPAH and 4 with HPAH. The age ranged from 29 to 55 years except for one patient who was 6 years old diagnosed with HPAH.

Chemicals and antibodies

All chemicals including MCT were purchased from Sigma Aldrich, St Louis, MO. Antibodies: caveolin-1α (sc894), PPARγ (sc7273), HSP90 (sc13119) purchased from Santa Cruz laboratories, Santa Cruz, CA. PY-STAT3 (Tyr705, 9145), Bcl-xL (2764), p-Erk (Thr202/Tyr204, 4370), and Erk (4695) from Cell Signaling, Beverley, MA, β actin (A5441) and α-actin (C6198) from Sigma, caveolin-2 (610684), eNOS (610297) and STAT3 (610190) from BD Transduction, Palo Alto, CA.

Measurement of right ventricular systolic pressure

Rats anesthetized with pentobarbital (60 mg/kg, *ip*), were ventilated through a tracheostomy (roughly equivalent to 70-80 breaths/min)[6]. A thoracotomy was performed; and right ventricular systolic pressure (RVSP) measured with a small needle attached to a tubing (PE50). After perfusing the lungs with normal saline, heart and lungs were removed. Right lung was frozen and stored at -80℃. The heart and the left lung were kept in 10% buffered formaldehyde.

Estimation of right ventricular hypertrophy

The ratio of the right ventricle (RV) and the left ventricle including septum (LV) was used to assess right ventricular hypertrophy (RVH)[6,7]. In addition, the ratio of RV (mg)/final body weight (FBW, g) and the ratio of LV (mg)/FBW (g) were calculated.

Estimation of protein expression

Proteins (50-100 μg) from lung supernatants were used to examine the expression of proteins of interest[6,7]. The antibodies used were caveolin-1 (1:5000), Caveolin-2 (1:500), PPARγ (1:100), PY-STAT3 (1:200), Bcl-xl (1:200), p-Erk (1:2000), eNOS (1:400), or HSP90 (1: 3000). Loading protein was evaluated using β actin (1:10000), STAT3 (1: 2000) or Erk

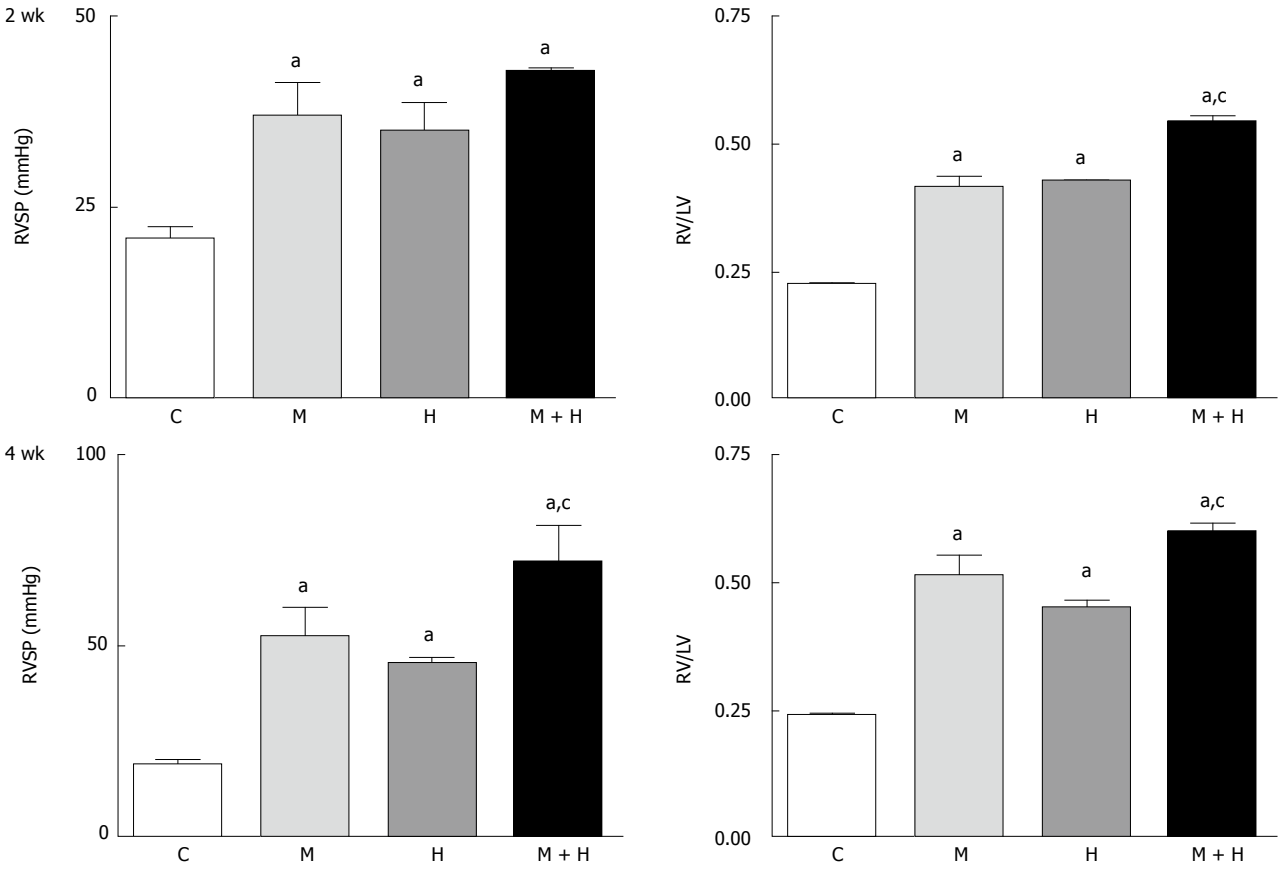

Figure 1 This figure depicts right ventricular systolic pressure and right ventricular hypertrophy in controls, monocrotaline, hypoxia and monocrotaline + hypoxia at 2 (*n* = 5-8) and 4 wk (*n* = 6-10). $^{a}P < 0.05$ *vs* C, $^{c}P < 0.05$ *vs* M and H. RVSP: Right ventricular systolic pressure; C: Controls; M: Monocrotaline; H: Hypoxia; M + H: Monocrotaline + hypoxia.

(1:2000) as appropriate. Protein bands visualized by chemiluminescence are expressed as % normal.

Lung histopathology and double immunofluorescence

Five to 6 μm lung sections were cut from the paraffin blocks, which were processed form the lung tissue preserved in 10% formaldehyde. Hematoxylin/eosin and elastic van Gieson stains were used for histopathological evaluation. Double immunofluorescence study (on all sections) was carried out at New York Medical College Facility, using caveolin-1 and α-actin antibodies as described previously[6,7]. Immunofluorescence was evaluated using a laser scanning confocal microscope.

Statistical analysis

The data are expressed as means ± SEM. Differences among multiple means were determined by one way Anova analysis using SPSS program. Specific differences were determined using Scheffe's test with < 0.05 as significant.

RESULTS

Weight gain

At 2 wk (*n* = 5-8), the weight gain in the MCT and hypoxia groups was lower compared with the controls (controls, 63 ± 3 g; MCT, 38 ± 3 g^{a}; hypoxia, 39 ± 2 g^{a}). In the MCT + hypoxia group, there was a further reduction in the weight gain (6 ± 7 g^{a,c}). There was no mortality in any of the groups. $^{a}P < 0.05$ *vs* controls, $^{c}P < 0.05$ *vs* MCT and hypoxia groups.

At 4 wk (*n* = 7-11), the mortality in the MCT and the MCT + hypoxia groups were 22% and 30% respectively, but none in the hypoxia alone group. Weight gain in the hypoxia group was comparable to the controls (97 ± 7 g *vs* hypoxia 94 ± 4 g, *P* = NS). The weight gain in the MCT group was significantly reduced (68 ± 7 g^{a}) and a further reduction was noted in the MCT + hypoxia group (45 ± 5 g^{a,c}). $^{a}P < 0.05$ *vs* controls, $^{c}P < 0.05$ *vs* MCT.

Hemodynamic data

At 2 wk, RVSP and RV/LV ratio were significantly higher in the MCT, hypoxia and MCT + hypoxia groups compared with the controls (Figure 1, top panel); with a further increase at 4 wk (Figure 1, bottom panel). The ratios of RV (mg)/FBW (g) confirmed increased RVH in the MCT + hypoxia groups at 2 and 4 wk compared with the MCT and hypoxia alone groups. RV (mg)/FBW (g) ratio: 2 wk; C, 0.5 ± 0.01, MCT, 1.02 ± 0.57^{a}, Hypoxia, 1.19 ± 0.057^{a}, MCT + Hypoxia, 1.54 ± 0.04^{a,c}, 4 wk; C, 0.55 ± 0.019, MCT, 1.15 ± 0.56^{a},

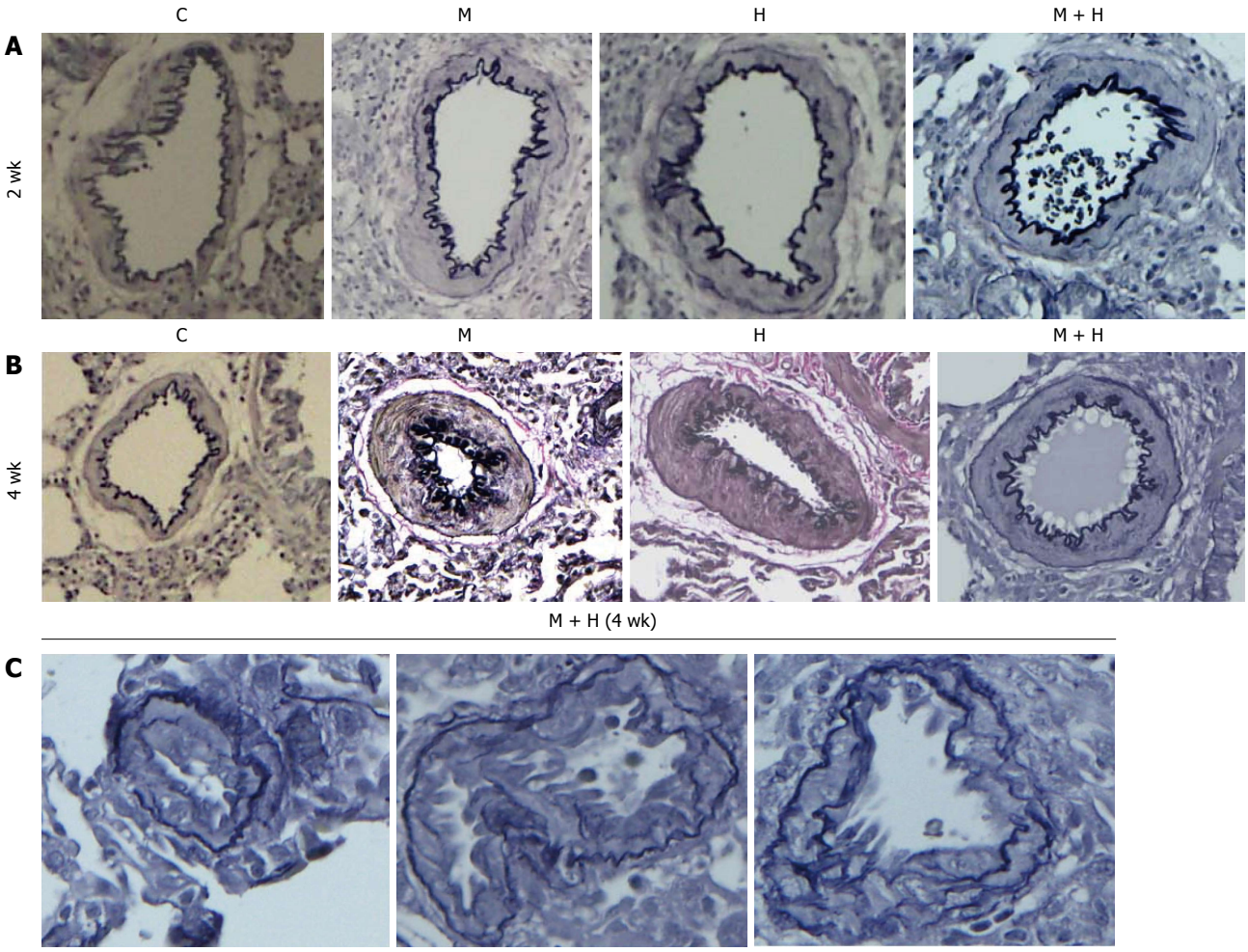

Figure 2 Pulmonary arteries (experimental groups). A and B: Pulmonary arteries (size 200-317 μm) from the controls and different experimental groups (elastic van Gieson stain): At 2 and 4 wk, arteries from MCT (M), hypoxia (H) and MCT + hypoxia (M + H) exhibit increased medial wall thickening compared with the control (C). Magnification = × 100; C: Arteries (size 100-155 μm) from 4 wk M + H group showing the presence of neointima. Fragmentation of internal elastic lamina can be seen in these arteries. Magnification = × 400.

hypoxia, 1.05 ± 0.08[a], MCT + hypoxia, 1.59 ± 0.01[a,c]. [a]$P < 0.05$ *vs* C, [c]$P < 0.05$ *vs* MCT or hypoxia group. The LV (mg)/FBW (g) ratio, however, was not different in any of the experimental groups compared with the controls (data not shown).

Histopathology

Experimental groups: Increased pulmonary arterial medial wall thickening is present in all the experimental groups at 2 and 4 wk (Figure 2A and B). Panel C shows neointima in small arteries at 4 wk in the MCT + hypoxia group.

Humans: Pulmonary arteries from IPAH and HPAH patients show varying degrees of medial wall thickening, neointima and luminal narrowing (Figure 3).

Caveolin-1 and caveolin-2 expression

The expression of both caveolin-1 and caveolin-2 was significantly reduced in the MCT and MCT + hypoxia groups at 2 wk. In the hypoxia alone group, caveolin-1 expression was not reduced; however, the caveolin-2 expression was slightly but significantly reduced compared with the controls (Figure 4).

At 4 wk, caveolin-1 and caveolin-2 were significantly reduced in the MCT group. In the hypoxia group, the expression of caveolin-1 was comparable to the controls; however, the expression of caveolin-2 was reduced, but not as low as seen in the MCT group. Importantly, in the MCT + hypoxia group, caveolin-1 expression was significantly increased compared with the MCT group (81% ± 3.9% *vs* 17% ± 3.6%, $P < 0.05$), although still low compared to the controls (81% ± 3.9% *vs* 100% ± 0%, $P < 0.05$). However, despite an increased caveolin-1 expression in this group, caveolin-2 showed a further reduction (Figure 4).

Localization of caveolin-1

Experimental groups: At 2 wk post-MCT, only 23% ± 0.87% of arteries exhibited the presence of endothelial caveolin-1. Consistent with previous observations[7]; in the current study, the endothelial caveolin-1 loss at 2 wk was not associated with an increased caveolin-1 expression in SMC. The MCT + hypoxia group showed a further reduction in the endothelial caveolin-1 expression (11% ± 1%). A few

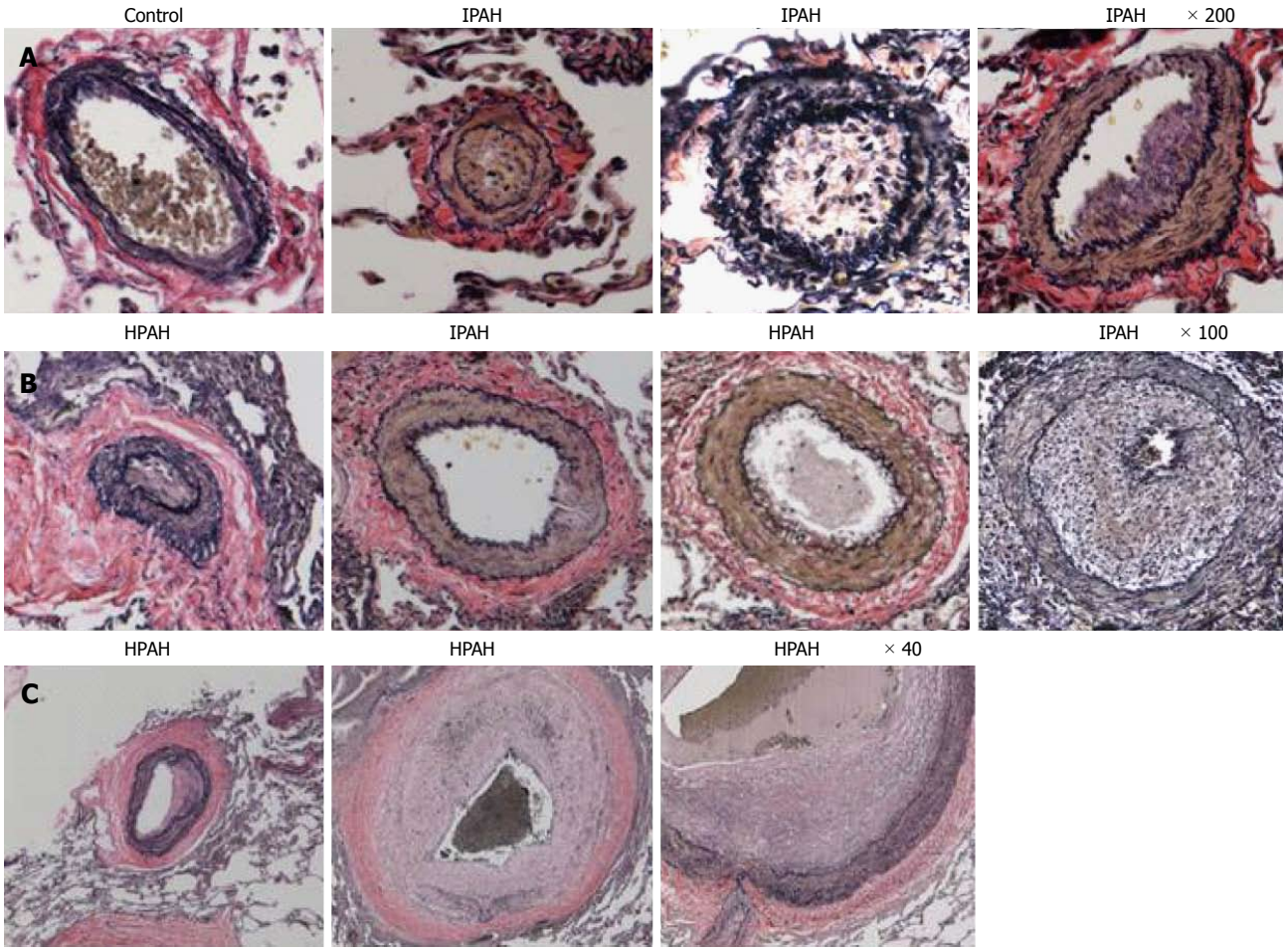

Figure 3 Pulmonary arteries (Human). A and B: Pulmonary arteries (size 134-323 μm) from a control, IPAH and HPAH patients. Control artery is thin walled. The arteries from patients exhibit varying degrees of muscular thickening, neointima and significant narrowing of the lumen; C: Larger arteries exhibiting vascular remodeling, extensive neointima formation and narrowing of the lumen.

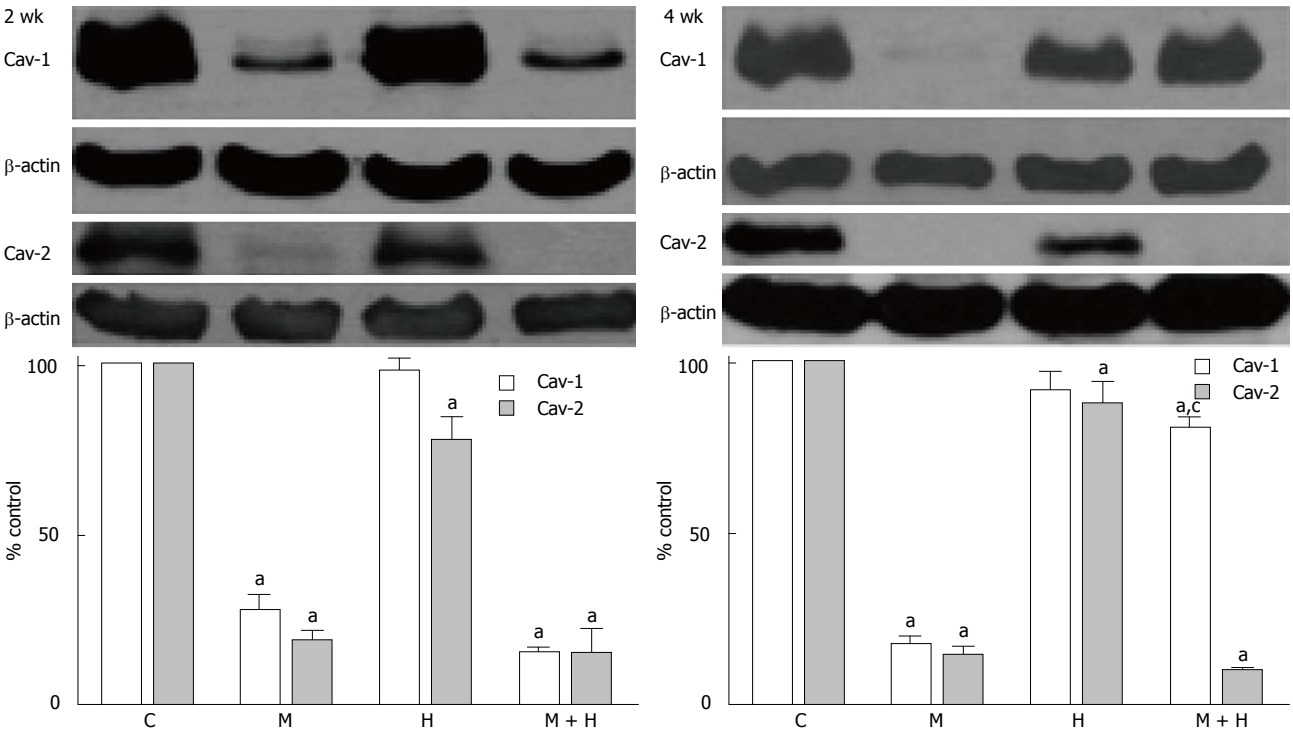

Figure 4 Western blots and bar graphs showing the expression of caveolin-1, caveolin-2 and β actin in controls, monocrotaline, hypoxia and monocrotaline + hypoxia at 2 (*n* = 3-6) and 4 wk (*n* = 5-8). [a]*P* < 0.05 *vs* C, [c]*P* < 0.05 *vs* M. C: Controls; M: Monocrotaline; H: Hypoxia; M + H: Monocrotaline + hypoxia.

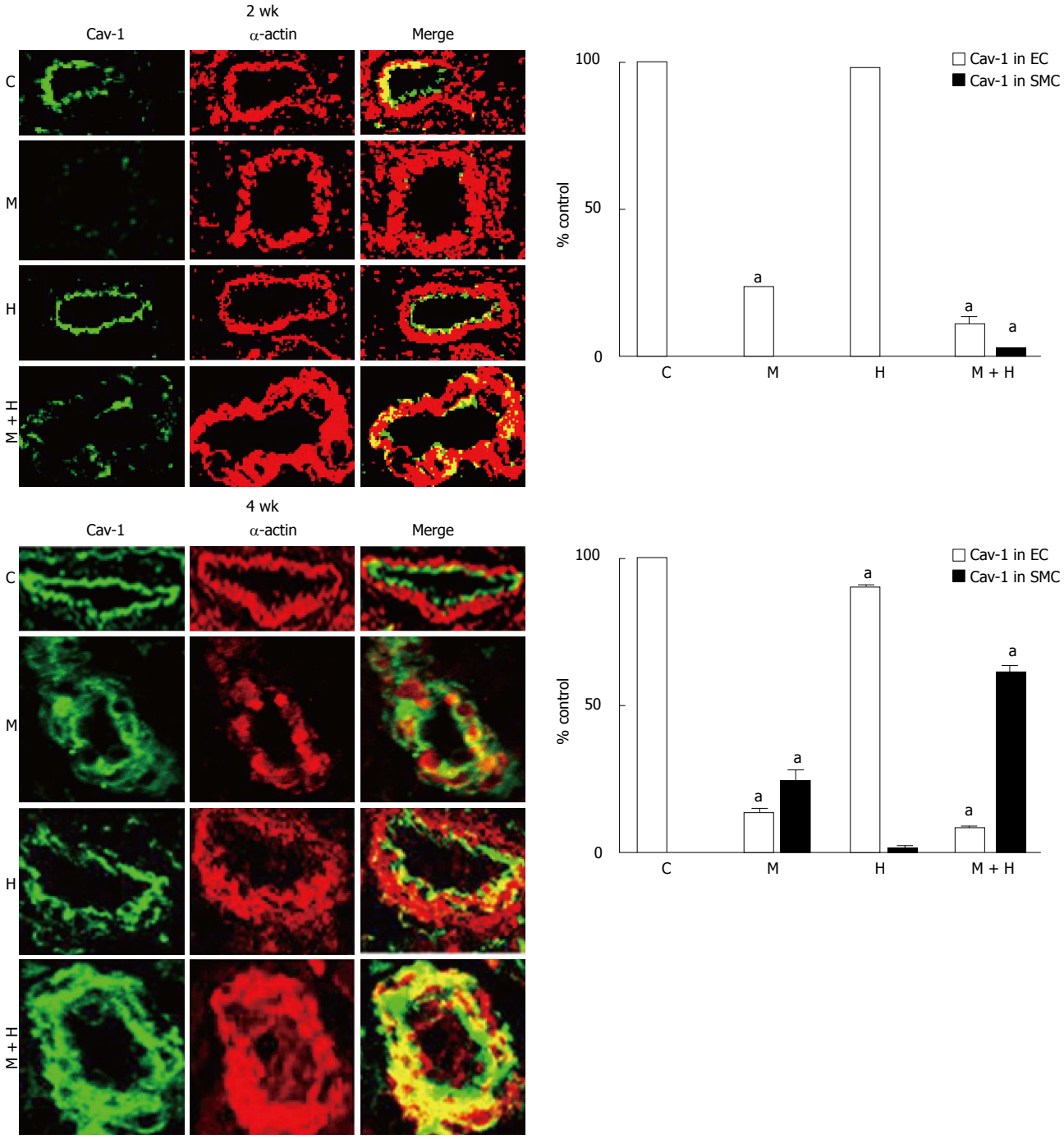

Figure 5 Immunofluorescence study depicting the expression of caveolin-1 (green) and smooth muscle α actin (red) in pulmonary arteries from controls, monocrotaline, hypoxia and monocrotaline + Hypoxia groups at 2 and 4 wk. The accompanying bar graphs (n = 4-5) shows the % arteries exhibiting the presence of caveolin-1 in endothelium (EC) and in smooth muscle layer (SMC). [a]$P < 0.05$ *vs* C. C: Controls; M: Monocrotaline; H: Hypoxia; M + H: Monocrotaline + hypoxia.

arteries displaying endothelial caveolin-1 loss exhibited increased expression of caveolin-1 in SMC (2.9% ± 0.25%). Expression of endothelial caveolin-1 in the hypoxia group, however, was not different compared with the controls (Figure 5, top panel).

At 4 wk, in the MCT and MCT+ hypoxia groups, endothelial caveolin-1 was expressed in 13% ± 1.4% and 8% ± 0.79% of arteries respectively. In the MCT group, increased caveolin-1 expression in SMC was observed in 24% ± 3.5% of arteries. Importantly, in the MCT + hypoxia group, 61% ± 2% of arteries displayed increased caveolin-1 in SMC, consistent with the observed increase in total caveolin-1 expression in the lungs. However, the neointimal layer revealed scant expression of caveolin-1. Interestingly, in the hypoxia group, there were a few arteries with endothelial caveolin-1 loss (90% ± 0.89% *vs* C, 100% ± 0%, $P < 0.05$); and a smaller number of arteries (1.2%

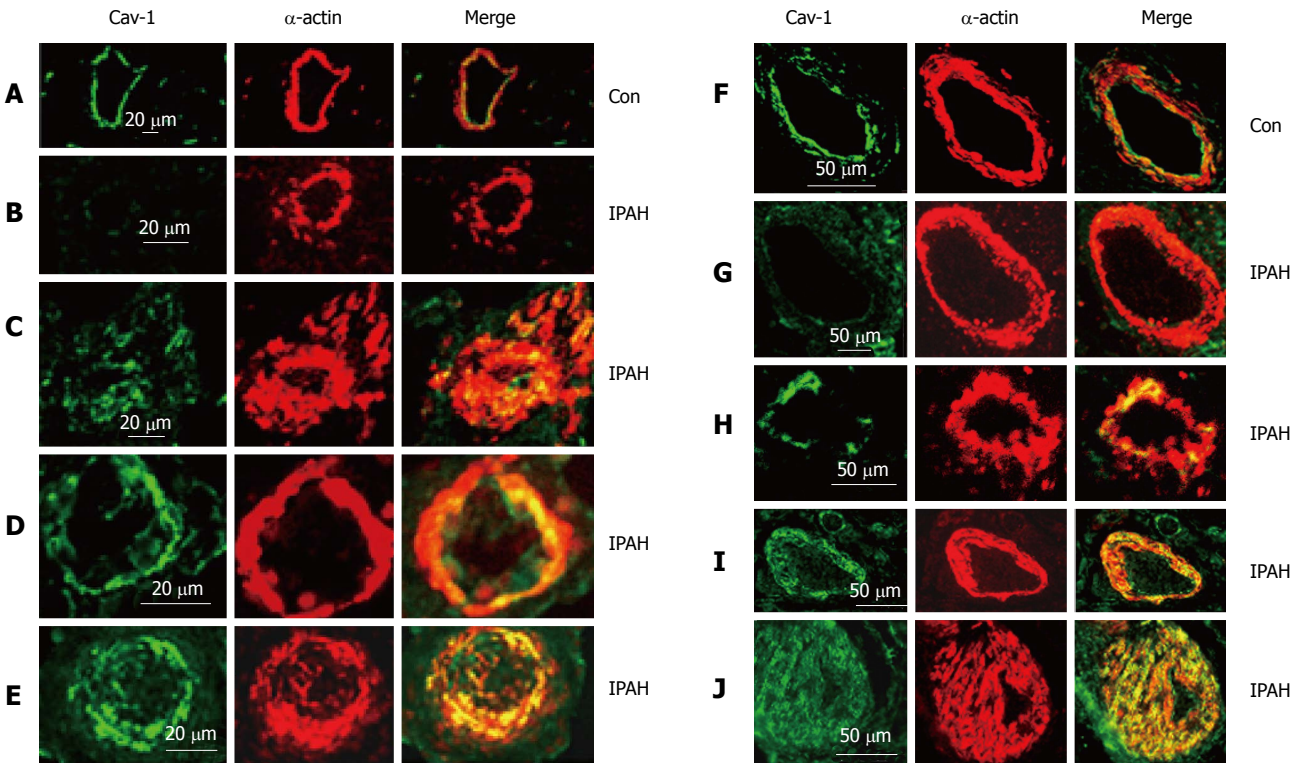

Figure 6 Immunofluorescence study showing the expression of caveolin-1 (green) and smooth muscle α-actin (red) in pulmonary arteries from the controls (A and F), and from the patients with idiopathic pulmonary arterial hypertension (B-E) and with heritable pulmonary arterial hypertension (G-J). In controls, endothelial caveolin-1 is well preserved and there is no enhanced expression of caveolin-1 in smooth muscle layer. Two arteries each from patients, IPAH (B and C), HPAH (G and F) show loss of endothelial caveolin-1 in B and G, and the appearance of increased expression of caveolin-1 in SMC in C and H. The next panels D, E, I and J from 4 different patients show loss of endothelial caveolin-1 and enhanced expression of caveolin-1 in SMC. PAH: Pulmonary arterial hypertension; IPAH: Idiopathic PAH; HPAH: Heritable PAH; SMC: Smooth muscle cells.

± 0.58%) with endothelial caveolin-1 loss, exhibited an increased caveolin-1 expression in SMC (Figure 5, bottom panel).

Human lungs: The control pulmonary arteries showed well preserved caveolin-1 in the endothelial layer. Arteries from IPAH and HPAH patients showed varying degrees of alterations in caveolin-1 expression not unlike what was noted in the 4 wk MCT + hypoxia group, such as endothelial caveolin-1 loss, increased caveolin-1 expression in SMC and the presence of neointima (Figure 6).

Caveolin-1 and PPARγ expression

At 2 wk, caveolin-1 loss in the MCT and MCT + hypoxia groups was accompanied by an increase in the expression of PPARγ ($P < 0.05$ *vs* controls, Figure 7). Since our previous studies had shown caveolin-1 loss at 48 h after MCT injection, we investigated the expression of PPARγ and caveolin-1 at 48 h ($n = 4$) and 1 wk ($n = 4$). At 48 h post-MCT, caveolin-1 expression was reduced to 56% ± 1.4% ($P < 0.05$ *vs* controls) associated with a PPARγ expression of 118% ± 9% (P = ns *vs* control). At 1 wk post-MCT, a further reduction in caveolin-1 (38% ± 1%) was associated with an increase in the expression of PPARγ (203% ± 22%, $P < 0.05$ *vs* control). No alterations were observed in the expression of PPARγ in the 2 wk hypoxia group (Figure 7).

At 4 wk, in the MCT group, a reciprocal increase in PPARγ expression accompanied the caveolin-1 loss. Importantly, in the MCT + hypoxia group, the increased total caveolin-1 expression in the lungs correlated with a reduction in the expression of PPARγ. In the hypoxia group, the expression of caveolin-1 was slightly decreased (90% ± 0.89%), and the PPARγ expression, however, was not altered (Figure 7).

Proliferative and anti-apoptotic pathways

As shown in Figure 8, both at 2 and 4 wk, the activation of p-Erk and PY-STAT3, and increased Bcl-xL expression were present in all experimental groups.

eNOS and HSP90 expression

Although eNOS expression in the 2 wk-post MCT group was not significantly reduced compared with the controls, the expression of HSP90, however, was reduced ($P < 0.05$ *vs* controls). Expression of eNOS was increased in the hypoxia group, but the HSP90 expression was unaltered. In the MCT + hypoxia group, an increased eNOS expression, and a normal HSP90 expression were observed (Figure 9).

At 4 wk, in the MCT group, eNOS and HSP90 levels were reduced. In the hypoxia and MCT + hypoxia

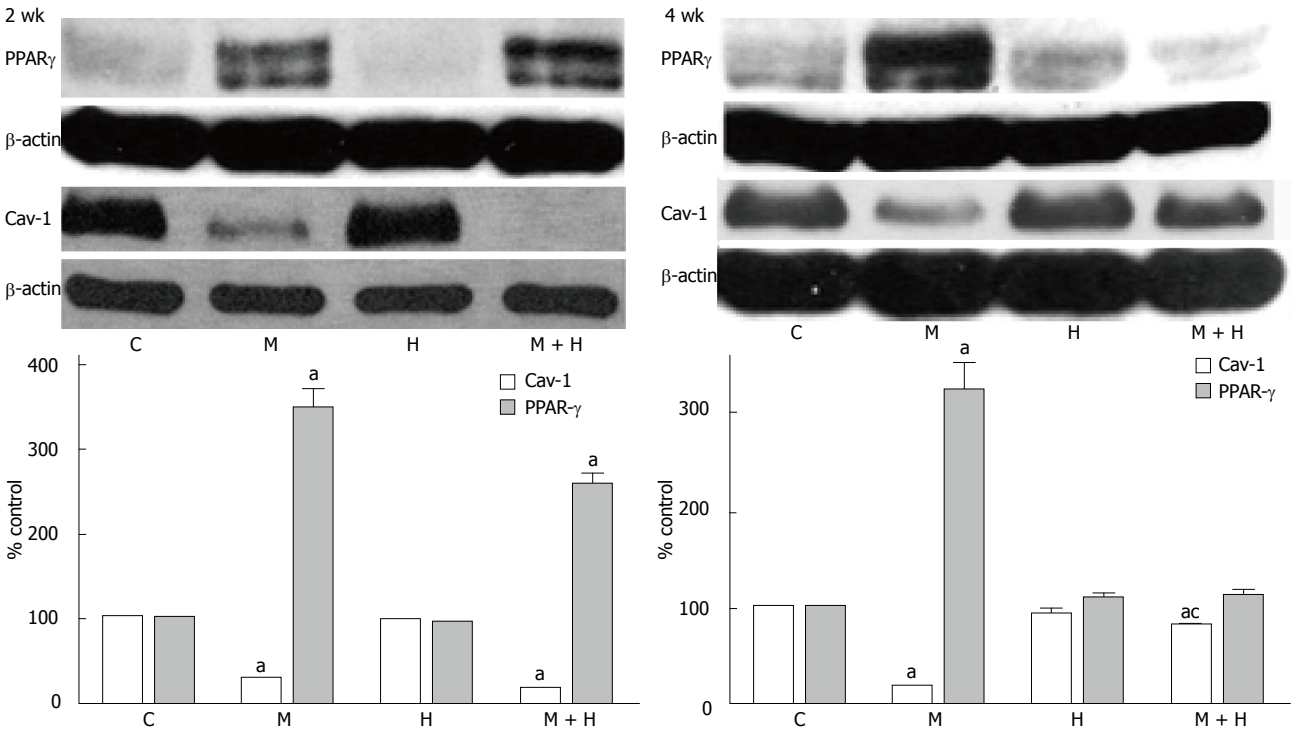

Figure 7 Representative western blots and bar graphs depicting the expression of caveolin-1 and peroxisome proliferator-activated receptor γ in controls, monocrotaline, hypoxia and monocrotaline + hypoxia groups at 2 (*n* = 5-8) and 4 wk (*n* = 5-8). $^{a}P < 0.05$ *vs* C, $^{c}P < 0.05$ *vs* M. C: Controls; M: Monocrotaline; H: Hypoxia; M + H: Monocrotaline + hypoxia; PPAR: Peroxisome proliferator-activated receptor.

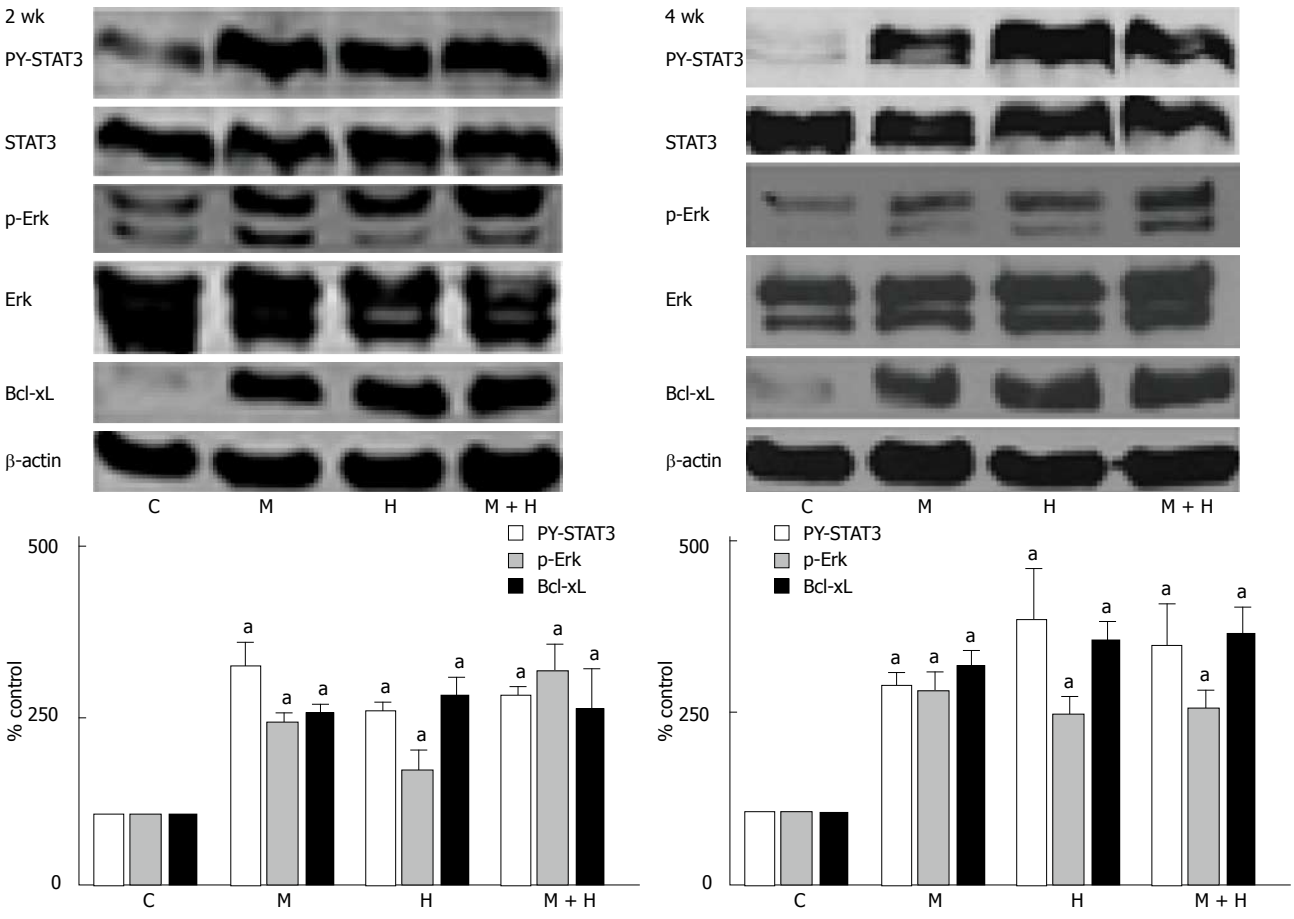

Figure 8 Representative western blots and bar graphs depicting the expression of PY-STAT3, p-Erk and Bcl-xL in controls, monocrotaline, hypoxia and monocrotaline + hypoxia at 2 (*n* = 4-7) and 4 wk (*n* = 5-8). STAT3, Erk and β-actin were used to assess the protein loading. $^{a}P < 0.05$ *vs* C. C: Controls; M: Monocrotaline; H: Hypoxia; M + H: Monocrotaline + hypoxia.

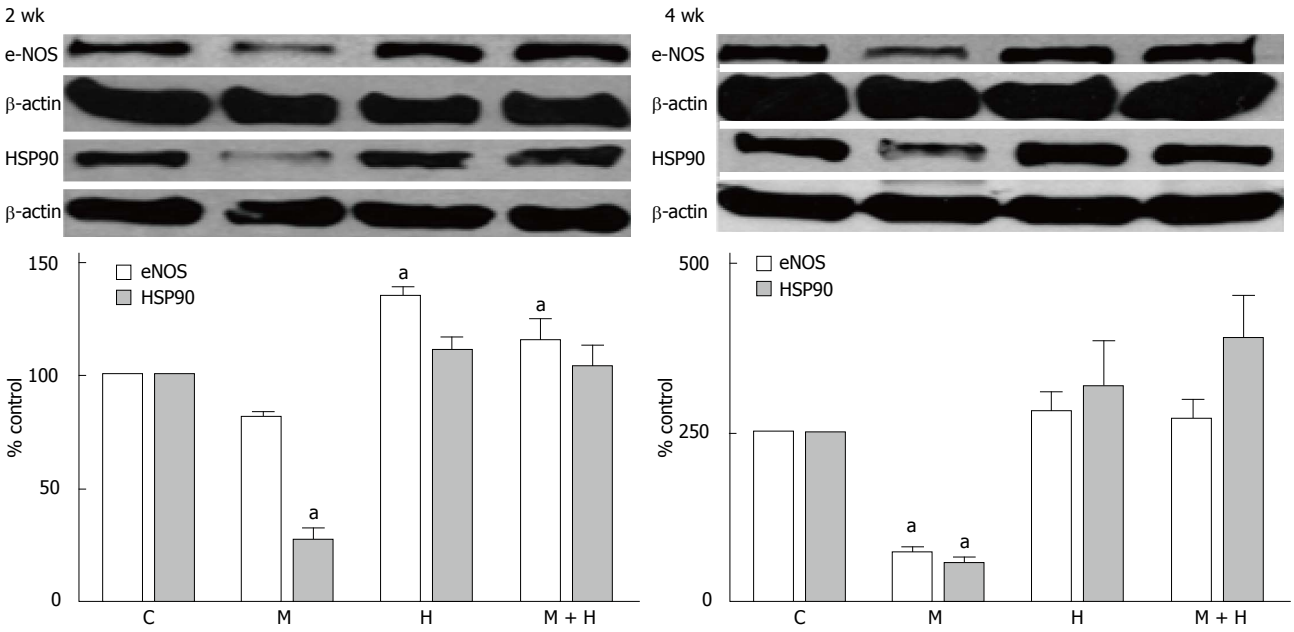

Figure 9 Representative western blots and bar graphs depicting the expression of endothelial nitric oxide synthase, HSP90 and β-actin in controls, monocrotaline, hypoxia and monocrotaline + hypoxia at 2 (*n* = 3-6) and 4 wk (*n* = 4-7). [a]*P* < 0.05 *vs* C. C: Controls; M: Monocrotaline; H: Hypoxia; M + H: Monocrotaline + hypoxia.

groups, eNOS and HSP90 levels were not altered (Figure 9).

DISCUSSION

The significant aspect of our study is the progressive disruption and loss of endothelial caveolin-1, activated proliferative pathways leading to PH in the MCT model. By 4 wk, a further reduction in endothelial caveolin-1 is accompanied by an increased caveolin-1 expression in SMC, observed in 24% of the arteries. The total caveolin-1 expression, however, remained significantly low. Exposure of MCT-treated rats to hypoxia accelerated the disease process. An increased number of arteries exhibited augmented caveolin-1 expression in SMC associated with an increase in total caveolin-1 expression. Importantly, some of the arteries exhibiting an increased caveolin-1 expression in SMC displayed neointima with scant caveolin-1. Furthermore, lung sections from patients with IPAH as well as HPAH showed similar changes, *i.e.*, endothelial caveolin-1 loss, increased caveolin-1 in SMC. Neointimal lesions were seen only in arteries with increased caveolin-1 expression in SMC.

Neointima and plexiform lesions have been described in rodent PH models such as Sugen + hypoxia and pneumonectomy + MCT[20,21,28]. In the Sugen + hypoxia model, the initial EC apoptosis is followed by cellular proliferation and angiogenesis deregulation resulting in plexiform lesions with significantly reduced caveolin-1 expression[29,30]. The reduced expression of caveolin-1 in plexiform lesion is supported by the electron microscopic examination showing a lack of caveolae[31]; the total caveolin-1 protein levels in the lungs, however, are not decreased[32]. *In-vitro* studies have shown that in response to cyclic stretch, caveolin-1 in SMC shifts to non-caveolar sites, mediates Erk activation and participates in cell proliferation. Interestingly, SMC not expressing caveolin-1 fail to proliferate when subjected to cyclic stretch[33,34]. It is likely, that the extensive damage and/or loss of EC, leads to the exposure of SMC to direct shear stress and pressure, resulting in the caveolin-1 shift from caveolae to non-caveolar sites, thus altering caveolin-1 function.

In the hypoxia group, at 2 wk, there was no endothelial caveolin-1 loss, indicating that there was no physical disruption of EC. During hypoxia, caveolin-1 forms a tight complex with eNOS[19,35], leading to the dysfunction of both factors. Removal of hypoxia[36,37] or eNOS/caveolin-1 complex disruption attenuates PH[38]. At 4 wk, the total caveolin-1 expression in the lungs was not altered, but immunofluorescence studies revealed a small loss in endothelial caveolin-1 accompanied by 1.2% of arteries exhibiting increased caveolin-1 expression in SMC. It is noteworthy that in infants with respiratory distress syndrome or bronchopulmonary dysplasia, PH in the absence of EC disruption, does not lead to endothelial caveolin-1 loss or increased caveolin-1 expression in SMC. However, accompanying inflammation results in endothelial cell membrane disruption and endothelial caveolin-1 loss with subsequent increased caveolin-1 expression in SMC[16]. These studies suggest that the endothelial disruption and the endothelial caveolin-1 loss may be necessary for the increased caveolin-1 expression in SMC.

Caveolin-2 loss concomitant with caveolin-1 loss has been shown in the experimental models of PH,

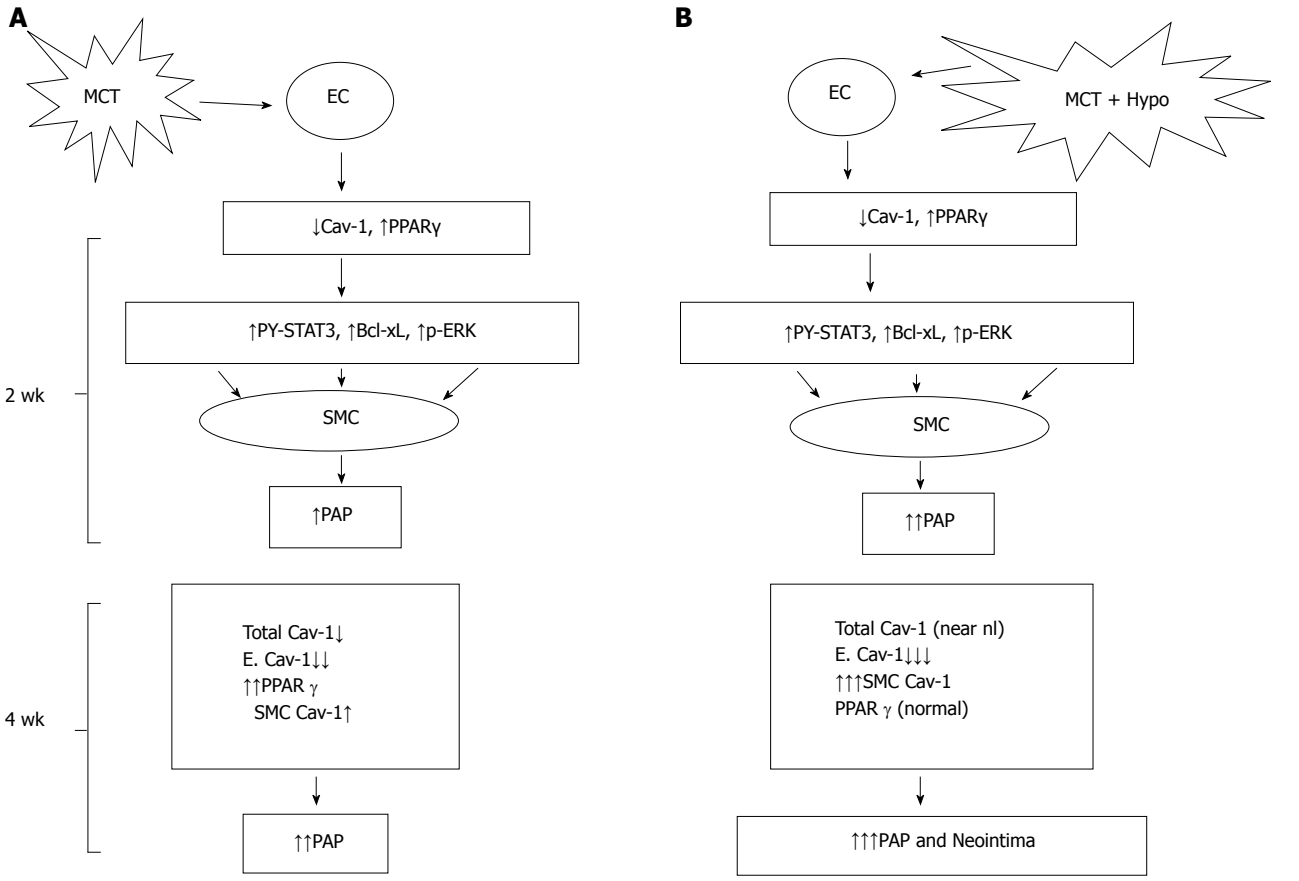

Figure 10 Monocrotaline injury to endothelial cells resulting in the loss of caveolin-1 and the activation of the proliferative pathways (PY-STAT3, Bcl-xL, p-ERK) leading to PH at 2 wk; and a reciprocal relationship between caveolin-1 and peroxisome proliferator-activated receptorγ expression (A) and MCT + hypoxia (MCT + Hypo) accelerates the disease process (B). At 4 wk, there is a further loss of endothelial caveolin-1 (E. Cav-1) and enhanced expression of cav-1 in smooth muscle cells (SMC), however, the total cav-1 levels remain low (17% vs C, 100%). These alterations are accompanied by a further increase in pulmonary artery pressure. Panel B shows MCT + hypoxia (MCT + Hypo) accelerates the disease process. At 2 wk, extensive endothelial caveolin-1 is accompanied by the activation of proliferative pathways and PH (higher pulmonary artery pressure compared with MCT alone group). At 4 wk, a further loss of E. Cav-1 is accompanied by significantly increased expression of caveolin-1 in SMC compared with MCT alone group. At this stage total caveolin-1 level is closer to normal (81% vs C, 100%), and neointimal lesions can be seen. MCT: Monocrotaline; EC: Endothelial cells; PPAR: Peroxisome proliferator-activated receptor.

and the rescue of caveolin-1 restores caveolin-2 expression[10,39]. Caveolin-2 is expressed in a number of cell types including EC and SMC, and it colocalizes with caveolin-1 and necessitates caveolin-1 for its transport to caveolae[23]. However, caveolin-2 is not necessary for caveolar localization of caveolin-1; but the co-expression of caveolin-1 and 2 results in a more efficient formation of caveolae[40,41]. In the present study, MCT-treated rats exhibited a significant loss of caveolin-2 concomitant with the loss of caveolin-1. In the MCT + hypoxia group at 4 wk, despite an increase in the total caveolin-1 expression, a significant loss of caveolin-2 was present, which supports the view that the major part of caveolin-1 in SMC may not be localized in caveolae. In the hypoxia group, despite the presence of caveolin-1, some loss of caveolin-2 was observed, suggesting that a part of caveolin-1 may not be available for caveolin-2 localization.

All experimental groups (MCT, hypoxia and MCT + hypoxia) at 2 and 4 wk revealed the activation of PY-STAT3, pERK1/2 and Bcl-xL. Caveolin-1 is a well known inhibitor of pro-proliferative and anti-apoptotic factors[11,42]; and the rescue of caveolin-1 as a preventive measure in the MCT model, inhibits the activation of proliferative pathways and attenuates PH[9,10]. Interestingly, in the presence of caveolin-1 in hypoxia groups and MCT + hypoxia group at 4 wk, proliferative pathways were activated; which strongly suggest that caveolin-1 is dysfunctional in these groups.

In the 4 wk MCT group, the expression of eNOS and HSP90 was significantly reduced, but was normal in the MCT + hypoxia groups. In addition, caveolin-1 expression in native EC and in neointimal cells was sparse in the latter group. Strong eNOS expression and low caveolin-1 expression have been reported in the plexiform lesions[39,43], besides, oxidant stress is a critical feature in patients with IPAH[44]. The major cause of PH in caveolin-1 knockout mice is thought to be eNOS uncoupling and subsequent oxidative and nitrosative stress; and PH is attenuated by caveolin-1 re-expression, eNOS inhibition or treatment with superoxide dismutase mimetic[45,46]. Furthermore, EC from patients with IPAH show caveolin-1 degradation induced by sustained eNOS and Src signaling[47]. It

is important to note, that caveolin-1 regulates eNOS-derived NO and superoxide, and NOX activity. Caveolin-1 sequestrates uncoupled eNOS, inhibits superoxide formation and prevents eNOS oxidase activity[48,49]. These observations support a pivotal role for caveolin-1 in preventing oxidative and nitrosative stress.

Protein and mRNA expression of PPARγ is described to be low in IPAH, Sugen + hypoxia[26] and the shunt[27] models of PH, but not in chronic obstructive pulmonary disease patients[26]. PPARγ, a ligand-activated transcription factor belongs to the nuclear hormone superfamily. In several cell systems, PPARγ has been shown to upregulate caveolin-1 expression[24,25,50]. In the present study, PPARγ levels revealed an inverse relationship with caveolin-1 in the MCT groups; initial low endothelial and total ceveolin-1 levels were associated with increased PPARγ levels. At 4 wk in the MCT + hypoxia group, an increase in total caveolin-1 was associated with a decrease in PPARγ levels. The increased expression of PPARγ may be a compensatory mechanism to upregulate the caveolin-1 expression during the initial phase of PH associated with significantly reduced caveolin-1 levels. In the hypoxia group, however, the PPARγ levels were not altered. A thiazolidinedione (TZD) compound (PPARγ activator) has been reported to attenuate hypoxia-induced PH[51]. Some of the TZD compounds are reported to have cholesterol disruptive function independent of PPARγ[52]. Interestingly, cholesterol lowering statins in the hypoxia model of PH has been shown to disrupt the tight complex of eNOS and caveolin-1 resulting in the restoration of eNOS function and the attenuation of PH[38]. Recent studies have shown that increased PPARγ expression portends poor prognosis in some forms of cancer[53,54]. In view of these observations, increasing PPARγ levels as a therapeutic measure in PH is of some concern. It is possible that PPARγ activation may be beneficial in some forms of PH or at some stage during the disease; or a selective increase in PPARγ expression in EC may be useful. In any case, further studies are necessary to ascertain the roles of PPARγ and caveolin-1, and their interrelationship in PH.

In conclusion, addition of hypoxia to MCT-treated rats results in an acceleration of the disease process. Extensive endothelial damage, progressive endothelial caveolin-1 loss, and increased caveolin-1 expression in SMC accompanied by an augmented total caveolin-1 protein expression in lungs is followed by neointima formation. In addition, caveolin-1 and PPARγ revealed an inverse relationship (Figure 10). Importantly, lung sections from IPAH and HPAH patients showed similar alterations in caveolin-1 expression, *i.e.*, endothelial caveolin-1 loss and increased caveolin-1 expression in SMC. Both in humans and the MCT + hypoxia group, neointimal lesions were observed only in the arteries exhibiting increased caveolin-1 expression in SMC. Since increased caveolin-1 expression in SMC has been shown to be actively pro-proliferative, this alteration in caveolin-1 expression may be a prelude to neointima formation. In the hypoxia group, in the absence of endothelial disruption or the endothelial caveolin-1 loss, there was neither an increased expression of caveolin-1 in SMC nor neointima. These results suggest that the endothelial cell integrity may be an important factor that determines the course of the disease.

ACKNOWLEDGEMENTS

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COMMENTS

Background

Neointima formation in pulmonary hypertension (PH) portends poor prognosis. Despite major advances in the field, the mechanism/s of pathogenesis is not yet clear, which makes the therapeutic measures a challenge.

Research frontiers

Caveolin-1, a membrane protein plays a significant role in pulmonary vascular homeostasis and in the pathogenesis of PH.

Innovation and breakthroughs

An important aspect of the study is that caveolin-1 plays a dual role in the pathogenesis of PH, *i.e.*, as an anti-proliferative and a pro-proliferative factor. This change in function of caveolin-1 is similar to what has been reported in cancer. Loss of endothelial caveolin-1 leads to the activation of proliferative pathways, vascular remodeling and PH. As the disease progresses, the resulting extensive endothelial caveolin-1 loss associated with endothelial cell damage is followed by an enhanced expression of caveolin-1 in smooth muscle cells (SMC). The authors have shown that by subjecting monocrotaline-treated rats to hypoxia accelerates the disease process; by 4 wk, a large number of arteries exhibit enhanced expression of caveolin-1 in SMC. This caveolin-1 becomes pro-proliferative and facilitates cell proliferation, cell migration and neointima formation. In the experimental models and humans, neointima is observed only in the arteries exhibiting extensive endothelial damage and enhanced expression of caveolin-1 in SMC.

Application

The results would lead to further research in the role of caveolin-1 in SMC in PH, and in assessing the effects of modulation of caveolin-1 expression.

Peer-review

The present investigation is well written and interesting.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Renal function assessment in atrial fibrillation: Usefulness of chronic kidney disease epidemiology collaboration *vs* re-expressed 4 variable modification of diet in renal disease

Rami Riziq-Yousef Abumuaileq, Emad Abu-Assi, Andrea López-López, Sergio Raposeiras-Roubin, Moisés Rodríguez-Mañero, Luis Martínez-Sande, Francisco Javier García-Seara, Xesus Alberte Fernandez-López, Jose Ramón González-Juanatey

Rami Riziq-Yousef Abumuaileq, Emad Abu-Assi, Andrea López-López, Sergio Raposeiras-Roubin, Moisés Rodríguez-Mañero, Luis Martínez-Sande, Francisco Javier García-Seara, Xesus Alberte Fernandez-López, Jose Ramón González-Juanatey, Cardiology Department, University Clinical Hospital of Santiago de Compostela, 15706 Santiago de Compostela, Spain

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Correspondence to: Rami Riziq-Yousef Abumuaileq, MD, Cardiology Department, University Clinical Hospital of Santiago de Compostela, A choupana s/n, 15706 Santiago de Compostela, Spain. drrami2012@hotmail.com
Telephone: +34-981-950778
Fax: +34-981-950534

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Abstract

AIM: To compare the performance of the re-expressed Modification of Diet in Renal Disease equation *vs* the new Chronic Kidney Disease Epidemiology Collaboration equation in patients with non-valvular atrial fibrillation.

METHODS: We studied 911 consecutive patients with non-valvular atrial fibrillation on vitamin-K antagonist. The performance of the re-expressed Modification of Diet in Renal Disease equation *vs* the new Chronic Kidney Disease Epidemiology Collaboration equation in patients with non-valvular atrial fibrillation with respect to either a composite endpoint of major bleeding, thromboembolic events and all-cause mortality or each individual component of the composite endpoint was assessed using continuous and categorical $\geqslant$ 60, 59-30, and < 30 mL/min per 1.73 m^2 estimated glomerular filtration rate.

RESULTS: During 10 ± 3 mo, the composite endpoint occurred in 98 (10.8%) patients: 30 patients developed major bleeding, 18 had thromboembolic events, and 60 died. The new equation provided lower prevalence of renal dysfunction < 60 mL/min per 1.73 m^2 (32.9%),

compared with the re-expressed equation (34.1%). Estimated glomerular filtration rate from both equations was independent predictor of composite endpoint (HR = 0.98 and 0.97 for the re-expressed and the new equation, respectively; $P < 0.0001$) and all-cause mortality (HR = 0.98 for both equations, $P < 0.01$). Strong association with thromboembolic events was observed only when estimated glomerular filtration rate was < 30 mL/min per 1.73 m^2: HR is 5.1 for the re-expressed equation, and HR = 5.0 for the new equation. No significant association with major bleeding was observed for both equations.

CONCLUSION: The new equation reduced the prevalence of renal dysfunction. Both equations performed similarly in predicting major adverse outcomes.

Key words: Atrial fibrillation; Anticoagulants; Follow-up studies; Kidney; Prognosis

Core tip: In atrial fibrillation, renal dysfunction entails more adverse events. Limited data exist on the performance and prognostic value of the re-expressed Modification of Diet in Renal Disease equation *vs* the new Chronic Kidney Disease Epidemiology Collaboration equation in atrial fibrillation. We compared the performance of both equations at predicting major outcomes in patients with non-valvular atrial fibrillation. The study encouraged the use of the new equation as it decreased the prevalence of patients with renal dysfunction, in a real world cohort of patients with non-valvular atrial fibrillation and at the same time showed similar prognostic impact like the re-expressed equation.

Abumuaileq RRY, Abu-Assi E, López-López A, Raposeiras-Roubin S, Rodríguez-Mañero M, Martínez-Sande L, García-Seara FJ, Fernandez-López XA, González-Juanatey JR. Renal function assessment in atrial fibrillation: Usefulness of chronic kidney disease epidemiology collaboration *vs* re-expressed 4 variable modification of diet in renal disease. *World J Cardiol* 2015; 7(10): 685-694 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i10/685.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i10.685

INTRODUCTION

Renal dysfunction is a common comorbidity observed in patients with atrial fibrillation (AF). Patients with AF and renal dysfunction are more likely to develop thromboembolic (TE) events compared to those with AF and normal renal function[1,2]. The presence and severity of renal dysfunction is also a recognized predictor in the bleeding risk scores used commonly to estimate the hemorrhagic risk in anticoagulated patients with AF[3,4].

Therefore, accurate assessment of renal function is of paramount importance as it will help inform the decision making process aiming for optimizing the management of patients with AF. Current recommendations advocate the estimation of renal function by means of estimated glomerular filtration rate (eGFR) using the validating prediction equations instead of serum creatinine[5].

Until recently, the two most commonly used creatinine based equations estimating GFR were the 4 variable Modification of Diet in Renal Disease (MDRD-4) Study[6] and the Cockcroft-Gault (C-G) equation[7]. The MDRD-4 equation was re-expressed to be used in the current era of standardized serum creatinine assay, whereas the C-G equation was not updated, and its use is not recommended currently[8]. More recently, a new equation, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[9], has been proposed as an alternative equation to replace the widely used re-expressed MDRD-4 formula in routine clinical use, on the basis that it estimates measures of GFR more accurate than the re-expressed MDRD-4 equation.

Several studies have demonstrated the higher accuracy of the new CKD-EPI at estimating the true renal function, thus enabling it to provide better clinical risk prediction in different disease contexts[10-12]. However, it is currently unknown if the better estimates from the new CKD-EPI would be translated into better risk prediction in the particular context of patients with AF, since very few patients in the derivation cohort of the new CKD-EPI formula had AF[9].

In this study, we aimed to comparatively evaluate the re- expressed MDRD-4 and the new CKD-EPI formulas at predicting the occurrence of major adverse outcomes in a real world cohort of patients with non-valvular AF (NVAF) who are recently on vitamin K antagonists (VKA).

MATERIALS AND METHODS

Patient's sample

Retrospectively, we identified all consecutive patients of ⩾ 18 years of age with a confirmed diagnosis of AF on VKAs attending outpatient cardiology consultations of a tertiary hospital between January 2011 and February 2013. Only patients who fulfilled the following criteria were included in this study: Patients with permanent or paroxysmal AF recently started on VKAs (*i.e.,* not more than 8 mo passed since the beginning of their VKAs therapy), and who have regular visits for INR measurements. Patients with prosthetic valve (n = 452), rheumatic heart disease (n = 43), active cancer (n = 41), dementia (n = 26), and/or interrupted vitamin K antagonist > 3 d (n = 73) were excluded. Thus, the final analyzed cohort consisted of 911 patients. A detailed medical history was recorded for each patient, and the basal clinical characteristics at study entry together with information on follow up were carefully

gathered by cardiologists.

The vast majority of patients were on acenocoumarol (93%; and the remaining patients were on warfarin).

The study was approved by the Clinical Research Ethics Committee of our hospital.

Calculation of eGFR

For each patient, Serum creatinine was measured by the modified kinetic Jaffe method in a single clinical laboratory in our institution. All creatinine measurements were performed with an isotope dilution mass spectroscopy (IDMS)-traceable enzymatic assay that has previously been shown to provide very reliable eGFR results compared with the measured GFR[13]; these measurements were analyzed automatically using the ADVIA 2400 Chemistry System (Siemens Diagnostics, Tarrytown, NY, United States).

We calculated the eGFR using the IDMS-traceable version of the MDRD-4 equation[8]: 175 × [standardized serum creatinine (mg/dL)]$^{-1.154}$ × age$^{-0.203}$ × (0.742 if female) × (1.212 if black).

The new CKD-EPI equation was also used[9]: 141 × (minimum of standardized serum creatinine (mg/dL)/κ or 1)$^{\alpha}$ × [maximum of standardized serum creatinine (mg/dL)/κ or 1]$^{-1.209}$ × 0.993^{age} × (1.018 if female) × (1.159 if black). Where κ is 0.7 for females and 0.9 for males and α is -0.329 for females and -0.411 for males.

We categorized the eGFR obtained from each formula into three categories: ⩾ 60 mL/min per 1.73 m^2 (normal or mild renal dysfunction), 30-59 mL/min per 1.73 m^2 (moderate renal dysfunction) and < 30 mL/min per 1.73 m^2 (severe renal dysfunction). No patients were on renal replacement therapy.

Endpoints and definitions

Patients were followed up to 1-year after the enrolment. The primary endpoint of the present study was a composite endpoint of major bleeding, TE complications, or death; whichever comes first. The secondary endpoint was each individual component of the composite endpoint.

Data on major bleeding, and TE complications were gathered from the cardiology clinic visits and records, and through hospital files as well as through primary care centers reports.

We used the 2005 International Society on Thrombosis and Haemostasis (ISTH) criteria to define major bleeding[14]. Thus, a major bleeding event was adjudicated if one of the following criteria was met: fatal bleeding and/or symptomatic bleeding in a critical area or organ (*e.g.,* such as intracranial, intraspinal, intraocular, retroperitoneal, atraumatic intraarticular, pericardial, or intramuscular with compartment syndrome); and/or bleeding causing drop of hemoglobin of ⩾ 2 g/dL, or leading to transfusion of ⩾ 2 units of whole blood or packed red blood cells.

A TE complication was defined as the occurrence of ischemic stroke, transient ischemic attack, or peripheral embolism (including fatal TE events). Diagnosis of stroke or transient ischemic attack required an acute neurological deficit lasting for more or less than 24 h, respectively, which could not be explained by other causes and with at least 1 image test (computed tomography or magnetic resonance) compatible with the diagnosis, as well as confirmation from a neurologist. A diagnosis of peripheral embolism was defined as non-central nervous system embolism leading to an abrupt vascular insufficiency associated with clinical or radiographic evidence of arterial occlusion in absence of another mechanism such as atherosclerosis, instrumentation, or trauma.

Statistical analysis

Qualitative data were expressed as frequencies and percentages while quantitative data were summarized as mean and standard deviation. Comparison between qualitative data was performed using the χ^2 test or the Fisher exact test, as appropriate. The *t*-Student test was used to compare quantitative data.

The relationship between the primary endpoint and eGFR according to both formulas was evaluated using separate Cox proportional hazard regression models. The candidate variables to construct the multivariate Cox models were those variables presented $P < 0.10$ in the univariate Cox analysis, or those co-variables of recognized prognostic value in the medical literature. Once the initial Cox models had been established, they were simplified by stepdown elimination. Thus, the final Cox models to determine the adjusted effect of eGFR on the composite endpoint, included: age, sex, previous stroke, basal hemoglobin, chronic obstructive pulmonary disease, diabetes mellitus, congestive heart failure or left ventricular ejection fraction ⩽ 40%, history of malignant disease and coronary artery disease.

The association between eGFR formulas and the individual endpoints of either major bleeding or TE events was determined using competing-risks regression based on Fine and Gray's proportional subhazards models. The Fine and Gray models were adjusted for HAS-BLED score[4] in the case of testing the relationship between eGFR formulas and major bleeding, and for CHA_2DS_2-VASc score[15] in the case of testing the relationship between eGFR formulas and TE events. For all-cause mortality, we used a Cox regression model. Once the initial Cox model for predicting all-cause mortality had been established, it was simplified by stepdown elimination; and finally included the following covariables: age, sex, diabetes mellitus, and history of malignant disease, previous stroke, basal hemoglobin, and congestive heart failure or ejection fraction ⩽ 40%.

The discriminatory capacity of each formula at predicting either the primary or secondary endpoint was determined by calculating the c- statistic. We used

Table 1 Baseline characteristics *n* (%)

Age (yr)	73 ± 11
Men	605 (66.4)
Systolic blood pressure at study entry	139 ± 28
Hypertension	678 (74.4)
Current smoking	77 (8.5)
Diabetes mellitus	220 (24.1)
Heart failure	343 (37.7)
Peripheral arterial disease	92 (10.1)
History of stroke or TIA	103 (11.3)
Coronary artery disease	127 (13.9)
COPD	183 (20.1)
CHA2DS2-VASc:	
= 0	62 (6.8)
⩾ 1	849 (93.2)
⩾ 2	772 (84.7)
History of malignancy	135 (14.8)
HAS-BLED	
0	47 (5.2)
1	160 (17.6)
2	365 (40.1)
3	261 (28.6)
4	69 (7.6)
5	6 (0.7)
6	3 (0.3)
Alcohol consumption ⩾ 40 g/daily	81 (8.9)
Prior bleeding	115 (12.6)
Anemia	178 (19.5)
Abnormal liver function[1]	9 (1)
PINRR	58% ± 18%

[1]Defined as cirrhosis or elevated liver transaminases enzymes > 3 times higher than the upper limit of normal and elevated total billirubin > 2 times higher than the upper limit of normal. CHA2DS2-VASc: Congestive heart failure, hypertension, age ⩾ 75, diabetes mellitus, stroke, vascular disease, female sex category; COPD: Chronic obstructive pulmonary disease; HAS-BLED: Uncontrolled Hypertension: systolic > 160 mmHg, Abnormal renal/liver function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, elderly > 65 years, drugs/alcohol concomitantly; TIA: Transient ischemic attack; PINRR: Percentage of INRs in therapeutic range.

the Delong test to compare the c-statistic values from each formula.

The calibration of the model was assessed with the Grønnesby and Borgan goodness-of-fit test. This test determines how closely the predicted event rate approximates the observed event rate over a range of scores. A significant value of *P* indicates a lack of fit.

The estimated coefficients were expressed as the hazard ratio (HR) with the respective 95%CI. A 2-sided $P < 0.05$ was considered statistically significant for all analyses.

Finally, we also assessed the incremental prognostic value of using one equation over another; using the concept of net reclassification improvement (NRI) as described by Pencina *et al*[16], to determine whether the reclassification of patients by one of the formulas regarding to each other, would result in a more accurate risk estimation.

All the analyses were performed with STATA 13, and by using the MedCalc statistical software version 12.2.1.

The study was reviewed by our expert Biostatistic Emad Abu-Assi, MD, PhD.

RESULTS

Mean age was of 73 ± 11 years, male patients constitute 66.4% of the studied population. Baseline characteristics are summarized in Table 1.

Assessment of renal function according to the formula used

The mean eGFR was higher when computed by the new CKD-EPI than with the re-expressed MDRD-4 (69.8 ± 23, 67.2 ± 19 mL/min per 1.73 m^2), respectively ($P < 0.0001$ for comparison).

There was lower prevalence of eGFR < 60 mL/min per 1.73 m^2 with the new CKD-EPI than with the re-expressed MDRD-4 (32.9% *vs* 34.1%).

Events throughout the follow-up

During a follow up of 10 ± 3 mo, the composite endpoint occurred in 98 (10.8%) patients: 30 (3.3%) patients developed major bleeding, 18 (2%) had TE events, and 60 (6.6%) patients died.

Relation with the composite endpoint

The rate of the composite endpoint increased monotonically from the higher to the lower eGFR categories for both formulas (Figure 1).

Significant association was observed between the eGFR using both formulas as continuous variables and the composite endpoint. The adjusted hazard ratios of eGFR by each formula on the composite endpoint were: 0.98 (95%CI: 0.967-0.988) and 0.97 (95%CI: 0.963-0.987) for the re-expressed MDRD-4 and the new CKD-EPI, respectively (Table 2).

Similarly, the eGFR as a categorical variable was a strong independent predictor of the occurrence of the composite endpoint regardless of the formula used (Table 3).

The discriminative capacity of both formulas at predicting the composite endpoint, were quite similar, regardless of the eGFR was used as continuous (0.683 *vs* 0.695 for the re-expressed MDRD-4 and the new CKD-EPI, respectively; $P = 0.748$) or categorical variable (0.632 *vs* 0.639 for the re-expressed MDRD-4 and the new CKD-EPI, respectively; $P = 0.45$) (Table 4).

Relation with major bleeding

There was a step increase in the major bleeding rate, as the eGFR declines, independently of the formula used to calculate the eGFR (Figure 1).

After adjusting for HAS-BLED bleeding risk score, the re-expressed MDRD-4 eGFR as well as the new CKD-EPI eGFR, as continuous variables, showed a tendency to predict major bleeding: HR for both formulas = 0.98 (95%CI: 0.965-1.000; $P = 0.07$) (Table 2).

No significant association was observed between

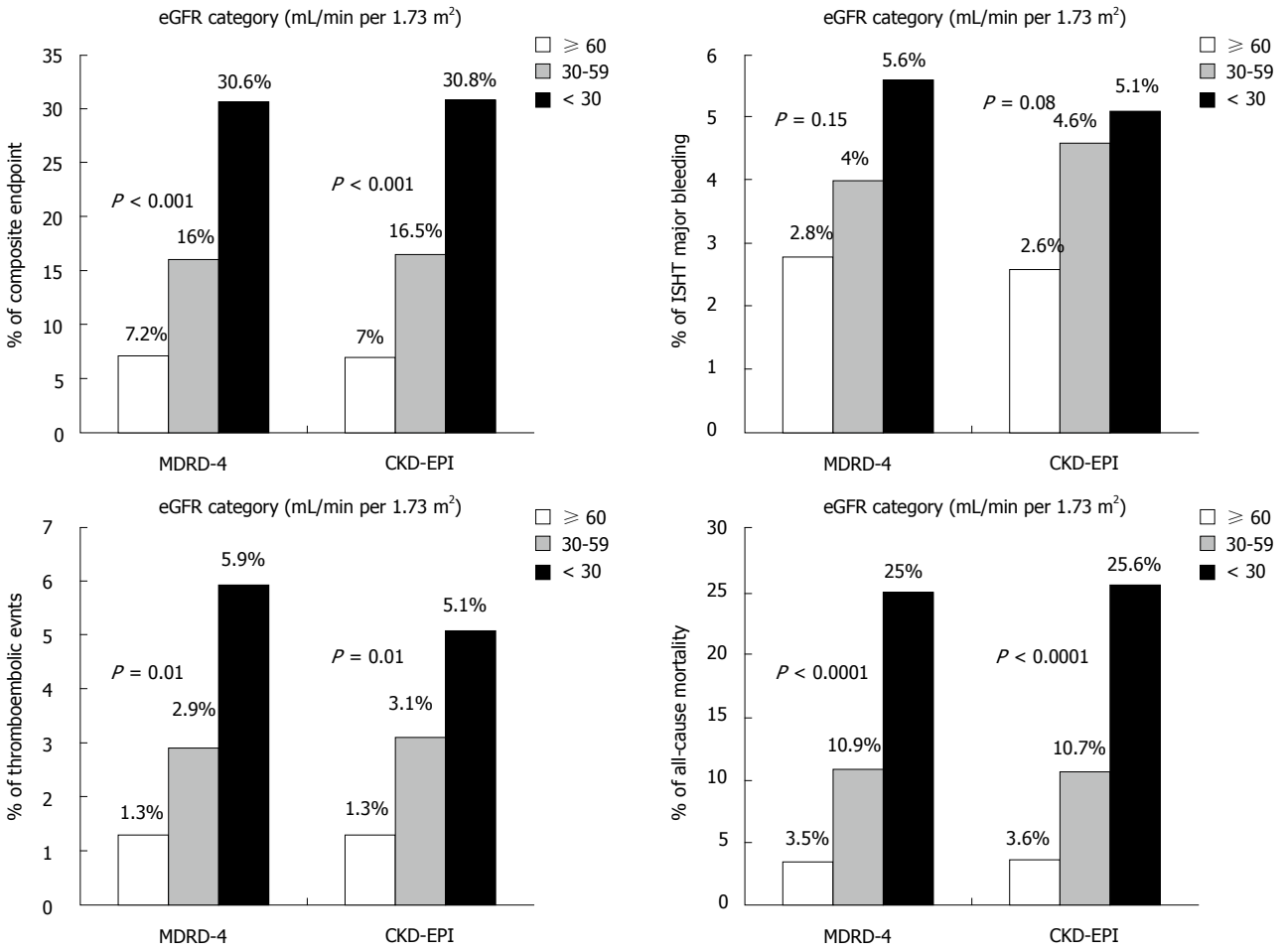

Figure 1 Distribution of major cardiovascular events according to the categories of estimated glomerular filtration rate using the re-expressed Modification of Diet in Renal Disease-4 and the new Chronic Kidney Disease Epidemiology Collaboration equations. eGFR: Estimated glomerular filtration rate; MDRD-4 indicates: Four variables Modification of Diet in Renal Disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration.

Table 2 Unadjusted and adjusted effect (HR) on outcomes of continuous estimated glomerular filtration determined by the re-expressed Four variables Modification of Diet in Renal Disease and the new Chronic Kidney Disease Epidemiology Collaboration equations

	MDRD-4		CKD-EPI	
n **(%)**	**Unadjusted HR (95%CI)**	**Adjusted HR (95%CI)**	**Unadjusted HR (95%CI)**	**Adjusted HR (95%CI)**
Composite endpoint, 98 (10.8)	0.97 (0.958-0.977)	0.98[1] (0.967-0.988)	0.96 (0.955-0.975)	0.97[1] (0.963-0.987)
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Major bleeding, 30 (3.3)	0.97 (0.951-0.985)	0.98[2] (0.965-1.000)	0.97 (0.949-0.984)	0.98[2] (0.965-1.000)
P value	< 0.0001	0.07	< 0.0001	0.07
Thromboembolism, 18 (2)	0.98 (0.959-1.003)	0.98[3] (0.965-1.000)	0.97 (0.948-0.996)	0.98[3] (0.965-1.001)
P value	0.09	0.15	< 0.0001	0.22
All-cause mortality, 60 (6.6)	0.96 (0.948-0.973)	0.98[4] (0.965-0.995)	0.96 (0.947-0.971)	0.98[4] (0.965-0.995)
P value	< 0.0001	< 0.0001	0.02	0.001

[1]Adjusted for age, sex, previous stroke, basal hemoglobin, chronic obstructive pulmonary disease, diabetes mellitus, congestive heart failure or left ventricular ejection fraction ⩽ 40%, history of malignant disease and coronary artery disease; [2]Adjusted for HAS-BLED risk score [Hypertension (uncontrolled: systolic >160 mmHg)], abnormal renal function, abnormal liver function, stroke, bleeding history or predisposition (anemia), labile international normalized ration (INR), elderly > 65 years, and Drugs/alcohol concomitantly); [3]Adjusted for CHA2DS2-VAScscore [Cardiac failure or dysfunction, Hypertension, Age_75 years (doubled), Diabetes, Stroke (doubled), Vascular disease, Age 65 to 74 years, and Sex category (female)]; [4]Adjusted for age, sex, diabetes mellitus, history of malignant disease, previous stroke, basal hemoglobin and congestive heart failure or ejection fraction ⩽ 40%. CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; MDRD-4: 4-variable Modification of Diet in Renal Disease Study equation.

categorical eGFR from both formulas and major bleeding, either in the unadjusted or by using the adjusted competing-risk models (Table 3).

At predicting major bleeding, the discriminative ability of the continuous re-expressed MDRD-4 eGFR was modest: 0.666; quite similar to that obtained from

Table 3 Unadjusted and adjusted effect (HR) on outcomes of categorical estimated glomerular filtration rate determined by the re-expressed four variables Modification of Diet in Renal Disease and the new Chronic Kidney Disease Epidemiology Collaboration equations

		MDRD-4		CKD-EPI	
n (%)		Unadjusted HR (95%CI)	Adjusted HR (95%CI)	Unadjusted HR (95%CI)	Adjusted HR (95%CI)
Composite endpoint, 98 (10.8)	⩾ 60		1.00 (Reference)		
	30-59	2.43 (1.592-3.703)	1.7[1] (1.11-2.78)	2.51 (1.642-3.827)	1.8[1] (1.1-2.8)
		P < 0.0001	*P* = 0.02	*P* < 0.0001	*P* = 0.02
	< 30	6.99 (3.585-13.649)	3.3 (1.6-6.9)	7.4 (3.871-14.125)	3.6 (1.8-7.4)
		P < 0.0001	*P* = 0.001	*P* < 0.0001	*P* < 0.0001
Major bleeding, 30 (3.3)	⩾ 60		1.00 (Reference)		
	30-59	1.53 (0.715-3.260)	1.01[2] (0.46-2.25)	1.87 (0.883-3.948)	1.2[2] (0.58-2.75)
		P = 0.30	*P* = 0.95	*P* = 0.1	*P* = 0.58
	< 30	3.56 (0.811-15.580)	1.03 (0.22-4.95)	3.65 (0.827-16.074)	1.1 (0.25-5.35)
		P = 0.09	*P* = 0.93	*P* = 0.08	*P* = 0.9
Thromboembolism, 18 (2)	⩾ 60		1.00 (Reference)		
	30-59	2.04 (0.734-5.649)	1.4[3] (0.49-4.15)	2.13 (0.767-5.917)	1.4[3] (0.50-4.25)
		P = 0.17	*P* = 0.15	*P* = 0.15	*P* = 0.50
	< 30	8.01 (1.664-38.555)	5.1 (1.04-25.4)	7.84 (1.625-37.825)	5 (1.0-24.9)
		P = 0.009	*P* = 0.045	*P* = 0.01	*P* = 0.04
All-cause mortality, 60 (6.6)	⩾ 60		1.00 (Reference)		
	30-59	3.34 (1.909-5.827)	2.6[4] (1.4-2.7)	3.14 (1.793-5.481)	2.4[4] (1.3-4.5)
		P < 0.0001	*P* = 0.002	*P* < 0.0001	*P* = 0.005
	< 30	10.64 (4.843-23.359)	4.9 (2.0-11.9)	10.89 (5.122-23.166)	5.2 (2.2-12.3)
		P < 0.0001	*P* < 0.0001	*P* < 0.0001	*P* < 0.0001

[1]Adjusted for age, sex, previous stroke, basal hemoglobin, chronic obstructive pulmonary disease, diabetes mellitus, congestive heart failure or ejection fraction ⩽ 40%, history of malignant disease and coronary artery disease; [2]Adjusted for HAS-BLED risk score [Hypertension (uncontrolled: systolic > 160 mmHg)], Abnormal renal function, abnormal liver function, stroke, bleeding history or predisposition (anemia), labile international normalized ration (INR), elderly > 65 years, and drugs/alcohol concomitantly); [3]Adjusted for CHA2DS2-VAScscore [Cardiac failure or dysfunction, Hypertension, Age_75 years (doubled), Diabetes, Stroke (doubled), Vascular disease, Age 65 to 74 years, and Sex category (female)]; [4]Adjusted for age, sex, diabetes mellitus, history of malignant disease, previous stroke, basal hemoglobin and congestive heart failure or ejection fraction ⩽ 40%. CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; MDRD-4: 4-variable Modification of Diet in Renal Disease Study equation.

Table 4 Calibration and discrimination abilities of the re-expressed four variables Modification of Diet in Renal Disease and the new Chronic Kidney Disease Epidemiology Collaboration equations

			MDRD-4	CKD-EPI	*P* value
Composite endpoint	Calibration, χ^2 (*P* value)		1.7 (0.79)	3.5 (0.48)	
	c-statistic (95%CI)	eGFR continuous	0.683 (0.629-0.737)	0.695 (0.643-0.747)	0.748
		eGFR categorical	0.632 (0.600-0.664)	0.639 (0.607-0.670)	0.452
Major bleeding	Calibration, χ^2 (*P* value)		5.9 (0.20)	5.4 (0.25)	
	c-statistic (95%CI)	eGFR continuous	0.666 (0.581-0.751)	0.677 (0.596-0.759)	0.8548
		eGFR categorical	0.550 (0.443-0.658)	0.571 (0.465-0.679)	0.7872
Thromboembolism	Calibration, χ^2 (*P* value)		0.13 (0.99)	1.9 (0.76)	
	c-statistic (95%CI)	eGFR continuous	0.616 (0.584-0.648)	0.644 (0.612-0.675)	0.2736
		eGFR categorical	0.617 (0.585-0.649)	0.622 (0.590-0.654)	0.7582
All-cause mortality	Calibration, χ^2 (*P* value)		0.83 (0.94)	1.5 (0.82)	
	c-statistic (95%CI)	eGFR continuous	0.715 (0.684-0.744)	0.722 (0.691-0.750)	0.5227
		eGFR categorical	0.679 (0.647-0. 709)	0.678 (0.646-0.708)	0.911

eGFR: Estimated glomerular filtration rate; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; MDRD-4: 4-variable Modification of Diet in Renal Disease Study equation.

using the continuous new CKD-EPI eGFR: c-statistic = 0.677 (*P* = 0.85).

When eGFR was considered as a categorical variable, the discriminative capacity of each formula at predicting major bleeding was of 0.550 and of 0.571 for the re-expressed MDRD-4 and the new CKD-EPI, respectively (*P* = 0.79) (Table 4).

Relation with thromboembolic event

As shown in Figure 1, the distribution of the TE event rate in the different eGFR categories, demonstrated a consistent gradient of risk, regardless of the formula used.

After adjusting for the CHA_2DS_2-VASc risk score, no significant association was observed between eGFR as a continuous variable and TE events: HR = 0.98 (95%CI: 0.965-1.000) and 0.98 (95%CI: 0.965-1.001), for the re-expressed MDRD-4 and the new CKD-EPI, respectively (Table 2).

When eGFR was considered as a categorical variable, only significant association existed between eGFR < 30 mL/min per 1.73 m^2 and the TE complications, after

controlling for CHA_2DS_2-VASc score: HR = 5.1 (95%CI: 1.04-25.4) for the re-expressed MDRD-4, and HR = 5.0 (95%CI: 1.0-24.9) for the new CKD-EPI (Table 3).

The discriminative power of GFR estimates determined by both formulas was also modest. For continuous eGFR, the c-statistic values were of 0.616 and 0.644 for the re-expressed MDRD-4 and the new CKD-EPI, respectively, (P = 0.27), and for categorical eGFR, the c-statistic values were 0.617 and 0.622 when using the re-expressed MDRD-4 and the new CKD-EPI, respectively, (P = 0.76) (Table 4).

Relation with all-cause mortality

The rate of all-cause mortality increased progressively from the higher to the lower eGFR values for both formulas (Figure 1).

Continuous eGFR calculated by either the reexpressed MDRD-4 or the new CKD-EPI was an independent predictor of all-cause mortality; adjusted HR = 0.98; (P < 0.01) (Table 2).

A strong association was also found between categorical eGFR and all-cause mortality after adjusting for several confounders (Table 3).

Good discrimination was obtained from continuous eGFR: c-statistic = 0.715 for the re-expressed MDRD-4 and 0.722 for the new CKD-EPI (P = 0.52).

The discriminative power of eGFR as a categorical variable in terms of c-statistic was: 0.679 and 0.678 when using the re-expressed MDRD-4 and the new CKD-EPI, respectively, (P = 0.91) (Table 4).

Estimated GFR from both formulas demonstrated good calibration for the major cardiovascular events with P value > 0.1 (Table 4).

The NRI analysis did not significantly favor the new CKD-EPI over the re-expressed MDRD-4 whether for predicting the composite endpoint, major bleeding and all-cause mortality (NRI = 2.13%, 4.35%, and 0.9%, with P = 0.27, 0.19, and 0.7, respectively).

However, at predicting the TE event, the NRI favored the new CKD-EPI formula with NRI of 1% (95%CI: -0.08 to +2.0, P = 0.07) indicating a strong tendency to reclassify better the patients according to their risk of developing TE event, compared with the re-expressed MDRD-4.

DISCUSSION

In this real world cohort of patients with NVAF on VKAs, the new CKD-EPI formula classified lower percentage of patients as having eGFR < 60 mL/min per 1.73 m^2 than the re-expressed MDRD-4 equation did. This means that the use of the new CKD-EPI formula results in lower prevalence of renal dysfunction. We also found that renal dysfunction assessed either by the re-expressed MDRD-4 or the new CKD-EPI was strongly associated with the composite endpoint of major bleeding, TE event and all-cause mortality, and with all-cause mortality, as well.

Patients with NVAF are often elderly with multiple comorbidities which require pharmacotherapy of growing complexity, and this makes the reliable estimation of renal function to be undeniably a critical issue. Moreover, the availability of the new oral anticoagulants have renewed the great interest toward the accurate evaluation of renal function in patients with NVAF[17,18].

Up to our knowledge, this is the first study comparing the prognostic performance of the re-expressed MDRD-4 and the new CKD-EPI formulas used for estimating GFR in a real world population of patients with NVAF on VKAs who have a full range of eGFR.

In this cohort, the new CKD-EPI formula classified lower percentage of patients as having eGFR < 60 mL/min per 1.73 m^2 (32.9% with new CKD-EPI *vs* 34.1% with re-expressed MDRD-4). This reasonable ability of the new CKD-EPI formula to reduce the rate of patients with renal dysfunction could be highly appreciated by the clinicians in daily clinical practice which usually needs close attention to the status of renal function to reach the optimal management, and more safe use of renally excreted medications and nephrotoxic contrast agents, in patients with NVAF. Our finding is consistent with that found in the derivation cohort of the new CKD-EPI[9] and to the findings obtained from multiple studies in different clinical settings[12,19-21].

In our analysis, renal dysfunction determined by GFR estimates using both formulas was a significant predictor of the composite endpoint and all-cause mortality. Similar findings have been shown in previous study used the MDRD-4[22], but until now, no study has compared the prognostic usefulness of these formulas in a real world patients with NVAF. In this study, we did not find any significant difference in the prognostic impact between the new CKD-EPI and the re-expressed MDRD-4 at predicting major adverse cardiovascular outcomes.

In our analysis, we found that both formulas with the eGFR as a continuous variable and after controlling for HAS-BLED risk score[4], showed a tendency to predict major bleeding. Previous association between renal dysfunction and major bleeding were found in AF studies[22,23]. However, the prior tendency was lost when the eGFR using both formulas was tested as categorical variables; this may be explained by the small number of events (30 events, 3.3%) that could limit the detection of significant relationship from the data.

TE prevention remains the primary cornerstone in the management of patients with NVAF. In dealing with this great aim, there are conflicting data about the ability of renal dysfunction to predict this major catastrophe. Several studies demonstrated significant association between reduced eGFR and TE event[22-24], conversely, in other studies, decreased eGFR did not show significant relationship with TE event[25,26]. These differences could be explained by the differences in the formula used to estimate GFR, sample size, patients

characteristics (*i.e.,* from a real world or clinical trial population), and/or the disparities in duration of follow up between the studies. Therefore, there is a strong need for further evaluation of that uncertainty in a real world population. Regarding this important issue, in our real world cohort of patients with NVAF, and after adjusting for the CHA_2DS_2-VASc risk score[15] there was a significant association between eGFR as categorical variable and TE event only when the eGFR was < 30 mL/min per 1.73 m^2 (*i.e.,* severe renal dysfunction category) with similar prognostic impact of both the re-expressed MDRD-4 and the new CKD-EPI. Furthermore, the NRI analysis showed a tendency of the new CKD-EPI to reclassify better the patients according to their risk of developing TE event, compared with the re-expressed MDRD-4.

It should be kept in mind that the eGFR formulas were designed to most accurately estimate renal function and not to predict major adverse outcomes. Indeed, the relative performance of the two different GFR estimating equations in our study can be explained by their respective compositions (*i.e.,* the difference of mathematical modeling and how specific variables are coded and weighted by each equation). Also, the relative variance in performance between both formulas can be explained by the differences in their respective derivation populations. The MDRD-4 formula was originally developed in patients with established renal dysfunction[6]; for this, the re-expressed MDRD-4 formula may be less applicable to patients from the real world with full range of GFR. In contrast, the new CKD-EPI equation could be more precise in our community-based cohort of patients with NVAF, as the new CKD-EPI was developed in population with and without renal dysfunction[9].

Although, many laboratories are preparing their installation to use the new CKD-EPI equation instead of the re-expressed MDRD-4 formula according to the current guideline[27] and a consensus document[28], however, old habits die hard. Our assessment of the prognostic performance of both formulas in the particular clinical context of AF might be of great importance as it could help convince the clinicians and mitigate the doubts and obstacles regarding the adoption of the new CKD-EPI.

Really, patients with NVAF and renal dysfunction continue to represent a complex management problem in relation to decision making for thromboprophylaxis. With respect to the overall concept, the data obtained from our analysis, state that the new CKD-EPI formula reduced the prevalence of patients with renal dysfunction (*i.e.,* eGFR < 60 mL/min per 1.73 m^2), and at the same time continued to have prognostic impact similar to that of the re-expressed MDRD-4 equation at predicting the major adverse events. Taken together, our notable results from a real world cohort encourage the use of the new CKD-EPI equation to assess renal function in patients with NVAF and reinforce the current recommendation[9,27,28] for the use of the new CKD-EPI formula in all clinical situations.

It is clear that our study presents an analysis of a modest sized cohort of patients with NVAF on VKAs from the real world, and the prevalence of patients with eGFR < 60 ml/min/1.73 m^2 was just reduced by 1.2% when using the new CKD-EPI formula. However, our cohort might give a good reflection of the general population with millions of patients having NVAF, in whom the percentage of 1.2% would be highly significant.

Limitations

The main limitation of our study is its retrospective design, but it has interesting strong points as it reflects real world practice by enrolment of consecutive patients with NVAF who have full range of eGFR and were attending our outpatient cardiology clinics with the advantage of careful follow up and data collection by cardiologists.

The sample size might be another limitation of our study that could limit the likelihood of detecting small effects or significant relationships from the data. Important to mention here that we did not have the direct measured GFR, so we cannot determine the extent to which the two formulas reflect the GFR as determined by the gold standard method. However, eGFR is the practical way to estimate renal function which has been used in several patient populations. The fact that we have only one serum creatinine measure for every patient could limit the verification of the acute *vs* chronic nature of the renal dysfunction in some patients, but this limitation was present in several related studies[23-25]. The lack of cystatin C data might be considered a limitation of our study. However, it should be taken into account that all the creatinine measurements in our study cohort were performed with the IDMS-traceable enzymatic assay method, which has been shown to provide very reliable eGFR results[13] and is considered the standard method to assess renal function[29].

Finally, all of the enrolled patients in our cohort have Caucasian race, so the applicability of our findings in other populations with different races should be addressed in other studies.

The new CKD-EPI reduced the prevalence of patients with renal dysfunction, in a real world cohort of patients with NVAF on VKAs. Renal dysfunction reflected by GFR estimates from the re-expressed MDRD-4 or the new CKD-EPI was an independent predictor of the composite endpoint and all-cause mortality. Both formulas had similar prognostic impacts regarding the prediction of composite endpoint, major bleeding, TE events and all-cause mortality. Our analysis indicates that the more widespread adoption of the new CKD-EPI instead of the re-expressed MDRD-4 may improve the management of patients with NVAF.

COMMENTS

Background

Renal dysfunction is a frequent comorbidity seen in patients with atrial fibrillation. Moreover, renal dysfunction is a strong predictor of thromboembolic event and also of bleeding event (when the patients are anticoagulated). This reflects the need for more accurate estimate of renal function to guarantee the optimal management of patients with atrial fibrillation. The standard way to assess renal function is the glomerular filtration rate. Among the available equations to estimate the glomerular filtration rate are: the re-expressed Modification of Diet in Renal Disease equation which is still the commonly used equation by many laboratories all over the world and the new Chronic Kidney Disease Epidemiology Collaboration equation which has been recently proposed to be used instead of previous equation in daily practice as the new equation has an assumed ability to reduce the prevalence of patients with renal dysfunction and better reclassification of patients. There is limited information about the performance of both equations in patients with atrial fibrillation.

Research frontiers

The authors think that the new Chronic Kidney Disease Epidemiology Collaboration equation to estimate glomerular filtration rate must have a wide diffusion as an alternative to the re-expressed Modification of Diet in Renal Disease equation. In this paper the authors provide support to the hypothesis, reporting the superiority of the new Chronic Kidney Disease Epidemiology Collaboration equation over the re-expressed Modification of Diet in Renal Disease equation in the clinical context of patients with atrial fibrillation on anticoagulation.

Innovations and breakthroughs

The results derived from our analysis, state that the new Chronic Kidney Disease Epidemiology Collaboration equation reduced the prevalence of patients with renal dysfunction (*i.e.,* estimated glomerular filtration rate < 60 ml/min per 1.73 m^2), and at the same time continued to have the prognostic impact similar to the re-expressed Modification of Diet in Renal Disease equation at predicting the major adverse events. Although there are still some concerns about the performance of the new equation in subgroups of elderly and obese patients, the study from a real world cohort encourages the cardiologists to use of the new Chronic Kidney Disease Epidemiology Collaboration equation to assess renal function in patients with atrial fibrillation and increase the confidence to use it in all clinical situations.

Applications

The millions of patients with atrial fibrillation will get benefit and better management if there is wide spread adoption of the new Chronic Kidney Disease Epidemiology Collaboration equation instead of the re-expressed Modification of Diet in Renal Disease equation, giving the ability of the new equation to correctly reclassify patients in comparison with the re-expressed equation.

Terminology

The Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) was published in May 2009 as a reliable tool to estimate glomerular filtration rate. It was developed in an effort to create an equation more accurate than the re-expressed Modification of Diet in Renal Disease equation. Researchers pooled data from multiple studies to develop and validate this new equation. They used 10 studies that included 8254 participants, randomly using 2/3 of the data sets for development and the other 1/3 for internal validation. Sixteen additional studies, which included 3896 participants, were used for external validation. The CKD-EPI equation performed better than the Modification of Diet in Renal Disease equation, as the prevalence of chronic kidney disease was 11.5% *vs* 13.1% according to the National Health and Nutrition Examination Survey data in the United States of America.

Peer-review

First of all I would like to congratulate the authors with their achievement. In this retrospective study including relatively limited sample size of Caucasian subjects, the findings encourage the use and application of the new CKD-EPI equation for assessment not only of renal function in patients with non-valvular atrial fibrillation but also in all clinical situations. For the first time, Abumuaileq RRY *et al* evaluated the re- expressed MDRD-4 and the new CKD-EPI formulas at predicting the occurrence of major adverse outcomes in a real world cohort of patients with non-valvular atrial fibrillation on anticoagulation. The study was well conducted and clinically relevant.

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CASE REPORT

Percutaneous pulmonary valve implantation in a single artery branch: A preliminary experience

Massimo Chessa, Gianfranco Butera, Luca Giugno, Angelo Micheletti, Diana G Negura, Mario Carminati

Massimo Chessa, Gianfranco Butera, Luca Giugno, Angelo Micheletti, Diana G Negura, Mario Carminati, Pediatric and Adult Congenital Heart Centre, IRCCS-Policlinico San Donato-University Hospital, San Donato Milanese, 20097 Milan, Italy

Author contributions: Chessa M, Butera G and Carminati M designed research; Giugno L, Micheletti A and Negura DG performed research; Giugno L, Micheletti A and Negura DG analyzed data; Chessa M wrote the paper.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment, according with the National Low.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Correspondence to: Dr. Massimo Chessa, MD, PhD, FSCAI, FESC, Pediatric and Adult Congenital Heart Centre, IRCCS-Policlinico San Donato-University Hospital, via Morandi 30, San Donato Milanese, 20097 Milan, Italy. massimo.chessa@grupposandonato.it
Telephone: +39-2-52774531
Fax: +39-2-52774459

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Abstract

To describe preliminary experience of percutaneous pulmonary valve implantation, in a single pulmonary branch position. Two procedures in 2 patients from a single center are described, where implantation of percutaneous valves within a single pulmonary artery branch was technically successful. The procedural indication was pulmonary valve regurgitation and/or residual stenosis. The 2 patients were symptomatic. An Edwards Sapien™ valve (Patient 1), and a Medtronic Melody™ valve (Patient 2) were implanted. Both pts were discharged with an excellent valve function. In this report it is underlined that this modality is technically feasible and may be considered an option in patients with congenital heart defect under special circumstances.

Key words: Congenital heart disease; Tetralogy of fallot; Pulmonary atresia; Percutaneous pulmonary valve; Grown-ups with congenital heart diseases

Core tip: Today MelodyVR valve (Medtronic, Minneapolis, MN) and the SAPIENTM transcatheter heart valve valve (Edwards Lifesciences LLC, Irvine, CA) are available to use in patients with a conduit connecting the right ventricle to the main pulmonary arteries (PA). However, given the anatomic variability of the right ventricular outflow tract and the concomitant occurrence of branch PA disease frequently encountered in this patient population, alternative approaches to valve replacement needs to be explored. In order to solve this problem we use a different approach implementing percutaneous pulmonary valve implantation, in a single pulmonary branch position.

Chessa M, Butera G, Giugno L, Micheletti A, Negura DG, Carminati M. Percutaneous pulmonary valve implantation in a single artery branch: A preliminary experience. *World J Cardiol* 2015; 7(10): 695-699 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i10/695.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i10.695

INTRODUCTION

The first successful percutaneous pulmonary valve implantation (PPVI) was described by Bonhoeffer *et al*[1], Currently two balloon expandable transcatheter valves are available for PPVI: the MelodyVR valve (Medtronic, Minneapolis, MN) and the SAPIENTM transcatheter heart valve (Edwards Lifesciences LLC, Irvine, CA).

Both valves are indicated in patients with a conduit connecting the right ventricle to the main pulmonary arteries (PA). However, given the anatomic variability of the right ventricular outflow tract (RVOT) and the concomitant occurrence of branch PA disease frequently encountered in this patient population, alternative approaches to valve replacement have been explored[2-4]. We report our preliminary experience of PPVI in a single pulmonary branch.

CASE REPORT

Two patients (Table 1) underwent PPVI in a single branch between 2013 and 2014.

Patient 1 had a complete repair of tetralogy of Fallot in 2001, followed by a re-operation in August 2003 for residual ventricular septal defect (VSD) + right ventricular outflow tract obstruction (RVOTO). During the second intervention a trans-annular patch + VSD closure + Tricuspid Valve plasty and patch enlargement of a hypoplastic left pulmonary artery (LPA) were performed. In 2006, the patient underwent a cardiac catheterization that showed absence of the LPA, confirmed in 2013 with a cardiac magnetic resonance (CMR) patient 2 had a left modified Blalock-Taussig Shunt, and then at age 1 year, underwent a complete correction of her PA-VSD + MAPCAs unifocalization and FreeStyle 14 mm RV-PA conduit implantation. The conduit was changed when she was 5-year-old using a 23 mm FreeStyle conduit associated with PA bifurcation plasty.

A CMR showed a dilated RV (EDVI = 157 mL/m^2) with severe pulmonary valve regurgitation (PVR) (RF = 43%), moderate RVOTO (peak gradient 40 mmHg), preserved RV systolic function, and absence of the right pulmonary artery (RPA) branch.

Informed consent was obtained for both two patients. Femoral venous and arterial accesses were obtained under general anesthesia. A complete left and right heart catheterization was done in both pts.

The RV angiography in patient 1 showed a dilated RVOT with a diameter of 32 mm (Figure 1A-C), too large for classical PPVI. At the origin of the RPA, there was the evidence of a waist (Figure 1D) with a diameter of 24 mm; a prestenting of the PA branch was made with a 43 mm ANDRA XXL stent mounted on 25 mm × 50 mm Crystal Balt balloon (Figure 1E).

The RV angiography in patient 2 showed a dilated, calcified, and moderate stenotic conduit (Figure 2A and B). A prestenting of the conduit was made using 2 premounted covered CP stents (45 mm length, 8 zigs and dilated with a BiB balloon 22 mm × 55 mm) (Figure 2C-E). The final stented conduit had a proximal angle which was considered not perfectly suitable (Figure 2F) for a traditional implant of the percutaneous valve. The valve was implanted at the origin of the LPA were the landing zone was better.

After a standard valve preparation including a thorough washing protocol and crimping protocol, a 26 mm Edwards Sapien valve in patient 1, and a 22 mm MelodyVR valve in patient 2 were deployed.

There were no immediate procedure-related complications. The trans-thoracic echo (TTE) performed the day after showed excellent valve function with no regurgitation and no stenosis. Therefore the patient 2 complained, 48 h later, about an abrupt onset of chest pain, shortness of breath, and hypoxia. She immediately underwent a computed tomography angiograpy (CTA) and the final diagnosis was micro pulmonary embolismi with evidence of filling defects in the distal pulmonary branches in the latero-basal segment of the lower lobe + in the inferior segment of the lingula.

She was immediately transferred to the ICU and she was started on Eparine.

The patient recovered immediately, the saturation became normal and she was discharged at home 10 d after.

At the last follow-up (range 6-12 mo) both pts were asymptomatic, with neither pulmonary valve regurgitation nor residual stenosis.

Patient 1 repeated the CMR 1 year after and the EDVI was 123 mL/m^2.

DISCUSSION

Robb *et al*[5], reported an animal study, with a Melody valve implantation in the pulmonary artery branches. The Authors demonstrated that RVOT dilation and distortion consistent with transanular patch repair of ToF could be reliably mimicked with an animal model. Secondly, it was shown that PPVI into the right and left branch PAs resulted in a significant reduction in pulmonary regurgitation, with preserved biventricular function demonstrated by MRI and catheterization.

Qureshi *et al*[6], reported a case of a transcatheter placement of the Melody valve in the proximal left pulmonary artery of a patient with acquired right pulmonary artery occlusion.

In our patients the valve was inserted in the only pulmonary artery branch available either because the RVOT was judged to be too large to allow a PPVI in a regular position, or the previous implanted conduit had a better angle (after prestenting) in the distal part, in the area in which there was a conjunction with the left pulmonary artery.

The complication experienced by Patient 2 could have occurred to the fact that some microemboli originate in the lower part of the conduit just under the pulmonary valve. Since this zone in which there is a

Table 1 Patients' characteristics

Patient	DoB	Weight (kg)	Diagnosis	Surgery	L/R PAB	PVR (yes/no)	RVOTO (yes/no)	Previous Caths (year)	CMR/ CT scan	Valve	Prestening	Compli-cations	Follow-up
1st (male)	June-2000	43	ToF	TAP	RPAB	Yes	No	2006	CMR	Sapien 26	Yes	No	12-mo
2nd (female)	July-1994	56	PA-VSD-MAPCAs	RV-PA conduit FreeStyle 14 mm and 23 mm (1999)	LPAB	Yes	Yes (moderate)	2012	CMR	Melody 22	Yes	Yes	6-mo

PA-VSD-MAPCAs: Pulmonary arteries-ventricular septal defect-major aortopulmonary collateral arteries; TAP: Trans-annular patch; RVOTO: Right ventricular outflow tract obstruction; CMR: Cardiac magnetic resonance; PVR: Pulmonary valve regurgitation.

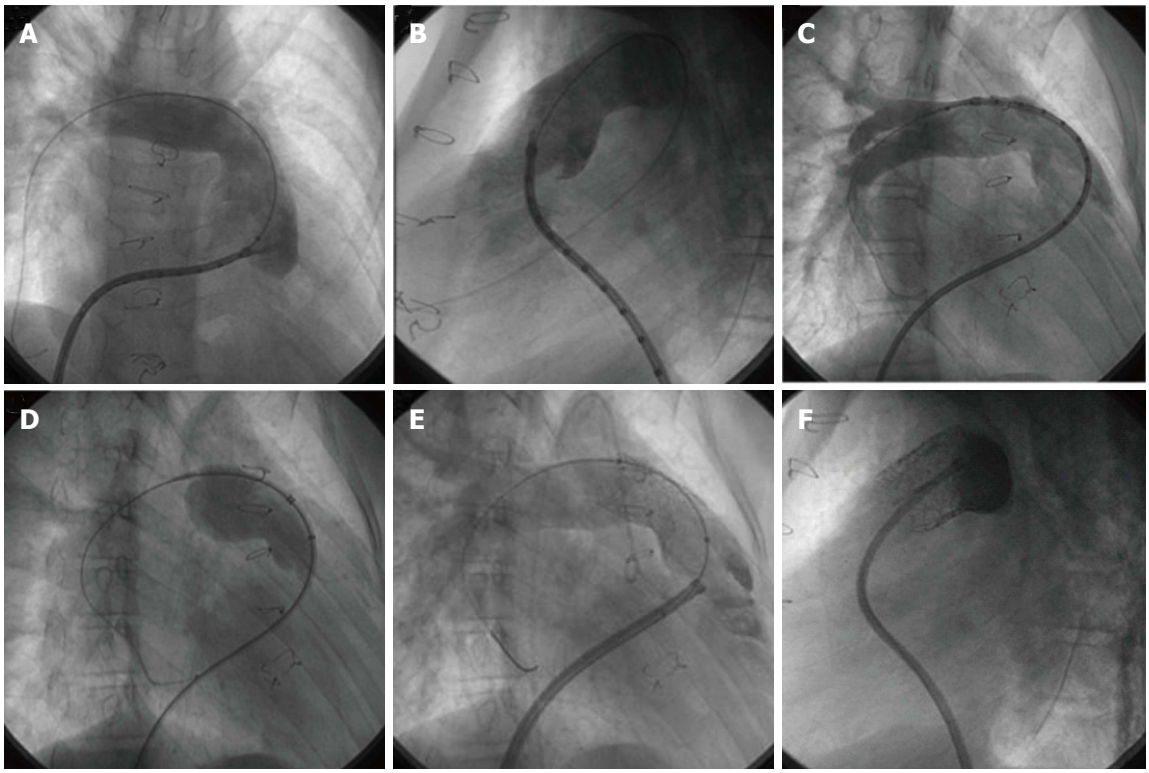

Figure 1 Angiograms in anterior-posterior, LL, right anterior oblique projections of the percutaneous pulmonary valve implantation using a Melody valve. A-C: Injections in the RV and origin of the RPA showing a dilated RVOT and stenosis at the origin of the RPA; D: Evidence at the balloon sizing of a waist at the origin of the RPA; E: Pre-stenting of the PA branch with a 43 mm ANDRA XXL; F: Good final result after Valve implantation with no evidence of PVR. PPVI: Percutaneous pulmonary valve implantation; RVOT: Right ventricular outflow tract; PA: Pulmonary arteries; RPA: Right pulmonary artery; AP: Anterior-posterior.

flow is not contractile, it could have favored a slowing down of the flow, creating a favorable condition for the formation of microemboli. It is difficult to support the idea that these emboli were related to the large sheaths used, because the episode was 48 h later. The pt was anticoagulated with a Heparine bolus of 100 IU/kg and the ACT was > 200 during all the procedure-time: She was just on antiplatelet therapy (ASA: 100 mg as in our protocol), after the procedure. Anticoagulation prophylaxis *vs* standard platelet antiaggregation should be take into consideration for these specific pts.

This brief report shows that PPVI can be performed in Pts with a congenital heart defect, and with a single pulmonary artery branch.

The use of a percutaneous valve in a branch pulmonary artery is not to be proposed for all the pts with a large or with a complex RVOT anatomy; what can be underlined with this report is that this modality is technically feasible and it may be considered as an option in high-risk patients under special circumstances.

COMMENTS

Case characteristics

Patient 1: Dyspnea and low stress tolerance; and Patient 2: Chest pain, shortness of breath, and hypoxia.

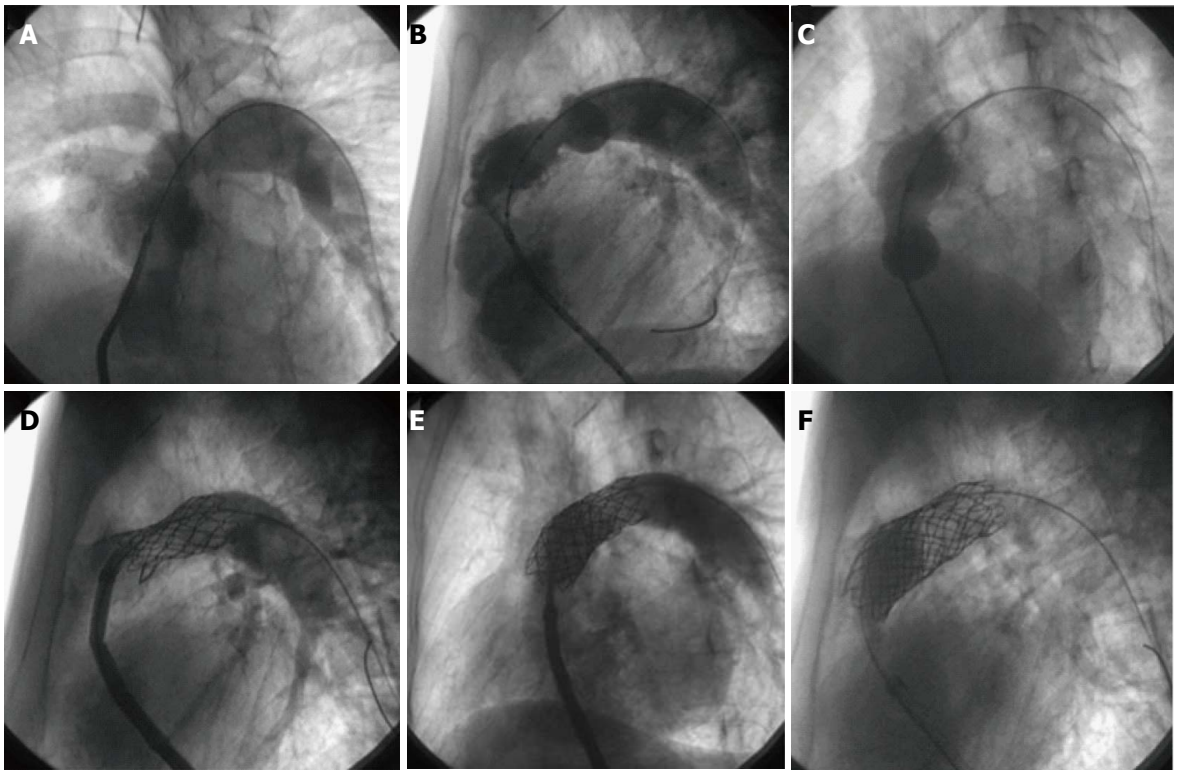

Figure 2 Angiograms in anterior-posterior, LL, projections of the percutaneous pulmonary valve implantation using a Sapien valve. A, B: Injections in the RV showing a dilated and calcified conduit with some degree of stenosis at the PA edge; C: A compliant AGA 34 mm ASD sizing balloon was inserted into the Freestyle conduit showing the stenotic area; D: Prestenting of the conduit using a premounted covered CP stent 45 mm; E: Second covered CP stent was implantation; F: After a re-dilation by utilizing an ATLAS 22 × 40 balloon dilatated at 14 Atm a final stented conduit was obtained with a proximal angle nor suitable for a PPVI in a regular position. PPVI: Percutaneous pulmonary valve implantation; RV: Right ventricular; PA: Pulmonary arteries.

Clinical diagnosis

Patient 1: Hypoplasia of left pulmonary branch; and Patient 2: Occlusion of right pulmonary branch.

Laboratory diagnosis

Patient 1: WBC: 7650/mm^3 (N: 66%; L: 18%; M: 10%). Hb: 13.3 g/dL, Hct: 38.4%; PLT 216000/mm^3; PCR: 2.8 mg/dL; PCT: 0.05 ng/mL; Patient 2: Hb: 10.2 g/dL, Hct: 31.1%; RBC: 3.78 × 10^6 U/L; INR: 2.12.

Imaging diagnosis

Patient 1: Cardiac magnetic resonance (MR), chest X-ray, echocardiography; Patient 2: Cardiac TC multi-slide, cardiac MR, chest X-ray, echocardiography.

Pathological diagnosis

Patient 1: The right ventricular angiography showed a dilated right ventricular outflow tract with a diameter of 32 mm and at the origin of right pulmonary artery there was an evidence of a waist with a diameter of 24 mm; Patient 2: A cardiac RM show a dilated right ventricle (EDVI = 157 mL/m^2) with severe pulmonary valve regurgitation (PVR) (RF = 43%), moderate right ventricular outflow tract obstruction (RVOTO) (peak gradient: 40 mmHg), preserved RVC systolic function.

Treatment

Patient 1: 26 mm Edward Sapien Valve implantation in left pulmonary branch; Patient 2: 22 mm MelodyVR valve implantation.

Related reports

Patient 1: ToF s/p complete surgical repair and following surgical treatment of residual Ventricular septal defect + RVOTO with trans annular patch (TAP) + VSD closure + Tricuspid valve plasty and patch enlargement of hypoplastic left pulmonary artery (LPA). In 2006 a cardiac catheterization showed the absence of LPA, confirmed in 2013 with a cardiac MR that show hypoplasia of left pulmonary branch; Patient 2: pulmonary atresia + VSD and MAPCAs s/p a left modified Blalock-Taussing shunt (MBTS) and following complete correction with unifocalization and 14 mm RV-PA conduit implantation that five years later was change with a 23 mm FreeStyle conduit + PA bifurcation plasty.

Term explanation

PPVI is the Percutaneous Pulmonary Valve Implantation that can be done in case of congenital heart disease in a GUCH population (Grown Ups with Congenital Heart Diseases) with RVOTO and/or PVR.

Experiences and lessons

Valve implantation in pulmonary branches could be a useful approach in high-risk patients under special circumstances.

Peer-review

This article provided the innovative approach to a particular kind of diseases in critical patients and gave the good results at the last follow up.

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CASE REPORT

Intra-His bundle block in 2:1 atrioventricular block

Seung Pyo Hong, Yon Woong Park, Young Soo Lee

Seung Pyo Hong, Yon Woong Park, Young Soo Lee, Division of Cardiology, Department of Internal Medicine, Daegu Catholic University Medical Center, Daegu 705-718, South Korea

Author contributions: Hong SP, Park YW and Lee YS designed and wrote the report; Lee YS performed the electrophysiological study and pacemaker implantation.

Institutional review board statement: This is a clinical case report. The patient related identification information has been avoided according to the policy of Daegu Catholic University Medical Center Institutional Review Board.

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Correspondence to: Young Soo Lee, MD, Division of Cardiology, Department of Internal Medicine, Daegu Catholic University Medical Center, 33 Duryugongwon-ro 17-gil, Nam-gu, Daegu 705-718, South Korea. mdleeys@cu.ac.kr
Telephone: +82-53-6503441
Fax: +82-53-6213166

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Abstract

Intra-hisian atrioventricular (AV) block is not a common phenomenon, but it is important for the development of advanced or complete AV block. We observed a 77-year-old female patient with the 2:1 AV block due to an intra-hisian block. In this case we tried to detect the block site, but an alternating pattern of the AH conduction was noted on the His-electrogram in the electrophysiological study (EPS). The cause of the confusing finding might have been the instability of the catheter to record a His potential. We could detect a splitting of the His-electrogram with an intra-hisian block after minimal manipulation of the catheter. The authors' observations suggest that catheter stability is important for a precise recording in the EPS and radiofrequency catheter ablation procedure.

Key words: Cardiac electrophysiologic techniques; Bundle of His; Atrioventricular block

Core tip: Intra-hisian atrioventricular (AV) block associated with 2:1 AV block is an uncommon phenomenon, but it is important for the development of complete AV block. We observed a 77-year-old female with 2:1 AV block due to an intra-hisian block. An alternating pattern of the AH conduction was noted on the His-electrogram. The cause of that confusing finding might have been the instability of the catheter for recording the His potential. We could detect a splitting of the His-electrogram with intra-hisian block after minimal manipulation of the catheter. The authors' observations suggest that catheter stability is important for a precise recording.

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INTRODUCTION

Catheter stability is very important for achieving a precise

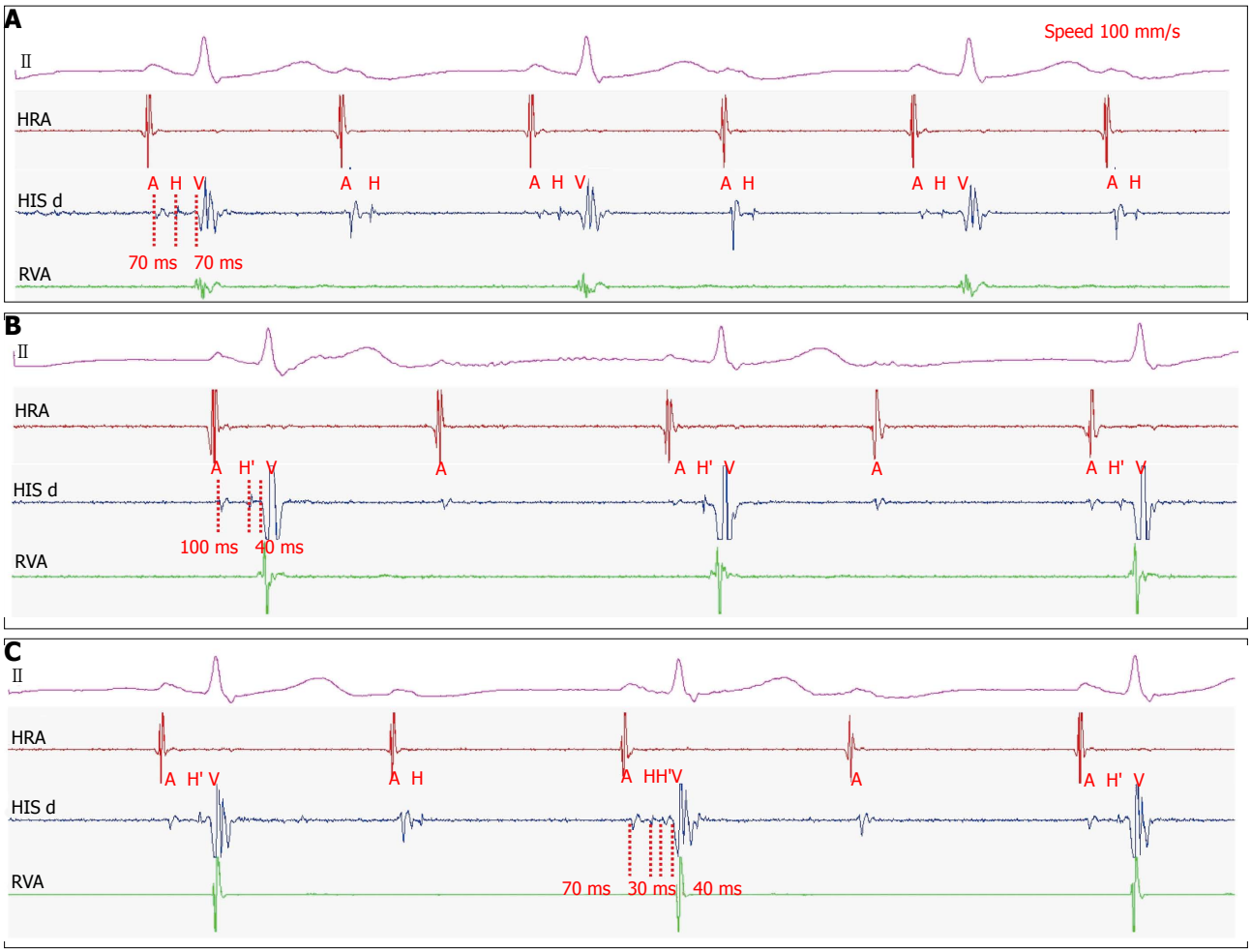

Figure 1 Surface electrocardiograms with intra-cardiac electrocardiograms in sweep speed of 100 mm/s. Surface ECG shows 2:1 atrioventricular block with a right bundle branch block. A: Intra-cardiac ECG was located the right ventricular apex (RVA), His bundle (HIS), and right ventricular apex (RVA). Surface ECG revealed 2:1 atrio-ventricular block. Intra-cardiac ECG revealed infra-hisian block and short A-H interval (70 ms); B: Intra-cardiac ECG revealed supra-hisian block and long A-H interval (100 ms); C: The intracardiac ECG shows the splitting of the His potential (H-H') during AV conduction and only the proximal activation of His potential during the AV block, which means an intra-hisian block. A: Potential of right atrium; H: Potential of proximal His bundle; H': Potential of distal His bundle; V: Potential of right ventricle; ECG: Electrocardiogram.

electrophysiological study (EPS); 2:1 atrioventricular (AV) block is usually a disease of various levels such as supra-, intra- and infra-hisian[1,2]. We report a case with some confusion caused by the instability of the catheter used for the His-electrogram recording during 2:1 AV block due to an intra-hisian block.

CASE REPORT

A 77-year-old female complained of dizziness. Her past history had hypertension, and had been taking dihydrophyridine calcium channel blockers for 10 years. The chest radiograph showed cardiomegaly. The echo-cardiography revealed a normal function and wall motion. A resting electrocardiogram (ECG) revealed 2:1 AV block and right bundle branch block. After an intravenous injection of atropine and an exercise treadmill test, the ECG exhibited persistent 2:1 AV block.

The patient underwent an EPS to determine the position of the conduction block. EPS catheters located in right ventricle, His bundle and right atrium. Firstly, we found an infra-hisian block with a normal A-H interval (70 ms) and long H-V interval (70 ms) on the intracardiac ECG (Figure 1A), and then a supra-hisian block with longer A-H interval (100 ms) and shorter H-V interval (40 ms) (Figure 1B). After a while, another intracardiac ECG revealed a splitting of the His potential (H-H') in the A-V conduction and only the proximal activation of the His potential during A-V block, which meant an intra-His bundle block (Figure 1C).

The patient was performed the implantation of permanent pacemaker as a result of the intra-His bundle block. The patient has no other symptoms until now.

DISCUSSION

In our case, we tried to detect the block site but an alternating pattern of the AH conduction was noted in the His-electrogram during the EPS. The cause of

this confusing finding might have been due to the instability of the catheter to record a His potential. However, the His potential was distinct in the first recoding of the His bundle (Figure 1A). In addition, the His potential spontaneously moved and the AH interval prolonged during the AV conduction, which concluded that the catheter was not stable.

A consistent intracardiac electrogram reflects a stable catheter position. Among the several catheters used during the EPS, the catheter used for the His-electrogram recoding is the most unstable in a beating heart. Catheter stability is important for obtaining a precise recording during the EPS and radiofrequency catheter ablation procedure. Instability of catheters can contribute to a misdiagnosis and procedural complications especially with AV nodal reentrant tachycardia. Recently, a remote magnetic navigation system is able to provide better catheter stability during the radiofrequency catheter ablation procedure[3]. However, that system cannot be used during diagnostic procedures such as in our study because of the high cost. Fortunately, we could detect the splitting of the His-electrogram with an intra-hisian block after a minimal manipulation of the catheter.

Intra-hisian AV block during 2:1 AV block is not a common phenomenon, but it tends to develop into advanced or complete AV block[4]. We should try to obtain a precise His electrogram and find the exact block site when conducting an EPS for 2nd degree high grade AV block.

COMMENTS

Case characteristics

A 77-year-old female complained of dizziness.

Clinical diagnosis

Intra-hisian atrioventricular (AV) block detected a splitting of the His-electrogram after a correction of the catheter instability during the electrophysiological study (EPS).

Differential diagnosis

Second-degree Mobitz 1 block with 2:1 AV is easily misdiagnosed as general two to one conductive rate regardless of block site.

Laboratory diagnosis

The laboratory test results were unremarkable.

Imaging diagnosis

The intracardiac electrocardiogram revealed splitting of the His potential of the AV conduction during the EPS.

Treatment

A permanent pacemaker was implanted as a result of intra-hisian AV block.

Related reports

To best of our knowledge, intra-hisian AV block is an uncommon phenomenon.

Term explanation

Intra-hisian AV block in His-electrogram exhibited a splitting of the His potential during the AV conduction and only the proximal activation potential of the His bundle during the AV block, which meant an intra-His bundle block.

Experiences and lessons

The authors should try to achieve a precise recording of the His electrogram and find the exact block site when conducting an EPS for 2nd degree high grade AV block.

Peer-review

It is an interesting case report.

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CASE REPORT

Late endocarditis of Amplatzer atrial septal occluder device in a child

Neerod K Jha, Laszlo Kiraly, John SK Murala, Csaba Tamas, Haitham Talo, Hazem El Badaoui, Magdi Tofeig, Malaika Mendonca, Sameer Sajwani, Mary A Thomas, Sura Ahmed Al Doory, Mohammad D Khan

Neerod K Jha, Laszlo Kiraly, John SK Murala, Csaba Tamas, Department of Pediatric Cardiac Surgery, Sheikh Khalifa Medical City (managed by Cleveland Clinic), Abu Dhabi, United Arab Emirates

Haitham Talo, Hazem El Badaoui, Magdi Tofeig, Mohammad D Khan, Department of Pediatric Cardiology, Sheikh Khalifa Medical City (managed by Cleveland Clinic), Abu Dhabi, United Arab Emirates

Malaika Mendonca, Department of Critical Care, Sheikh Khalifa Medical City (managed by Cleveland Clinic), Abu Dhabi, United Arab Emirates

Sameer Sajwani, Sura Ahmed Al Doory, Department of Cardiology, Latifa Hospital, Dubai, United Arab Emirates

Mary A Thomas, Department of Pathology, Sheikh Khalifa Medical City (managed by Cleveland Clinic), Abu Dhabi, United Arab Emirates

Author contributions: Jha NK, Kiraly L, Murala JSK, Tamas C, Talo H, El Badaoui H, Tofeig M and Khan MD designed the report and written the manuscript; Mendonca M, Sajwani S, Thomas MA and Al Doory SA analysed the data and revised it with special reference to the specialty involved; all authors were involved in the patient management.

Institutional review board statement: None.

Informed consent statement: The authors state that all involved persons (subjects or legally authorized representative) gave their informed consent (written or verbal) prior to study inclusion.

Conflict-of-interest statement: There is no conflict-of- interest in this review.

Correspondence to: Dr. Neerod K Jha, MD, Cardiac Surgeon, Department of Pediatric Cardiac Surgery, Sheikh Khalifa Medical City, District-Karamah, Karamah Street, P.O. Box 51900, Abu Dhabi, United Arab Emirates. njha@skmc.ae
Telephone: +971-50-6107532
Fax: +971-2-8193489

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Abstract

Bacterial endocarditis following atrial septal defect closure using Amplatzer device in a child is extremely rare. We report a 10-year-old girl who developed late bacterial endocarditis, 6 years after placement of an Amplatzer atrial septal occluder device. Successful explantation of the device and repair of the resultant septal defect was carried out using a homograft patch. The rare occurrence of this entity prompted us to highlight the importance of a closed long-term follow up, review the management and explore preventive strategies for similar patients who have multiple co-morbidities and a cardiac device. A high index of suspicion is warranted particularly in pediatric patients.

Key words: Endocarditis; Atrial; Septal; Device

Core tip: Bacterial endocarditis following atrial septal defect closure using Amplatzer device in a child is extremely rare.

We report a 10-year-old girl who developed late bacterial endocarditis, 6 years after placement of an Amplatzer atrial septal occluder device. This case report demonstrates the need for long-term follow up of patients with intracardiac device especially those with multiple co-morbidities or who have vulnerability to infection due to poor general condition or extreme of age such as our's. A high index of suspicion for device complication is required if there is a sepsis or embolic phenomenon found. Incomplete endothelization of the prosthetic devices may be linked to the endocarditis and needs to be explored.

Jha NK, Kiraly L, Murala JSK, Tamas C, Talo H, El Badaoui H, Tofeig M, Mendonca M, Sajwani S, Thomas MA, Al Doory SA, Khan MD. Late endocarditis of Amplatzer atrial septal occluder device in a child. *World J Cardiol* 2015; 7(10): 703-706 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i10/703.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i10.703

INTRODUCTION

Bacterial endocarditis of intracardiac devices including Amplatzer atrial septal occluder in pediatric population is extremely rare. In view of the limited experience, this is an opportunity to highlight such cases in order to review management and explore preventive strategies for a successful outcome in future. We, therefore, presenting herewith a child with multiple co-morbidities who developed late bacterial endocarditis of an Amplatzer device following closure of secundum atrial septal defect and underwent successful management.

CASE REPORT

A 10-year-old girl was referred to us with a high-grade intermittent fever of 2 wk duration being treated for septic shock and altered sensorium. She also had a pyopericardium which was drained. She required ventilatory and minimal inotropic support. She had generalised anasarca, bilateral mild pleural effusions and generalised muscle spasticity. Additionally, there was cellulitis on the chest wall requiring surgical debridement. Blood and wound cultures were positive for Streptococcus Pyogenes, sputum for *Pseudomonas* and urine for *Escherichia coli*. She was managed with appropriate antibiotics. Other medications included diuretics, anti-convulsive therapy and ACE-inhibitors. She was known to have global developmental delays associated with cerebral atrophy and epilepsy with clonic seizures. In the past, at the age of 4 years, she underwent device closure of atrial septal defect in a hospital abroad. However, the details of the procedure were not available.

Routine blood tests revealed elevated markers of infection in addition to evidence of hepato-renal dysfunction. Computerized tomography of the chest revealed minor effusions in the pleural cavities and an Amplatzer device in the atrium.

A two dimensional echocardiogram confirmed moderately depressed biventricular function, thickened pericardium and a prosthetic device in the inter-atrial septum with attached mobile vegetation (Figure 1). Other cardiac structures were normal.

It was suspected that the atrial septal occluder device was infected and possibly was a source of multiple systemic embolization and persistent bacteraemia. Therefore, we proceeded for surgical removal of the device.

Surgery was performed through the median sternotomy under systemic heparinization, standard cardiopulmonary bypass using aortic and bicaval cannulation, aortic cross-clamping and cardioplegic arrest at moderate systemic hypothermia *via* right atrial approach. The pericardium was thickened and adherent all around featuring constrictive pericarditis. The Amplatzer device's surface was partially covered with the soft tissue (endothelized) with patchy bare areas. However, there was no active vegetation found (Figure 2). Other cardiac structures including valves were grossly normal. After explantation of the device, a resultant atrial septal defect was repaired using a patch obtained from the pulmonary homograft.

The patient had a slow but steady recovery. The post-operative echocardiography showed improved cardiac function without residual defects. There was a constant decline in the levels of inflammatory markers. She recovered fully except that generalized spasticity is still persistent. The histopathological examination of the tissue attached to the device showed evidence of severe acute and chronic inflammation in the connective tissue (Figure 3). However, a stain for fungal organism and culture of the tissue within the device was negative. Further studies to investigate the tissue infection within the soft tissue such as biofilm study or electron microscopy was not available.

DISCUSSION

Transcatheter occlusion technique using Amplatzer device has become a preferred approach for atrial septal defects in selected patients. The common complications associated with occluding devices are mal-positioning or migration of device, thromboembolism, arrhythmias or endocarditis[1-5]. Bacterial endocarditis of an Amplatzer septal occluder device in the pediatric population is very rare. However, few reports have described early and late endocarditis associated with such device in adult population[2-4].

Early device infection could be due to inoculation of organisms during implantation. However, hematogenous infection is the primary source of late endocarditis. In our patient, the source of infection could have been cellulitis or respiratory infection. In addition, there was purulent pericarditis. This combination suggests a hematogenous spread of infection leading to prosthesis endocarditis. In the only published report in a 4-year-old

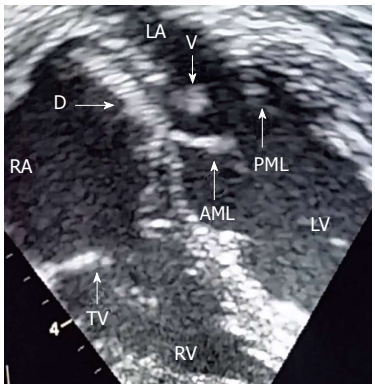

Figure 1 Two dimensional echocardiography image showing a large mobile vegetation (V) attached to the lower surface of an Amplatzer device (D) near the mitral valve. AML: Anterior mitral leaflet; PML: Posterior mitral leaflet; LV: Left ventricle; RV: Right ventricle; LA: Left atrium; TV: Tricuspid valve; RA: Right atrium.

Figure 2 Explanted Amplatzer device showing embedded soft tissue and bare metal surface.

child, authors have proposed incomplete endothelization of the device as a mechanism of late endocarditis[3]. Upon closer look of the explanted device, we also have noticed gross evidence of incomplete endothelization in the form of exposed metallic surface of the device in places without soft tissue coverage.

There are no established guidelines for the management of late endocarditis involving intra cardiac devices. We suggest that intensive management involving prolonged antibiotic therapy, monitoring of inflammatory markers and frequent blood cultures may be the first step. However, surgery is warranted if there is evidence of septal perforation, dehiscence, fistula formation, vegetation or embolization[6,7]. The relative indication may include persistent positive blood cultures in spite of maximal medical therapy[6,7].The homograft patch may be preferred choice for repair of resultant septal defect after explantation of the device in this situation presumably due to resistant nature of the homograft tissue against infection and better antibiotic penetration as compared to synthetic materials. Bovine pericardium may be an alternative.

In the clinical and experimental studies, it has been

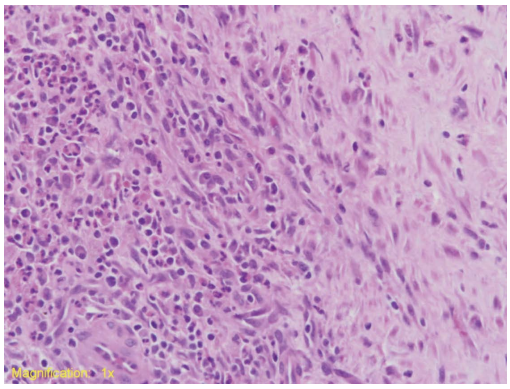

Figure 3 Microphotograph of the tissue within the explanted device showing connective tissue with dense, mixed, acute and chronic inflammatory infiltrates.

demonstrated that it takes 3-6 mo for complete neo-endothelization of the device[3]. Therefore, appropriate length of bacterial endocarditis prophylaxis for patients with atrial septal device closure was arbitrarily determined and usually extends from 6 mo to 1 year after implantation[2-4]. We hope that in future, additional investigations, imaging techniques or biochemical markers will allow identification of patients with incomplete endothelization who warrant long-term endocarditis prophylaxis.

This case report demonstrates the need for long-term follow up of patients with intracardiac device especially those with multiple co-morbidities or who have vulnerability to infection due to poor general condition or extreme of age. A high index of suspicion for device complication is required, if there is a sepsis or embolic phenomenon found. An intensive medical or surgical management and a prolonged follow up is warranted for a successful outcome.

COMMENTS

Case characteristics

A 10-year-old girl presented with late bacterial endocarditis of a cardiac Amplatzer device in addition to multiple co-morbidities and pancarditis.

Clinical diagnosis

Late bacterial endocarditis of cardiac prosthesis with cellulitis and pancarditis.

Differential diagnosis

Primary endocarditis of cardiac device, septicemia, pericarditis.

Laboratory diagnosis

Leucocytosis and mildly elevated hepato-renal function markers.

Imaging diagnosis

CT scan of the chest and an echocardiograhy showed evidence of a cardiac prosthesis (Amplatzer atrial septal occluder device) with a large vegetation.

Pathological diagnosis

The histopathology of the soft tissue attached to the explanted cardiac device showed the presence of acute and chronic inflammatory infiltrates. In addition, gross examination of the explanted Amplatzer device confirmed the presence of

bare metal exposed to the surface and the blood within the device.

Treatment
The patient was treated with intravenous antibiotics according to the culture and sensitivity reports of the blood, urine and pericardial fluids. Additionally, explantation of the atrial septal occluder device was done on cardiopulmonary bypass on urgent basis in order to remove the source of persistent bacteraemia and to avid thromboembolism.

Related reports
The biofilm and electron microscopic studies were not available which may have a precise-diagnostic value to prove the presence of specific infection within the device.

Term explanation
Cardiopulmonary bypass is a term used to commonly indicate open heart surgery using a cardiopulmonary bypass machine with an oxygenator.

Experience and lessons
This case report not only represents a very rare occurrence of the late endocarditis of the cardiac device in association with pancarditis and multiple co-morbidities in a child but also provides authors an opportunity to focus their attention on the mechanism and prevention of this pathology for a better outcome in future. They have substantiated the hypothesis of the role of exposed bare metal surface and deficient soft tissue coverage (incomplete endothelization) as a cause of prosthetic endocarditis. This fact not only guides us to explore the preventive measures while designing cardiac devices in future but also to be aware of a possibility of endocarditis in similar patients with multiple comorbidities and low resistance especially in pediatric population in order to have a cautious long-term follow-up and antibiotic prophylaxis.

Peer-review
The case report is very interesting and well described.

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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: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
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REVIEW

Translational research of adult stem cell therapy

Gen Suzuki

Gen Suzuki, Division of Cardiovascular Medicine, University at Buffalo, Clinical and Translational Research Center, Buffalo, NY 14203, United States

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Correspondence to: Gen Suzuki, MD, PhD, Associate Professor of Medicine, Division of Cardiovascular Medicine, University at Buffalo, Clinical and Translational Research Center, 875 Ellicott Street, Buffalo, NY 14203, United States. gsuzuki@buffalo.edu
Telephone: +1-716-8292710
Fax: +1-716-8292665

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Abstract

Congestive heart failure (CHF) secondary to chronic coronary artery disease is a major cause of morbidity and mortality world-wide. Its prevalence is increasing despite advances in medical and device therapies. Cell based therapies generating new cardiomyocytes and vessels have emerged as a promising treatment to reverse functional deterioration and prevent the progression to CHF. Functional efficacy of progenitor cells isolated from the bone marrow and the heart have been evaluated in preclinical large animal models. Furthermore, several clinical trials using autologous and allogeneic stem cells and progenitor cells have demonstrated their safety in humans yet their clinical relevance is inconclusive. This review will discuss the clinical therapeutic applications of three specific adult stem cells that have shown particularly promising regenerative effects in preclinical studies, bone marrow derived mesenchymal stem cell, heart derived cardiosphere-derived cell and cardiac stem cell. We will also discuss future therapeutic approaches.

Key words: Congestive heart failure; Adult stem cells; Mesenchymal stem cell; Cardiosphere-derived cell; Cardiac stem cell

Core tip: Cell-based therapy emerged as a new approach to restore damaged heart function. Although cell therapy in experimental animal models is promising, beneficial effects in clinical trials are variable. This review summarizes recent preclinical and clinical applications on three specific adult stem cells (bone marrow derived mesenchymal stem cell, heart derived cardiosphere-derived cells and cardiac stem cell) and discuss about future approaches.

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INTRODUCTION

The prevalence of congestive heart failure secondary to chronic coronary artery disease is increasing in spite of

Table 1 Clinical Trials of mesenchymal stem cells, cardiosphere-derived cells and cardiac stem cells in heart disease

Trial name	Study design	No. of patients	Delivery method	Cell dose	End point evaluation	Follow-up period	Outcome
MSCs							
Chen *et al*[10]	Randomized, controlled study	MSC n = 34 Control n = 35	Intracoronary	48-60 × 10^9 cells	Echocardiography	3 and 6 mo	LVEF↑
POSEIDON[28]	Randomized, Pilot study	MSC n = 30 Auto *vs* Allo	Intramyocardial (transendocardial)	20, 100, 200 × 10^6 cells	Cardiac CT	12 mo	LVEF↔ LVEDV↓
PROMETHEUS[29]	Randomized, Pilot study	MSC n = 6 No control	Intramyocardial (transepicardial)	20, 200 × 10^6 cells	MRI	18 mo	LVEF↑ Scar size↓
C-CURE[30]	Randomized, controlled study	MSC n = 21 Control n = 15	Intramyocardial (transendocardial)	7 × 10^6 cells	Echocardiography	6 and 24 mo	LVEF↑ LVESV↓
CDCs							
CADUCEUS[36,37]	Randomized, controlled study	CDC n = 17 Control n = 8	Intracoronary	12.5-25 × 10^6 cells	MRI	6 and 12 mo	LVEF↔ Scar size↓
ALCADIA	Pilot study	CDC n = 6 No control	Intracoronary	25-30 × 10^6 cells	MRI	12 mo	LVEF↑ Scar size↓
TICAP[38]	Randomized, controlled study	CDC n = 7 Control n = 7	Intracoronary	2-3 × 10^6 cells	MRI	18 mo	LVEF↑
CSCs							
SCIPIO[50]	Randomized, controlled study	CSC n = 20 Control n = 13	Intracoronary	1 × 10^6 cells	Echocardiography MRI	12 mo	LVEF↑ Scar size↓

Auto: Autologous; Allo: Allogeneic; MSCs: Mesenchymal stem cells; CSCs: Cardiac stem cells; CDCs: Cardiosphere-derived cells; CT: Computed tomography; MRI: Magnetic resonance imaging.

recent advances in medical and device therapies that delay the progression of disease[1]. Currently available medical interventions attenuate neurohormonal activation (*e.g.*, renin-angiotensin-aldosterone system, sympathetic nervous system, and arginine vasopressin), reducing myocyte apoptotic cell death, reducing interstitial connective tissue proliferation and attenuating the progression of myocyte cellular hypertrophy. However, none of the current therapies are effective in reversing myocyte loss and cellular abnormalities associated with myocyte contractile performance which are impaired in the failing heart. Recent investigations have demonstrated that there is an endogenous cardiac repair system that arises from resident cardiac stem cells regulating cardiac engraftment by maintaining a low level of myocyte proliferation, regeneration and cell death[2]. Nevertheless, the regenerative capacity of this endogenous stem cell pool is limited.

Expansion of adult stem cells *ex vivo* can stimulate the heart to induce endogenous or exogenous cell based repair. Cell-based therapy has emerged as a promising therapy to regenerate the failing heart through its potential to repair dead myocardium and improve left ventricle (LV) function[3-5]. Although clinical trials have demonstrated the safety and feasibility of using bone marrow-derived stem cells [Bone marrow mononuclear cells (MNCs) or mesenchymal stem cells (MSCs)] or heart-derived stem cells [cardiac stem cells (CSCs) or cardiosphere-derived cells (CDCs)] in humans with MI who do not have severe heart failure, the long term clinical efficacy of this approach is variable with a small improvement in LV function[6-8]. Although the biological action of adult stem cells *in vivo* is still controversial, for now, the beneficial effects of adult stem cells are considered to be associated with the secretion of paracrine factors rather than direct differentiation of *de novo* cardiac cells[9]. Accordingly, stem cells secrete multiple growth factors and cytokines which reduce scar volume and myocyte apoptosis, increase myocyte proliferation and activate endogenous CSCs to produce new myocytes. Therefore, current research using adult stem cells has focused on optimizing cell based therapy that effectively improves LV function and decreases disease progression. This would have a major impact on the survival and quality of life in patients with ischemic heart disease as well as reduce healthcare expenditures related to recurrent hospitalizations from advanced disease. In this review we will discuss three types of adult stem cells, MSCs, CDCs and CSCs, which are involved in the early phase of clinical trials (Table 1) and address current problems and future directions (Table 2).

MSCS IN ISCHEMIC CARDIOMYOPATHY

MSCs arise from a small proportion of bone marrow mononuclear cells (0.001%-0.01% of nucleated cells in the bone marrow). Although it has been reported that MSCs can be differentiated into cardiomyocytes and vascular-like structures[10-14], actual *in vivo* differentiation is infrequent. Moreover, current approaches using direct myocardial injection or intracoronary infusion of cells in the infarcted region result in a low myocardial retention of stem cells[15]. Thus, most of the beneficial effects derived from MSCs are considered to be related to a paracrine mechanism. MSCs produce a wide variety of cytokines, chemokines and growth factors, and many are involved in restoring cardiac function or regenerating myocardial tissue. Factors such as basic fibroblast

Table 2 Alternative strategies of stem cell therapy

Enhancement of cell survival, mobilization and paracrine secretion
Pharmacology (Statins, *etc.*)
Genetic modification (Akt and Ang1, VEGF and SDF-1, HO-1, bFGF/IGF-1/BMP2)
Preconditioning (Hypoxia, TLR3 stimulation)
Combination of different cell types or delivery approaches
MSCs and CSCs
Stop-flow (infarct area) and global intracoronary infusion (viable area)
Others
Cell infusion immediate after revascularization (allogeneic MSCs, CDCs, *etc.*)
Repeated cell infusion
Stimulation of exosome release
Direct exosome (or microRNAs) injection
Cell therapy in hypertrophied myocardium or dysfunction due to congenital heart disease

MSCs: Mesenchymal stem cells; CSCs: Cardiac stem cells; CDCs: Cardiosphere-derived cells; VEGF: Vascular endothelial growth factor; SDF: Stromal cell-derived factor; bFGF: Basic fibroblast growth factor; IGF: Insulin-like growth factor; BMP2: Bone morphogenetic protein 2; HO-1: Heme-oxygenase 1; Ang1: Angiopoietin 1.

growth factor (bFGF), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β, and stromal cell-derived factor (SDF)-1 inhibit LV remodeling[16] and apoptosis, stimulate proliferation of endogenous myocytes and angiogenesis, activate endogenous CSCs[4] and mobilize bone marrow progenitor cells to the heart[17]. Importantly, MSC are immunoprivileged because they do not express MHC class II molecules therefore they escape immune-rejection, release immunomodulatory factors and inhibit T-cell proliferation. Allogeneic cells can be expanded *ex vivo* and stored to use in patients[18,19]. This would allow for "off-the-shelf" treatment of patients with severe LV dysfunction, without the need to wait for cell processing and expansion[19].

A large number of preclinical investigations have been performed using MSCs, and demonstrate a significant beneficial effect on cardiac structure and function[13,20-23]. In a large animal model, Quevedo *et al*[18] demonstrated that administration of allogeneic MSCs to a swine model of chronically infarcted myocardium resulted in improvements in regional contractility and myocardial blood flow, as well as engraftment, differentiation and enhanced survival. Williams *et al*[24] assessed serial cardiac MRI in animals with post-MI LV remodeling and showed progressive scar size reductions, improvements in ejection fraction (EF) and reverse LV chamber remodeling in animals receiving allogeneic MSCs as compared to controls[24]. Mesenchymal precursor cells (MPCs) are subpopulation of MSCs expressing the STRO-3 cell surface marker. MPCs are highly proliferative and secrete abundant paracrine factors. Houtgraaf *et al*[25] demonstrated that slow infusion of allogeneic MPCs (12.5 to 37.5 million cells) to an bovine model with acute MI improved regional and global function and reduced scar volume and LV remodeling. Interestingly, MPC infusion in the infarct-related coronary artery caused myocyte cell size reduction in the infarcted and remote regions. Based on these data a clinical trial is currently ongoing (NCT01781390, phase Ⅱ) that investigates the safety of MPCs in patients with *de novo* anterior MI.

We have demonstrated that slow infusion of allogeneic MSCs into the three major coronary arteries in swine with hibernating myocardium increased regional cardiac function in both the ischemic left anterior descending (LAD) artery and remote regions (wall thickening: LAD: 24% to 43%, Remote: 60% to 85%, $P < 0.05$)[17]. Intracoronary MSCs (icMSCs) significantly increased cKit+/CD133 positive cells (or bone marrow-derived progenitor cells) in the bone marrow and circulation corresponding to the increase in myocardial localization of cardiac progenitor cells (cKit+/GATA4 or Nkx2.5+). icMSCs also induced myocytes to enter the cell cycle and increased the production of small cardiac myocytes indicating the presence of cardiac regeneration. Although some laboratories have identified rare myocytes arising from MSCs in swine[18], our own studies using multiple reporter genes could not identify cardiac myocytes differentiating from labeled MSCs[26]. Thus, cardiac regeneration after icMSCs is related to a bone marrow-derived progenitor cell mediated endogenous cardiac repair mechanism.

Chen *et al*[10] administered 48-60 billion bone marrow derived MSCs by intracoronary injection into 34 patients and reported a 13% increase in EF compared to placebo groups at 3-6 mo follow-up. The Percutaneous Stem Cell Injection Delivery Effects on neo-myogenesis (POSEIDON) trial, by Hare's group, tested the ability of autologous and allogeneic MSCs (20, 100 and 200 million cells) in patients with ischemic cardiomyopathy to promote cardiac recovery following transendocardial stem cell injection[27,28]. Using multidetector computed tomography and biplane left ventriculography, this study reported a 32% reduction in scar size in allogeneic MSCs group *vs* a 35% reduction in autologous MSCs groups without improvement of LV EF. Subgroup analysis demonstrated that 20 million MSCs improvement in LV EF and LVEDV. Furthermore, autologous MSCs showed improvement in the 6 min walk test and allogeneic MSCs reduced LVEDV. Additionally, allogeneic MSCs did not stimulate a donor specific alloimmuno reaction. Thus, this study clearly demonstrates the importance of cell injection site and the safety of using allogeneic MSCs in patients. The Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS) trial investigated injection of autologous MSCs (20-200 million cells) into akinetic or hypokinetic areas in hearts that were unsuitable for surgical revascularization during coronary artery bypass graft surgery (CABG)[29]. Cardiac MRI analysis demonstrated that MSC injection increased EF by 9.4% as well as increased scar reduction by 48%

and contractile improvement in dysfunctional areas where surgical reperfusion was not performed[29]. Although this study lacked a placebo control group and had a limited patient number (6 patients), it demonstrates the potential benefits of injection of MSCs directly into non-revascularized myocardium. The Cardiopoietic stem Cell therapy in heart failure (C-CURE) trial tested the ability of a "cardiogenic cocktail" to enhance the therapeutic benefits of autologous MSCs[30]. Bartunek *et al*[30] pretreated MSCs with growth factors to enhance their cardioprotective functions. Twenty-one patients with class 2 or 3 heart failure received over 700 million cardiogenic cocktail treated MSCs by electromechanically guided endomyocardial injections. No adverse events or systemic toxicity was observed. Moreover, in LV EF, end-systolic volume and the 6-min walking test were significantly improved. Subsequently, the Safety and Efficacy of Autologous Cardiopoietic Cells for Treatment of Ischemic Heart Failure (CHART-1) trial is currently ongoing. This study is investigating the efficacy and safety of Bone Marrow-derived Mesenchymal Cardiopoietic Cells for the Treatment of Chronic Advanced Ischemic Heart Failure. The safety and efficacy of MSCs and modified MSCs in patients have been confirmed. In the future, randomized controlled trials involving a large population of patients are anticipated.

CDCS IN ISCHEMIC CARDIOMYOPATHY

Smith *et al*[31] expanded in culture tissue from percutaneous myocardial biopsies to form cardiospheres as the basis for cardiac stem cell expansion. They selected floating cardiospheres (out-growing cells) for culture and expanded them in a monolayer to isolate what is termed CDCs. Cardiospheres and CDCs express antigens specific for stem cells (cKit, CD90, CD105 and the absence of CD34 and CD45) as well as proteins vital for cardiac contractile (Nkx2.5, GATA4) and electrical function (Cx43)[32]. This defines cardiospheres and CDCs as a population of cardiac progenitor cells. Cardiospheres are heterogeneous groups of cells that contain not only adult CSCs, which are capable of long-term self-renewal and cardiomyocyte differentiation, but also vascular cells and differentiated progenitor cells[33]. Preclinical investigations were exclusively reported from Marban's group, they demonstrated that administration of CDCs in an experimental acute MI model reduced LV remodeling, improved contractility and reduced the infarct size without improvement in cardiac function[5]. Specifically they show that injection of 10 million of autologous CDCs to a swine model of infarcted myocardium resulted in a significant reduction in infarction size (approximately 5%) compared to a 2.4% reduction in placebo with no change in global function[5]. Malliaras *et al*[34] showed that injection of 12.5 million of allogeneic CDCs significant reduced scar size (3.6%) and preserved EF in a swine model of MI compared to no reduction in scar size (0.4%) and deterioration of EF (approximately 9.9%) in placebo. Lee *et al*[33] compared the effects of CDCs and their precursor cells, cardiospheres, in a swine MI model. They found that the effects on infarct reduction and preservation of EF were similar in both CDCs and cardiospheres whereas there was improved hemodynamics and regional function and preservation of LV chamber remodeling (all quantified by serial cardiac MRI) in animals receiving cardiospheres.

We previously demonstrated that slow infusion of CDCs into the three major coronary arteries (total dose 30 million CDCs) in swine with hibernating myocardium improved regional function in ischemic LAD (wall thickening: 23% to 51%, $P < 0.05$) as well as in the normal right coronary artery (RCA) regions (68% to 107%, $P < 0.05$) and global function (EF: 54% to 71%, $P < 0.05$)[35]. Quantitative histochemical analysis demonstrated that CDCs increased myocyte nuclear density and significantly reduced myocyte cellular hypertrophy in hibernating LAD and normal RCA regions indicating viable myocardium is a main therapeutic target.

The cardiosphere-derived autologous stem cells to reverse ventricular dysfunction (CADUCEUS) involved 25 patients who were given 12.5-25 million autologous CDCs[36] after successful percutaneous coronary intervention. The CDCs were expanded for approximately 36 d in culture from right ventricular endomyocardial biopsies taken 2-4 wk after acute MI. After expansion CDCs were injected into the previously stented coronary artery between 6-12 wk after heart attack. Despite the lack of improvement in left ventricular EF or patient reported outcomes, the scar reduction was 28% and 46% at 6 and 12 mo respectively and regional wall thickening was significantly improved in treated patients by 7.7%[37]. Serious adverse events were also reported to be three times higher in the treated group, however due to the relatively small number of patients enrolled, this trial cannot ascertain to the safety of CDCs. The autologous human cardiac-derived stem cell to treat ischemic cardiomyopathy (ALCADIA) trial investigated CDCs expanded from cardiac (endomyocardial) tissue isolated during CABG. This trial combined the use of stem cells, bioengineered scaffolds and biologics to create a hybrid therapy. CDCs were cultured for 1 mo before intracoronary injection followed by placement of a gelatin sheet containing bFGF over the injection site. Six months after therapy, cardiac MRI indicated a 12.1% increase in EF, a 3.3% reduction in infarct size and a significant improvement in wall motion as well as maximum aerobic exercise capacity. Since this study enrolled only 6 subjects, study is anticipated to enroll larger patients.

The transcoronary Infusion of Cardiac Progenitor Cells in Patients with Single Ventricle Physiology trial involved in 14 patients who had hypoplastic left heart syndrome. Tissue was isolated from the right atrium of patients receiving stage 2 (Glenn) or stage 3 (Fontan) surgeries[38]. Cardiospheres were expanded from this right atrium tissue for 2-3 wk in culture. CDCs (2-3

million autologous cells, n = 7) were injected into the 3 major coronary arteries 1 mo after surgery[38]. At 18 mo post injection, cardiac echo and MRI indicated an increase in right ventricular EF from 46.9% to 54.0% (P = 0.0004) compared to no change in EF (46.7% to 48.7%, P-ns) in control. This was a small study (only 7 patients received CDCs) but indicates that viable and dysfunctional myocardium can be treated with autologous CDCs. Although CDCs are beneficial in patients with heart disease, CDCs have many characteristics that overlap with MSCs[39]. Therefore, it is necessary to identify the similarities and differences in biological responses of both MSCs and CDCs prior to further proceeding with clinical applications.

CSCS IN ISCHEMIC CARDIOMYOPATHY

Several investigators have demonstrated the presence of small clusters of Sca-1+, cKit+ or a side population cells (multipotent stem cells identified by the ability to efflux Hoechst dye) in the cardiac atria and apex[40-42]. These cells were named CSCs and are most abundant during postnatal cardiac development after birth. Progeny of CSCs acquire a cardiomyocyte phenotype therefore resident CSCs are optimal candidates for cardiac regeneration studies. CSCs are self-renewing, can replace senescent and apoptotic CSCs *via* mobilization of BM-derived stem cells, and participate in maintaining the CSC pool in the heart[43-45]. In adulthood, the cells are quiescent and reside within the heart. Following ischemic injury, activation by paracrine signals induces CSCs to divide. Nevertheless, their proliferative potential is limited and the extent of the myocardial injury (*e.g.*, necrosis and fibrosis following MI) is frequently too large to be compensated by new cardiomyocytes formed from dividing resident CSCs[40]. In the normal organism the heart retains a pool of CSCs that regulate cardiac homeostasis by maintaining a low level of myocyte proliferation, regeneration and cell death[2]. It is well known that CSCs are a rare population in the myocardium making their isolation and cultivation difficult and time-consuming. Since these cells are located in the heart and are primed for cardiac repair, protocols to enhance their endogenous activity or expand these cells *in vitro* before re-implanting them in the heart are currently being tested. A limited number of animal studies indicate that the administration of CSCs can slow left ventricular remodeling and cardiac improve function after ischemic injury[40,46,47]. Welt *et al*[48] demonstrated that intramyocardial injection of autologous CSCs in a canine infarct model with permanent LAD occlusion resulted in the preservation of global function (31% to 33%) and reduced LV remodeling compared to functional deterioration (35% to 26%, P < 0.05) and LV remodeling in vehicle animals[48]. Bolli *et al*[49] demonstrated that administration of autologous CSCs to a swine model of chronically infarcted myocardium resulted in improvements in regional and global contractility (45.4% to 51.7%, P < 0.05) as well as engraftment and differentiation of injected CSCs[49].

The stem cell infusion in patients with ischemic cardiomyopathy (SCIPIO) trial isolated autologous CSCs during CABG[50]. SCIPIO involved 23 patients who had experienced MI in the past and exhibited an EF of under 40%. One million of cKit positive and lineage negative CSCs were isolated with magnetic beads from cultures of right atrial appendage tissue and administered *via* intracoronary infusion 1 mo after CABG. Twelve months after the treatment, infarct size was decreased by 30.2%, regional wall thickening was increased by 18% and left ventricular EF was increased by 8.2%. The benefits of treatment continued to increase and left ventricular EF was increased by 12% after 2 years[51]. Although studies have shown the beneficial effects of CSCs on the infarcted myocardium, their biological actions in the heart are still controversial[52]. Further studies are necessary to clarify the significance of CSCs in clinical applications.

FUTURE DIRECTIONS

Based on current achievements in experimental large animal studies and clinical trials of cell-based therapies, it is evident that cell therapies still require significant progress to be registered in the daily practice of modern medical therapies. The following strategies are solutions to overcome current limitation of cell-based therapies.

PRECONDITIONED MSCS

Since the safety and efficacy of MSCs has been demonstrated by clinical work, there has been an increasing interest on enhancing the benefits of MSC therapy. For example, combining MSC and pharmacotherapy[53], genetically modifying MSCs[54-56] and pre-conditioning MSCs[57] are approaches that are being explored to augment MSC-mediated cardiac repair. MSCs transfected to overexpress Akt or cell survival protein promoted myocardial protective function[16,55]. Furthermore, MSCs engineered to express combinations of gene products such as Akt and angiopoietin-1 (Ang1) have also shown functional benefits in experimental animal models[58]. MSCs overexpressing VEGF and SDF-1 improved cardiac function by activating Akt pathway[54]. MSCs transfected to express heme-oxygenase 1 (HO-1), an enzyme that improves MSC tolerance to hypoxia, infused into a cardiac ischemia-reperfusion model improve EF and lower end systolic volume compared to controls[59]. MSCs pretreated with growth factors, bFGF, IGF-1 and bone morphogenetic protein 2 (BMP2), improved myocardial repair in a rat model of MI[60]. Those preconditioned MSCs improved engraftment and survival of transplanted cells. Although data are promising, the safety of these cells must be carefully evaluated before use in humans.

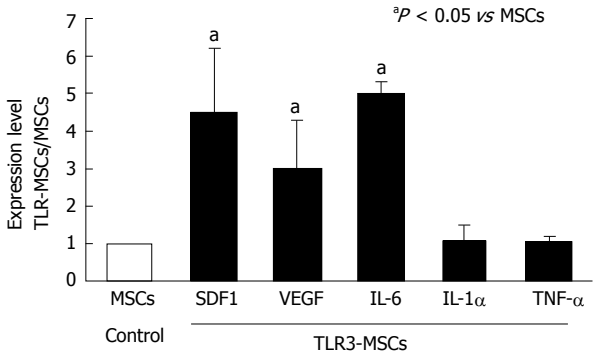

Figure 1 Toll-like receptor 3-mesenchymal stem cells enhance to secrete paracrine factors. RNA mimetic polyinosinic-polycytidylic acid [poly(I:C)] stimulated TLR3 system on MSCs. TLR3-MSCs secreted a variety of paracrine factors. RT-PCR detected significant upregulation of SDF1, VEGF and IL6 while inflammation related cytokines (IL-1α, TNFα) were downregulated. Injection of TLR3-MSCs in cardiomyopathy model improved cardiac function more than standard MSCs in association with increasing myocyte proliferation, reducing fibrosis and myocyte apoptosis. TLR3: Toll-like receptor 3; MSCs: Mesenchymal stem cells; SDF1: Stromal cell-derived factor-1; VEGF: Vascular endothelial growth factor; IL: Interleukin; TNF: Tumor necrosis factor.

PRECONDITIONED MSCS WITHOUT GENETIC MODIFICATION

As mentioned above, the currently used approaches to enhance stem cells are mostly through genetic modification. Thus, modified cells are not considered as a clinically relevant approach because genetically engineered stem cells may have increased unwanted long-term side-effects. We demonstrated that stimulation of toll-like receptor 3 (TLR3) produced many trophic factors without induction of inflammatory-related cytokines[26]. Poly (I:C) is structurally similar to double-stranded RNA and is known to interact with TLR3, which is expressed on the membrane of B-cells, macrophages, dendritic cells, MSCs and CDCs. Poly (I:C) directly reacts with the TLR3 receptor on the surface of MSCs/CDCs. Thus, after washing and collecting MSCs/CDCs after stimulation, poly (I:C) does not reside within the cells and does not affect the heart environment after injection of cells. Interaction of Poly (I:C) with TLR3 on MSCs causes secretion of the growth factors VEGF and the cytokine IL-6 without up-regulation of the inflammatory cytokines IL-1 and TNFα (Figure 1). Injection of TLR3 activated MSCs (TLR3-MSCs) in a non-ischemic cardiomyopathy model improved cardiac function more than standard MSCs in association with increasing myocyte proliferation, reducing fibrosis and myocyte apoptosis[61]. Activation of TLR3 on CDCs (TLR3-CDCs) stimulated the secretion of HGF, IGF1 and IL-6 without up-regulation of inflammatory cytokines. TLR3-MSCs or TLR3-CDCs are safe and feasible to use in the human heart. Further investigation is necessary to confirm the safety and feasibility to use in the heart.

MSCS AND CSCS

Combining MSC and CSC in post-MI treatment may further enhance the therapeutic effects of each cell type. Recent work by Williams *et al*[62] demonstrated that the combined use of 1 million human CSCs with 200 million human MSCs provided greater recovery, almost to baseline, in a swine model of anterior wall MI[62]. While all stem cell treated animals demonstrated improved LV EF compared to placebo controls, notably, animals receiving dual cell therapy had a 2-fold greater reduction in scar size (21.1% for CSC/MSC *vs* 10.4% for CSC alone or 9.9% for MSC alone) and had improved rates of pressure change during diastole. Overall left ventricular chamber dynamics were improved in both the dual therapy and CSC or MSC alone treated groups. Interestingly, CSC alone treated animals demonstrated better isovolumic relaxation as compared to controls, while MSC alone treated animals exhibited improved diastolic compliance, indicating that the enhanced effect of dual therapy on both systolic and diastolic function may be due to a synergistic effect between CSC and MSC targeted mechanisms.

REGIONAL INFUSION WITH STOP-FLOW *VS* GLOBAL INFUSION WITH SLOW INFUSION

Clinically applied techniques for cell delivery include endomyocardial injection using an injection needle or infusion of cells into a coronary artery supplying the infarcted region using a stop coronary flow technique. Although both approaches elicit significant improvements in cardiac function, they increase the risk of endomyocardial hemorrhage and MIs caused by stem cells plugs in the capillaries which could potentially limit the beneficial effects of cell-therapy. We previously demonstrated that slow infusion of MSCs into the three major coronary arteries without stop flow technique (global infusion) did not cause microembolization and stimulated prominent cardiac regeneration in ischemic as well as normally perfused RCA regions in swine with hibernating myocardium[17]. Likewise, intracoronary injection of autologous CDCs[35] without a stop-flow technique in swine with hibernating myocardium stimulated myocyte proliferation and regeneration in an ischemic LAD region as well as the normally perfused RCA regions. Subsequently, we applied the global infusion approach in an acute MI model, CDC infusion significantly improved cardiac function despite no changes in the size of infarction area. These results indicates that scar reduction and functional improvement are independent phenomenon[63]. Accordingly, the approach of stem cell injection in the entire heart is safe and feasible to improve LV dysfunction and our results indicate that normally perfused and viable myocardium could be the target for regenerative therapy. Alternatively, combining stop-flow infusion in the infarcted area with slow flow infusion into the viable myocardium may be a method to enhance therapeutic efficacy.

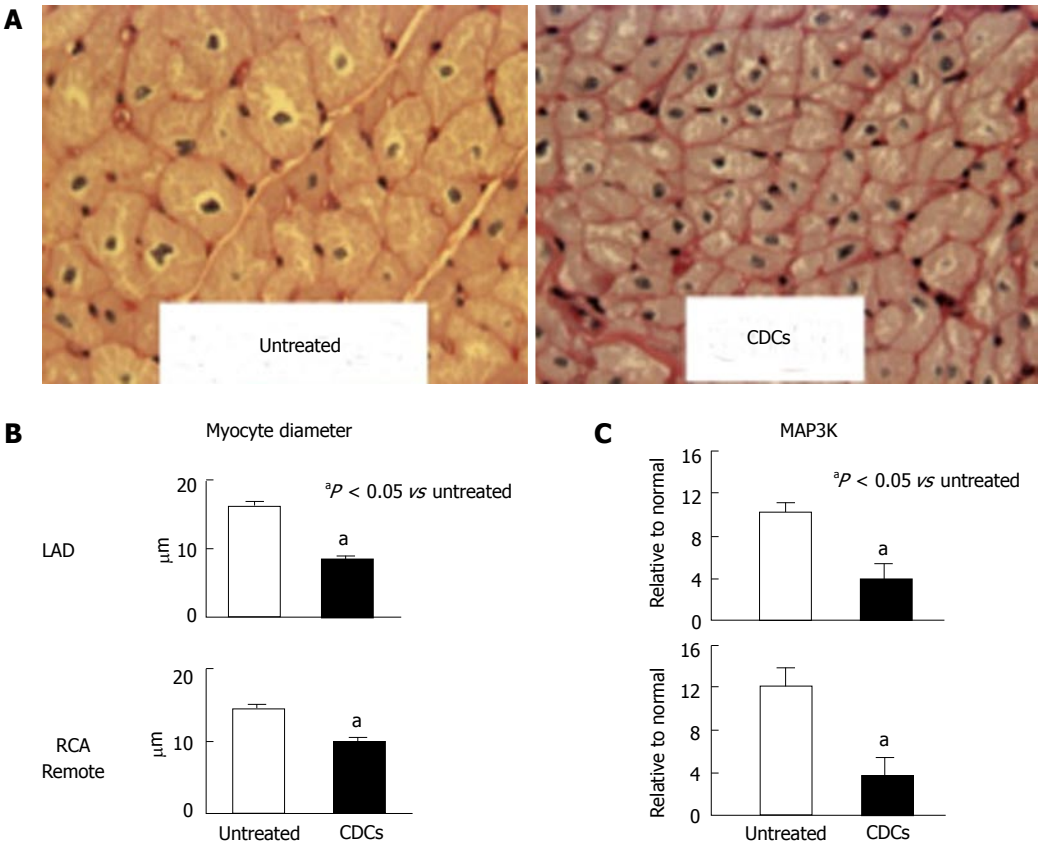

Figure 2 The effect of cardiosphere-derived cells on myocyte cell size and MAP kinase in the dysfunctional left anterior descending *vs* remote regions. A: Images (PAS staining) demonstrate that hypertrophied myocytes in untreated hibernating LAD became smaller after CDCs. Myofibrils were condensed indicating the production of healthy myocytes; B: Myocyte diameter was significantly reduced in hibernating LAD and remote regions; C: Corresponding to the morphological change, protein level of MAP3K was downregulated in LAD and remote regions. Data indicates CDCs induced myocyte regeneration and hypertrophy regression. CDCs: Cardiosphere-derived cells; LAD: Left anterior descending; RCA: Right coronary artery.

ALLOGENEIC CDCS INFUSION IMMEDIATE AFTER REPERFUSION

Allogeneic CDCs can escape direct recognition of helper T cells due to the lack of expression of MHC class Ⅱ antigen (SLA class Ⅱ on pig)[34,64] and therefore are immunoprivileged. Based on these observations, a recent clinical trial was initiated using allogeneic human CDC treatment in patients with chronic myocardial infarction (ALLSTAR trial). These allogeneic cells can be expanded *ex vivo* and stored for use at a future time[18]. This "off-the-shelf" treatment for patients with AMI immediately after revascularization is unique in that *ex vivo* expanded cells are available immediately for treatment and the patient does not need to wait for cell processing and expansion[19]. Recently, administration of CDCs immediately after reperfusion demonstrated the protective effects in swine with acute myocardial infarction[65]. Thirty minutes after ischemia-reperfusion, CDCs were injected into the infarct-related coronary artery and reduced the size of the infarct area and myocyte apoptosis in the border region. Although data were collected 48 h post CDCs injection, we recently demonstrated that functional improvement and myocyte regeneration were maintained up to 1-mo follow-up. These data indicate that the cardioprotective effects at early times were maintained. Previously bone marrow cell and endothelial progenitor cells injection in patients were performed within 7 d after AMI and demonstrated superiority to cell injection within 24 h[66,67]. Since stem cell homing factors (mobilization, migration and adhesion) are maximized between day 3 and day 7[68], these therapies are effective for stem cell homing. However, the inflammation caused by MI is already developed and the potential cardioprotective effects (*i.e.*, *via* anti-apoptotic effects or modulation of the inflammatory response) are limited when cells are delivered. Since CDCs secrete multiple cytokines (SDF-1, Akt)[69], growth factors (HGF, IGF-1, VEGF)[69,70] and exosomes[71,72], CDCs early after reperfusion might reduce the inflammatory response and protect the heart from functional deterioration due to reperfusion injury.

REPEATED INJECTION OF STEM CELLS

Since single injection of CDCs improved regional function and reduced scar volume[36,37], repeated injection of stem cells has been considered a more effective approach to regenerate myocardial tissue[73,74]. However, the initial infusion of cells activates and enhances the immune response[34,64] and the subsequent injected cells are quickly eliminated and ineffective. This quick

reaction is mainly associated with acquired/adaptive rather than innate immunity. Repeated infusion of autologous/allogeneic CDCs may overcome the limited functional recovery from a single injection[73,74]. However, the extent of immune activation caused by repeated injections is unclear and optimal immunosuppressive therapy is still undetermined. Development of efficacious CDC platforms administered with optimal immune suppression would circumvent barriers related to multiple injections of stem cells and allow the widespread application of "off-the-shelf" cell therapy to treat the large number of patients in need[64,75,76].

EXOSOME ACTIVATION AND MICRORNAS

The beneficial effects of adult stem cells are mainly associated with the secretion of paracrine factors rather than direct differentiation of *de novo* cardiac cells[9]. They secrete multiple growth factors and cytokines which reduce scar volume and myocyte apoptosis, increase myocyte proliferation and activate endogenous CSCs to produce new myocytes. Recently, it was reported that CDCs secrete exosomes and they play important roles for cardiac regeneration[71]. Exosomes transfer microRNAs from cell to cell and they inhibit inflammation and apoptosis and increase angiogenesis and myocyte proliferation. Therefore, a new method of treatment may focus on how to effectively stimulate secretion of exosomes from stem cells or may be directly injecting exosomes in the infarcted myocardium.

ANTI-HYPERTROPHIC EFFECT

Besides their regeneration potential, adult stem cells have other beneficial effects such as anti-apoptosis, anti-inflammation, extracellular matrix reduction, contractile alternation and anti-hypertrophy. Pathological cardiac hypertrophy in post MI remodeling is a major cause of mortality and morbidity including the risk of sudden cardiac death and heart failure in patients[77-81]. It is associated with increased interstitial fibrosis, cell death and cardiac dysfunction. LV assist devices used in heart failure patients as a bridge to heart transplantation not only improved peripheral circulation but also reversed the geometric remodeling of the heart and restored the function of the heart[82-85].

We demonstrated that global infusion of CDCs into hearts with chronically ischemic myocardium's improved myocardial function in the ischemic and remote regions[86]. CDCs significantly increased newly formed small myocytes. Interestingly, CDCs also reduced the cell size of pre-existing myocytes and hypertrophic signaling (mitogen activated kinases) in the ischemic and remote regions. Data indicate that CDCs have the potential to reverse cardiac hypertrophy (Figure 2). Future studies are necessary to determine whether hypertrophy regression is primary or secondary to myocardial regeneration and functional improvement.

CONCLUSION

Promising data derived from experimental models indicate the potential success of using cell based therapy in clinical applications. To overcome the current limitations in the field, development of new methods to enhance cardiac repair is necessary. In light of their proven safety profiles, MSC, CDC and CSC are prime candidates for cell based therapies. Recently, it was reported that a combination of CSCs and MSCs may be more effective than either one alone, and this approach is under investigation. Similarly, pre-conditioning MSC and CDCs are also promising approaches, and further investigation is anticipated. Optimizing the dose and method of delivery, as well as the timing for delivery are important variables that should be studied. It is anticipated that cell based therapies will become a mainstream treatment for heart diseases due to their potential ability to improve functional outcomes and decrease mortality.

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REVIEW

Vascular endothelial dysfunction and pharmacological treatment

Jin Bo Su

Jin Bo Su, INSERM U955, 94700 Maisons-Alfort, France

Jin Bo Su, Université Paris-Est Créteil Val-de-Marne, 94700 Créteil, France

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Correspondence to: Jin Bo Su, PhD, INSERM U955, Equipe 3, ENVA, 7 avenue du Général de Gaulle, 94700 Maisons-Alfort, France. jin-bo.su@inserm.fr
Telephone: +33-1-43967386
Fax: +33-1-43967399

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Abstract

The endothelium exerts multiple actions involving regulation of vascular permeability and tone, coagulation and fibrinolysis, inflammatory and immunological reactions and cell growth. Alterations of one or more such actions may cause vascular endothelial dysfunction. Different risk factors such as hypercholesterolemia, homocystinemia, hyperglycemia, hypertension, smoking, inflammation, and aging contribute to the development of endothelial dysfunction. Mechanisms underlying endothelial dysfunction are multiple, including impaired endothelium-derived vasodilators, enhanced endothelium-derived vasoconstrictors, over production of reactive oxygen species and reactive nitrogen species, activation of inflammatory and immune reactions, and imbalance of coagulation and fibrinolysis. Endothelial dysfunction occurs in many cardiovascular diseases, which involves different mechanisms, depending on specific risk factors affecting the disease. Among these mechanisms, a reduction in nitric oxide (NO) bioavailability plays a central role in the development of endothelial dysfunction because NO exerts diverse physiological actions, including vasodilation, anti-inflammation, antiplatelet, antiproliferation and antimigration. Experimental and clinical studies have demonstrated that a variety of currently used or investigational drugs, such as angiotensin-converting enzyme inhibitors, angiotensin AT1 receptors blockers, angiotensin-(1-7), antioxidants, beta-blockers, calcium channel blockers, endothelial NO synthase enhancers, phosphodiesterase 5 inhibitors, sphingosine-1-phosphate and statins, exert endothelial protective effects. Due to the difference in mechanisms of action, these drugs need to be used according to specific mechanisms underlying endothelial dysfunction of the disease.

Key words: Endothelial dysfunction; Endothelium-dependent vasodilation; Endothelial nitric oxide synthase; Inflammation; Nitric oxide; Pharmacological treatment; Reactive nitrogen species; Reactive oxygen species; Risk factors

Core tip: The endothelium is involved in the regulation of vascular tone and permeability, coagulation and fibrinolysis, inflammatory and immunological reactions

and cell growth. Cardiovascular risk factors cause vascular endothelial dysfunction through impairing endothelium-derived vasodilators, enhancing endothelium-derived vasoconstrictors, producing reactive oxygen species and reactive nitrogen species, activating inflammatory and immune reactions and promoting thrombosis. Among these mechanisms, a reduction in nitric oxide bioavailability plays a central role in the development and progression of endothelial dysfunction. A variety of currently used or investigational drugs exert endothelial protective effects according to specific mechanisms underlying endothelial dysfunction of the disease.

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INTRODUCTION

The endothelium is formed by a monolayer of endothelial cells. It constitutes a physical barrier between blood and tissues and regulates the exchange of molecules between blood and tissues. In addition, endothelial cells metabolize, synthesize and release a variety of substances, including vasoactive substances regulating vascular tone, blood pressure and local blood flow, the substances participating in coagulation, fibrinolysis and inflammatory and immunological reactions, reactive oxygen species (ROS) and reactive nitrogen species (RNS) involved in oxidation and nitrosylation of proteins and lipids, and growth factors promoting cell growth (Table 1). Any perturbation affecting the capacity and equilibrium of the endothelium as a physical barrier and to metabolize, synthesize and release these substances will cause endothelial dysfunction, which contributes to the development and progression of cardiovascular diseases. After summarizing the role of a number of endothelium-derived vasoactive substances and risk factors of endothelial dysfunction, this review focus on several categories of pharmacological substances that may be used for improving endothelial function.

Table 1 Some of endothelium-derived substances

Vasoactive substances
Endothelium-derived vasodilators
Adrenomedullin
Endothelium-derived hyperpolarizing factors
Kinins
Nitric oxide
Prostacyclin
Endothelium-derived vasoconstrictors
Angiotensin II
Endothelin-1
Vasoconstrictor prostanoids
Coagulation and fibrinolysis
Coagulation
Factor V
Heparan sulfate
Protein C
Protein S
Thrombomodulin
Tissue factor
von Willebrand factor
Fibrinolysis
Plasminogen activator inhibitor
Tissue plasminogen activator
Urokinase
Growth factors
Basic fibroblast growth factor
Insulin-like growth factor
Platelet-derived growth factor
Transforming growth factor
Inflammatory and immunological mediators
Cytokines
Interleukins
Monocyte chemoattractant protein 1
Transforming growth factor
Tumor necrosis factor-α
Adhesion molecules
Intercellular adhesion molecules
Platelet-endothelial cell adhesion molecules
Selectins
Vascular cell adhesion molecules
Reactive oxygen species and reactive nitrogen species
Reactive oxygen species
Hydrogen peroxide (H_2O_2)
Hydroperoxyl (HO_2)
Superoxide (O_2^-)
Reactive nitrogen species
Nitrite (NO_2^-)
Nitrogen dioxide (NO_2)
Peroxynitrite ($ONOO^-$)
Nitryl chloride (NO_2Cl)

ENDOTHELIUM-DERIVED VASOACTIVE SUBSTANCES

The endothelium releases a variety of vasoactive substances, including different vasodilators such as nitric oxide (NO), prostacyclin, kinins, and endothelium-derived hyperpolarizing factors (EDHF), vasoconstrictors such as endothelin-1 and PGH_2, and ROS. Among endothelium-derived vasodilators, NO occupies a central position because changes in the release of endothelial NO play a crucial role in the perturbation of vascular homeostasis and in the development of endothelial dysfunction associated with various cardiovascular disorders.

NO

NO is synthesized from the amino acid L-arginine by a family of enzymes, the NO synthase (NOS), through the L-arginine-NO pathway. Three isoforms of NOS have been identified. Neuronal NOS (nNOS), initially found in the nervous system, is also constitutively expressed in skeletal and cardiac muscles, vessels and many other tissues. Endothelial NOS (eNOS) is constitutively expressed mainly in endothelial cells, whereas the expression of inducible NOS (iNOS) can

be stimulated by diverse factors such as cytokines and endotoxin in different circumstances. The endothelium-derived NO release is primarily ensured by eNOS and complemented by nNOS expressed in vascular endothelial cells. Therefore, eNOS is primordial in the regulation of NO production by endothelial cells. The activity of eNOS depends on several factors, including eNOS mRNA and protein expression, the abundance of asymmetric dimethylarginine (ADMA, an endogenous eNOS inhibitor that competes with L-arginine for binding to eNOS)[1,2], the quantity and quality of cofactors such as tetrahydrobiopterin (BH4) and NADPH that are necessary for eNOS catalyzing NO production from L-arginine[3,4], its interaction with caveolin and heat shock protein 90 (hsp90)[5,6], and its translational modifications such as phosphorylation at different sites by multiple kinases or phosphatases [for example, phosphorylation at ser-1179 by phosphotidylinositol-3-kinase (PI3K)/protein kinase B (Akt) activates eNOS to initiate NO synthesis][7,8] and S-nitrosylation at cysteine residues[9]. In addition, excessive superoxide (O_2^-) and hydrogen peroxide (H_2O_2) production due to increased NAD(P)H oxidase[10,11] and eNOS uncoupling induced by changes in oxidized low density lipoprotein (OxLDL), caveolin-1, BH4, a switch from S-nitrosylation to S-glutathionylation and oxidation of the zinc-thiolate complex by peroxynitrite ($ONOO^-$) also affects effective eNOS activity[12-15].

NO released by eNOS participates in the regulation of vascular tone. NO activates soluble guanylate cyclase by binding to its ferrous heme, leading to the conversion of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP) that causes vascular smooth muscle relaxation. Moreover, NO exerts antiinflammatory, antiplatelet, antiproliferative and antimigration actions that contribute to the maintenance of an adequate environment for the endothelium. Lacking eNOS gene in mice induces insulin resistance, hyperlipidemia and hypertension[16]. NO released by nNOS also participates in the regulation of vascular tone, especially for the regulation of vascular tone in skeletal muscles. Altered nNOS activity and protein levels contribute to muscular damage due to sustained vasoconstriction in patients with Duchenne muscular dystrophy[17,18] and endothelial dysfunction in dogs with Duchenne muscular dystrophy[19].

Prostacyclin

Prostacyclin (also called PGI2) is generated from arachidonic acid by cyclooxygenase (COX) in endothelial cells. Activation of IP receptors by PGI2 activates adenylate cyclase to synthesize cyclic adenosine monophosphate (cAMP) from adenosine triphosphate, causing vascular smooth muscle relaxation. However, PGI2 synthesis can be inactivated by increased cytokines[20] and under certain conditions, PGI2 exerts vasoconstrictor action and contributes to endothelial dysfunction[21].

EDHF

The term "EDHF" describes a set of substances causing vascular myocyte hyperpolarization and spreading endothelial hyperpolarization to vascular myocytes, resulting in vascular myocyte relaxation, which is not affected by blocking NO or prostacyclin production[22]. Interestingly, in eNOS/COX-1 double-knockout mice, EDHF-mediated vasodilation plays a compensatory role for the absence of endothelial NO[23]. EDHF hyperpolarize myocyte membranes by opening calcium-activated potassium channels named as BKca, IKca, and SKca according to their conductance (big, intermediate, and small conductance), leading to K^+ efflux[24-26]. EDHF-mediated vasodilation also involves epoxyeicosatrienoic acids (EETs), gap junction, reactive oxygen species and hydrogen peroxide, depending on the vascular beds and vessel types[24,25,27]. Cytochrome P450 epoxygenase catalyzes the production of EETs from arachidonic acid in different vessels and EETs participate in, at least partly, the hyperpolarization and relaxation of myocytes in these vessels[28,29]. It is worth noting that EDHF-mediated vasodilation is a complex phenomenon, which involves multiple signaling pathways that may be not exclusive in response to different stimuli[24-26]. Altered EDHF signalings may account for endothelial dysfunction in some cardiovascular disorders as suggested by studies in animals. For example, a defect in connexins that compose gap junctions is partly responsible for impaired vasodilator responses in hypertensive rats[30] and in diabetic rats[31]. However, the role of EDHF in endothelial dysfunction in human cardiovascular diseases remains elusive. This may be related to the difficulty that the function of EDHF can only be deciphered after impairment of NO and prostacyclin-mediated responses.

Kinins

Kinins such as bradykinin can be generated in vessel walls, especially in endothelial cells that contain the components such as kinin precursors and kinin-generating enzymes, necessary for the production of kinins[32]. The biological effects of kinins are mediated by stimulation of constitutively expressed B2 receptors and inducible B1 receptors. In endothelial cells, activation of B2 receptors by bradykinin releases NO, prostacyclin, EDHF and tissue plasminogen activator, which exert diverse physiological and pathological actions on cardiovascular system, including regulation of coronary vascular tone and local blood flow of organs, coagulation, fibrinolysis, and water-electrolyte while[33,34]. Stimulation of B1 receptors by its agonists also induce NO-mediated vasodilation[35]. Due to very short half-life in the blood, bradykinin essentially plays an autocrine/paracrine role. Experimental studies have demonstrated a protective role of bradykinin B2 receptors on cardiovascular function. It is, at least in part, due to opposing effects of bradykinin B2 receptor activation on angiotensin Ⅱ AT1 receptor activation because of multiple cross-talks between the kallikrein-kinin system and the renin-

angiotensin system[36]. This explains the contribution of kinins to the cardiovascular protective effects of angiotensin-converting (ACE) inhibitors and angiotensin AT1 receptor blockers. Deletion of both B1 and B2 receptors in diabetic mice exacerbates nephropathy as indicated by increased oxidative stress, mitochondrial DNA deletions and renal expression of fibrogenic genes, suggesting a protective role of the kallikrein-kinin system on diabetic nephropathy[37]. However, these mice exhibit neither accelerated cardiac dysfunction nor ROS production, challenging the protective role of kinins in this setting[38]. Otherwise, kinins can increase endothelial permeability[39] and are involved in inflammatory responses by activating phospholipase A2 to release arachidonic acid that is used for the production of vasoconstrictor prostanoids, which may be harmful for endothelial function.

Adrenomedullin

Adrenomedullin (AM), a vasodilator peptide initially identified from human pheochromocytoma, can be secreted by vascular cells, especially by endothelial cells[40-42]. AM exerts its action in the cardiovascular system through receptor complexes composed of the calcitonin receptor-like receptor and receptor activity-modifying proteins. In vessels, the receptors for AM are expressed in both endothelial and smooth muscle cells[43,44]. AM-induces endothelium-dependent and -independent vasodilation, depending upon species and vascular beds[42]. AM-induced endothelium-dependent vasodilation is mediated by PI3K/Art/NO/cGMP pathway, the activation of cGMP-stimulated protein kinase G and/or the production of a vasodilator prostanoid (likely prostacyclin)[42,45,46], whereas AM-induced endothelium-independent vasodilation involves the opening of K^+ channels (calcium-activated K^+ channels or ATP sensitive K^+ channels, probably depending upon vascular beds) and the activation of cAMP-dependent protein kinase A[42,47]. In addition to vasodilator effect, AM was shown to inhibit angiotensin II-induced ROS generation by NAPDH[48], and AM -deficient mice developed insulin resistance due to increased ROS[49]. AM protects bone marrow-derived mononuclear cell and endothelial progenitor cells from apoptosis and exerts a vascular protective role[50,51]. AM also exerts an protective effect on endothelial barrier function and reduces endothelial permeability in response to inflammation and endotoxin[52-56]. Otherwise, AM posses angiogenesis property and participates in vascular remodeling [50,57,58]. Plasma AM levels were shown to be higher in many pathological situations such as arteriosclerosis, sepsis, essential or pulmonary hypertension and heart failure[52,59-63], whereas intracoronary AM levels were lower in patients with stable coronary disease[64] and in infants with brain damage after surgery under cardiopulmonary bypass[65]. Increased AM levels were interpreted as a compensatory mechanism to protect cardiac and vascular function[61,66]. Expression of receptors for AM was shown to be increased in rats with heart failure though its signification remains elusive[67,68]. Despite its protective role in the cardiovascular system, AM was shown to be involved in the growth of different tumors such as prostate, colorectal and bladder tumors, and AM and its receptors are potential targets for the treatment of these tumors[69-71].

Angiotensin Ⅱ

Endothelial cells express ACE and angiotensin AT1 and AT2 receptors. Once released, angiotensin Ⅱ immediately binds to these receptors and those expressed on smooth muscle cells. Although angiotensin Ⅱ causes both vasoconstriction *via* AT1 receptors and vasorelaxation by stimulating AT2 receptors, angiotensin Ⅱ-induced vasoconstriction is predominant in many circumstances. Moreover, angiotensin Ⅱ exerts multiple actions affecting endothelial function. Angiotensin Ⅱ upregulates endothelial receptors for OxLDL, stimulates OxLDL uptake, and enhances OxLDL-mediated ROS generation and endothelial cell apoptosis[72]. Angiotensin II increases receptors for vascular endothelial growth factors and matrix metalloproteinases (MMPs), which may account for increases in endothelial permeability and vascular remodeling[73-75]. Angiotensin Ⅱ increases the expression of plasminogen activator inhibitor type 1 (a natural inhibitor of tissue-type plasminogen activator and urokinase-type plasminogen activator) in endothelial cells[76], thereby favoring thrombosis. Angiotensin II favors inflammation by inducing COX-2 expression[74] and increasing cytokine tumor necrosis factor-alpha (TNF-α)[75]. Although angiotensin Ⅱ can upregulate eNOS and inducible NO synthase (iNOS) expression, it reduces eNOS-derived NO by promoting eNOS uncoupling through monocyte-dependent S-glutathionylation[77]. In addition, the activation of AT2 receptors also contributes to the angiotensin Ⅱ-induced vascular remodeling[78]. These actions of angiotensin Ⅱ may contribute to endothelial dysfunction as the renin-angiotensin system is activated, as is the case in atherosclerosis[79] and heart failure.

Endothelin-1

Although endothelin-1 can upregulate eNOS expression by enhancing eNOS mRNA stability *via* protein tyrosine kinases and protein kinase C-dependent pathways[80,81], endothelin-1 *via* type A endothelin receptors induces expression of adhesion molecules and neutrophil adhesion to endothelial cells, and promotes cytokine and ROS generation[82-84]. Elevated endothelin-1 blood levels can be seen in atherosclerosis[85], pulmonary hypertension[86] and heart failure[87,88], which may account for the development of endothelial dysfunction under these circumstances. Although the activation of Type B endothelin receptors generally induces vasodilation, these receptors appear to mediate endothelin-1-induced ROS production and contribute to endothelial dysfunction in obese rats[82].

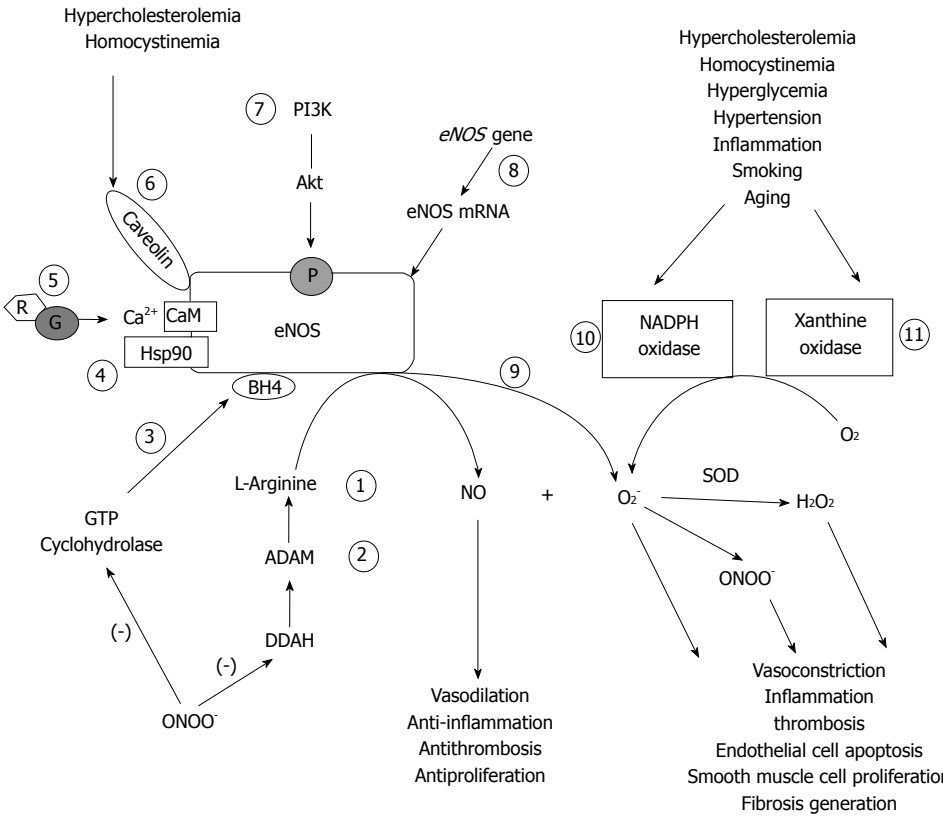

Figure 1 Mechanisms underlying the reduction in nitric oxide bioavailability involve both reduced nitric oxide production and increased nitric oxide scavenging. A reduction in NO production can be resulted from: (1) decreased L-arginine availability due to L-arginine deficiency and/or changes in L-arginine transporter; (2) accumulation of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial NO synthase (eNOS); (3) deficiency or modification of cofactor tetrahydrobiopterin (BH4); (4) altered interactions between eNOS and caveolin due to increased caveolin-1; (5) changes in receptor-coupled G proteins; (6) altered eNOS-heat shock protein 90 (Hsp90) interaction due to changes in Hsp90 abundance; (7) changes in calcium-independent phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)-mediated eNOS activation by tyrosine or serine phosphorylation; and (8) decreased eNOS expression due to reduced *eNOS* gene transcription and/or decreased eNOS mRNA stability. Increased NO scavenging by reactive oxygen species (ROS) and reactive nitrogen species (RNS) can be due to: (9) eNOS uncoupling related to changes in BH4, cavelin-1 and oxidized low density lipoproteins; (10) increased NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) expression and activity; and (11) increased xanthine oxidase expression and activity. CaM: Calmodulin; DDAH: Dimethylarginine dimethylaminohydrolase; SOD: Superoxide dismutase; NO: Nitric oxide.

Vasoconstrictor prostanoids

Endothelial cells can produce vasoconstrictor prostaglandin H2 (PGH2), thromboxane A2 (TXA2) and PGF2α. The production of these prostanoids is enhanced in hypertension, hypercholesterolemia, diabetes and vitamin E deficiency[89], which in turn upregulates NAPDH oxidase and type 4 and type 5 phosphodiesterases (PDE4 and PDE5)[90,91], resulting in increases in ROS production, cAMP and cGMP degradation, and vasoconstriction. TXA2 is also a potent activator of platelets, while activated platelets in turn release a large amount of TXA2 to promote thrombosis. Furthermore, TXA2 interacts with EDHF by inhibiting potassium channels, EETs and gap junction-mediated signaling pathways[27], which could account for the development of endothelial dysfunction.

RISK FACTORS CAUSING ENDOTHELIAL DYSFUNCTION

Clinically, endothelial dysfunction is characterized by impaired endothelium-dependent vasorelaxation in response to endothelium-dependent agonists such as acetylcholine and bradykinin, or to maneuvers that increase shear stress such as flow-mediated dilatation. Although mechanisms leading to endothelial dysfunction are multiple, a reduction in NO bioavailability is largely observed in many cardiovascular disorders. As shown in Figure 1, reduced NO bioavailability can be the consequence of decreased L-arginine availability[92], increased ADMA[1,2], altered interaction with hsp90[93] and phosphorylation of eNOS[94], as well as increased NO scavenging by excessive ROS generated by NADPH and xanthine oxidases[10,11,95] and eNOS uncoupling. It is worth noting that changes in caveolin-1[96], BH4[97], S-glutathionylation of eNOS[15,98] and OxLDL[12] are all involved in eNOS uncoupling. Importantly, a reduction in eNOS protein expression also leads to impaired eNOS activity and NO production, which can be observed in different cardiovascular diseases such as atherosclerosis, acute myocardial infarction and heart failure in animals and in humans[99-103]. However, mechanisms underlying endothelial dysfunction in different cardiovascular

disorders may be different, depending on risk factors contributing to the development of specific disease.

Hypercholesterolemia/atherosclerosis

Atherosclerosis is a chronic arterial disease involving the formation of multiple atheromatous plaques within arteries by accumulation of lipids due to the inability to remove LDL from macrophages. In this process, endothelial dysfunction related to hypercholesterolemia plays a pivotal role in the development of atherosclerosis. Hypercholesterolemia induces endothelial cell activation, leukocyte recruitment and adherence, platelet activation and adhesion within the vasculature, reflecting an inflammatory response and high thrombotic state that may cause endothelial dysfunction. Hypercholesterolemia increases superoxide and hydrogen peroxide production by increasing NAD(P)H oxidases[79], xanthine oxidase[95], and myeloperoxidase[104]. Increased superoxide reacts with NO, resulting in the formation of RNS and reduced eNOS-derived NO bioavailability. ROS induce oxidation of lipids, proteins and DNA, which cause cell damage, necrosis and cell apoptosis. Increased RNS induce nitrosylation reactions that modify the structure and function of proteins. Hypercholesterolemia increases caveolin-1 levels[105], also contributing to impaired eNOS activity. In addition, hypercholesterolemia disturbs reactions between oxygen radicals or enzymatic oxidation and lipoproteins, particularly LDL phospholipids and results in the production of oxidized phospholipids. These phospholipids contain arachidonic acid and bind to their membrane receptors, resulting in their accumulation within the cellular membrane, immune and inflammatory responses and ROS generation, which in turn induces eNOS uncoupling that impairs endothelium-dependent vasodilation induced by endogenous vasodilator such as kinins but enhances the role of endogenous vasoconstrictors such as angiotensin Ⅱ and endothelin-1, and promote endothelial dysfunction[106,107]. Therefore, endogenous vasoactive substances such as NO, prostanoids, ROS, RNS, AM, angiotensin Ⅱ, endothelin-1 and other substances interact and reduced NO bioavailability due to eNOS uncoupling is a key event contributing to the development of endothelial dysfunction and ultimately atherosclerosis.

Hyperhomocysteinemia

Homocysteine is a non-protein α-amino acid synthesized from methionine. Hyperhomocysteinemia is observed in patients with coronary disease and is correlated with endothelial dysfunction[108]. Homocysteine causes endothelial dysfunction through NO inhibition, vasoconstrictor prostanoid production, EDHF inhibition[109,110], angiotensin AT1 receptor activation, and ROS generation[111]. Homocysteine reduces eNOS activity by increasing asymmetric dimethylarginine production[112] and eNOS uncoupling *via* decreasing intracellular do novel synthesis of BH4[97], leading to decreased NO bioavailability and increased ROS generation. Furthermore, homocysteine downregulates eNOS expression in human endothelial cells[113], and induces endothelial loss, vascular deendothelialization and increases platelet adherence and consumption in baboons[114]. Homocysteine also increases ROS generation by phosphorylating NAPDH oxidase[115], and/or by increasing ACE activity *via* ACE homocysteinylation to generate angiotensin Ⅱ that activates NAPDH oxidase[116].

Hyperglycemia/diabetes mellitus

Endothelial dysfunction is associated with both insulin-dependent and independent diabetes mellitus. In this setting, hyperglycemia increases ROS generation through activating protein kinase C-mediated NAD(P)H oxidases[117] and peroxynitrite-mediated eNOS uncoupling[118], which also leads to reduced NO bioavailability. In addition, hyperglycemia increases iNOS expression and iNOS-derived NO and peroxynitrite production, leading to increased ROS and RNS levels and pancreatic islet endothelial cell apoptosis[119]. Moreover, hyperglycemia promotes platelet aggregation by increasing expression and circulating levels of endothelial adhesion molecules through protein kinase C-NFκB signaling pathway[120-122], and increases endothelial apoptosis[123]. All of these effects of hyperglycemia contribute to endothelial dysfunction observed in diabetes mellitus. Otherwise, increased release of vasoconstrictors such as prostanoids and endothelin-1 through protein kinase C-mediated pathway in response to hyperinsulinemia and hyperglycemia appears to precede changes in vascular complication or NO production[124,125]. Changes in EDHF also contribute to endothelial dysfunction, especially in type 2 diabetes as suggested in rat models in which an impaired EDHF-mediated vasorelaxation was observed before marked alteration in NO-mediated responses[126-129]. Therefore, altered NO bioavailability in type 2 diabetes appears to be a relatively late event worsening endothelial dysfunction.

Hypertension

An impaired endothelium-dependent vasorelaxation has been observed in patients with essential hypertension[130] and in several animal models of hypertension. This is related to a lower production of endothelium-derived vasodilators and/or over production of vasoconstrictors. An increased endothelin-1 production also plays a role in endothelial function, especially in pulmonary hypertension as lung is an important metabolic organ of circulating peptides such as adrenomodullin and endothelin-1. In this regard, pulmonary endothelin-1 extraction affects the incremental resistance of pulmonary vascular bed in response to increased cardiac work[131] and plasma endothelin-1 levels are closely related to clinical worsening of patients with pulmonary hypertension[132]. An impaired NO and EDHF-mediated vasorelaxation linked to an increased ADMA that inhibit eNOS and downregulates SKca in

endothelial cells has been reported in hypertensive patients and in spontaneous hypertensive rats[133]. Interestingly, alterations in EDHF appears to occur before alterations in NO pathways in different rat models of hypertension[134]. An reduced vasodilator response to AM has been observed in hypertensive patients[45]. However, an increased eNOS expression is generally observed in animal models of hypertension associated with angiotensin Ⅱ. In this case, angiotensin Ⅱ-induced oxidative stress and increases in the production of vasoconstrictor prostanoids and cytokines may account for the development of endothelial dysfunction. Furthermore, a reduced NO bioavailability has been reported in some models of hypertension. This appears to be linked to reduced substrate availability due to L-arginine deficiency and changed L-arginine transport[92] and to eNOS uncoupling due to oxidation of BH4 and/or S-glutathionylation, leading to increased ROS production[4,15]. Thus, although altered NO bioavailability may not be an initial event to induce endothelial dysfunction, it participates in its progression in hypertensive subjects.

Smoking

Endothelial dysfunction is one of the primary damages induced by cigarette smoke. Circulating cigarette toxins such as free radicals and reactive glycation products can react with endothelial cells and cause vascular impairment[135]. Cigarette smoking induces inflammatory state as indicated by elevation of white blood cells, adhesion molecules and cytokines, and increases ROS production and lipid peroxidation[136-141]. These mechanisms may contribute to impaired endothelium-dependent vasodilation observed in active smokers, even at young healthy adult, and in passive smokers[142,143]. However, despite a reduced NO bioavailability, eNOS expression has been shown to be increased in different endothelial cells or decreased in platelets in response to cigarette smoke[144,145]. Cigarette smoke extracts inhibits eNOS activity of pulmonary arterial endothelial cells through modifying eNOS phosphorylation pattern, which cannot be protected by antioxidants such as vitamin E and C[146,147]. In this setting, decreased NO bioavailability is probably the consequence of decreased eNOS activity due to modified eNOS phosphorylation and uncoupling as well as NO scavenging by increased ROS.

Inflammation

Endothelial cells produce inflammatory and immune mediators (Table 1) and undergo morphological modifications in response to inflammatory stimuli. The inflammatory and immune mediators increase endothelial permeability and promote adhesion of leukocyte to endothelial cells and interactions between chemokine receptors on leukocyte and proteoglycans on endothelial cells, leading to leukocyte transendothelial migration to inflammation sites. Inflammation induced endothelial dysfunction is often associated with impaired NO bioavailability. For example, typhoid vaccination induced an inflammatory response as indicated by increased cytokines and oxidative stress as well as a decreased endothelium-dependent vasodilation that was partially restored by antioxidant vitamin C[148]. In patients with viral myocarditis, acetylcholine induced a coronary vasoconstriction rather vasodilation[149]. Similar responses were also observed in mice with virus-induced myocarditis, which was attributed to reduced eNOS activity and expression[150]. In some autoimmune diseases, anti-endothelial antibodies cause abnormal immune activation that activates endothelial cells to release adhesion molecules and cytokines, leading to inflammation, increased permeability of the endothelium, thrombosis and cell apoptosis[151-153], which are, at least in part, responsible for endothelial dysfunction in this setting. Patients with rheumatoid arthritis have increased levels of cytokines and ADMA and impaired flow-mediated dilation[154]. Similarly, increased arterial stiffness is closely correlated with ADMA blood levels in systemic lupus erythematosus patients[155]. The increase in ADMA levels may account for reduced NO bioavailability in these autoimmune diseases.

In some cases, an over production of NO occurs in response to inflammation. Septic shock associated with a severe infection and sepsis is characterized by a profound hypotension, widespread endothelial injury and activation, multiple organ failure and death. In this setting, toxic microbe products, including endotoxins (bacterial membrane lipopolysaccharides, LPS) of gram-negative bacteria and analogous molecules in the walls of gram-positive bacteria and fungi, dramatically activate mononuclear cells to release cytokines[156] that upregulate bradykinin B1 receptors[157,158], inducible NO synthase[159] and COX-2[160], which increase NO and prostaglandin E2. In this regard, blocking or deleting bradykinin B1 receptors might yield benefits for the treatment of septic shock. However, experimental studies showed conflicting results regarding the role of kinins in septic shock in animals. Mice with overexpression of B1 receptors exhibited an increased susceptibility to develop septic shock and mice lacking B1 receptors or both B1 and B2 receptors had an enhanced resistance to LPS-induced sepsis[161-163], whereas mice lacking B1 receptors had an higher mortality in response to LPS[164] and additional B1 receptor blockade suppressed the beneficial effect of B2 receptor blockade[165]. Similarly, B2 receptor blockade showed no effect or amelioration in porcine sepsis[165,166]. Results regarding the role of NO, particularly iNOS in septic shock are also elusive. Experiments in rats and in human blood cells showed that iNOS expression is correlated with cell apoptosis in septic shock[167,168]. Selective iNOS inhibition improved hemodynamics and mortality in nondiabetic rats with LPS-induced sepsis but not in diabetic rats[169], whereas depletion of iNOS resulted in increased dysfunctional mitochondria, IL-

1β production and caspase-1 activation in response to LPS in myeloid cells from both mice and humans and increased NLRP3 inflammasome-mediated cytokine production and mortality in mice with LPS-induced sepsis, which was prevented by NLRP3 deficiency[170]. Although treatment with methylene blue that has the ability to scavenge NO and to inhibit NO synthase showed a transient and reproducible beneficial effect on systemic vascular resistance, arterial pressure and organ function in patients with septic shock, but its effect on mortality remains unknown[171,172].

Aging

Aging is accompanied by complex structural and functional modifications of the vasculature, leading to dysfunction of both the endothelium and smooth muscle cells. Changes in aged smooth muscle cells are characterized by changed migration, proliferative and apoptotic behavior, increased response to vasoconstrictors and decreased expression of Ca^{2+}-activated K^+ channels in coronary arteries[173,174]. Aged endothelial cells are associated with decreased NO synthesis and sensitivity to agonist and mechanic stimuli that promote eNOS expression but increased sensitivity to be apoptotic[175,176]. Loss of PI3K/Akt-dependent eNOS phosphorylation seems to be a main mechanism explaining the reduction in NO production in old rats[94]. In addition, aging of endothelial cells is associated with increased production of vasoconstrictor prostanoids, endothelin-1 and ROS[176-178]. ROS are mainly produced by mitochondrial respiratory chain and NADPH oxidases, although eNOS uncoupling my also contribute to increased ROS during aging[179].

METHODS FOR MEASURING ENDOTHELIAL DYSFUNCTION

In animals, endothelial dysfunction can be measured by examining vasodilator responses to endothelium-dependent substances such as acetylcholine, bradykinin and serotonin in comparison with responses to endothelium-independent molecules such as NO donor in the absence and presence of NOS inhibitor and COX inhibitor *in vivo*[180,181] and in isolated vessels[19,182].

The methods used in clinical practice to measure endothelial dysfunction are detailed elsewhere[183]. This includes invasive methods by using quantitative angiography and intracoronary Doppler wire within coronary circulation and non-invasive methods, including venous occlusion plethysmography to measure forearm blood flow, flow-mediated dilatation in brachial artery, and peripheral arterial tonometry measuring pulsatile volume changes in the distal digit[183].

In addition, some circulating biomarkers such as endothelin-1, E-selectin, von Willebrand factor, thrombomodulin, intercellular adhesion molecules and vascular cell adhesion molecules can also be analyzed to detect endothelial dysfunction, although none of them are specific[183].

CARDIOVASCULAR DRUGS IMPROVING ENDOTHELIAL FUNCTION

Experimental and clinical studies have shown that numerous currently used or investigational drugs can improve endothelial function, although they have different structure and mechanisms of actions.

ACE inhibitors and AT1 blockers

Since the success of ACE inhibitors in the treatment of heart failure and discovery of their multiple actions, ACE inhibitors and AT1 blockers are widely used to the treatment of hypertension, arthrosclerosis, diabetes and some autoimmune diseases. It is well established that ACE inhibitors can improve endothelial function in animals with heart failure[184] and in patients with coronary artery disease[185,186]. This effect is related to both reduction in angiotensin Ⅱ and increase in bradykinin accumulation. In addition, ACE inhibitors upregulate eNOS expression in animals[102,187]. The effect of ACE inhibitors on eNOS expression is mediated by bradykinin B2 receptors, which can be blocked by B2 receptors blockers[102,187]. ACE inhibitors and AT1 blockers also inhibit ROS production and COX-2-derived vasoconstrictors, which contribute to endothelial protective effects of these drugs[188]. It appears that the combination of both ACE inhibitor and AT1 blocker does not produce more beneficial effects on endothelial dysfunction than monotherapy in a murine model of atheroclerosis[189], whereas the combination of a stain with an ACE inhibitor or an AT1 blocker produces additive effects on systemic inflammation biomarkers[190]. Also, the combination of ramilapril with felodipine, an calcium channel blocker does not induce more effect on endothelium-dependent vasodilation than each drug alone but increases endothelium-independent vasodilation in spontaneous hypertensive rats[191].

Antioxidant agents

Several substances having very different molecular structure and proprieties, such as vitamin C and E, N-acetylcysteine and genistein exert antioxidant effects through different mechanisms.

Vitamin C can improve endothelium-dependent response in circumstances such as chronic smoking, diabetes mellitus, hypercholesterolemia and hypertension[136,192-195]. Vitamin C protects the endothelium by scavenging superoxide, which in turn prevents NO scavenging, lipid peroxidation, platelet and neutrophil activation, and adhesion molecule upregulation[136,196]. Vitamin C scavenges peroxidase-generated reactive nitrogen species and inhibits myeloperoxidase/H_2O_2/nitrite-mediated LDL oxidation[197]. Vitamin E also exerts endothelial-protective effects in smoking and hypercholesterolemia[194,198] but its effects in diabetes remains controversial[199,200]. Vitamin E acts as a lipid

soluble antioxidant, scavenging hydroperoxyl radicals in lipid milieu[201].

N-acetylcysteine is a non-essential amino acid, essentially used in the treatment of cough. However, experimental studies have demonstrated that N-acetylcysteine is a potent antioxidant. It acts on the production of glutathione, which protects the cardiovascular system from harmful effects of TNF-α that induces glutathione depletion and ROS production *via* NADPH oxidase and ceramide[202-206]. For example, N-acetylcysteine improves coronary and peripheral vascular endothelium-dependent responses in patients with or without atherosclerosis[203]. The effect of N-acetylcysteine on endothelial dysfunction is associated with inhibition of NADPH oxidase expression, leukocyte adhesion and inflammatory cytokine secretion[204]. In addition, N-acetylcysteine inhibits von Willebrand factor dependent platelet aggregation and collagen binding in human plasma and in mice[207], attenuates MMPs expression in microvascular endothelial cells and in rats[202,208], and inhibits caveolin-1 upregulation and improves endothelial barrier function in mice[209], which may also contribute to the endothelial protective effect of N-acetylcysteine. N-acetylcysteine interacts with endogenous and exogeneous vasodilators. For example, in patients with systemic sclerosis, N-acetylcysteine induces vasodilation in association with a reduction in plasma AM concentrations[210] and potentiates hypotensive effects of ACE inhibitors in hypertensive patients[211].

Genistein is a soya-derived phytoestrogen and exerts an antioxidant effect. Genistein attenuates endothelial dysfunction in hypertensive rats and hyperhomocysteinemic rats. This endothelial protective effect appears to be due to increases in eNOS activity and expression and decreases in cytokine and ROS generation[212-215]. Genistein also improves endothelium-dependent vasodilator response in healthy postmenopausal women, increases plasma nitrite/nitrate concentration but decreases plasma endothelin-1 levels[216]. In this regard, genistein may be useful for the treatment of endothelial dysfunction associated with atherosclerosis and hypertension.

Beta blockers

Some beta blockers, particularly the β1-selective beta blockers exert endothelial protective effects. Nebivolol, a β1-antagonist with β2,3-agonist property, improves endothelium-dependent vasodilator responses in patients with essential hypertension[217,218] and in smokers[219]. Nebivolol also improves endothelial function, which is associated with reduced vascular remodeling and expression of endothelin-1 and cytokines in rats with pulmonary hypertension and in endothelial cells taken from these rats[220]. The effect of Nebivolol on endothelial function appears to be mediated by increasing NO release and reducing prothrombotic blood levels of fibrinogen, homocysteine and plasminogen activator inhibitor-1, especially in smokers[218,219]. Carvedilol, a non-selective β1- and β2 antagonist with α-antagonist property, also improves endothelium-dependent responses in patients with essential hypertension but this seems to be related to its antioxidant capacity[218]. The combination of carvedilol with an ACE inhibitor produces more beneficial effect on endothelial function than each drug alone in hypertensive patients with obesity[221]. Thus, this type of beta blockers and its combination are suitable for the treatment of endothelial dysfunction associated with hypertension, atherosclerosis, and probably diabetes.

Dihydropyridine calcium channel blockers

Nicardipine and nifedipine protects against ROS-induced endothelial cell death and lose of glutathione in cultured cells[222]. Benidipine exerts an endothelial protective effect against OxLDL induced ROX generation in human endothelial cells[223]. Israpidine improves endothelial function in cholesterol-fed rabbit[224]. Thus, the endothelial protective effect of dihydropyridine calcium channel blockers is mainly mediated by their antioxidant actions related to reduction in lipid peroxidation and associated ROS generation[222,225]. In addition, some dihydropyridines such as, amlodipine, azelnidipine and nifedipine were shown to exert an antiinflammatory action as indicated by decreased C-reactive protein and interleukin-6 levels as well as leucocyte activation[226,227]. Amlodipine or in combination with an renin inhibitor improves endothelial dysfunction in hypertensive patients, which seems to be linked to its NO-releasing action and anti-inflammatory effect[181,228-230]. In addition, the combination of amlodipine with a statin induces more favorable vascular effects than each drug alone in rats with hypertension or diabetes[231,232]. Thus, in addition to hypertension, dihydropyridines may also be useful for the treatment of endothelial dysfunction in diabetes.

Phosphodiesterase-5 inhibitors

Phosphodiesterase-5 (PDE5) is a cytosolic enzyme localized in vascular smooth muscle, heart, skeletal muscle, platelet, placenta, brain, kidney, liver, pancreas, gastrointestinal tissues and lung[233]. In vasculature, the primary action of PDE5 is to degrade cGMP and thereby induces vasoconstriction. PDE5 inhibitors are a class of drugs used to improve erectile dysfunction. These drugs block PDE5-induced cGMP degradation, leading to tissue cGMP accumulation and vasodilation[234]. PDE5 inhibitors upregulate eNOS expression and thereby increase NO release[235,236], which may contribute to long-term vasodilator effects of PDE5 inhibitors. PDE5 inhibitors also exert other initially unexpected effects. For example, in mouse hind limb ischemia model, treatment with sildenafil not only improves blood flow recovery but also increases capillary density and endothelial progenitor cell mobilization[237]. In patients with vasculogenic erectile dysfunction, daily treatment with vardenafi reduces both arterial stiffness and plasma AM level[238]. These effects may also account

for the effects of chronic PDE5 inhibition. In addition to erectile dysfunction, PDE5 inhibitors can improve endothelial dysfunction in other circumstances. For example, PDE5 inhibition improves coronary and peripheral vascular endothelial function, and inhibits platelet activation in patients with coronary artery disease[239] or with congestive heart failure[240-242], and improves endothelium-dependent vasorelaxation in rats with experimental diabetes mellitus[243]. PDE5 inhibitors also improves erectile function in patients with systemic sclerosis and reduces plasma endothelin-1 concentration[244]. Similarly, PDE5 inhibitors improve Raynaud's phenomenon characterized by reduced blood flow to fingers and toes in response to cold and stress, probably through decreasing plasma endothelin-1 and improving microcirculation[245]. However, the mechanism underlying endothelin-1-reducing effect of PDE5 inhibitors remains to be determined.

Statins

Statins, inhibitors of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase are a class of drugs utilized to reduce hypercholesterolemia, especially LDL cholesterol. In 1994, pravastatin was shown to improve endothelium-dependent response of coronary and peripheral arteries in patients with hypercholesterolemia[246], which was confirmed later by other studies[247]. The beneficial effect of statins on endothelial function involves multiple mechanisms. Statins improving endothelial dysfunction is partly due to their lowering LDL cholesterol effect, while native LDL and OxLDL reduce eNOS expression[248,249] and increase levels of caveolin-1[250]. Statins also exert direct antioxidant effects on LDL to reduce electronegative form of LDL[251,252]. Statins increase NO bioavailability by activating eNOS *via* the PI3K/Akt signaling pathway[253], agonist-stimulated eNOS-hsp90 interaction[250], and BH4-mediated eNOS coupling. This latter was demonstrated in patients with atherosclerosis[254] and in rat model of insulin resistance of diabetes[231]. These studies showed that atorvastatin increased vascular BH_4 content and NO bioavailability and reduced O_2^- production *via* upregulating GTP-cyclohydrolase Ⅰ gene expression and activity. These effects occurred rapidly in patients with atherosclerosis and could be reversed by mevalonate, indicating a direct effect of vascular HMG-CoA reductase inhibition[254]. In addition, statins upregulate eNOS expression through enhancing eNOS mRNA stability. Indeed, statins increase eNOS mRNA polyadenylation through Rho-mediated changes in the actin cytoskeleton[255,256]. However, a study showed that statins can increase eNOS gene transcription by upregulating Kruppel-like factor 2 through inhibition of Rho pathway[257]. The effect of statins on eNOS expression may account for the long-term effect of stains on endothelial function. Statins also exerts antiinflammatory effects[258]. For example, atorvastatin treatment reduces proinflammatory cytokines (TNF-α, IL-1 and IL-6), intercellular adhesion molecules and C-reactive protein blood levels in hypercholesterolemic patients[259], while rapid withdrawal of statin treatment increases proinflammatory and prothrombotic biomarkers[260]. Statins were also shown vascular benefice in other inflammatory diseases such as rheumatoid arthritis[261]. Otherwise, statins increase circulating endothelial progenitor cells, likely through the PI3K/Akt pathway[262], which could contribute to long-term effects of statins on endothelial function.

Anther type of LDL-lowering drugs, the proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, may be expected to improve endothelial function. In humans, PCSK9 mutation is closely correlated with LDL cholesterol levels and inhibition of PSCK9 with a monoclonal antibody reduces LDL cholesterol levels[263] and enhances the LDL cholesterol-lowering effect of atorvastatin[264]. Studies in cells and animals have shown that PCSK9 is associated with inflammation and endothelial cell apoptosis. In mice, systolic inflammation and OxLDL upregulate PCSK9, whereas PCSK9 interacts with macrophage, leading to NF-κB activation[265,266]. Knockdown of PCSK9 with PCSK9 siRNA or induction of gain of function mutant D374Y-PCSK9 reduces expression of stress-response genes and specific inflammation pathways, inflammation pathway activation and OxLDL-induced endothelial apoptosis[266-268]. Nonetheless, the effects of PCSK9 inhibition on human endothelial function are not yet explored.

Angiotensin-(1-7)

Angiotensin-(1-7) is a metabolite of angiotensin I under the action of various enzymes, including neutral endopeptidase, prolylendopeptidase, aminopeptidase A and neprilysin[36]. It can also be generated from angiotensin Ⅱ by prolylcarboxypeptidase[269] and carboxypeptidase (ACE2)[270]. In endothelial cells, angiotensin-(1-7) activates eNOS *via* the Mas/PI3K/Akt pathway and inhibits angiotensin Ⅱ-induced NAD(P)H oxidase activation[271,272]. Chronic treatment with angiotensin-(1-7) improves renal endothelial dysfunction associated with apolipoprotein E-deficiency[273] and diet-induced obesity in mice[274], which is likely mediated by increasing NO release[275] and eNOS expression[276,277]. Otherwise, angiotensin-(1-7) restores vascular ACE2-angiotensin-(1-7)-Mas receptor axis function that impairs ROS production by angiotensin AT1 receptor-activated NAD(P)H oxidases in hypertensive or diabetic rats[278,279]. Angiotensin-(1-7) restores NO/cGMP production and migration, decreases NADPH oxidase activity, and enhances survival and proliferation of endothelial progenitor cells isolated from the blood of diabetic patients in a Mas/PI3K/Akt-dependent manner[280]. Interestingly, overexpression of angiotensin-(1-7) gene restores the vasoreparative function of endothelial progenitor cells in mice[280]. Despite these encouraging results in cells and in animals, the information regarding the effects of angiotensin-(1-7) on human endothelial function

remains lacking.

Bradykinin

As discussed above, endogenous bradykinin exerts multiple actions that affect endothelial function. It is worth noting that bradykinin as an investigational drug protects against ROS- and toxin-induced microvascular endothelial cell death[281], and chronic treatment with bradykinin not only preserves eNOS expression in dogs with pacing-induced heart failure[101], but also upregulates eNOS and nNOS expression in vessels and in the heart of dogs with dystrophin-deficiency cardiomyopathy[19,282]. However, due to the very short half-life and implication of bradykinin in the inflammation[283] and cancers[284,285], the clinical use of bradykinin remains a challenge.

eNOS transcription enhancer

Interestingly, specific targeting eNOS transcription with a chemical compound, AVE3085, increases eNOS expression but reduces oxidative stress and platelet activation, which is associated with improved endothelium-dependent relaxation and cardiac function in animals with different experimental diseases[286-289]. This compound also prevents the inhibitory effect of ADMA on endothelium-dependent vasodilation in human internal thoracic artery rings and in pig coronary artery rings[290,291]. Thus, this compound showed a potential for the treatment of endothelial dysfunction although its effects in human clinical situations remains to be demonstrated.

I_f inhibitor, ivabradine

I_f current is an inward current carried by Na^+ and K^+, activated by hyperpolarization and conducted by hyperpolarization-activated cyclic nucleotide-gated channels (f-channels)[292]. I_f current participates in the spontaneous depolarization during Phase 4 of the action potential and plays a crucial role in the pacemaker activity of pacemaker cells located in the sinus node and atrioventricular node. Inhibition of this current by ivabradine slows down heart rate and exerts cardioprotective effects[293-296], which may involves pleiotropic actions of ivabradine[297]. Among them, beneficial effects of ivabradine on the endothelium-dependent vasodilation and on the expression of eNOS expression in both animals and humans have been reported[298-300]. Nevertheless, the effects of ivabradine on human endothelial dysfunction are controversial. Several studies did not observe significant improvement in flow-mediated vasodilation by ivabradine in patients with microvascular angina pectoris[301] or stable coronary heart disease[302] and in patients with type Ⅱ diabetes[303]. In addition, in patients with stable of coronary disease without heart failure, the additional ivabradine plus standard treatment did not improve outcome but was associated with increased frequency of atrial fibrillation, questioning the utility of this drug in the treatment of stable coronary disease[304].

Sphingosine-1-phosphate

Sphingosine-1-phosphate (S1P), a signaling sphingolipid formed by sphingosine kinase in the blood and in tissues, regulates different biological responses such as angiogenesis, vascular permeability and trafficking of T- and B-cells. S1P enhances endothelial barrier function[305,306], stimulates endothelial NO release through Akt-mediated phosphorylation of eNOS[307], and reconstitutes high density lipoproteins[308]. S1P also has antiinflammatory properties and exerts protective effect against endotoxin-induced lung injury[309,310]. Moreover, S1P exhibits a potent effect on the differentiation of adipose-derived stem cells into endothelial-like cells and upregulation of eNOS in these cells[311]. All of these properties of S1P may contribute to its endothelial protective effects. Interestingly, an orally active of S1P analogue, FTY720 also shows similar effects[312]. Thus, S1P and analogues may be used to improve endothelial function, especially in atherosclerosis and acute lung injury where presents an impairment of endothelial barrier function[313].

CONCLUSION

Endothelial dysfunction is a common mechanism involved in many cardiovascular diseases, although in some diseases such as atherosclerosis, endothelial dysfunction plays a critical role in the development of diseases, whereas in others such as essential hypertension and type Ⅱ diabetes, endothelial dysfunction generally occurs as a complication but thereafter contributes to the development and progression of organ damages. Clearly, multiple mechanisms such as inflammation, increased ROS and RNS, cellular apoptosis, increased vasoconstrictor production, decreased vasodilator production and vascular remodeling are involved in endothelial dysfunction and a specific pathology may involve more or less them as described above. However, a decreased NO bioavailability appears to play a central role because in many pathologies such as atherosclerosis, diabetes, essential and pulmonary hypertension and heart failure except for septic shock where there is a overproduction of NO, a reduction in NO bioavailability occurs sooner or later in response to different risk factors. This may explain the beneficial effects of some drugs in the treatment of a variety of cardiovascular disorders. It appears that a drug with endothelium-protective property may yield more therapeutic benefits than that without such feature. For this reason, the evaluation of endothelium-improving action may be helpful for the development of a novel cardiovascular drug. Moreover, due to the differences in risk factors contributing to the different cardiovascular diseases and the differences in mechanisms of action, treatment of endothelial dysfunction with drugs needs to be carried out according to specific mechanisms

underlying endothelial dysfunction of the disease.

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REVIEW

Therapeutic modification of arterial stiffness: An update and comprehensive review

Ching-Fen Wu, Pang-Yen Liu, Tsung-Jui Wu, Yuan Hung, Shih-Ping Yang, Gen-Min Lin

Ching-Fen Wu, Department of Internal Medicine, Mennonite Christian Hospital, Hualien 97144, Taiwan

Pang-Yen Liu, Yuan Hung, Shih-Ping Yang, Gen-Min Lin, Division of Cardiology, Department of Internal Medicine, Tri-Service General Hospital, and National Defense Medical Center, Taipei 114, Taiwan

Tsung-Jui Wu, Gen-Min Lin, Department of Medicine, Hualien-Armed Forces General Hospital, Hualien 97144, Taiwan

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Correspondence to: Gen-Min Lin, MD, MPH, Department of Medicine, Hualien-Armed Forces General Hospital, No. 163, Jiali Rd. Xincheng Township, Hualien 97144, Taiwan. farmer507@yahoo.com.tw
Telephone: +886-340-38260601
Fax: +886-340-38261370

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Abstract

Arterial stiffness has been recognized as a marker of cardiovascular disease and associated with long-term worse clinical outcomes in several populations. Age, hypertension, smoking, and dyslipidemia, known as traditional vascular risk factors, as well as diabetes, obesity, and systemic inflammation lead to both atherosclerosis and arterial stiffness. Targeting multiple modifiable risk factors has become the main therapeutic strategy to improve arterial stiffness in patients at high cardiovascular risk. Additionally to life style modifications, long-term ω-3 fatty acids (fish oil) supplementation in diet may improve arterial stiffness in the population with hypertension or metabolic syndrome. Pharmacological treatment such as renin-angiotensin-aldosterone system antagonists, metformin, and 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors were useful in individuals with hypertension and diabetes. In obese population with obstructive sleep apnea, weight reduction, aerobic exercise, and continuous positive airway pressure treatment may also improve arterial stiffness. In the populations with chronic inflammatory disease such as rheumatoid arthritis, a use of antibodies against tumor necrosis factor-alpha could work effectively. Other therapeutic options such as renal sympathetic nerve denervation for patients with resistant hypertension are investigated in many ongoing clinical trials. Therefore our comprehensive review provides knowledge in detail regarding many aspects of pathogenesis, measurement, and management of arterial stiffness in several populations, which would be helpful for physicians to make clinical decision.

Key words: Arterial stiffness; Cardio-ankle vascular index; Pulse-wave velocity; Renin-angiotensin-aldosterone system antagonist

Core tip: Arterial stiffness has been recognized as

a marker of cardiovascular disease and associated with long-term worse clinical outcomes in several populations. Age, hypertension, smoking, and dyslipidemia, known as traditional vascular risk factors, as well as diabetes, obesity, and systemic inflammation lead to both atherosclerosis and arterial stiffness. Targeting multiple modifiable risk factors has become the main therapeutic strategy to improve arterial stiffness in patients at high cardiovascular risk.

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INTRODUCTION

Arteries provide not only blood flow conduits from the heart to peripheral organs, but also play a major role in hemodynamic cushioning, buffering the forward propagating flow from the heart, and the backward resistance by the peripheral arterioles, which maximize cardiovascular efficiency. Arterial stiffness characterized by higher intravascular distending pressure has been recognized as a marker of cardiovascular disease (CVD) and associated with long-term prognosis in several populations[1-4]. A recent meta-analysis including 17 longitudinal studies demonstrated that aortic stiffness was an independent predictor of incident CVD and all-cause mortality in the general population[4]. Therefore, evidence-based approaches for improving arterial stiffness are of clinical importance to reduce the hazards of subsequent CVD. This review article will discuss the latest knowledge of the pathological backgrounds, the measurements, and the effects of pharmacological and non-pharmacological interventions for arterial stiffness.

The pathophysiology of arterial stiffness

As a major component of the circulatory system, the arterial system can be functionally and structurally divided into two sub-systems: (1) the large elastic, conducting arteries (*e.g.*, the aorta, the carotid arteries, and the iliac arteries), which store blood ejected from the heart during systole, and expel blood to the peripheral tissues during diastole, thereby ensuring a steady blood flow irrespective of cardiac cycles or concurrent blood pressure; (2) resistance muscular arteries, especially those of the lower limb (*e.g.*, femoral, popliteal, and posterior tibial arteries), which are capable of altering vascular smooth muscle tone, allowing them to modulate the velocity of pressure wave that is conducted to the resistance muscular arteries from the central aorta[5]. The sites of aortic flow reflection are not simply anatomically determined, but also subjected to systemically structural and functional control. For example, the site of reflection is more central in the case of hypertension, atheromatous arteries or increased sympathetic activity[6].

The pressure waveform recorded at any site of aorta is the summation of the forward-traveling waveform generated by cardiac pumping force and the backward traveling wave, the "echo" wave reflected at peripheral sites. The summation result determines the cardiac afterload during systolic phase and the augmented backward coronary perfusion pressure during diastolic phase. When the arteries are compliant and elastic, the reflected wave merges with the incident propagating wave during diastole, thus augmenting the diastolic blood pressure and enhancing coronary perfusion[7]. On the contrary, when arteries are stiffer, pulse wave velocity increases, and both the incident and the reflected wave travel faster; therefore, the reflected wave merges with the incident wave at systole and increase systolic pressure and cardiac afterload, while, concomitantly, losing the augmented diastolic perfusion pressure[7] (Figure 1). The added part on systolic pressure and cardiac afterload was named aortic augmentation index [AIx, (second/first systolic peak) $\times$ 100%][8]. In the long term, increasing pulsatility causes stretching of load-bearing elastic lamellae and mechanical stress on the wall leading to vascular structural changes and stiffening. Hence, the harm of arterial stiffness is two-sided, negatively affecting the heart and blood vessels[9] (Figure 2).

Factors affecting arterial stiffness

Age is a main determinant of stiffness in large elastic arteries[7,10]. The stiffness of these arteries increases significantly after the age of 55 years. Aging causes the degeneration and remodeling of elastic components of arterial wall. At the cellular-molecular level, an age-related decrease in intra-cellular magnesium concentration is associated with increases in stiffness[10].

Most traditional cardiovascular risk factors and CVD have an adverse effect on arterial stiffness, *via* endothelial dysfunction and adverse vascular remodeling. Hypertension, diabetes, dyslipidemia, and insulin resistance, which contribute to atherosclerosis, have been involved in the process of arterial stiffening. In essential hypertension, the elastic properties of large arteries are impaired, although it is not clear whether the disease itself alters the intrinsic elastic properties or this is the ultimate final effect of increase in distending pressure[11,12]. Distending pressure as estimated by 24-h pulse pressure was another major factor additionally to older age contributing to the occurrence of arterial stiffness[13]. In patients with diabetes or metabolic syndrome, arterial stiffening is consistently observed across all age groups, even in childhood[14]. Insulin resistance is dose-dependent and positively correlated with arterial stiffness[15-17]. Chronic hyperglycemia and hyper-insulinemia may increase local activity of renin-angiotensin-aldosterone system (RAAS) and expression of angiotensin type I receptor in vascular tissue and thus promote the development of arterial wall hypertrophy

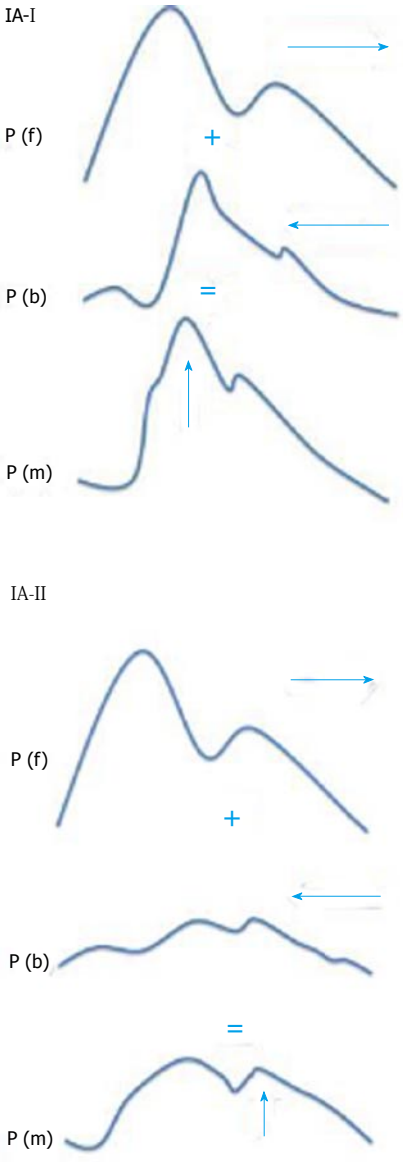

Figure 1 The central aortic pressure waveform is the summation of forward travelling wave, P (f) and the reflected backward-travelling wave, P (b). On the top graph IA-I, is an illustration of a stiff aorta or peripheral vasoconstriction, both P (f) and P (b) travel fast and the magnitude of the reflected wave is increased, thus augmenting the systolic pressure of summated central aortic pressure waveform, P (m). In graph IA-II, is another illustration of a distensible aorta or with vasodilatation. Length and thickness of horizontal arrows correspond to the waveform velocity and the magnitude of the reflected wave, respectively. Vertical arrows indicate point of merging of P (f) and P (b).

and fibrosis[18,19].

In addition hyperinsulinemia has proliferative effects, *via* unbalanced activities on growth-promoting mitogen activated kinase pathways and PI3-kinase-dependent signaling[20]. In pre-diabetic stage, impaired glucose tolerance enhances nonenzymatic glycation of proteins with covalent cross-linking of collagen and alters the mechanical properties of interstitial tissue of arterial wall[21,22].

Chronic kidney disease (CKD) is a well-known risk factor of arterial stiffness[23]. Several mechanisms have been proposed to explain the effect of CKD. For instance, upregulation of matrix metalloproteinases enhances collagen and elastin turnover through enzymatic cross-link degradation[24], causing weakening of the extracellular matrix[25]. Accumulation of advanced glycation end-products makes collagen stiffer as well[26]. In addition, CKD may cause endothelial dysfunction, which attributes to high oxidative stress, increased endothelin-1 concentrations and impairment of endothelial nitric oxide synthase and arterial relaxation[27]. Chronic inflammation and RAAS activation are also involved in the process of arterial stiffening in CKD[28,29]. CKD alters bone metabolism to promote vascular calcification by increasing osteoclast activity, fibroblast growth factor 23, osteoprotegerin which inhibit bone morphogenic proteins, and reducing pyrophosphate, Matrix G1a protein, and fetuin A levels[30].

Arterial elastic properties are impaired in young people with a family history of hypertension, diabetes or myocardial infarction[31]. It has been recognized that genetic factors may contribute to arterial stiffening as well. The latest advances in genome-wide association study have identified that some genetic variants and specific polymorphisms may affect arterial stiffness. The Framingham Heart Study showed that four regions of suggestive linkage were found in chromosomes 2, 7, 13, and 15 (LOD scores 2.0) for higher risk of arterial stiffness[32]. Potential candidate genes in these regions included the insulin-like growth factor-1 receptor, myocyte-specific enhancer factor 2A, chondroitin synthase (CHSY1), proprotein convertases (PACE4 and FURIN), b-adducin (ADD2), neurokinin-1 receptor (TACR1), a-2B adrenergic receptor (ADRA2B), and interleukin-6 (IL-6). Other candidate gene polymorphism, such as the renin-angiotensin-aldosterone genes, the Matrix and metalloproteinase genes, the endothelial cell-related genes, and the inflammatory genes, are all in undergoing investigations[33].

Lifestyle characteristics are important determinants of arterial stiffness. Cigarette smoking, including passive smoking and current smoking has an adverse impact on the arterial stiffness[34-36]. Elevated arterial stiffness has been found among patients with chronic obstructive pulmonary disease and inflammation, which are highly related to the adverse effect of smoking. Obesity, weight gain, lack of physical activity and high dietary intake of sodium chloride, which is associated with blood pressure elevation, can aggravate arterial stiffness[37-40]. Intake of caffeine, a neurotoxin has also been acknowledged of an unfavorable effect on arterial compliance[40]. Other risk factors such as chronic cytomegalovirus infection, has been known as a novel potential contributor to arterial stiffening[41]. Table 1 lists the main demographic, clinical and lifestyle characteristics that may influence arterial stiffness.

Measurement of arterial stiffness

A stiffer vessel will conduct the pulse wave faster than a

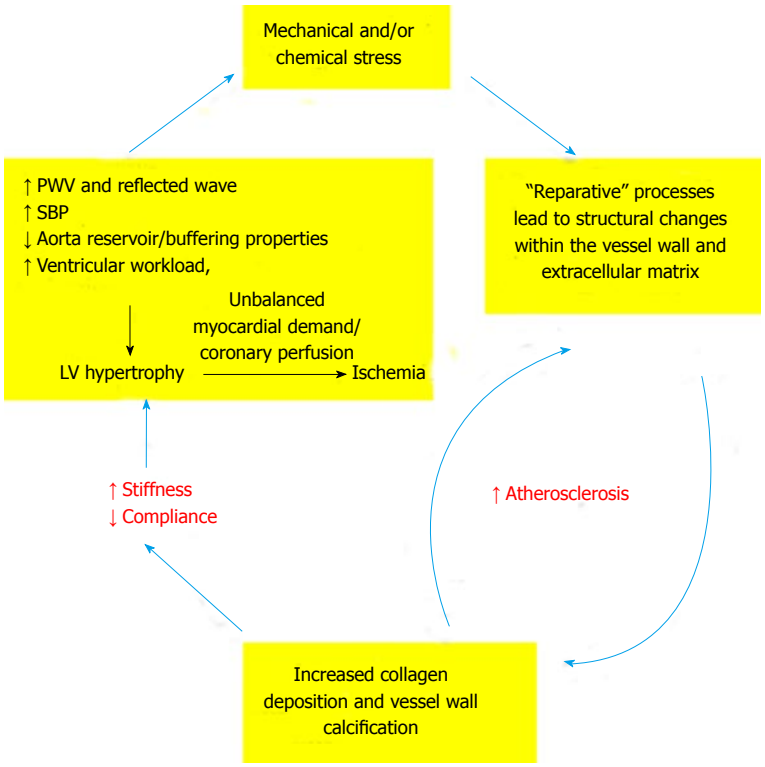

Figure 2 Aortic elastic properties may be altered by several processes, resulting in increased stiffness, decreased compliance, and encompassing the diseased ventricular-arterial coupling. Mechanical and chemical stress factors include hypertension, inflammation, advanced glycation end products, *etc.* LV: Ventricular; PWV: Pulse wave velocity; SBP: Systolic blood pressure.

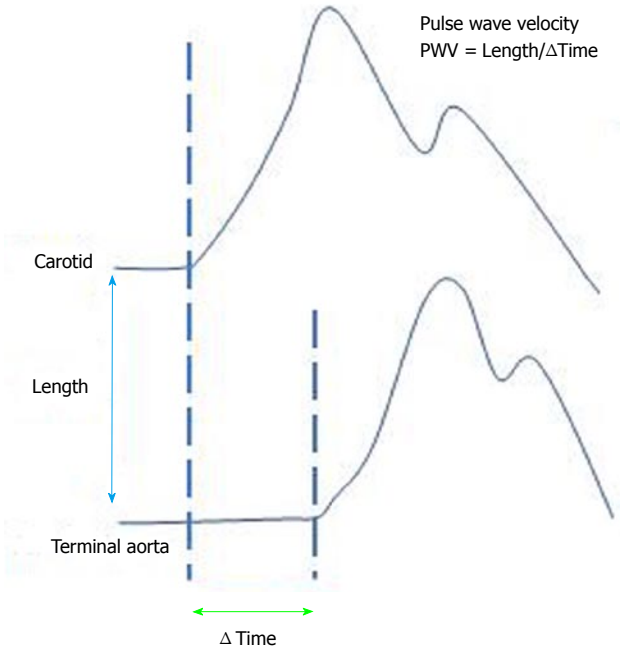

Figure 3 For practical purpose, femoral artery is counted as the terminal aorta. The measured distance is length. If ΔTime represents the time delay between the feet of the 2 waves, pulse wave velocity.

Table 1 Demographic, clinical, and lifestyle factors associated with arterial stiffness

Age[7]
Sex[110]
Established cardiovascular disease[3]
Potential risk factors for atherosclerosis
Hypertension[11]
Dyslipidemia[2]
Cigarette smoking[63]
Chronic obstructive pulmonary disease[111]
Diabetes[14]
Obesity[3]
Obstructive sleep apnea[106]
Menopause[110]
Polycystic ovarian syndrome[112]
Hypothyroidism[113]
Chronic kidney disease[23]
Endothelial dysfunction[27]
Systemic inflammation[99]
Cytomegalovirus infection[114]
Nutritional and lifestyle aspects
Caffeine[115]
Chronic alcohol consumption[116]
Sedentary lifestyle[58]
Resistance exercise training[61]
Genes variants
Genes of the Renin-Angiotensin-Aldosterone system[33]
Genes of the extracellular matrix proteins[33]

more distensible and compliant vessel. Arterial stiffness can be noninvasively evaluated by measuring pulse-wave velocity (PWV). The PWV is calculated by the distance (L) between the 2 vascular sites divided by the wave foot-to-foot time (ΔT) it takes for that forward wave to reach the end measuring point (Figure 3) Currently, PWV is the most validated measurement to noninvasively quantify arterial stiffness. It is considered the gold standard index to measure arterial stiffness, given its simplicity, reproducibility, accuracy, and strong prediction of adverse CVD events[42-44]. An increase in aortic PWV by 1 m/s corresponds to an age-, sex-, and risk factor-adjusted risk increase of 14%, 15% and 15% in total CVD events, CVD mortality, and all-cause mortality, respectively[5]. Nowadays, two kinds

of PWV were frequently used to evaluate arterial stiffness. Carotid-femoral PWV (cfPWV) measured by Doppler ultrasound is the most widely used measure of aortic stiffness and is regarded as the gold standard measure for evaluating arterial stiffness. Alternatively, brachial-ankle PWV (baPWV) measured by the Omron oscillometric/plethysmographic system has recently received attention because of its consistent association with CVD risk factors and its ease of use for large-scale population studies[42-44]. Based on the formula assumptions, cfPWV reflects the stiffness of descending aorta, while baPWV reflects the stiffness of both descending aorta and leg arteries. In a study conducted among healthy men aged 40-49, cfPWV strongly correlated with central PWV, and baPWV correlated with both central and peripheral PWVs[45]. The two indexes were highly correlated and the predictive values of these two PWVs were comparable[46]. Both cfPWV and baPWV have been reported to be independent predictors of subclinical coronary artery calcification, incident vascular events, incident heart failure, and all-cause mortality in the general population[47,48]. The main disadvantage of cfPWV is inevitably affected by blood pressure, which is an important confounder for CVD. In addition, cfPWV is often overestimated for the inaccurate measurement in the distance between the carotid and the femoral to measure the pulse wave[49]. Other methods for the PWV measurements include single-point, carotid–radial or femoral–tibial arterial segments. The predictive values of these more peripheral PWV measurements to incident vascular events remain unknown[50]. Aortic characteristic impedance standing for the minimal impedance for higher frequencies of pressure-and-flow harmonics and being proportional to PWV is an indirect technique, but this is rarely used alone now[51]. AIx, arterial wave reflection magnitude [(reflected/forward wave amplitude) × 100%], and pulse pressure amplification [(radial/aortic pulse pressure) × 100%], the analysis of pulse waveforms parameters of central arteries, have been associated with the development of end organ damage as well[52].

The stiffness parameter β is another measure of arterial stiffness. The equation for stiffness parameter β is ln(Ps/Pd) × D/ΔD, where Ps is the systolic blood pressure, Pd is the diastolic blood pressure, D is the diameter of the artery, and ΔD is the change in arterial diameter between Ps and Pd[53]. The stiffness parameter β is less affected by blood pressure; however it is limited by assessing a local segment of the artery, and becoming dependent on blood pressure for those with hypotension or moderate and severe hypertension[53]. Therefore, the cardio-ankle vascular index, CAVI, was developed to incorporate the stiffness parameter β[54]. The equation for CAVI is a $[(2\rho/\Delta P) \times \ln(Ps/Pd) \times PWV^2] + b$, where ρ is the blood viscosity, ΔP is Ps - Pd, PWV is the pulse wave velocity from the aortic origin to the ankle region *via* the femoral artery, and a and b are constants for converting a CAVI value to a value obtained by Hasegawa's method[55]. Theoretically, the CAVI is essentially intrinsic to the stiffness parameter β and thus less dependent of blood pressure than PWV. Table 2 summarizes the merits and disadvantages of different measurements of arterial stiffness.

Therapeutic modification of arterial stiffness

Lifestyle modification: Obesity is related to insulin resistance, hypertension, obstructive sleep apnea (OSA), and eventually arterial stiffness. A meta-analysis involving 20 studies (including 3 randomized controlled trials) revealed that modest weight loss (mean 8% of initial body weight) could improve PWV values by 32% in the collected 1259 participants[56]. In addition, weight reduction was found in association with decreased CAVI values in a cohort of 47 obese individuals in Japan[57]. Effects of exercise on arterial stiffness were extensively investigated. Physical activity was associated with 35% reduction in cardiovascular mortality and 33% reduction in all-cause mortality[58]. Almost 60% of the benefits are contributed by the reduction of body weight, blood pressure and serum lipids[59], and the other 40% may be explained by the improvement of vascular hemodynamics including endothelial function, arterial compliance and remodeling[60]. Whether mode and dose of exercise affecting arterial stiffness had been recently reviewed in a meta-analysis[61]. In total, forty-two studies and 1627 participants were included in the study, which concluded aerobic exercise, but not resistant exercise or combined aerobic and resistant exercise, improved PWV weighted mean difference (WMD): -0.63 m/s, 95%CI: -0.90 to -0.35, and AIx (WMD: -2.63%; 95%CI: -5.25 to -0.02). The benefits for improving arterial stiffness were greater in the peripheral index, baPWV (WMD: -1.01 m/s; 95%CI: -1.57 to -0.44) than in central index, cfPWV (WMD: -0.39 m/s; 95%CI: -0.52 to -0.27). There was dose-dependent relationship between exercise intensity (frequency of exercise sessions and absolute exercise intensity) and the improvement of AIx. Nevertheless, the exercise session duration was not significantly associated with the reduction of AIx[61]. In individuals with stiffer arteries (PWV ⩾ 8 m/s), aerobic exercise had a larger effect in reducing PWW. In addition, the benefits of aerobic exercise were documented in subpopulations with normal health, overweight/obese, pre-hypertension, hypertension, or CKD.

Smoking cessation has been proven to decrease aortic stiffness. In one 60 wk follow-up observational study, smoking cessation group had better arterial stiffness indices (central blood pressure, -7.1 ± 1.4 mmHg *vs* 1.2 ± 2.7 mmHg, $P < 0.01$; baPWV, -204 ± 64 cm/s *vs* -43 ± 72 cm/s, $P < 0.01$; reduced radial AIx, -6.4 ± 2.8% *vs* -1.0 ± 3.9%, $P < 0.01$)[62]. Another observational study also showed that smoking cessation was associated with improved arterial stiffness as evaluated by CAVI values[63]. Moreover, avoidance of second-hand smoke, such as workplace smoking bans,

Table 2 A summary of the advantages and disadvantages of different measurements for evaluating arterial stiffness

	Advantage	Disadvantage
cfPWV[42-44]	Reflects the stiffness of the descending aorta The gold standard measure for arterial stiffness	Largely affected by the change of BP Overestimated for the inaccurate measurement in the distance between the carotid and the femoral arteries
baPWV[116]	Reflects the stiffness of both the descending aorta and the leg artery High association with CV risk factors Ease of use for large-scale population studies	Largely affected by the change of BP Underestimates arterial stiffness in hypertensive patients with a history of cardiovascular events
hfPWV[117]	Strongly correlated with cfPWV moderately correlated with baPWV	Require a high level of proficiency in order to obtain accurate results
faPWV[117]	Moderately correlated with baPWV	The predictive value to incident vascular events remains unknown
pAIx[110]	Assessed non-invasively and peripherally, *e.g.*, carotid, and radial arteries Correlated well with the central AIx	Largely affected by the change of BP Not a valid surrogate of arterial compliance in the elderly and diabetic populations
The stiffness parameter β[53,54]	Independent of the change of BP	Assessing only a local segment of the artery Loss of the independence of BP for those with moderate to severe hypertension or hypotension
CAVI[118]	Independent of the change of BP A novel atherosclerotic index that incorporates PWV and BP measurements The coefficients of variation are small (< 4%), and does not require significant training	CAVI, as a cardiovascular risk marker has not to be investigated definitively in large prospective clinical trials

Ba: Brachial-ankle arteries; CAVI: Cardio-ankle vascular index; BP: Blood pressure; cf: Carotid-femoral arteries; fa: Femoral-ankle arteries; hf: Heart-femoral arteries; pAIx: Peripheral augmentation index; PWV: Pulse-wave velocity.

has been reported to improve PWV after introducing smoke-free workplaces[64].

Dietary and nutrient interventions: Several dietary modifications had been reported with beneficial effects on arterial stiffness. Among them, omega (ω)-3 fatty acids (fish oil) supplementation was mostly studied. In most of clinical trials, ω-3 fatty acids supplementation improved arterial stiffness, especially in the population with overweight, metabolic syndrome, diabetes or hypertension[65]. Aside from a study with acute ω-3 fatty acids administration in healthy participants, almost all ω-3 trials were long-term prescribed varying from 1.5 to 25 mo. In this acute fish-oil supplementation study, there were no immediate reductions in parameters of arterial stiffness[66]. The lowest daily dosage of long-chain polyunsaturated fatty acids (PUFAs) that documented an effect on arterial stiffness was 540 mg eicosapentaenoic acid (EPA) along with 360 mg docosahexaenoic acid (DHA) in overweight patients with hypertension[67]. Sjoberg *et al*[68] introduced 2, 4, and 6 g of fish oil supplementation per day into the diets of overweight or obese adults for 12 wk. Only the highest dose group (6 g of fish oil per day) revealed significant improvement in arterial distensibility, as measured by PWV. Among healthy subjects, Chong *et al*[69] reported a significant improvement in PWV and AIx immediately after a long chain ω-3 PUFA-rich meal containing 4.7 g of DHA and EPA. In a randomized controlled trial in Japan, highly purified EPA administration (1.8 g/d for 3 mo) significantly reduced both PWV and CAVI values in individuals with metabolic syndrome[70]. However, other two studies using smaller amount (1.7 g of EPA/ DHA per day for 12 wk and 1.8 g of EPA/ DHA per day for 12 mo) did not improve arterial stiffness among slightly overweight but relatively healthy subjects[71,72]. Accordingly, the benefits from ω-3 supplementation could be more evident using a comparable dose over a greater duration within an older age, more diseased populations.

Soy isoflavones was another nutrient, which has been studied frequently. Among five soy isoflavone interventional studies, four interventional studies showed an improvement in PWV or systemic arterial compliance in subjects taking soy isoflavone relative to their placebos[73-76], whereas one study reported no effect[77]. Notably, the majority of the soy interventions were conducted in postmenopausal women. In other studies with positive results, one study reported that consumption of alcoholic red wine might decrease AIx acutely relative to that after consumption of dealcoholized red wine[78], and a study showed that consumption of black tea flavonoids could reduce the digital volume pulse-stiffness index but not PWV[79]. Other dietary and nutritional interventions, nonetheless, reported no definite effect on arterial stiffness, such as garlic[80], conjugated linoleic acid[81], vitamins or folic acid[82-86] on PWV.

Among the minerals, salt plays a detrimental role. Consistent evidence suggest that 10-140 mmol sodium chloride supplementation per day would increase arterial stiffness in individuals with hypertension[87,88]. In a randomized clinical trial, salt reduction was associated with decreased pulse pressure across all ethnic groups including white, black and Asians, whereas PWV decreased only in blacks in response to salt reduction[87].

In addition, Gates *et al*[89] revealed that large elastic artery compliance was much improved in the older adults with systolic hypertension following only one-week of dietary sodium restriction.

Pharmacological therapy: Since blood pressure is the strongest modifiable factor directly leading to arterial stiffness, a number of clinical trials have been conducted to investigate the effect of antihypertensive medications on the change of arterial stiffness. Notably almost all classes of anti-hypertensive medications except diuretics and non-vasodilating beta-blockers such as atenolol could decrease arterial stiffness effectively[90,91]. Among all classes of anti-hypertensive medications, RAAS system antagonists have shown the best clinical results, probably due to their anti-fibrotic properties[91]. With regard to other modifiable risk factors, 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) could decrease arterial stiffness by lowering low-density lipoprotein cholesterol concentrations, the effect of anti-inflammation, and stabilizing the atheroma plaques[92,93]. In patients with diabetes, glycemic control with oral anti-diabetic agents with metformin and glitazone were reported to improve arterial stiffness[94,95]. Using high dose of RAAS antagonists was extremely effective in attenuating the severity of arterial stiffness in diabetic patients with hypertension[96]. Notably, pharmacological modifications to these traditional vascular risk factors have been confirmed to improve arterial stiffness evaluated by PWV or CAVI[97]. In patients with chronic inflammatory disease such as rheumatoid arthritis, several anti-inflammatory agents have been tested, but until now, only antibodies against tumor necrosis factor-alpha have been shown to improve arterial stiffness, independently of adequate blood pressure control[98,99]. In menopausal women, although the effect of sex hormone replacement therapy on arterial stiffness is uncertain, one study showed that using raloxifene, a potent selective estrogen receptor modulator may lead to positive result[100]. The phosphate binder, sevelamer was found to improve arterial stiffening in patients with end-stage renal disease[101]. Alagebrium, an advanced glycation end-products crosslink breaker, has shown to improve arterial stiffness in animal studies despite the effect was missing in a small group of older individuals[102,103]. However, further clinical trials were not conducted because of financial problems of the developing company. Currently, some ongoing trials are conducted to evaluate the effect of antidiabetic pharmacological therapy including metformin and alogliptin, the dipeptidyl peptidase 4, on the improvement of arterial stiffness in obese children and adolescents, and in adult individuals with type 2 diabetes, respectively[104,105].

Device and interventional therapy: It is well known that OSA is related to obesity and correlated with several CVD risk factors, such as hypertension and metabolic syndrome, which contributes to adverse clinical outcomes. A meta-analysis involving 15 articles, investigated the effect of continuous positive airway pressure (CPAP) on arterial stiffness in 615 patients with OSA. A significant improvement of all indices of arterial stiffness was observed after CPAP treatment (SMD = -0.74; 95%CI: -1.08 to -0.41). Neither the proportion of compliance nor the duration of CPAP use altered the outcomes after CPAP treatment[106].

Enhanced external counterpulsation (EECP), using pneumonic cuffs over the legs to inflate and deflate according to the cardiac cycle, is a non-invasive modality for treatment of symptomatic patients with coronary artery disease not amenable to revascularization procedures. In a randomized clinical trials conducted in 42 patients with coronary artery disease, central arterial stiffness and AIx were reduced following 17- and 35-sessions respectively, as well as peripheral arterial stiffness was reduced following 35 sessions in the EECP treatment group as compared with the placebo[107].

Since autonomic nervous system is involved in the pathogenesis of hypertension, its modification such as renal sympathetic denervation, and baroreflex activation therapy could attenuate arterial stiffness by improving arterial stiffness indices and central hemodynamics in patients with resistant hypertension[108,109]. However, these studies were conducted in patients with resistant hypertension, and the result may not be simply extrapolated to all the patients with arterial stiffness.

CONCLUSION

Arterial stiffness has been recognized as a marker of CVD and associated with long-term prognosis in several populations. Older age, hypertension, cigarette smoking, and dyslipidemia, known as traditional vascular risk factors, as well as diabetes, obesity, and systemic inflammation contribute to arterial stiffness. Targeting multiple modifiable risk factors has become the main therapeutic strategy to improve arterial stiffness in patients at high cardiovascular risk. Additionally to life style modifications, long-term ω-3 fatty acids intake in diet may improve arterial stiffness in the population with hypertension or metabolic syndrome. Pharmacological treatment such as RAAS antagonists, metformin, and HMG-CoA reductase inhibitors were useful in individuals with hypertension or diabetes. In obese people with OSA, weight reduction, aerobic exercise, and CPAP treatment may improve arterial stiffness as well. In specific populations such as with chronic inflammatory disease, a use of antibodies against tumor necrosis factor-alpha could work effectively. Other therapeutic options such as renal sympathetic nerve denervation for patients with resistant hypertension remains under investigated clinically. Therefore this comprehensive review provides knowledge in detail regarding the aspect of pathogenesis, measurement, and management of arterial stiffness in several populations, which

would be helpful for physicians to make clinical decision.

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MINIREVIEWS

Visualization of catheter ablation for atrial fibrillation: Impact of devices and anatomy

Mark A Benscoter, Paul A Iaizzo

Mark A Benscoter, Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455, United States

Mark A Benscoter, Department of Engineering, Mayo Clinic, Rochester, MN 55905, United States

Paul A Iaizzo, Department of Surgery, University of Minnesota, Minneapolis, MN 55455, United States

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Correspondence to: Paul A Iaizzo, PhD, Department of Surgery, University of Minnesota, 420 Delaware Street SE, B172 Mayo, MMC 195, Minneapolis, MN 55455, United States. iaizz001@umn.edu
Telephone: +1-612-6247912
Fax: +1-612-6242002

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Abstract

Endocardial access to the left atrium is commonly achieved to treat patients with atrial fibrillation, using different device delivery systems for cardiac ablation. But the large variation in human anatomy presses the limits of existing medical devices. In this unique study, we directly visualized the device-tissue interface in fresh reanimated human hearts using Visible Heart® methodologies. Our goal was to better understand any opportunities to improve therapeutic approaches. The visual images obtained in this study (also featured in this article) allow a more intimate grasp of the key steps required in various ablation procedures, as well as some limitations of current device designs. These images show the potential risks of conducting transseptal punctures and the difficulties of placing catheter tips in certain scenarios (*e.g.*, when creating circumferential lesions); they also demonstrate potential problems that could occur while attempting to place catheter tips on such anatomies like the mitral isthmus. In our analysis of these images, we focus on where enhancements are needed to refine device functionality.

Key words: Atrial fibrillation; Cryogenic catheter ablation; Radiofrequency ablation; Transseptal puncture

Core tip: Visible Heart® methodologies are utilized to directly visualize a functional human heart anatomy and key steps in the cardiac ablation procedure to emphasize limitations of current device delivery systems. Specifically, these images illustrate potential risks of transseptal punctures as well as the challenges faced by clinicians when placing catheter tips in certain scenarios, considering the wide variation in human anatomy. The focus is on where enhancements are

needed to refine device functionality and improve therapeutic approaches.

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INTRODUCTION

For many years, ablation (either radiofrequency or cryogenic) has been used to treat patients with atrial fibrillation (AF)[1-3]. But variations in cardiac anatomy have consistently influenced therapeutic success[4-8]. Different medical device designs have been developed for creating effective lesions in such varied anatomic structures[9-12]. However, in order to apply therapies for left atrium (LA) targets, navigation is first required into the right atrium (RA) and then across the septum.

Ablating the anatomic locations within the left heart was initially made feasible by a modified Cox's maze procedure[13-16]. In such a procedure, each step requires an intimate understanding of the endocardial anatomy[17]. Importantly, the inappropriate placement of devices in any ablation procedure can result in significant unintended consequences, including the creation of ineffective lesions (no transmurality), the need for subsequent ablation procedures, and/or cardiac tamponade during transseptal punctures[18-20]. In an effort to reduce the incidence of such unintended consequences, ablation is commonly performed with the assistance of imaging tools such as fluoroscopy or echocardiography. Imaging tools not only help eliminate unintended consequences such as perforation, but also help ensure occlusion of pulmonary veins (PVs). In addition, the use of fluoroscopy, angiography, and noncontact mapping has improved the quality of the images[21]. However, no imaging method allows one to directly visualize the device-tissue interface or to take into consideration the impact of accuracy on heart rhythm[21,22].

In this unique study, we used Visible Heart® methods to directly visualize the device-tissue interface in fresh human hearts reanimated in a clear Krebs-Henseleit buffer (Sigma-Aldrich Corporation, St. Louis, MO, United States), as previously described[22,23]. Our goal was to better understand any opportunities to improve therapeutic approaches during the key steps of various ablations procedures. The visual images obtained in our study (and featured in this article) allow a more intimate grasp of the steps required as well as any limitations of current device designs.

In particular, the images reveal the interaction of ablation technology with human tissue, providing a sense of the spatial relationship between the device and anatomic structures. In our analysis of these images, we focused on where enhancements are needed to refine device functionality. For purposes of analysis, we separated the key steps of ablation procedures into 3 distinct image sets, based on the device used and the anatomy: (1) navigating the RA; (2) conducting transseptal punctures from the RA to the LA; and (3) creating lesions and reaching the key anatomic locations in the LA with different types of ablation devices. Delineating the limitations of current devices and pinpointing the major anatomic challenges should prove to be of great importance for both practicing physicians and medical device designers[23].

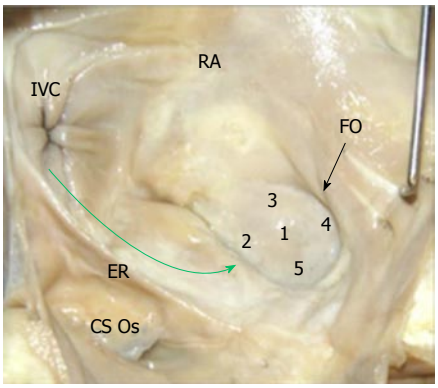

Figure 1 Fossa ovalis and transseptal punctures. All of the 5 locations shown on the fossa ovalis (black arrow) are transseptal puncture possibilities, and the path of device delivery to the fossa ovalis is shown (green arrow). CS Os: Coronary sinus ostium; ER: Eustachian ridge; FO: Fossa ovalis; IVC: Inferior vena cava; RA: Right atrium.

NAVIGATING THE RA

Success in navigating the RA has been limited, given the challenging anatomies of key RA structures combined with the limitations of current device designs. Endocardial cardiac ablations of the atria commonly originate *via* access from the inferior vena cava (IVC). An introducer, at the groin, is inserted into the femoral vein and then advanced into the RA. The IVC serves as a low-pressure return path of deoxygenated blood to the RA. Thus, the IVC is a suitable starting point for endocardial procedures because it eliminates risks associated with device introduction. Key ablation procedure structures in the RA include the fossa ovalis (FO), coronary sinus (CS), and right atrial appendage (RAA).

FOSSA OVALIS

The FO serves as the access point for ablation of the LA. As devices enter the RA, the Eustachian valve of the IVC forms a bridge between the IVC and the Eustachian ridge (ER) (Figure 1).

The FO is also a structure that causes devices to bind or become lodged, and device tips can catch on the compliant membrane of the valve[24-26]. Because the

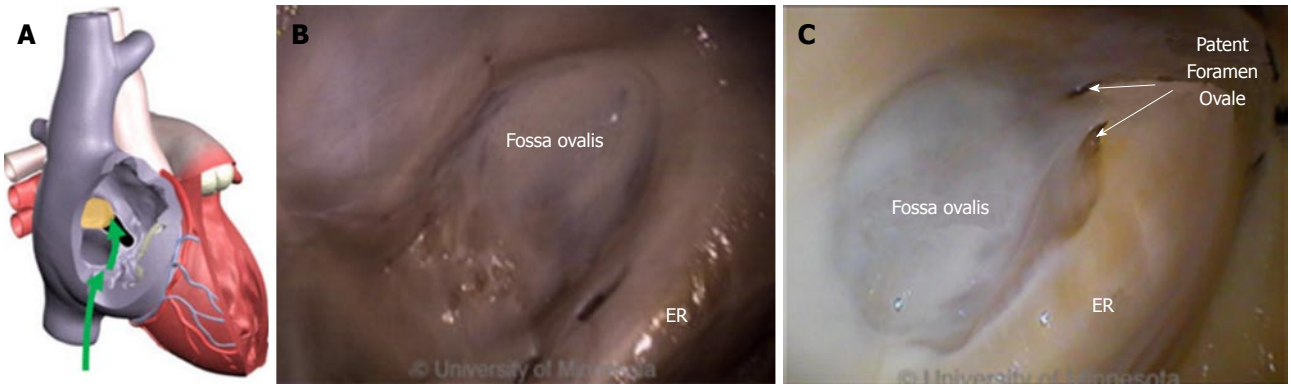

Figure 2 Anatomy of fossa ovalis and Eustachian ridge related to transseptal punctures. A: Inferior vena cava approach to transseptal punctures (green arrows); B: Image of fossa ovalis and Eustachian ridge (ER); C: Image of a patent foramen ovale in the fossa ovalis (two white arrows), with the ER adjacent to the fossa ovalis.

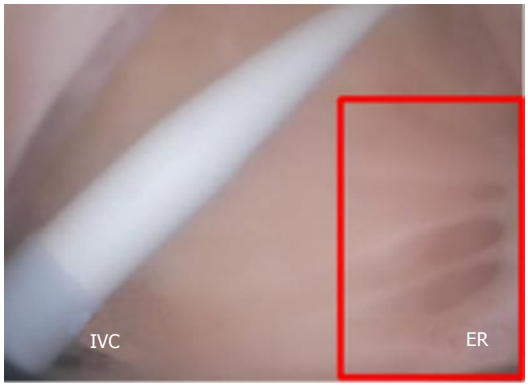

Figure 3 Catheter dilator and sheath in the right atrium. Pectinated muscles border the Eustachian ridge (ER, red rectangle). IVC: Inferior vena cava.

FO and the IVC are located on the superior aspect of the ER, the ER can serve as a guide to facilitate device delivery to the FO, allowing the device to glide along the valve and onto the ER (Figure 2B and C).

The ER is a prominent rise between the FO and the CS ostium (Os)[27-29]. The superior and posterior margins of the FO are enfolded to produce the prominent muscular protrusion on the endocardial surface. The FO lies next to the aorta, in some cases making transseptal punctures difficult[30]. Bordered by septal tissue and the ER, the FO is typically slightly recessed (Figure 2B and C). These structures can either facilitate or inhibit the operation of a medical device, either directing it in the intended direction or preventing it from being placed in the desired location.

Current catheter delivery systems often face challenges in reaching the FO and conducting transseptal punctures. Pectinated muscles adjoin the ER, which itself is pronounced and moves with each contraction. The pectinated muscles adjacent to the FO are composed of undulations that are capable of restraining the tip of a dilator or catheter (Figure 3). Dilator tip dimensions are sized to only allow a transseptal needle to pass. This small tip size also increases the chance of binding in these muscles if the tip is placed incorrectly.

During transseptal punctures, the FO is manipulated extensively. Both its size and thickness contribute to changes in the amount of compliance when force is applied (Figure 4). A large amount of compliance in the FO, coupled with a lack of compliance in the much thicker septal wall, can result in concentration of the transseptal force on the FO.

Dilator tips enable practitioners to confirm anatomic location by tenting the FO. Once tenting is achieved, a transseptal needle can be advanced through the FO (Figure 5A and B). The large amount of tenting that is usually required and the compliance of the membrane draw into question how much force the FO is able to tolerate before the procedure fails. Though necessary to perform transseptal punctures, FO tenting-combined with excess extension of the transseptal needle tip into the LA - can result in cardiac tamponade.

The very close proximity of the FO to both the right superior pulmonary vein and the right inferior pulmonary vein makes it challenging to reorient device tips after transseptal punctures (Figure 6).

Devices whose total deflection is limited, or whose deflection is located more proximally in the shaft, result in tip changes that make it nearly impossible to orient the device in a way that facilitates catheter introduction into the right PVs. Consequently, the FO needs to be manipulated more. Additionally, an incorrect puncture site location can increase the difficulty of introducing a catheter into the right PVs.

Once a transseptal puncture is complete, the tissue is stretched over the outside diameter of the dilator and onto the outside diameter of the sheath (Figure 5B). This transfer of force, the overall diameter of the sheath, and manipulation of the device in the LA can all contribute to the possibility of tearing the FO. As the sheath is deflected and the device is introduced into the LA, the resulting forces on the sheath push and pull the FO. If these forces become excessive, the FO can tear (Figure 7).

This step in the procedure prompts additional consideration of the use of the transseptal needle in the

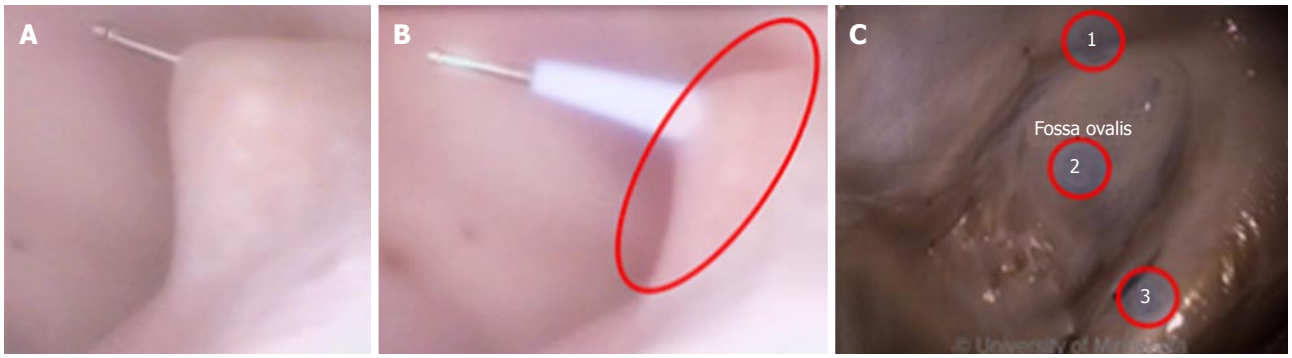

Figure 4 Fossa ovalis manipulation during transseptal punctures. A: Deformation of the fossa ovalis at the point of needle puncture in the left atrium; B: Transseptal puncture at the time of dilator insertion, with deformation of the fossa ovalis (red oval) and a tight fit between the dilator and the fossa ovalis; C: Image of the septal ridge (red circle 1) around the fossa ovalis, the thin and highly compliant nature of the fossa ovalis (red circle 2), and the Eustachian ridge (red circle 3).

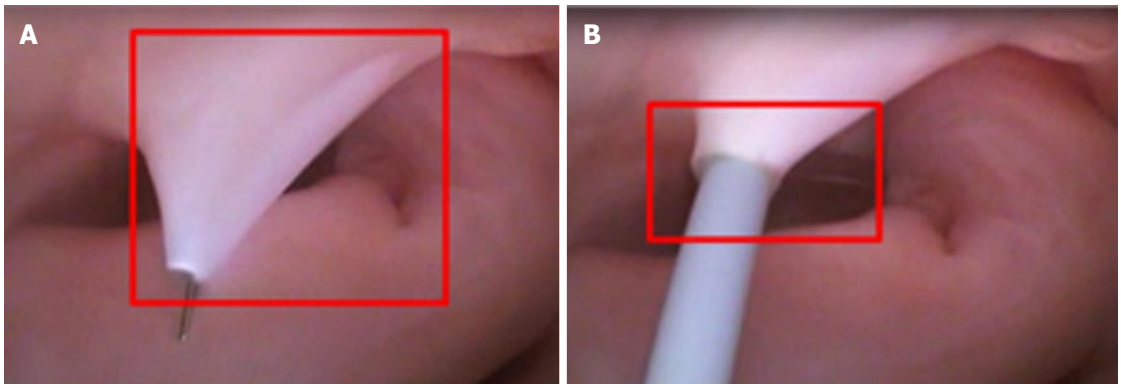

Figure 5 Tenting of the fossa ovalis. A: View from the left atrium of the fossa ovalis at the point of needle puncture (red square); B: Simultaneous view from the right atrium at the point of needle puncture (red rectangle).

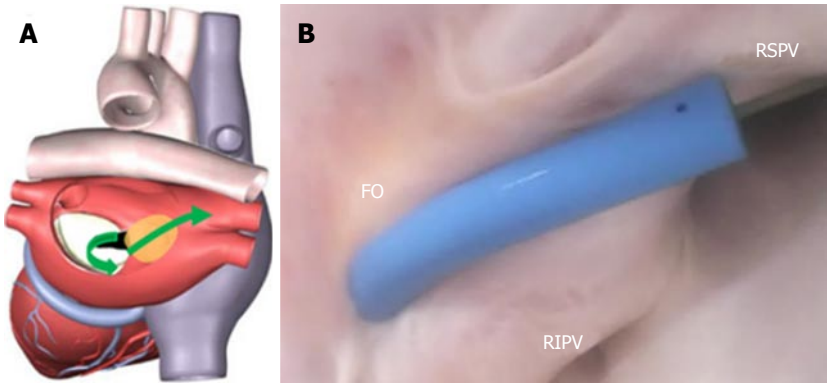

Figure 6 Fossa ovalis anatomy and device delivery for transseptal punctures. A: Path of device delivery to the pulmonary vein originating from the fossa ovalis (green arrows); B: Left atrial sheath placement after a transseptal puncture, with a guidewire introduced into the right superior pulmonary vein (RSPV). FO: Fossa ovalis; RIPV: Right inferior pulmonary vein.

LA. The transseptal needle extends beyond the tip of the dilator. The amount of extension is dictated by the interference fit of the diameter on the needle shaft to the internal diameter reducer in the dilator tip. Given the large amount of needle extension and the relative thickness of the FO, future device designs must improve the needle tip to reduce the risk of cardiac tamponade, while still preserving the ability to achieve successful punctures. Clearly, anatomic variations can have an impact on the ability to conduct transseptal punctures as well as possible complications.

Such variability in anatomic structures - combined with current device limitations in sheath size and in needle, length, and deflection capabilities - will require continued advancements in order to decrease the risk to patients. Device developers must continue to collaborate with electrophysiologists. A partnership between engineers and health care providers is critical

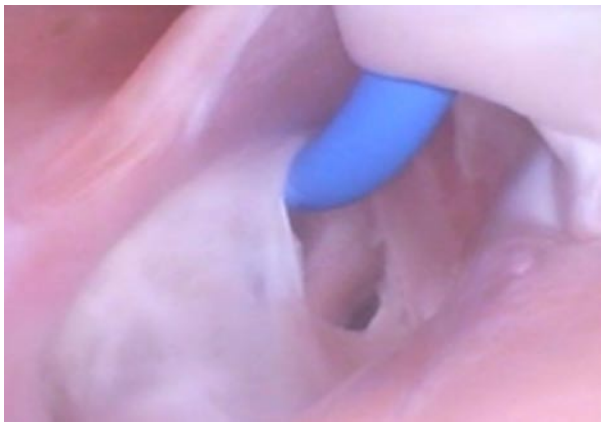

Figure 7 Tearing of the fossa ovalis. Example of a potential complication as a result of catheter navigation performed in a swine, showing the fossa ovalis tearing due to excessive deflection force from a delivery sheath attempting to navigate into a pulmonary vein.

for improving patient outcomes.

CORONARY SINUS

Arrhythmia ablation procedures commonly involve the CS[6,31]. Its ostium is located on the opposing side of the ER. In addition, the thebesian valve is located at the CS ostium (Figure 8). Inferior to the CS ostium, anatomic structures can be of various shapes and sizes[32].

Clearly, anatomic factors can increase the complexity of device delivery. The CS ostium resides in a deep pocket that is bordered by the ER, making catheter tip placement challenging. The location of the CS ostium relative to the IVC, along with the size of the ostium, can also present challenges.

To enter the CS, devices must have a high degree of deflection; furthermore, the region of deflection must have a small radius. With devices whose deflection is located more proximally in a stiff shaft and whose diameter is 8-Fr or larger, it will be more difficult to orient the tip so that it aligns with the CS ostium (Figure 9). Further design work is needed to develop devices that can deflect in a small radius, allowing the catheter tip to be oriented in such a way that it can align with the CS ostium.

RIGHT ATRIAL APPENDAGE

Right atrial ablation is required in instances of AF in which the cycle length recorded in the RAA is shorter than is the cycle length recorded in the left atrial appendage (LAA). The RAA location near the ostium of the IVC prompts the need to deflect devices (Figure 10A). The appendage can be a large structure; it is composed of very thin tissue as well as pectinated muscle and a sagittal band (Figure 10B and C).

Given the thin tissue of these anatomic structures, devices need to have very smooth tips that do not focus force into a point. With devices that have a rigid shaft, the risk of perforating the RAA is greater, because of the higher transfer of force. In contrast, with devices that have a compliant tip at the distal end, the tip can bend, thereby lessening the chance of perforating the RAA.

ABLATING LEFT ATRIAL STRUCTURES: PV, MI, AND LAA ROOFLINE

The LA has a venous component, along with a vestibule and an appendage. The additional 4 venous orifices serve as corners to the atrium. The vestibule surrounds the mitral ostium. The LAA is typically a small extension that originates adjacent to the mitral valve annulus and the left PVs.

In the atrial areas, the anterior wall behind the aorta is commonly thin and can be torn during transseptal punctures[33]. The thicker parts of the LA are on the superior wall[34]. The ostium of the right PVs are adjacent to the plane of the atrial septum. The tissue that makes this transition is smooth. The target of PV isolation is a muscular sleeve that extends into each vein and ends inside the sleeve; the role of the sleeve has been reviewed in other studies[35,36]. The organization of electrical activity from the PV is well known[37,38].

The smooth wall of the LA facilitates a uniform drag of the catheter tip against the tissue. The size of the LA is conducive to catheter tip placement against the tissue surface[27]. But the formation of a small gap is possible; complex cardiac navigation systems do provide some guidance as to gap location, yet it might not be sufficient.

LA ablation can occur in a number of different locations and can be prompted by continuous electrical activity, with a minimum duration of 100 ms[39] and either fractionated or fragmented electrical activity[40].

LEFT ATRIAL PULMONARY VEINS

Pulmonary vein isolation is currently considered as a key step in treating patients with all forms of AF. Of note, the muscle sleeves in the ostial opening of all 4 PVs emit ectopic beats. Electrical isolation of each vein is now the standard of care for treating AF, using either cryogenic or radiofrequency ablation[28-30]. In electrical isolation procedures, both ablation and diagnostic devices are used around and inside the PVs including guidewires, balloons, diagnostic catheters, and focal ablation catheters.

The ostia of the right PVs are adjacent to the FO. The ostial opening of the PV is a smooth surface. The close proximity of the PV ostia to the FO, and the sharp angle between them, make it difficult to orient a catheter through the puncture site and into the PV (Figure 11).

The ostia can comprise ridges and are adjacent to each other on opposing sides of the atrium. The shape and orientation of the PV can vary; other anatomic

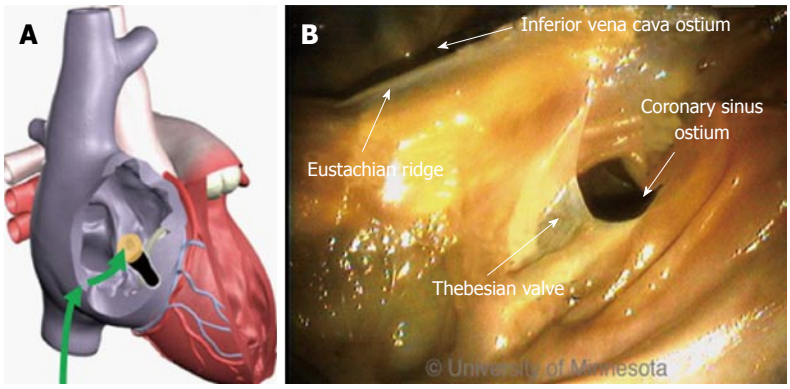

Figure 8 Coronary sinus and ablation procedures. A: Device approach originating from the inferior vena cava into the coronary sinus ostium (green arrows); B: Regional anatomy in the area of the coronary sinus is bordered by the thebesian valve, including the inferior vena cava catheter introduction point.

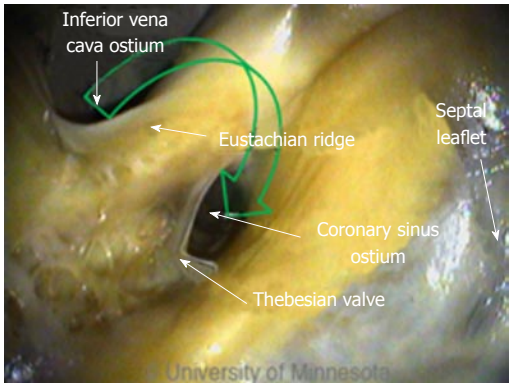

Figure 9 Deflection of devices in the coronary sinus. Green arrow depicts the device approach originating from the inferior vena cava; devices in this region require a high degree of deflection.

structures can be atypical. Variations can include differences in ostial size and the existence of a common shared ostium. All of these factors can affect the effectiveness of the devices used to electrically isolate the tissue.

The PVs interface with guidewires, sheaths, balloons, and focal ablation catheters. The location of the transseptal puncture can have a dramatic effect on the ability to place the catheter tip in the ostium of the PV, especially because the distance from the FO to the ostium is short. In addition, the orientation of the opening of the PV is directed in a way that can result in the need to twist the guidewire to allow it to migrate inside the PV (Figure 12A). This anatomic orientation of the FO and the PV illustrates the importance of a sheath that has a small radius of deflection at the tip in order to facilitate guidewire insertion.

Catheter placement into the PV is also affected by the contour of the catheter tip. The angle of the catheter's approach might require anatomic guidance to properly position the tip (Figure 12B). This device-tissue interface shows the importance of a smooth, contoured tip.

The complete insertion of the ablation catheter is affected by its size and by the size of the atrium. The proximity of the FO to the PV can limit the ability to have both the sheath and the catheter in the chamber (Figure 12C). Limiting the distance of the therapeutic region of the catheter would provide greater latitude for use of the entire system in the atrium. Operation of these devices on the right side of the atrium is one of the more challenging steps of the procedure.

Performing the same steps on the left side of the atrium requires different device performance. The orientation of the FO to the left PV ostia (as compared with the right side of the atrium) is more conducive to device delivery. Guidewire introduction is typically facilitated by the nearly linear orientation of the FO to the PV ostium (Figure 13A), which allows the guidewire to be placed and lodged in the PV (Figure 13B).

The alignment of the FO and left PVs allows for easy catheter introduction into the LA and sufficient room to operate the device, thereby reducing the stress on the FO and lessening the demands on the sheath (Figure 13C). For balloon-based devices, which require more area to operate than do focal ablation catheters, the alignment of the FO and left PVs is of particular importance.

Once the catheter is delivered into the PVs, therapy delivery remains challenging. For example, balloon-based therapeutic devices are larger, with only a limited amount of deflection ability, so they require more room to operate. Given the orientation of the transseptal puncture to the ostium of the PV, the balloon is able to fully occlude the vein. However, uniform cooling may not be achieved, because the balloon's orientation is limited by the FO's orientation to the PV's muscular sleeve (Figure 14).

These anatomic challenges accentuate the importance of having an acute distal deflection segment on the ablation device, in order to improve catheter tip orientation to, and alignment with, the PV ostium. Such challenges also jeopardize the ability of the sheath to maintain its placement in the LA. Decreasing the length of the distance between the distal tip of the sheath and the proximal end of the balloon would allow more sheath to be retained in the LA. The sheath must have a very distal deflection control with an acute radius of deflection. The smooth wall of the atrium facilitates placement of the ablation catheter.

If additional lesions are necessary beyond PV isolation, they can be created in the LA in the form of a linear lesion along the roofline or a mitral isthmus (MI)

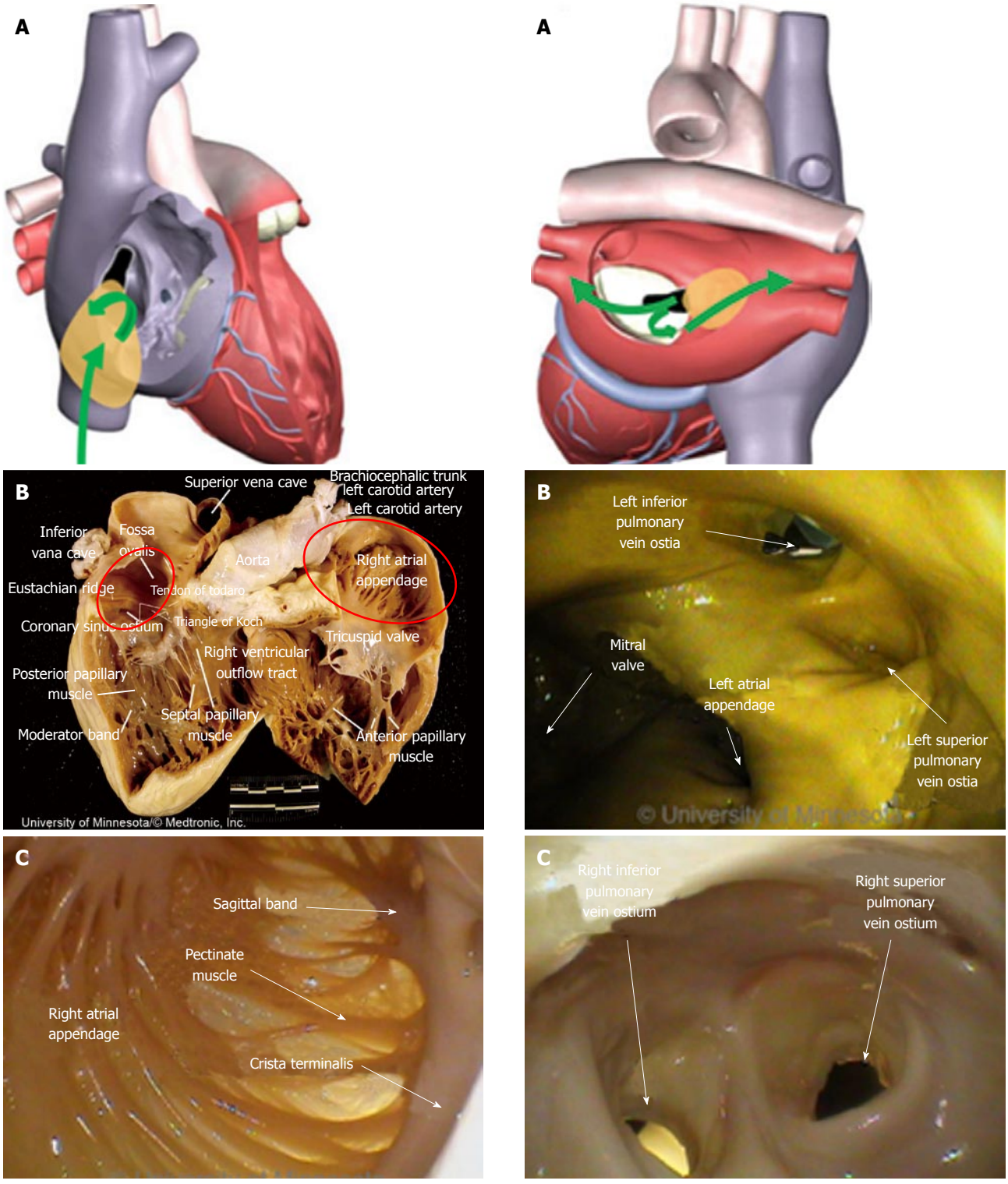

Figure 10 Right atrial appendage and transseptal punctures. A: Approach to the right atrial appendage through the inferior vena cava (green arrows); B: Image shows the large size of the right atrial appendage (red oval, right side) and pectinated muscles (red oval, left side); C: Image of the pectinated muscles and thin tissue.

Figure 11 Pulmonary veins and ablation devices. A: Directions of device introduction originating from the fossa ovalis into the left atrial pulmonary anatomy (green arrows); B: Image shows the left pulmonary vein ostium; C: Image of the right pulmonary vein.

lesion, or *via* ablation of the LAA.

LEFT ATRIAL APPENDAGE

Many times, the LAA is a site for the deposition of thrombus. A stroke is a possibility if the thrombus is able to dislodge and travel to a part of the vasculature that supplies blood to the brain. The LAA is oriented on the opposing side of the LA from the FO, making device delivery less challenging (Figure 15A).

For achieving and retaining device placement, the

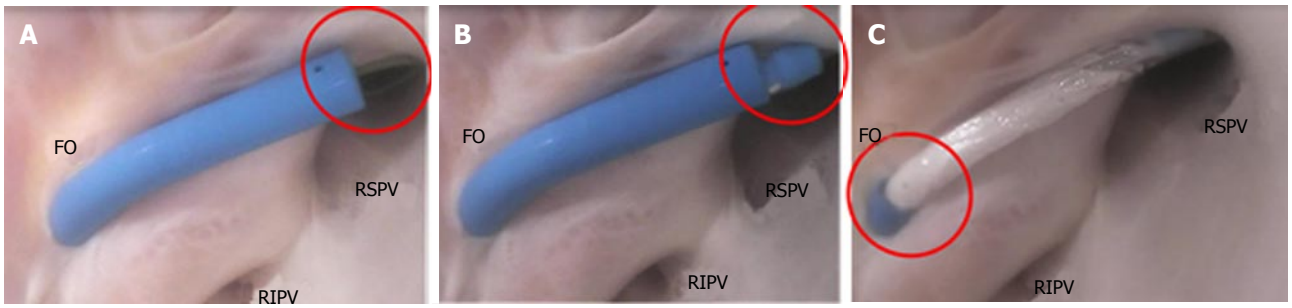

Figure 12 Device placement in the right pulmonary veins. A: Catheter sheath and the use of a guidewire for placement into the right superior pulmonary vein (RSPV, red oval); B: Introduction of the catheter tip at the pulmonary vein ostium (red oval); C: Retraction of the catheter sheath to the fossa ovalis (FO) and the introduction of a balloon catheter (red oval). RIPV: Right inferior pulmonary vein.

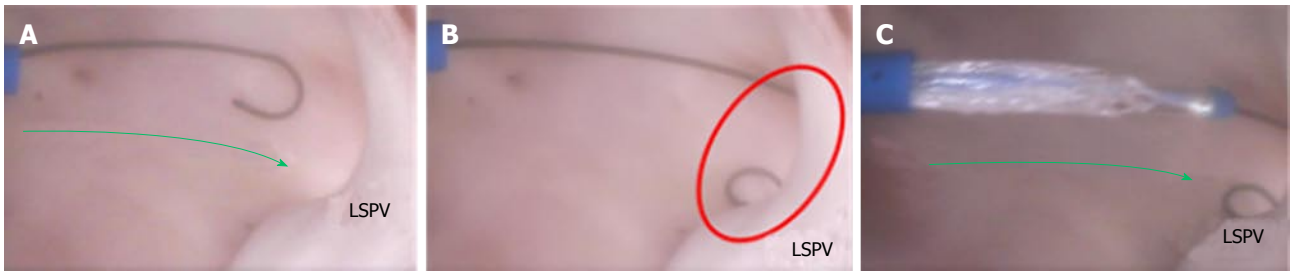

Figure 13 Device placements in the left pulmonary veins. A: Catheter sheath and the use of a guidewire for placement into the left superior pulmonary vein (LSPV, green arrow); B: Placement of the guidewire into the pulmonary vein ostium (red oval); C: Introduction of the balloon catheter across the left atrium (green arrow).

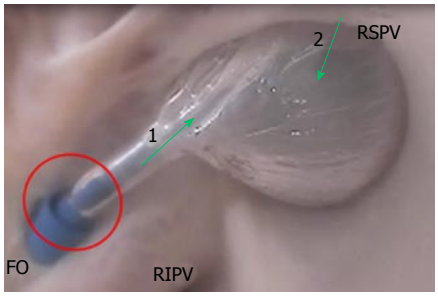

Figure 14 Balloon-based left atrial right superior pulmonary vein occlusion. The sheath is retracted to accommodate the ablation catheter (red circle). The catheter orientation (green arrow 1) is not aligned with the pulmonary vein ostium orientation (green arrow 2). FO: Fossa ovalis; RIPV: Right inferior pulmonary vein; RSPV: Right superior pulmonary vein.

opening of the LAA can be challenging. The ability to place a focal ablation device at the ostial opening is complicated by the presence of prominent ridges in the ostial area of the LAA. Focal devices for performing point-by-point ablation around the opening are difficult to operate. Alternatively, devices that encircle the LAA, or that occlude it, preclude the need to create point-by-point lesions and remove the complexity of attempting to place a catheter tip on a ridge structure.

Devices that deploy into the LAA and then place the therapeutic region at the opening are able to encircle the opening (Figure 16). Focal ablation devices need the ability to apply sufficient force on the tissue for lesion generation, without slipping into the pectinated muscles of the LAA interior.

Dynamically shaped ablation devices that could occlude the LAA would have an advantage, as they might be able to maintain position and sufficient force for lesion generation.

MITRAL ISTHMUS AND ROOFLINE ABLATION

The MI is a region of tissue that borders the annulus of the mitral valve and the LAA, and then rises over the ridge toward the left PV (Figure 17A). This is a common area to create a contiguous lesion in which it helps to terminate conduction patterns in patients with AF in whom PV isolation is not sufficient[27,31,32].

The creation of the MI or roofline linear lesion is affected by even a minor amount of anatomic movement of the MI with each contraction, making catheter tip placement on the ridge difficult. Any anatomic movement changes the force on the catheter tip and can contribute, at times, to a temporary loss of sufficient contact for lesion creation. The ability to maintain tissue contact is a byproduct of the amount of force on the catheter tip. For MI linear lesion creation, given the orientation of the FO in relation to the mitral valve annulus (MVA), the catheter tip must be able to reach to the MVA, and the deflection must be able to place force on the catheter tip (Figure 17B). The presence of a ridge is an additional complicating factor; the shape of the ridge can be pointed, making it difficult for the catheter to be placed on it.

To ensure necessary contact when creating a linear lesion, a focal catheter may be used against a

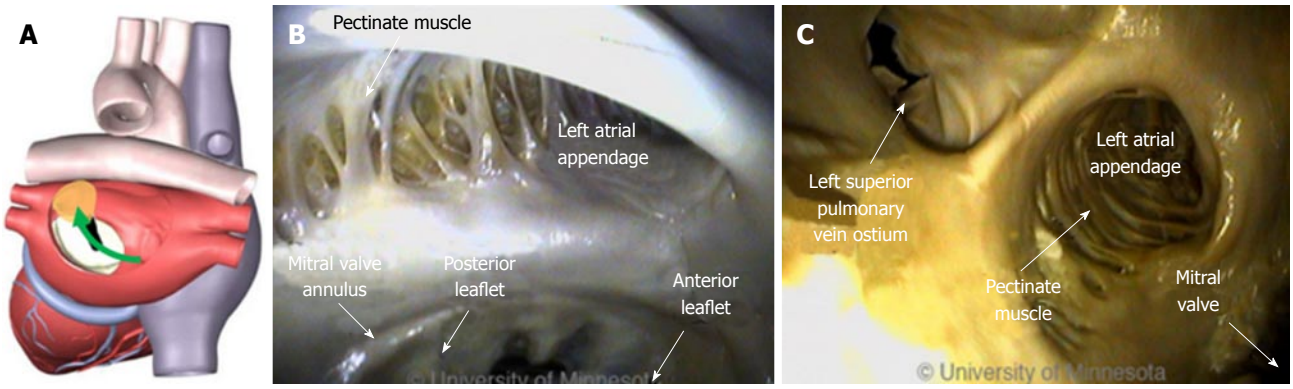

Figure 15 Left atrial appendage and device delivery. A: Approach to the left atrial appendage from the fossa ovalis (green arrow); B: Position of the left atrial appendage relative to the mitral valve annulus and the presence of pectinated muscle; C: Position of the left atrial appendage relative to both the mitral valve and the left superior pulmonary vein.

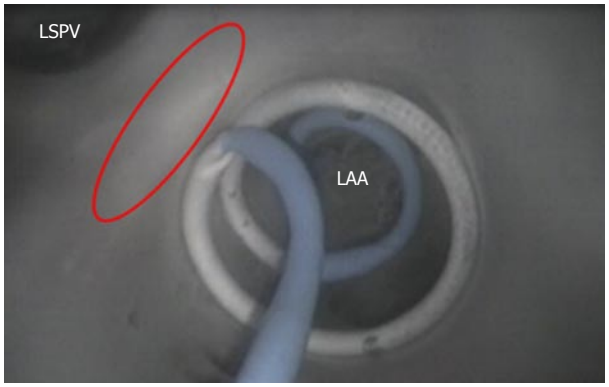

Figure 16 Device encircling the left atrial appendage. Image shows the catheter shaft circulating around the left atrial appendage (LAA) ostium; the red oval depicts the ridge between the LAA and left superior pulmonary vein (LSPV).

supporting structure, such as another catheter (Figure 18A) or the wall of the atrium (Figure 18B).

A focal ablation catheter has the advantage of adaptability. This device design could be extended to include repositioning of electrodes, softening of the tip, and better deflection capabilities - all of which could widen application across an array of atrial anatomies, resulting in an improvement in energy delivery.

CONCLUSION

Understanding how ablation devices interface with tissue and anatomic structures can make a crucial difference in their therapeutic application. Anatomic structures vary from person to person. Within each person, the endocardial surface changes shape with each heartbeat and can prompt catheter migration, making it difficult to know exactly where the device was placed and what is happening at the device-tissue interface. By using Visible Heart® methods to directly visualize the device-tissue interface in fresh reanimated human hearts, we assembled and analyzed an array of illuminating images, providing a critical aid to clinicians and medical device designers alike. For examples of

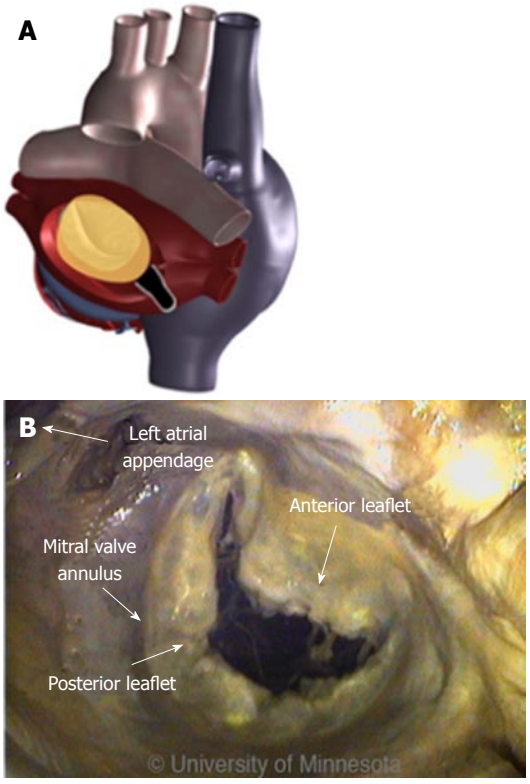

Figure 17 Mitral isthmus and roofline ablation. A: Diagram shows the mitral valve in the left atrium; B: Various structures, including the mitral valve annulus, which serve as the starting point for creating a linear lesion in the mitral isthmus.

functional anatomies of the human heart, refer to the free-access website, "The Atlas of Human Cardiac Anatomy" (www.vhlab.umn.edu/atlas).

The tools that have traditionally been used to treat patients with AF have numerous limitations, all of which lengthen ablation procedure time and increase the likelihood of disease recurrence. Future research in this field needs to focus on reducing the risks of transseptal procedures, increasing catheter mobility, enhancing the anatomic precision of catheter tip placement, and improving imaging capabilities. Studies must investigate

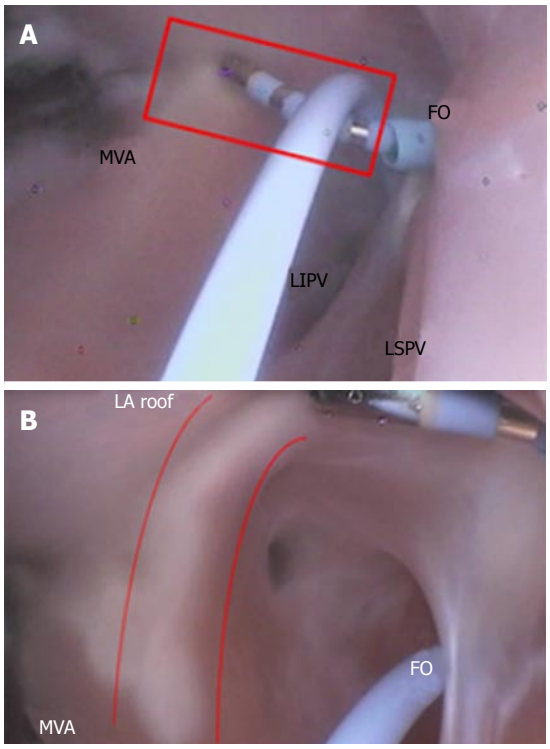

Figure 18 Focal ablation catheter. A: Mitral isthmus lesion creation originating at the mitral valve annulus (MVA, red rectangle); B: Creation of a roofline linear lesion (red curved lines). FO: Fossa ovalis; LA: Left atrial; LIPV: Left inferior pulmonary vein; LSPV: Left superior pulmonary vein.

methods for improving transseptal punctures, reaching targeted anatomies with therapeutic devices, and assessing the effectiveness and quality of lesions at the point of their creation.

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MINIREVIEWS

Coarctation of the aorta: Management from infancy to adulthood

Rachel D Torok, Michael J Campbell, Gregory A Fleming, Kevin D Hill

Rachel D Torok, Michael J Campbell, Gregory A Fleming, Kevin D Hill, Division of Pediatric Cardiology, Department of Pediatrics, Duke University Medical Center, Durham, NC 27705, United States

Author contributions: Torok RD, Campbell MJ, Fleming GA and Hill KD contributed to the manuscript literature review, data compilation, and writing.

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Correspondence to: Kevin D Hill, MD, MSCI, Associate Professor of Pediatrics in the Division of Pediatric Cardiology, Division of Pediatric Cardiology, Department of Pediatrics, Duke University Medical Center, 2400 Pratt Street, Room 7582, Box 3850, Durham, NC 27705, United States. kevin.hill@duke.edu
Telephone: +1-919-668466
Fax: +1-919-6687058

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Abstract

Coarctation of the aorta is a relatively common form of congenital heart disease, with an estimated incidence of approximately 3 cases per 10000 births. Coarctation is a heterogeneous lesion which may present across all age ranges, with varying clinical symptoms, in isolation, or in association with other cardiac defects. The first surgical repair of aortic coarctation was described in 1944, and since that time, several other surgical techniques have been developed and modified. Additionally, transcatheter balloon angioplasty and endovascular stent placement offer less invasive approaches for the treatment of coarctation of the aorta for some patients. While overall morbidity and mortality rates are low for patients undergoing intervention for coarctation, both surgical and transcatheter interventions are not free from adverse outcomes. Therefore, patients must be followed closely over their lifetime for complications such as recoarctation, aortic aneurysm, persistent hypertension, and changes in any associated cardiac defects. Considerable effort has been expended investigating the utility and outcomes of various treatment approaches for aortic coarctation, which are heavily influenced by a patient's anatomy, size, age, and clinical course. Here we review indications for intervention, describe and compare surgical and transcatheter techniques for management of coarctation, and explore the associated outcomes in both children and adults.

Key words: Coarctation of the aorta; Cardiac surgery; Cardiac catheterization; Balloon angioplasty; Stents

Core tip: This review explores both surgical and transcatheter approaches for the treatment of coarctation of the aorta and examines outcomes of these techniques in children and adults.

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INTRODUCTION

Coarctation of the aorta was first described by Morgagni in 1760[1], and in its simplest form refers to congenital narrowing of the proximal thoracic aorta. While aortic coarctation most commonly occurs as a discrete stenosis in the juxtaductal position, it may also be associated with long segment narrowing, hypoplasia of the transverse aortic arch, or stenosis of the abdominal aorta[2-4]. Coarctation of the aorta accounts for 5%-7% of all congenital heart disease[5], with an incidence of approximately 3 cases per 10000 births[6]. Coarctation may be seen in isolation or with additional cardiac lesions, such as bicuspid aortic valve, ventricular septal defect, patent ductus arteriosus, transposition of the great arteries, atrioventricular canal defects, or left-sided obstructive heart defects, including hypoplastic left heart syndrome[7-11]. Crafoord was the first to perform a successful surgical repair of aortic coarctation in 1944[12]. Since then, various surgical and transcatheter approaches have been developed, allowing patients to have significantly improved outcomes. Here, we briefly review the presentation and diagnosis of aortic coarctation and then focus on surgical and transcatheter approaches with their most recent associated outcomes.

DIAGNOSIS

Clinical presentation

Coarctation can present at any age. Neonates with ductal dependent or "critical coarctation" often present with heart failure, acidosis, and shock following closure of the ductus arteriosus. Without prompt medical resuscitation and surgical intervention, death may occur rapidly[13,14]. Prenatal diagnosis can prevent these sequelae by allowing for intervention before ductal closure. However, prenatal diagnosis of coarctation is challenging due to the presence of the ductus arteriosus and limited blood flow across the aortic isthmus in utero[13]. In the United States, fewer than 1 in 4 patients with isolated coarctation requiring neonatal intervention are diagnosed prenatally[15,16]. Moreover, approximately 30% of neonates with coarctation remain undiagnosed upon discharge after delivery[17]. For these reasons, many physicians advocate for newborn pulse oximetry screening programs, which increase the likelihood of detecting lesions like coarctation before ductal closure[18,19]. Additionally, coarctation must be suspected in infants with other left-sided obstructive heart lesions and may be diagnosed in infants with chromosomal defects, especially those with Turner syndrome and Jacobsen syndrome[20].

Patients with less severe coarctation may not be diagnosed until later in childhood when a murmur is heard or hypertension noted. In these patients, collateral vessels develop from the internal thoracic and subclavian arteries, thyrocervical trunks, and vertebral and anterior spinal arteries to supply blood to the descending aorta[21,22]. For those who enter adulthood undiagnosed, hypertension is the most common presenting symptom[23]. Others may complain of frequent headaches or claudication of the lower extremities with exertion. In these patients, the most telling exam findings suggestive of coarctation are diminished and/or delayed lower extremity pulses and a systolic pressure gradient between the upper and lower extremities[13,23]. However, for patients with extensive collateral blood flow, femoral pulses and lower extremity blood pressures may only be minimally diminished[24].

Evaluation

Chest X-ray is often nonspecific in young patients. In older patients, an anterior-posterior film may show indentation of the aorta at the site of coarctation with pre- and post-stenotic dilation of the aorta, creating the classic "3 sign". Notching of the posterior fourth to eighth ribs due to dilated intercostal arteries may also be seen in older patients[24,25]. Electrocardiogram is typically normal in infants, but in older children and adults, left ventricular hypertrophy is common due to ventricular pressure overload[24].

Transthoracic echocardiography can assess the presence and severity of aortic coarctation and any associated cardiac defects and is the diagnostic gold standard in neonates and infants (Figure 1). Although transthoracic echocardiogram remains the initial test of choice for coarctation, in larger children and adults[24], echocardiographic windows may be suboptimal. When this is the case, a computed tomography scan or magnetic resonance imaging (MRI) provides excellent anatomic detail at the site of coarctation, and these modalities are commonly used to create three-dimensional images for interventional planning (Figure 2). MRI has the additional benefit of defining and quantifying collateral vessel flow. Although cardiac catheterization was frequently used for diagnosis of coarctation in the past, it is now typically reserved for therapeutic intervention or in those cases where hemodynamic data is additive to the diagnostic evaluation[24,25].

TREATMENT

Without intervention, the outcome for patients with coarctation of the aorta is poor. In his classic 1970 natural history study, Campbell examined autopsy and clinical records of 465 patients with coarctation who survived beyond one year of age. The mean age of death was 34 years, with 75% mortality at 43 years of age. Causes of death included congestive heart failure (26%), aortic rupture (21%), bacterial endocarditis (18%), and intracranial hemorrhage (12%)[26]. Fortunately, several treatment options are now available, including surgical and transcatheter interventions. Guidelines regarding indications for

Table 1 Notable studies and guideline statements in the treatment and outcome of coarctation in adults and children

Ref.	*n*	Follow-up	Outcome
Cowley *et al*[60]	36	Mean 14 yr	Randomized trial comparing BA and surgery for native coarctation in children. Aortic aneurysm developed in 35% of BA patients and none of the surgical patients
Carr[81]	846	Mean 36 mo for catheter-based group and 7.8 yr for surgical group	Meta-analysis comparing catheter *vs* surgical intervention for adults with coarctation. Higher risk of restenosis and need for reintervention found in catheter-based group
Forbes *et al*[68]	578	Median 12 mo	Retrospective multicenter analysis at intermediate follow-up after stent placement for coarctation. Exceeding a balloon:coarct ratio of 3.5 and prestent BA increased risk of aortic wall injury
Warnes *et al*[24]	-	-	ACC/AHA guidelines for management of coarctation in adults
Holzer *et al*[67]	302	3-60 mo	Prospective analysis of acute, intermediate, and long-term follow-up after stent placement for coarctation using CCISC registry. At long-term follow-up, recoarctation in 20% of patients, 4% required unplanned reintervention, and 1% had aortic wall injury
Feltes *et al*[27]	-	-	AHA guidelines for transcatheter intervention in children with coarctation
Forbes *et al*[69]	350	Mean 1.7 yr	Multicenter observational study comparing surgery, BA, and stent placement for native coarctation in children using CCISC registry. Significantly lower acute complication rates in stent group but higher planned reintervention rates. Hemodynamic and arch imaging outcomes superior in stent and surgical patients compared to BA group
Harris *et al*[55]	130	3-60 mo	Prospective, multicenter analysis of short and intermediate outcomes for BA in native and recurrent coarctation in children. Trend toward increased acute aortic wall injury and restenosis in native coarctation patients
Sohrabi *et al*[75]	120	Mean 31.1 mo	Randomized clinical trial comparing covered and bare CP stents for native coarctation in adolescents and adults. Trend of increased rates of restenosis and lower rates of pseudoaneurysm in bare stent group
Meadows *et al*[70]	105	2 yr	Prospective, multicenter, single-arm study assessing safety and efficacy of CP stent in children and adults with coarctation. Two year follow-up of 86% showed 23 fractured stents with no significant clinical effects, 6 aortic aneurysms, 19 repeat catheter interventions, and no surgical interventions

BA: Balloon angioplasty; ACC: American College of Cardiology; AHA: American Heart Association; CCISC: Congenital Cardiovascular Interventional Study Consortium; CP: Cheatham platinum.

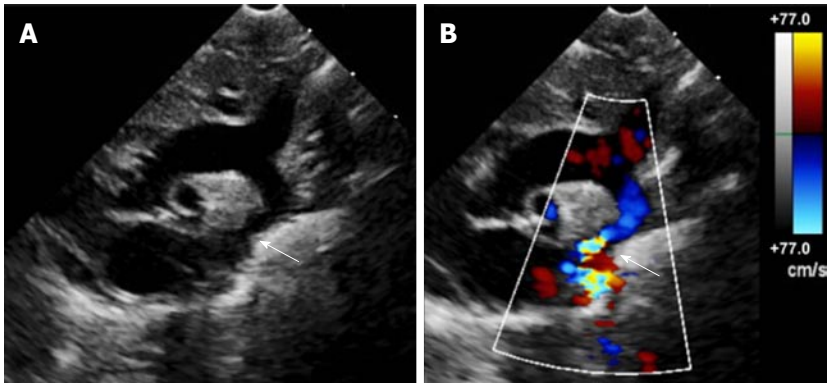

Figure 1 Echocardiogram of coarctation. A: Two-dimensional transthoracic echocardiogram image obtained from the suprasternal notch in an 11-day-old infant demonstrating discrete coarctation (arrow); B: Color Doppler of the same image with aliasing of flow at the site of coarctation (arrow).

intervention exist for both children and adults with coarctation (Tables 1 and 2), which include a peak-to-peak gradient ⩾ 20 mmHg or lesser gradients when there is significant anatomic evidence of narrowing on imaging with extensive collateral flow[24,27]. Other factors that may be considered include the presence of systemic hypertension, additional cardiac defects and/or single ventricle physiology, left ventricular hypertrophy, or elevated left ventricular end diastolic pressure[24,27-29].

Surgical repair

The first surgical technique described for coarctation of the aorta was resection with end-to-end anastomosis by Crafoord in 1944[30] (Figure 3A). Early studies showed recoarctation rates in over half of patients with this technique, and the use of a circumferential suture line was thought to be a major contributor[31,32].

Vosschulte described prosthetic patch aortoplasty as an alternative technique for coarctation repair in 1961. In this approach, the ductal tissue is ligated and divided, a longitudinal incision across the coarctation is made, and a prosthetic patch is sutured in place to enlarge the stenotic region (Figure 3B). This technique can be applied to longer segments of coarctation, avoids a circumferential suture line, and minimizes aortic mobilization and ligation of intercostal arteries[33]. While recoarctation rates of 5%-12%[34] were lower compared to the resection and end-to-end anastomosis technique, aortic aneurysm was a long-term concern, with rates between 18%-51% of patients[35-38]. Using

Table 2 Executive summary on the diagnosis and treatment of coarctation in children and adults

Diagnosis
Accounts for 5%-7% of congenital heart disease diagnoses
Neonates often present with heart failure, acidosis, and shock with critical coarctation
Less severe coarctation often detected during evaluation for hypertension or murmur in the older child or adult
Diminished or delayed lower extremity pulses and a systolic pressure gradient between the upper and lower extremities are the most useful exam findings
Transthoracic echocardiogram is initial test of choice; CT and MRI useful if echocardiogram inconclusive and for surgical planning
Treatment
Surgical repair
Extended end-to-end anastomosis typically preferred surgical method, as it avoids prosthetic material, allows resection of the coarctation, and has a wider incision that is less prone to restenosis
Surgical repair typically preferred over transcatheter approaches in the infant and young child with native coarctation, patients requiring repair of associated cardiac defects, or in those with complex coarctation anatomy
Balloon angioplasty
Often the preferred intervention for recurrent coarctation
Concern for recoarctation and aneurysm formation in native coarctation
Endovascular stent
Provides structural support and decreased rates of aortic wall injury and aneurysm compared to balloon angioplasty
Covered stents may protect against shear stress and subsequent restenosis, though care must be taken to avoid overlying vital branch vessels
Use of stents in small children controversial due to need for large sheath size and limitations in accommodating for somatic growth
Patient follow-up
Lifelong follow-up with at least annual cardiology visits and repeat imaging every 5 yr to assess coarctation site
High suspicion and aggressive treatment of baseline and exercise-induced hypertension
Future perspectives
Further long-term data analysis needed to determine optimal intervention based on patient anatomy, size, and age

CT: Computed tomography; MRI: Magnetic resonance imaging.

more distensible polytetralfluroethylene patch material instead of Dacron was initially promising[34] but still showed a 7% risk for aortic aneurysm and a 25% risk of recoarctation[39].

Subclavian flap aortoplasty was a modified approach reported by Waldhausen and Nahrwold in 1966. Here, the left subclavian artery is ligated and divided, and a longitudinal incision down the proximal left subclavian artery is extended beyond the area of coarctation. The proximal left subclavian stump is then folded down to enlarge the area of coarctation (Figure 3C). This technique avoids a circumferential suture line and the use of prosthetic material, which may allow for improved growth, and it can be used for repair of long segment coarctation[40,41]. Although still occasionally used by surgeons, one of the main reservations of this approach has been the need to sacrifice the left subclavian artery. This can create a subclavian steal phenomenon, with retrograde flow down the vertebral artery, and it has been associated with decreased length and muscle bulk of the left upper extremity, as well as claudication with exercise[42,43].

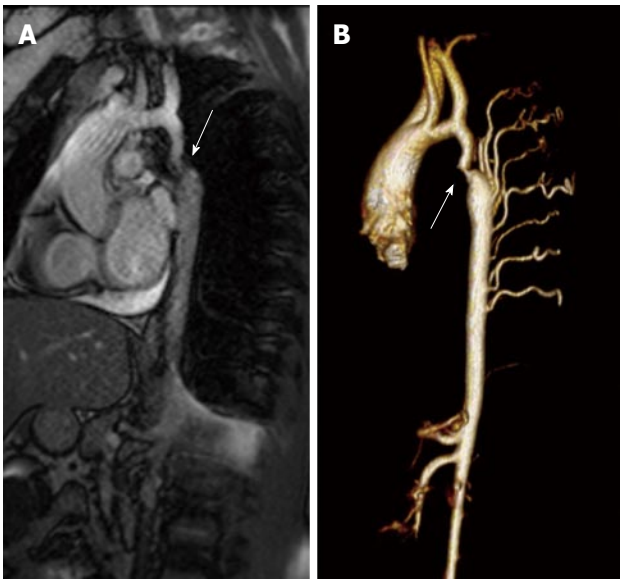

Figure 2 Magnetic resonance imaging of coarctation. A: Magnetic resonance image (steady-state free precession) in a sagittal projection demonstrating transverse arch hypoplasia and long segment coarctation of the aorta distal to the left subclavian artery (arrow) in a 12-year-old male; B: Three-dimensional reconstruction of a gated contrasted angiogram for the same patient, which demonstrates transverse arch hypoplasia, coarctation at the aorta at the distal transverse aortic arch and isthmus (arrow), and dilated intercostal arteries (collaterals).

Coarctation resection and replacement with an interposition graft was described by Gross[44] in 1951. After aortic cross clamp, the stenotic tissue is excised, and either a homograft or Dacron tube graft is sutured into the aorta. This approach is rarely used in the current era, as it is not ideal for pediatric patients due to growth limitations. However, occasionally it is an appropriate technique for adult patients with coarctation, especially those with aneurysm, long segment coarctation, or recoarctation after primary repair[45].

In 1977, Amato *et al*[46] described a modification to Crafoord's resection and end-to-end anastomosis technique, where a broader, longitudinal incision and anastomosis are created across the proximal aorta (Figure 3D). The extended end-to-end anastomosis still avoids the use of prosthetic material and allows resection of the coarctation and residual ductal tissue, but the wider incision is less prone to restenosis and enables enlargement of the transverse aorta, which is particularly helpful in neonates[46,47]. In the present era, extended end-to-end anastomosis is typically the preferred technique for surgical repair, especially in small children, due to low mortality rates and low rates of restenosis, ranging between 4%-11%[47-50].

Balloon angioplasty: Native coarctation

Surgical therapy was the only treatment option for coarctation until 1982, when the use of balloon angioplasty was described by Lock *et al*[51]. Several studies since then have shown balloon angioplasty to be a relatively effective acute intervention for native

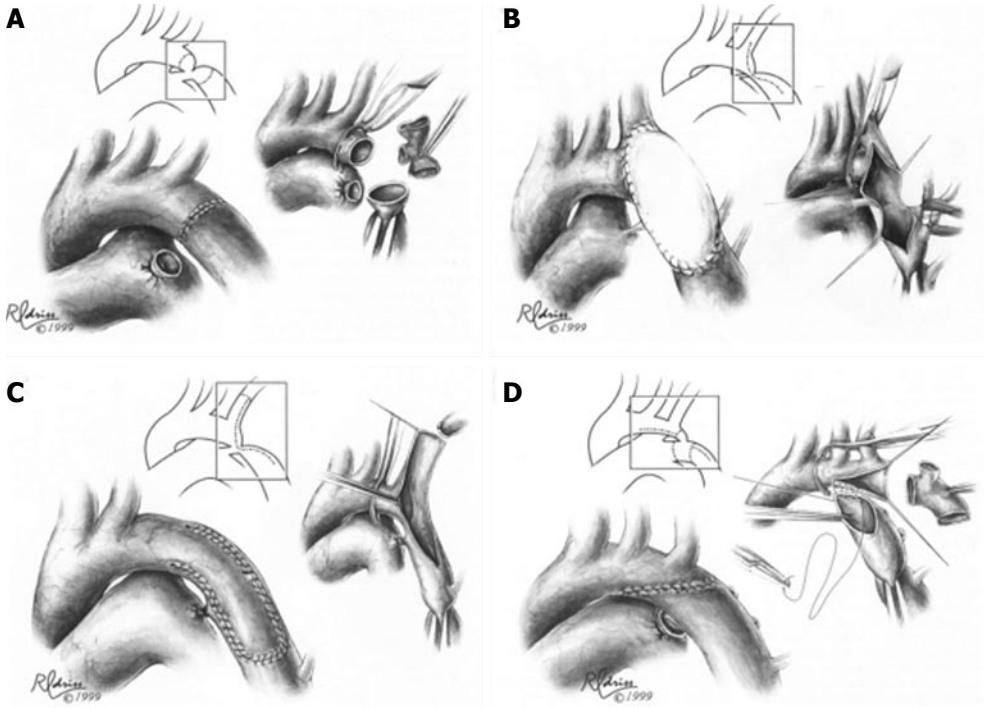

Figure 3 Surgical techniques in coarctation repair. A: Resection and simple end-to-end anastomosis. The coarctation is resected, and an end-to-end, circumferential anastomosis is created; B: Patch aortoplasty. An incision is extended across the coarctation, and a patch is sutured in place to enlarge the stenotic region; C: Subclavian flap aortoplasty. The left subclavian artery is ligated and divided. A longitudinal incision is extended from the proximal left subclavian artery beyond the area of coarctation, and the proximal left subclavian stump is folded down to enlarge the area of coarctation; D: Resection with extended end-to-end anastomosis. The coarctation is resected using a broad, longitudinal incision, and an oblique anastomosis is constructed between the undersurface of the transverse arch and the descending thoracic aorta. Figures adapted and reprinted with permission from the *Journal of Cardiac Surgery*[30].

coarctation, with rates of recoarctation ranging from 8%-32%[52-54]. In a report from the prospective, multicenter Congenital Cardiovascular Interventional Study Consortium (CCISC), 34 patients undergoing balloon angioplasty for native coarctation had adequate intermediate follow-up data at 18 to 60 mo post-intervention. In these patients, the rate of recoarctation was 15%[55]. A second concern with native coarctation angioplasty is aneurysm formation. Histologic and intravascular ultrasound studies have demonstrated the mechanism of angioplasty involves tearing of the intima and media[56-59]. Although some of these tears may heal, disruption of vascular integrity is believed to contribute to a relatively high incidence of aneurysm formation. This was demonstrated in a single center randomized trial comparing balloon angioplasty *vs* surgical repair of coarctation in older children (ages 3 years to 10 years). In this study with mean follow-up of 14 years after intervention, 35% of the balloon angioplasty patients developed aneurysm, compared to none of the surgical patients[60]. Similarly, the 2014 CCISC observational study showed 24% of patients with native coarctation developed aortic aneurysm at intermediate follow-up after balloon angioplasty[55].

Balloon angioplasty: Recurrent coarctation

In contrast to native coarctation, balloon angioplasty is often the preferred intervention for recurrent coarctation in children[27]. Acute success rates for this procedure range from 80%-93%[61]. Reported rates of aortic wall injury are low (1%-2%), and the longer term risk of aneurysm is believed to be ameliorated by scar tissue at the site of the recoarctation, which limits the degree of vascular disruption. However recoarctation rates remain a concern, with a broad range between 6%-53% described[62,63].

Likely the most fragile patient population to develop recurrent coarctation is children with hypoplastic left heart syndrome or other single right ventricle lesions. These patients are at risk for significant morbidity and mortality with recoarctation due to exacerbation of atrioventricular valve regurgitation and ventricular dysfunction[64]. The Pediatric Heart Network Single Ventricle Reconstruction trial was a large, multicenter, prospective study examining the outcome of infants with single right ventricle lesions after randomization to either right ventricle-pulmonary artery shunt or a modified Blalock-Taussig shunt at the time of Norwood procedure[65]. The incidence and timing of intervention for recoarctation in the first 12 mo after randomization was assessed, and 97 of 549 patients (18%) underwent intervention for recoarctation, which was most commonly performed at the time of pre-stage II cardiac catheterization by balloon angioplasty. Balloon angioplasty achieved adequate short term results, but 39% of patients required reintervention for recoarctation, compared to 5% of the patients who underwent surgical reintervention. Reassuringly,

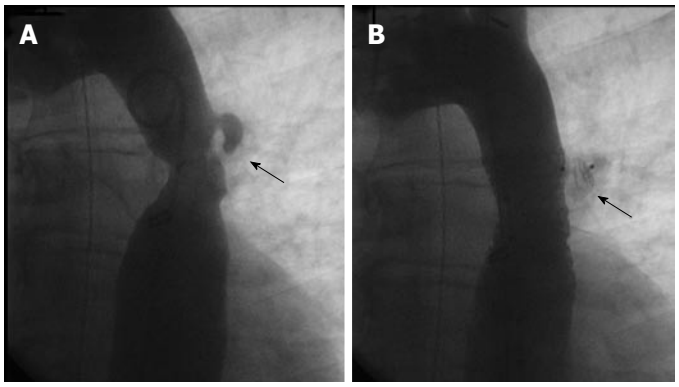

Figure 4 Endovascular stent placement for coarctation. A: Angiogram (LAO 30°, caudal 30°) demonstrating a discrete coarctation and intercostal aneurysm (arrow) in a 45-year-old male; B: Angiogram in the same projection after endovascular bare metal stent placement showing no significant residual stenosis. The intercostal aneurysm was successfully occluded with an Amplatzer Vascular Plug II (arrow).

with catheter or surgical intervention, the presence of recoarctation did not affect survival rates in this tenuous patient population[64].

Endovascular stent placement

In 1991, the use of endovascular stents for the treatment of coarctation was first reported[66], adding another dimension to the utility of transcatheter treatment for coarctation. Endovascular stents are inserted using balloon catheters but do not require overdilation of the vessel wall. Stents also offer structural support, thereby decreasing the rates of aortic wall injury and restenosis observed with balloon angioplasty alone[5] (Figure 4). Several studies have assessed the utility of endovascular stent placement in the treatment of coarctation[67]. Retrospective analysis of acute procedural data from 17 institutions from 1989 to 2005 showed successful stent placement without a significant residual gradient or serious complication in 553 of 565 (97.9%) patients. The overall complication rate was 14.3%, with aortic wall complications (aneurysm, intimal tear, or dissection) contributing 3.9%[68]. In a subsequent study, acute and long-term data regarding endovascular stent placement for coarctation were obtained from the CCISC. Here, the acute results of stent placement were successful without a significant blood pressure gradient or need for reintervention in 249/260 (96%) of cases. During follow-up spanning between 3 to 60 mo, recoarctation was seen in 20% of the 164 patients with follow-up imaging, and 4% of patients required unplanned repeat interventions. Aortic wall complications consisting of dissection or aneurysm were seen in 1% of the 302 total cases[67].

In a multicenter observational study comparing the outcomes of surgical, balloon angioplasty alone, and endovascular stent placement for coarctation using the CCISC registry, patients undergoing stent placement had significantly lower complication rates compared to balloon angioplasty or surgical patients. There was no significant difference among treatment groups for unplanned reintervention rates (4%-7%) at a mean of 1.7 years of follow-up, but those who underwent stent placement were more likely to undergo planned reintervention. Aortic wall injury was more likely to occur in patients who underwent balloon angioplasty alone, at a rate of 9.8% compared to none in the endovascular stent group[69]. In an attempt to more rigorously assess the effectiveness and safety of endovascular stent placement *vs* surgical repair, a Cochrane review was attempted in 2012, but it was determined that there was insufficient data available to identify the best treatment modality[5].

The Coarctation of the Aorta Stent Trial (COAST) has been an influential prospective study examining the effectiveness and safety of endovascular stent placement in children and adults with coarctation. Currently, no endovascular stent has been granted FDA approval for use in the aorta, and initially biliary stents were used off-label for treatment of coarctation. NuMED (Hopkinton, NY) created the Cheatham Platinum (CP) stent for specific use in the aorta, and in 2007, the COAST trial was designed as a prospective, multicenter, single-arm clinical study to assess the safety and efficacy of this stent for the treatment of coarctation[6].

Results for up to two years from CP stent placement are currently available from the COAST trial. CP stent implantation was attempted in 105 patients ranging from 8 to 52 years of age. All but one implantation was successfully completed, with no significant adverse events acutely. No patient had a significant gradient between the ascending and descending aorta after stent placement, and 99% had a gradient < 20 mmHg at one month. There was 89% follow-up at one year after stent placement and 86% follow-up at two years. At two years, 90% of patients maintained a blood pressure gradient < 20 mmHg between the upper and lower extremities. To date, 23 fractured stents have been identified, though none have led to decreased stent integrity, stent migration, aortic wall injury, or hemodynamic obstruction. Aortic aneurysm was diagnosed in 6 patients, one of which resolved without intervention. No patient has required surgical reintervention, and a total of 19 patients have undergone repeat transcatheter intervention, either due to aortic wall injury or dilation of the initial stent[70].

Thus far, the CP stent is felt to be a safe and effective treatment option for coarctation in older children and adults with native or recurrent coarctation. Stent fracture is common but thus far has been clinically insignificant. Reintervention is also common, either due

to planned dilation of smaller stents or aortic wall injury. Follow-up for the COAST trial is planned for up to 60 mo after stent placement and will provide further insight regarding these issues[6].

The use of endovascular stents in small children remains controversial due to the challenges in accommodating for significant somatic growth and the requirement for relatively larger sheath sizes. The ideal stent would be deployed through a small sheath but retain the ability to be dilated to an adult vessel diameter. This technology does not yet exist and still would require multiple interventions for stent dilation throughout a patient's lifetime. Additionally, neonates often have transverse arch hypoplasia, which does not easily lend itself to endovascular stent placement. Some small, single center studies have had positive short-term results with endovascular stent placement in young children, but further follow-up and investigation is needed[71,72].

Covered endovascular stents represent the latest transcatheter innovation and were first used for the treatment of coarctation in 1999. The fabric within the stent provides additional structural support, creates a protective barrier at the site of stent placement, and can help decrease shear stress. All of these factors theoretically reduce the risk of acute vascular trauma as well as longer term aneurysm formation. When aortic aneurysm or stent fracture occurs with bare stent placement, covered stents are often used as a rescue therapy. Covered stents may also be the initial transcatheter intervention of choice, especially in the setting of complex anatomy of the coarctation or in older patients with more friable and calcified aortic wall tissue. However, covered stents require larger sheath sizes, which limits their use in small children. Additionally, care must be taken to avoid stent occlusion of significant aortic branches, including paraspinal branches off of the descending aorta, which can be difficult to identify[73].

Initial smaller studies examining the use of covered stent placement in aortic coarctation were promising, with no reported aneurysms[73,74]. More recently, a randomized clinical trial was performed comparing bare CP stent *vs* covered CP stent placement in 120 patients. Both groups had no acute procedural complications, and at an average follow-up of 31.1 mo, the bare CP stent group had a statistically nonsignificant increase in the rate of recoarctation (6.7% *vs* 0%) and a nonsignificant lower incidence of pseudoaneurysm (0% *vs* 3.3%), compared to the covered CP stent cohort. The two cases of pseudoaneurysm in the covered stent group occurred at the proximal end of the stent, and both were successfully treated with a second covered stent with no further complications[75]. Further investigation into the safety and efficacy of covered stents is on-going with the COAST Ⅱ trial. This trial was initiated in 2010, hoping to provide information that will support FDA premarket approval of the covered CP stent in preventing aortic wall injury in high risk patients with coarctation as well as treatment of existing aortic wall injury related to complications from previous interventions for coarctation. Results are expected in the near future, but at this time, covered CP stents are not available for the treatment of coarctation in the United States outside of use in the COAST Ⅱ trial[70].

MANAGEMENT ALGORITHM

With many different options, deciding on the optimal treatment strategy for coarctation can be complicated, and there is no comprehensive evidence-based standard of care or algorithm. Guidelines from the American College of Cardiology and the American Heart Association provide some insight, but the level of evidence supporting these recommendations is suboptimal (Level B or C for all recommendations)[24,27]. In general, management is dictated by the age at presentation, complexity of the coarctation, and whether or not the coarctation represents a native *vs* recurrent obstruction. For the infant or young child presenting with native coarctation, most centers prefer surgical repair due to the long-term risk of aneurysm after balloon angioplasty, the need for redilation with stent placement, and the limitations imposed by small arteries unable to accommodate larger sheath sizes[60]. However, balloon angioplasty can be considered as a palliative strategy to stabilize neonates presenting in extremis and considered too sick for immediate surgical intervention[27]. Surgical repair may also be more appropriate in patients with complex coarctation anatomy, including those with transverse arch obstruction, tortuous segments of recoarctation, distortion of adjacent arterial branches, or when repair of associated cardiac defects is required[24].

In the older child, adolescent, or adult presenting with a simple, juxtaductal, native coarctation, stent placement is considered a reasonable approach, offering a less invasive alternative to surgical intervention and good long-term outcomes[24,27,76]. Only stents expandable to an adult size should be used, in an effort to avoid later surgical intervention[27].

For recurrent coarctation in the younger child, it is reasonable to consider initial balloon angioplasty, as aneurysm is less of a long-term concern than with native coarctation[27]. Balloon angioplasty is variably successful, and surgical reintervention may be required when there is incomplete relief of obstruction[55]. Stent placement can also be considered for recoarctation in older children and adolescents when the stent can be dilated to near adult size, thus avoiding the need for multiple redilations[27].

PATIENT FOLLOW-UP

Patients with repaired or unrepaired coarctation must be followed by a cardiologist throughout their lifetime. For those who have undergone repair, this follow-up should be at least annually, with specific attention paid to baseline or exercise-induced hypertension[24].

Hypertension is endemic in patients with aortic coarctation, even if no residual coarctation exists, and it must be appropriately treated[77,78]. The etiology of such high rates of baseline and exercise-induced hypertension remains unclear but may be due to any combination of underlying arteriopathy, decreased aortic wall compliance, abnormal streaming of blood flow, or renal abnormalities[77].

Additionally, imaging of the repaired coarctation should be performed at least every 5 years, or sooner based on original anatomy and symptoms, to assess the coarctation repair site for complications like aortic aneurysm or recurrent stenosis[24]. For repaired patients with a normal upper to lower extremity blood pressure gradient, normotension at rest and with exercise, and no evidence of aneurysm or associated heart defects, exercise is encouraged, and only activities with a large static component should be avoided[79]. Finally, per the 2007 American Heart Association guidelines, endocarditis prophylaxis is not routinely recommended beyond the first six months after surgical or transcatheter intervention, barring a previous history of infectious endocarditis[80].

CONCLUSION

In the seventy years since the first description of surgical intervention for aortic coarctation by Crafoord, tremendous progress has been made in the treatment and outcomes for these patients. Modifications of various surgical techniques have led to low mortality and morbidity rates, even in the smallest patients. Transcatheter balloon angioplasty and subsequently endovascular stent placement have expanded treatment options and provided less invasive approaches for repair in some patients. Still, both surgical and transcatheter approaches retain risks for adverse events and subsequent patient morbidity. As examined in this review, much effort has been spent investigating the intervention which yields the best patient outcomes, but further long-term data assessment is needed. The aortic coarctation patient population is a fascinating and heterogeneous one, and considering an individual patient's clinical presentation, anatomy, size, and age will most certainly continue to heavily influence treatment approach.

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Neoatherosclerosis: Coronary stents seal atherosclerotic lesions but result in making a new problem of atherosclerosis

Hidenori Komiyama, Masamichi Takano, Noritake Hata, Yoshihiko Seino, Wataru Shimizu, Kyoichi Mizuno

Hidenori Komiyama, Masamichi Takano, Noritake Hata, Yoshihiko Seino, Cardiovascular Center, Chiba Hokusou Hospital, Nippon Medical School, Chiba 270-1613, Japan

Wataru Shimizu, Cardiology, Nippon Medical School, Tokyo 113-0022, Japan

Kyoichi Mizuno, Mitsukoshi Health and Welfare Foundation, Tokyo 160-0023, Japan

Author contributions: Komiyama H wrote the manuscript; Takano M provided advice and revised the manuscript; Hata N, Seino Y, Shimizu W, and Mizuno K also provided intellectual advice.

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Correspondence to: Masamichi Takano, MD, Cardiovascular Center, Chiba Hokusou Hospital, Nippon Medical School, 1715 Kamakari, Inzai, Chiba 270-1613, Japan. takanom@nms.ac.jp
Telephone: +81-476-991111
Fax: +81-476-991908

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Abstract

Chronic inflammation of the native vessel wall with infiltration of lipid-laden foamy macrophages through impaired endothelium results in atherosclerosis. Percutaneous coronary intervention, including metallic stent implantation, is now widely utilized for the treatment of atherosclerotic lesions of the coronary artery. Bare-metal stents and the subsequently developed drug-eluting stents seal the atherosclerosis and resolve lumen stenosis or obstruction of the epicardial coronary artery and myocardial ischemia. After stent implantation, neointima proliferates within the stented segment. Chronic inflammation caused by a foreign body reaction to the implanted stent and subsequent neovascularization, which is characterized by the continuous recruitment of macrophages into the vessel, result in the transformation of the usual neointima into an atheromatous neointima. Neointima with an atherosclerotic appearance, such as that caused by thin-cap fibroatheromas, is now recognized as neoatherosclerosis, which can sometimes cause in-stent restenosis and acute thrombotic occlusion originating from the stent segment following disruption of the atheroma. Neoatherosclerosis is emerging as a new coronary stent-associated problem that has not yet been resolved. In this review article, we will discuss possible mechanisms, clinical challenges, and the future outlook of neoatherosclerosis.

Key words: Neoatherosclerosis; Percutaneous coronary intervention; Drug-eluting stent; Atherosclerosis

Core tip: Percutaneous coronary intervention, including metallic stent implantation, causes chronic inflammation of the coronary artery and neovascularization, which involves the continuous recruitment of macrophages

into the vessel. The phenomenon of stent neointima transformation from normal neointima to atherosclerotic lesions is now recognized as neoatherosclerosis, which causes in-stent restenosis and acute thrombotic occlusion. Neoatherosclerosis is now emerging as a new atherosclerosis-related problem that has not yet been solved. In this review, we will discuss possible mechanisms, clinical challenges, and the future outlook of neoatherosclerosis.

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INTRODUCTION

Atherosclerosis is caused by chronic inflammation at the site of damaged vascular endothelium and lipid-laden foamy macrophages derived from infiltration of monocytes into the arterial wall, and it results in coronary stenosis and thrombotic obstruction after atherosclerotic plaque disruption[1]. Percutaneous coronary intervention (PCI) is now widely accepted worldwide for the treatment of coronary artery disease due to atherosclerosis. In 1977, PCI by plain old balloon angioplasty (POBA) was performed for the first time by Gruntzig[2] to treat angina pectoris. In 1986, Sigwart *et al*[3] implanted a self-expandable stainless-steel stent to prevent acute occlusion and chronic restenosis caused by intimal dissection after balloon dilatation and elastic recoil of the coronary artery, respectively. In 1994, randomized clinical trials showed that bare-metal stent (BMS) implantation was superior to POBA with regard to short-term procedural success and long-term arterial patency[4,5]. However, in-stent restenosis (ISR) occurred in approximately 20%-30% of cases, causing the long-term failure of PCI that was bestowed the title of the "Achilles' heel" of PCI. According to pathological investigations, the primary pathogenesis of ISR is neointimal hyperplasia due to migration and proliferation of vascular smooth muscle cells (VSMCs) from the media. In the 2000s, the drug-eluting stent (DES) was introduced to prevent inhibition of neointimal hyperplasia and ISR of the BMS. Application of the DES to coronary artery disease has dramatically reduced the incidence of ISR in the clinical setting[6,7]. The so-called "first-generation DESs" were composed of a stainless steel stent platform and was coated with durable polymer-releasing anti-proliferative drugs. Although the first-generation DES, the sirolimus-eluting stent (SES) and paclitaxel-eluting stent (PES), decreased ISR, they are associated with a steady increase in very late stent thrombosis (VLST; > 1 year post-stent implantation) due to delayed re-endothelialization or a hypersensitivity reaction to the stent polymer[8]. Therefore, the next-generation DES were developed with new technology; specifically, the main feature of these DES was the inclusion of a biocompatible or biodegradable polymer to reduce vessel inflammation and a thin stent strut for normalization of rheological flow around the strut to diminish thrombogenicity. The second-generation DES, namely, zotarolimus-eluting stents, everolimus-eluting stents, and biodegradable polymer-coated biolimus-eluting stents, showed reduced incidences of VLST[9-11]. Nevertheless, the placement of second-generation DES was found to cause acute coronary syndrome originating from the stent segment[12].

Although metallic coronary stents, BMS, and DES resolve the problem of coronary lumen stenosis or occlusion in the acute phase after their implantation, they potentially cause new problems in the chronic phase, such as late ISR and VLST. It is now understood that some of these phenomena arise from the new pathogenic concept of "neoatherosclerosis", which is defined as the phenomenon of the transformation of stent neointima from normal neointima to an atherosclerotic lesion. We will review basic and clinical studies concerning topical problems of neoatherosclerosis that are associated with coronary stenting.

VASCULAR RESPONSE AFTER PCI

Mechanical injury of the vessel wall cannot be avoided by PCI, such as balloon dilatation and stent implantation. PCI procedures cause denudation of the coronary artery endothelium, resulting in exposure of the myointima, fissures in the atheromatous plaque, and overstretching of the circumferential vessel layers[13]. The endothelium regulates vascular tone, controls inflammation, maintains lipid and tissue-fluid homeostasis, and possesses antithrombotic properties[14]. The vascular endothelium protects against thrombus formation and blood coagulation through its production of nitric oxide, prostacyclin, tissue plasminogen activator, heparin-like molecules, tissue-factor pathway inhibitor, thrombomodulin, and other molecules[14]. Perturbation of the normal endothelium function by PCI is related to the pathogenesis of atherosclerosis and results in accelerated formation of atheromatous lesions[13]. Incomplete re-endothelialization in the coronary vascular wall induces thrombotic events after stent implantation in the early, late (> 1 mo, ⩽ 1 year post-stenting), or very late phase[15]. Denudation of the endothelium after PCI causes VSMCs to be exposed to blood flow directly, which modulates the proliferation and viability of the VSMCs[16,17]. Although BMS implantation is superior to POBA with respect to procedural success and long-term target lesion patency[4,5], dysfunction of the regenerated endothelium is more pronounced after stent implantation than after ballooning[18]. Any interventional procedure, even POBA, causes denudation of the endothelium and is associated with the same risk of very late thrombosis as BMS[19],

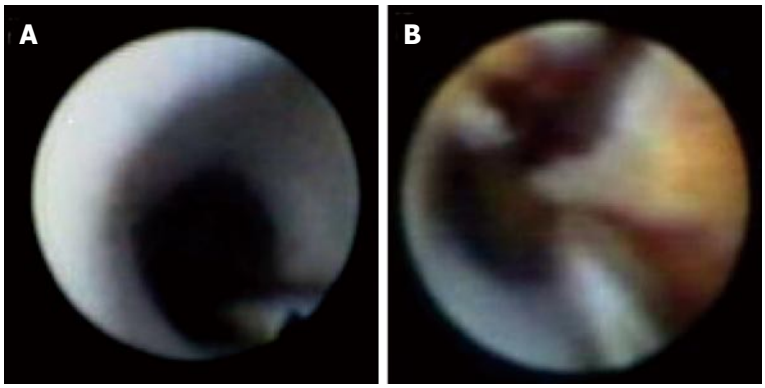

Figure 1 Coronary angioscopy images of ordinary neointima and neoatherosclerosis. A: Coronary angioscopy reveals ordinary neointima as a white and smooth membranous structure; B: The neointima appears as atheromatous and yellow, occasionally disrupted with thrombus formation.

and the regenerated endothelial cells are not structurally and functionally normal[20]. Stent implantation into the vessel leads to perturbations in blood flow, and flow reversal and disturbed shear stress around the stent strut promote vascular inflammation and injury[21,22]. The thickness of the stent struts determines the size of blood flow recirculation, which is associated with thrombogenicity within the stent segment[23]. Compared with the BMS, the first-generation DES, namely, SES and PES, which incorporated anti-proliferative drugs and durable polymers, were associated with dramatic reductions in the proliferation of the neointimal hyperplasia and ISR[6,7]. However, an increased risk of VLST was observed for these first-generation DES compared with BMS[24,25]. Autopsy studies showed that a lack of re-endothelialization with > 30% of the stent strut uncovered per cross-section was a strong predictor of late stent thrombosis (LST) and VLST[26]. Moreover, the polymer-induced type Ⅳ hypersensitivity reaction is one of the mechanisms of LST or VLST associated with SES. In contrast, excessive fibrin deposition and consequent stent mal-apposition (detachment of the stent struts from the coronary arterial wall) are associated with thromboses in the case of PES[26,27]. The new stent technology of second-generation DES involved minimization of vessel injury and normalization of micro-rheology around the stent strut, thinner struts, and the use of a biocompatible or biodegradable polymer[28]. The pathophysiology of LST and VLST is multifactorial, as mentioned above. However, other mechanisms are possibly linked to stent thrombosis. LST or VLST after placement of BMS and DES is an unresolved problem, and the new pathological concept of neoatherosclerosis is another mechanism of stent failure. It is understood that the pathophysiology and development of neoatherosclerosis differ between BMS and DES.

NEOATHEROSCLEROSIS IN BMS

Neointimal hyperplasia associated with BMS was considered to be stable, with peaks at 6 mo and 1 year after stenting during a 3-year follow-up[29]. However, extended follow-up of BMS showed that late luminal re-narrowing beyond 4 years was common[30]. Moreover, one-third of patients implanted with BMS who had restenosis presented with acute myocardial infarction or unstable angina 5 years after the index procedure that was not clinically benign[31]. Some reports have documented the occurrence of ACS due to the disruption of neoatherosclerosis after BMS implantation[32].

The findings of a histopathological study suggested the mechanism of the catastrophic late events after BMS implantation[33]. This study, which assessed nineteen stented coronary arteries obtained from 19 patients autopsied after non-cardiac death 2-7 years post-BMS implantation, showed that after more than 4 years of stenting, there was prominent infiltration of lipid-laden macrophages with strong collagen-degrading matrix metalloproteinase expressing ruptured and vulnerable plaque accompanied by thrombi around the struts evoked by remarkable foreign-body inflammation[33]. Regenerated endothelium after PCI forms poor endothelial cell junctions and expresses reduced numbers of antithrombotic molecules and nitric oxide, which contributes to neoatherosclerosis[15,18,34]. Neoatherosclerosis is now recognized as chronic inflammation in the vessel wall caused by the stent itself and subsequent neo-vessel formation, which causes continuous recruitment of macrophages and forms unstable lesions called thin-cap fibroatheroma (TCFA) that contribute to disruption of neointima and thrombus formation, leading to VLST[15].

Serial angioscopic observation at baseline, 6 to 12 mo, and ⩾ 4 years after BMS implantation revealed changes in the smooth white intima characterized by atheromatous yellow plaque with vulnerable features, such as surface disruption and thrombus formation, during the study period (Figure 1)[35]. In addition, the atheromatous transformation was correlated with ISR[35]. Optical coherence tomography (OCT) is a near-infrared light-based imaging modality with high-resolution that can accurately characterize tissue components *in vivo*[36]. Although there are no data regarding the angioscopic findings and histopathologic correlation in intimal tissue, an OCT study showed that the angioscopic yellow neointima likely corresponds to foamy macrophages infiltrating into the fibrous cap and underlying lipid accumulation, as well as that

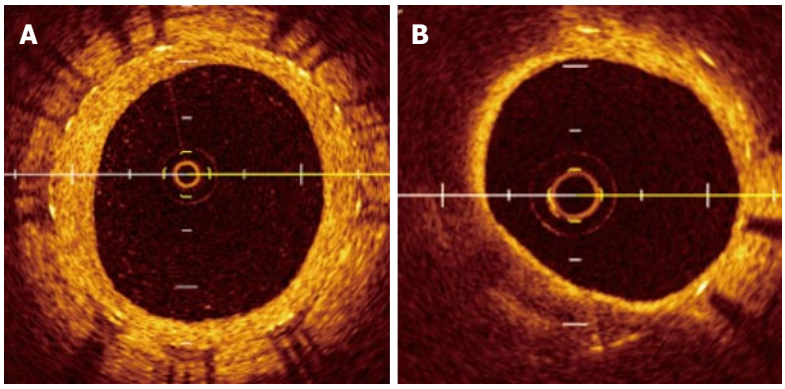

Figure 2 Optical coherence tomography images of common neointima (A) and neoatherosclerosis (B). A: Common neointima is recognized by its high-signal intensity and homogeneous region inside stent struts; B: The neointima has a diffuse border and marked attenuation.

the intensity of yellow likely signifies the thickness of the fibrous cap and amount of necrotic core[37]. OCT observation of BMS segments was performed in the early phase (< 6 mo) and late phase (≥ 5 years) after BMS implantation[38]. The normal neointima proliferated homogeneously, and the lipid-laden intima was not observed in the early phase. In the late phase, the lipid-laden intima was found in 67% of cases (Figure 2)[38]. Additionally, pathological characteristics, such as intimal disruption and thrombus formation, appeared (38% and 52% of cases, respectively). There was a similar incidence of peri-stent neovascularization in the 2 phases. However, the location of neovascularization was different between the two phases. Intra-intima neovascularization was more prevalent in the late phase than the early phase (62% and 0%, respectively; $P < 0.01$) and in segments with lipid-laden intima compared with non-lipidic segments (79% and 29%, respectively; $P = 0.026$)[38]. There are few reports showing that neoatherosclerosis of BMS increases ACS, clinically diagnosed as VLST. Therefore, further careful follow-up of neoatherosclerosis after BMS implantation is needed.

NEOATHEROSCLEROSIS IN DES

Chronic inflammation and insufficient functional endothelialization induce neoatherosclerosis inside both BMS and DES, causing ISR and thrombosis in the late phase[39]. In intravascular ultrasound (IVUS) analyses of VLST, neointimal rupture was observed within the stent segment in 43.5% of the DES and all of the BMS[40]. OCT also indicated that ruptured atherosclerosis and thrombosis in BMS and DES was the most common mechanism of definite VLST presenting as myocardial infarction with ST-segment elevation[41].

Pathological analysis of human coronary arteries with stented segments showed that unstable lesions, such as TCFA or intimal rupture, were associated with shorter implant durations for first-generation DES (1.5 ± 0.4 years) compared with BMS (6.1 ± 1.5 years). These results indicate that neoatherosclerosis in first-generation DES is more frequent and occurs earlier than that in BMS[39]. Pathology of second-generation everolimus-eluting cobalt chromium stents implanted < 3 years showed less uncovered strut area and milder inflammation compared with first-generation DES. However, neoatherosclerotic changes were confirmed even in second-generation DES, and there was no significant difference in neoatherosclerosis between first-generation DES and second-generation DES[42]. Neoatherosclerosis occurs more rapidly in DES than BMS, possibly because the eluted drug prevents endothelial cell proliferation, viability, and migration, which allows infiltration of lipid-laden foamy macrophage into the vessel, thereby accelerating atherosclerotic changes[43-46].

In first-generation DES, angioscopic follow-up of SES at baseline, 6 mo, and 2 years after implantation showed that neointimal growth inside the SES progressed heterogeneously, uncovered struts persisted in 20% of the patients for up to 2 years, and subclinical thrombus formation was not a rare phenomenon[47]. Although uncovered stent struts on angioscopic images do not correspond to incomplete re-endothelialization, uncovered struts may play a role in promoting atherosclerosis. An angioscopic follow-up study demonstrated that the neointima at baseline changed into a lipid-rich atherosclerotic and yellow neointima at 10 mo, with intramural thrombi being more frequently detected on newly formed yellow neointima[48]. Serial angioscopic findings up to 2 years after SES implantation showed that neointimal coverage was completed by 3 to 6 mo in BMS, whereas SES demonstrated the presence of thrombi and yellow plaques as long as 2 years after implantation[49]. The long-term vascular response was evaluated by serial angioscopic follow-up at 2 and 5 years after SES implantation, and incomplete neointimal stent coverage and the prevalence of latent thrombus within the SES segments did not decrease from 2 to 5 years[50].

In-stent neoatherosclerosis was recognized as an important mechanism of DES failure, especially late after implantation, regardless of its generation[51]. OCT was performed on a total of 50 lesions with angiographic in-stent restenosis (30 stable and 20 unstable angina patients, median follow-up time of 32 mo). Patients with unstable angina had a thinner fibrous cap and a higher incidence of TCFA, including intimal rupture and thrombi, than those with stable angina[51]. A direct comparison of the characteristics of neointimal

Table 1 Summary of each type of stent

Stent type	BMS	First-generation DES	Second-generation DES	BRS
Strut thickness	Thick	Thick	Thin	Thick
Incorporated drug	None	Rapamycin derivatives/ paclitaxel	Rapamycin derivatives	None/ rapamaycin derivatives
Polymer	None	Durable	Durable/ biodegradable	None/ biodegradable
Inflammation	Not available Foreign-body inflammatory reaction[33]	Strong	Slightly	Slightly[65]
Onset of neoathero-sclerosis	After 4 yr[39]	SES 70 d[42] PES 120 d[42]	CoCr EES 270 d[42]	Not available

Data modified from Inoue *et al*[33], Nakazawa *et al*[39], Otsuka *et al*[65]. DES: Drug-eluting stent; BRS: Bio-resorbable scaffold; SES: Sirolimus-eluting stent; PES: Paclitaxel-eluting stent.

hyperplasia and its time course between BMS and DES using OCT showed that lipid-rich neoatherosclerosis develops within stent segments earlier (< 9 mo) in DES than in BMS (≥ 48 mo), and the majority of ISR lesions developed lipid-laden neointima in both groups by 48 mo[52]. Morphological analysis of first-generation DES-ISR by OCT revealed that early (< 1 year) ISR showed a speckled pattern; in contrast, very late ISR (> 3 years) exhibited a pattern more similar to that of TCFA[53]. Angiographic and integrated backscatter IVUS analysis of ISR lesions after SES and BMS implantation showed that focal angiographic restenosis was predominantly present in the SES group, whereas diffuse restenosis was more common in the BMS group. The neointimal tissue in SES-related ISR lesions consisted of a significantly larger percentage of lipid tissue and a smaller percentage of fibrous tissue compared with that in BMS-related ISR lesions[54]. Characterization of neointimal tissue approximately 9 mo after DES implantation by OCT revealed that heterogeneous lesion type can be helpful in predicting outcomes regardless of DES generation[55]. Second-generation 40 zotarolimus, 36 everolimus, and 35 biolimus stents were not more protective against neoatherosclerosis compared with the first-generation 65 SES and 36 PES[12]. Table 1 summarizes the characteristics of each type of stent with regard to neoatherosclerosis. Taken together, continuous follow-up is required to clarify clinical events after DES regardless of its generation.

CONFRONTING NEOATHEROSCLEROSIS

There are drugs and mechanical interventions available to treat neoatherosclerosis. In the clinical setting, univariate analysis revealed that smoking and angiotensin-converting enzyme inhibitor or angiotensin Ⅱ inhibitor usage were associated with the presence of neoatherosclerosis[56]. Chronic kidney disease and > 70 mg/dL of low-density cholesterol at OCT follow-up were independent predictors of neoatherosclerosis[12]. Whether interventions addressing these risk factors and aggressive lipid-lowering therapy can improve neoatherosclerosis should be assessed in prospective trials.

Regarding PCI, OCT observation at 9 mo following treatment for DES-ISR using a paclitaxel-coated balloon to avoid repeated stenting showed a heterogeneous pattern in the neointima with speckled structures consistent with macrophage infiltration and a lipid pool consistent with neoatherosclerosis, indicating insufficient treatment of DES-ISR[57]. New stent technologies that accelerate endothelial healing through the use of a thinner stent strut, biodegradable polymer with contraluminal drug coating (Synergy™; Boston Scientific, Ultimaster™; Terumo), or luminal surface coating with CD34 antibody (COMBO™; Orbusneich Medical Technologies) to capture endothelial precursor cells, rendering the stents free from neoatherosclerosis, are expected in future clinical trials[58].

Complete bio-absorption of the vascular scaffold [bio-resorbable scaffolds (BRS)] a few years after implantation, which potentially reduces late adverse events such as VLST provoked by neoatherosclerosis in the stent caused by the permanent presence of a polymer and metallic artificial implant[59,60], can restore endothelial function[61,62]. IVUS analysis of ABSORB BVS revealed a significant plaque media reduction without a significant change in the vessel wall area (plaque regression)[61]. Nevertheless, unresolved problems remain regarding BRS. If overstretched, BRS can lose their radial strength, leading to stent fracture[63]. BRS demonstrated a higher probability of procedural side branch occlusion in small side branches compared with everolimus-eluting metallic stents[64]. Moreover, most of the data on BRS use are derived from relatively small and non-randomized studies with short or mid-term follow-up, and further studies are warranted to determine the real-world efficacy and safety of BRS[59]. The features of each stent are summarized in Table 1.

CONCLUSION

Cardiologists have been combatting coronary atherosclerosis through stent implantation and preventive medicine. Neoatherosclerosis is now emerging as a new problem that has not yet been solved. Although coronary stenting resolves the problem of atherosclerotic lesion-induced myocardial ischemia, it results in a new problem of neoatherosclerosis. New stent technology or drugs may solve this problem in the future.

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MINIREVIEWS

Epigenetic regulation in cardiac fibrosis

Li-Ming Yu, Yong Xu

Li-Ming Yu, Yong Xu, Laboratory of Cardiovascular Disease and Molecular Intervention, Department of Pathophysiology, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

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Correspondence to: Yong Xu, PhD, Laboratory of Cardiovascular Disease and Molecular Intervention, Department of Pathophysiology, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, Jiangsu Province, China. yjxu@njmu.edu.cn
Telephone: +86-25-86862888
Fax: +86-25-86862888

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Abstract

Cardiac fibrosis represents an adoptive response in the heart exposed to various stress cues. While resolution of the fibrogenic response heralds normalization of heart function, persistent fibrogenesis is usually associated with progressive loss of heart function and eventually heart failure. Cardiac fibrosis is regulated by a myriad of factors that converge on the transcription of genes encoding extracellular matrix proteins, a process the epigenetic machinery plays a pivotal role. In this mini-review, we summarize recent advances regarding the epigenetic regulation of cardiac fibrosis focusing on the role of histone and DNA modifications and non-coding RNAs.

Key words: Cardiac fibrosis; Epigenetics; Endothelial cell; Fibroblast

Core tip: Cardiac fibrosis contributes to the increased accidence of sudden cardiac death, heart failure and arrhythmia. The molecular mechanisms underlying cardiac fibrosis remain obscure. Seminal studies have revealed complex pathways associated with cardiac fibrosis. How histone/DNA modifying enzymes and microRNAs fine-tune these events are actively pursued by investigators. This review provides an overview on recent advances regarding the epigenetic regulation of fibrosis.

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INTRODUCTION

The term "epigenetics" was coined in 1953 by Waddington CH and the following decades have witnessed great progress achieved in this field[1]. By consensus epigenetics is defined as stably inheritable phenotypes stemmed from changes of chromatin

without alterations in primary DNA sequences[2]. The nucleosome, the fundamental unit of eukaryotic chromatin, is composed of an octamer of four core histones (H2A, H2B, H3, H4) surrounding 147 bp of DNA. The amino-terminal tails of histones serve as a platform for diverse posttranslational modifications including methylation[3], acetylation[4,5], ubiquitination[6,7], O-linked N-acetylglucosamine (GlcNAc)[6], phosphorylation[5] and sumoylation[8] on specific residues catalyzed by histone-modifying enzymes. These covalent modifications are dynamic[7] and modulate gene regulation in a combinatorial manner upon exposure to different stimulus[5,9,10]. Histone modifications manipulate gene activation/repression by influencing the accessibility of transcriptional factors to chromatin or by recruiting and/or occluding of non-histone proteins, mostly co-factors, in contrast to promoter CpG island methylation for gene silencing[11]. Proper function of the epigenetic machinery, or lack thereof, is implicated in mammalian development[12], carcinogenesis[4] and cardiovascular diseases (CVDs).

Fibrosis or scarring in different organs, including the lungs[13], the kidneys[14], the liver[15], and the heart, is characterized by deposition of extracellular matrix (ECM) components, such as collagens, laminins and fibronectin, caused by diverse insults. Fibrosis can be deemed as erroneous ECM "turnover", *i.e.*, imbalance between ECM production (increased) and ECM degradation (reduced). Collagen is the most abundant component of the ECM in the heart including five types (types Ⅰ, Ⅲ, Ⅳ, Ⅴ and Ⅵ) identified in the myocardium. Among these, types Ⅳ and Ⅴ collagens are components of the basement membrane, while types Ⅰ and Ⅲ collagen are the main constituents of the ECM[16,17]. A number of different cell types in the heart are responsible for collagen synthesis: All cardiac collagen types are produced by fibroblasts, whereas endothelial cells synthesize all types except type Ⅵ. Degradation of collagen is mediated by both intracellular and extracellular pathways, the latter involving matrix metalloproteinase (MMPs) and tissue inhibitors of MMPs (TIMPs)[18].

Fibrosis is an evolutionarily conserved process that serves to facilitate host defense and wound healing. Deregulated fibrosis, however, is invariably associated with loss of organ function. For instance, cardiac fibrosis is correlated with elevated mortality in dilated cardiomyopathy[19], which is the most common cardiomyopathy globally and directly correlates with sudden cardiac death, heart failure and arrhythmia[20-22]. Despite numerous progress made in identifying molecular mechanisms and/or factors that contribute to hypertrophy over the past decades, the mechanistic underpinnings of cardiac fibrosis is poorly understood. Although an extensive body of evidence suggests that cardiac fibroblast may participate in the pathogenesis of cardiac fibrosis, other cell types involved remain to be determined, especially endothelial cells and macrophages[23-26]. This review summarizes our current understanding of the involvement of epigenetic machinery in cardiac fibrosis and attempts to identify some of the previously unaddressed questions that require further investigation. We only briefly discuss the pathways and transcriptional factors involved in cardiac hypertrophy because models used to study cardiac hypertrophy and fibrosis often overlap and excellent reviews on cardiac hypotrophy are available elsewhere[27,28].

SIGNALING CASCADE IN CARDIAC FIBROSIS

Cardiac fibrosis usually appears in patients with hypertrophic cardiomyopathy, hypertension and/or diabetes mellitus, suggesting that cardiac fibrosis may be secondary to these conditions[29-33]. Myocardial infarction (MI), aging, and mutation in cardiac fatal genes such as Mhy7, Troponin T and BNP can also trigger cardiac fibrosis[34-38]. Studies in animal models have revealed a convoluted network of signaling cascades and transcriptional factors. A body of evidence suggests that the calcineurin–nuclear factor of activated T cells (NFAT) circuit, the β-adrenergic–receptor signaling pathway, and the IGF-Akt signaling pathway all contribute to cardiac fibrosis by modulating the activities of such transcription factors as serum response factor, myocyte enhancer factor (MEF), and kruppel-like factor during development and in response to pathophysiological stimuli[29,30,39-43]. Meanwhile, evidence from different groups shows that extracellular-regulated kinases Erk1 and Erk2 (Erk1/2), downstream effectors of the mitogen-activated protein kinase cascades, play a prominent role in cardiac hypertrophy and fibrosis. ERK activation mediated by auto-phosphorylation at Thr188 enhances TAC-induced cardiac hypertrophy and fibrosis[26,39,40].

TGF-β is believed to play the most central role in cardiac fibrosis based on the fact that TGF-β is activated in different models of cardiac fibrosis, which in turn facilitates the synthesis of ECM proteins and contributes to endothelial-mesenchymal transition (EndMT)[33,44-47]. Meanwhile, TGF-β represses ECM degradation by suppressing the expression of MMPs[48] and by augmenting the levels of protease inhibitors such as plasminogen activator inhibitors and TIMPs[44,49]. TGF-β drives fibrotic process by binding to the heterodimeric membrane receptor, which results in phosphorylation and subsequently nuclear translocation of SMAD family of transcription factors[50]. Thus, inhibition of the specific cellular receptors, kinases and other mediators involved in the activation of TGF-β pathway may provide effective therapeutic targets for cardiac fibrosis.

HISTONE MODIFYING ENZYMES IN CARDIAC FIBROSIS

Numerous enzymes that catalyze specific residues

of core histones have been implicated in cardiac hypertrophy and fibrosis. For instance, p300, a histone acetyltransferase, accelerates left ventricular remodeling after MI[9,10]. Inactivation of Ezh2, the catalytic subunit of the Polycomb repressor complex 2 responsible for histone H3K27 methylation (H3K27me3), induces cardiac fibrosis[3,51]. These histone modifying enzymes influence cardiac fibrosis *via* the interaction with sequence-specific transcriptional factors to manipulate fibrosis-associated gene activation or repression. For example, p300 and GATA-4 synergistically activate GATA-4-dependent transcription of the *ET-1* and *ANF* genes[10] and Ezh2-mediated H3K27me3 on the promoter zones directly represses fetal gene expression[51].

Trivedi *et al*[52] show that the mice deficient in Hdac2, a class Ⅰ histone deacetylase (HDAC), are resistant to isoproterenol-induced cardiac hypertrophy and fibrosis. Mechanistically, Hdac2 deletion leads to the derepression of inositol polyphosphate-5-phosphatase f (Inpp5f). Consequently, glycogen synthase kinase 3β (GSK3β) is constitutively activation thereby causing the inactivation of cardiac fetal genes[52]. However, the authors did not address whether fibrosis is independent of GSK3β or GSK3β is responsible for both cardiac hypertrophy and fibrosis. Olson and colleagues report that class Ⅱ HDACs interact with MEF2 and repress its activity, acting as signal-responsive repressors of transcription of cardiac fetal genes[53]. This observation is verified by several complementary studies. First, inhibition of class Ⅰ and Ⅱ HDACs by trichostatin A (TSA) protects the mammalian heart from pressure overload-induced cardiac fibrosis and attenuates hypertrophy-associated protein expression[51]. Zhang *et al*[53] show that calmodulin binding transcription activator 2 (CAMTA2), transcriptional coactivator for Nkx2-5, is repressed by an interaction with class II HDAC. Activation of PKC/PKD signaling leads to phosphorylation of class Ⅱ HDACs, creating docking sites for 14-3-3 proteins to exclude HDACs from the nucleus and relieving the inhibition of CAMTA2, which proceeds to activate cardiac hypertrophy and fibrosis[54]. Recently, our laboratory has identified a histone H3K4 trimethylation-dependent pathway that contributes to cardiac fibrosis. Specifically, we have discovered that SET1, an H3K4me3 modifying enzyme, induces the transcription of endothelin (ET-1) in vascular endothelial cells. Once released into the circulation, ET-1 then serves as an angiocrine factor to induce cardiac fibrosis in response to chronic angiotensin Ⅱ infusion or mechanic stretch[55].

Histone modifying enzymes can communicate with each other or other branches of the epigenetic machinery to modulate cardiac fibrosis. A study by Eom *et al*[56] further highlights the role of crosstalk between HDACs and HATs and post-translational modifications of these proteins in cardiac hypertrophy and fibrosis. These authors propose that the acetylation status of HDAC2 and by extension its activity in regulating cardiac fibrosis is controlled by p300/CBP-associated factor and HDAC5[56]. Weng *et al*[57] have found that the H3K4 methyltransferase complex (COMPASS) can forge a dialogue with chromatin remodeling proteins BRG1 and BRM to transactivate ET-1, which in turn invokes a pro-fibrogenic response in the heart; depletion of either COMPASS or BRG1/BRM alleviates Ang Ⅱ-induced cardiac fibrosis in mice[57].

Overall, although there is abundant evidence supporting a role for histone modifying enzymes in cardiac fibrosis, the dataset appears to be fragmental with many outstanding issues awaiting resolution. For instance, what is the genome-wide role for any given histone modifying enzyme in cardiac fibrosis? How are different histone modifying enzymes are recruited to the chromatin? Is there a unique histone signature that defines cardiac fibrosis? How to differentiate histone modifications and non-histone protein modifications? These lingering questions will have to be addressed in future studies.

MICRORNA INVOLVED IN CARDIAC FIBROSIS

MicroRNAs (miRNAs), usually 20-30 nucleotide in length, are one major form of small non-coding regulatory RNAs that also include short interfering RNAs (siRNAs) and piwi-interacting RNAs (piRNAs)[58]. In general, miRNAs act to silence gene expression by targeting specific mRNA at the posttranscriptional level. MiRNA expression profiles are widely used in cancer classification, diagnosis, therapy and prognosis[59], but mounting evidence shows that circulatory miRNAs, such as miR-29a and miR-21, may also be used as a diagnostic marker for cardiac fibrosis[60,61]. Numerous studies aimed to investigate the potential impact of miRNAs in the heart have demonstrated a key role for miRNAs in cardiac fibrosis in response to multiple injury stimulus.

It has been demonstrated that mice depleted of miR-212/132[62], miR-25[61,63], or miR-29[61] are protected from pressure-overload-induced cardiac fibrosis while miR-101[64] and miR-24[65] regulate fibrosis after MI. Knockdown of miR-133a[66] and cardiac-specific overexpression of miR-195 induces spontaneous cardiac hypertrophy and fibrosis. Thum *et al*[26] have shown that miR-21 silencing in fibroblasts decreases ERK-MAP kinase activity and curbs interstitial fibrosis. Follow-up studies have shown several different but not mutually exclusive mechanisms underlying the pro-fibrotic effect of miR-21. For instance, Roy *et al*[67] have found that miR-21 regulates fibroblast MMP-2 *via* targeting phosphatase and tensin homologue (PTEN). Alternatively, miR-21 also partly influences TGF-β-mediated EndMT *via* the PTEN/Akt pathway[68]. Conceivably, miR-21 might elicit a range of different pathways responsible for cardiac fibrosis at multiple levels. Cardiac-specific miR-208, transcribed from the a-myosin heavy chain (*a-MHC*) gene locus, regulates

stress-dependent fibrosis by negatively modulating expression of thyroid hormone receptor associated protein 1[69]. The role of miR-208 in cardiac fibrosis is further supported by the observation that inhibition of miR-208 by antisense oligonucleotide improves cardiac function and attenuates remodeling[70].

Sometimes miRNAs and their targets form feedback (forward or backward) loops to manipulate downstream pathophysiological events. For instance, da Costa Martins *et al*[41] have reported that pressure overload activates the calcineurin/NFAT axis to stimulate the expression of miR-199b. MiR-199b, once transcribed, targets dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1a (Dyrk1a), which activates NFAT by phosphorylating and thereby excluding NFAT from the nucleus. Conceivably, reduced levels of Dyrk1a as a result of miR-199b activation will release NFAT from the cytoplasm, which will lead to increased expression of miR-199b[41].

Cardiac- and skeletal muscle–enriched miR-22 regulates cardiomyocyte hypertrophy and cardiac fibrosis in response to stress *via* targeting Sirt1 and Hdac4[71], supporting the possibility that microRNAs could communicate with other epigenetic factors by directly influencing their abundances. Meanwhile, miRNAs could also suppress fibrotic genes transcription. MiR-133 and miR-30 could reduce production of collagens by directly down-regulating connective tissue growth factor (CTGF) through specific binding to its 3′ untranslated region (3′-UTR)[72]. MiR-101a can restrain interstitial fibrosis in post-infarct rats by targeting c-Fos to repress downstream effectors of TGF[64]. Intriguingly, miR-18/19 and miR-34a dampen age-related cardiac remodeling by negatively regulating the CTGF and thrombospondin-1[73] expression and directly targeting protein phosphatase 1 nuclear-targeting subunit[38], respectively.

DNA METHYLATION IN CARDIAC FIBROSIS

Patterns of mammalian DNA methylation vary in time and space. Similar to histone modifications, levels of DNA methylation are dependent on the balance of methyltransferases (DNMTs) and demethylases. In general, DNA methylation modulates gene transcription *via* changing chromatin conformation and/or influencing the interplay between DNA and proteins[74,75]. Based on the structural and functional differences, the enzymes responsible for DNA methylation identified so far include two categories: DNMT1 and DNMT3a/3b. DNMT1 is responsible for maintenance of DNA methylation using hemimethylated DNA strand as substrate[76], while DNMT3a/3b catalyze de novo DNA methylation operating on two un-methylated "clean" DNA strands[77].

A recent investigation by Xu *et al*[78] showed that TGF-β induces aberrant methylation of RASAL1 (a Ras-GTPase) promoter and subsequently down-regulation of RASAL1, resulting in elevated Ras-GTP activity to enhance EndMT and cardiac fibrosis. Mechanistically, this process is associated with ten eleven translocation family enzyme (TET3)-mediated RASAL1 promoter hydroxymethylation (or demethylation) and reversal of EndMT[78]. A recent study indicates that mice with cardiac-specific knockout of DNMT3b, predominantly expressed in the heart, exhibit extensive interstitial fibrosis and myo-sarcomeric disarray[79]. Further exploration suggests that dysregulation of DNA methylation-induced alternatively spliced myh7 transcript may be accountable for these phenotypes, which is similar to the aforementioned effects of miR-208 derived from myh6[60].

Methylation of DNA is not an isolated event but instead forges crosstalk with non-coding RNAs and histone modifications. For instance, Wang *et al*[80] show that lysine demethylase (LSD1) interacts and demethylates DNMT1 to increase DNMT1 stability, indicating that LSD1 may coordinately modulate histone and DNA methylation by acting directly on both histones and Dnmt1. Meanwhile, DNMT3a/b are recruited to tri-methylated H3-K9 positions *via* interacting with heterochromatin protein 1 (HP1a)[81], synergistically silencing transcription at the pericentric satellite repeats[82]. Whether these interactions and/or cooperations function in the heart remain elusive. Dakhlallah *et al*[83] demonstrated that in lung fibroblasts from patients with idiopathic pulmonary fibrosis, there was a negative correlation between increased DNA methylation-induced repression of miR-17-92 cluster and DNMT1 expression. Several miRNAs from the miR-17-92 cluster, most prominently miR-19b, directly regulated DNMT1 expression by targeting seed sequences in the 3-UTR in a negative feedback loop. To further study whether this system function *in vivo*, Dakhlallah *et al*[83] use a classical murine model of pulmonary fibrosis. After the initiation of fibrosis, treatment with 5-aza-2-deoxycytidine in bleomycin-challenged mice alleviated lung fibrosis by decreasing *DNMT-1* gene expression while restoring miR-17-92 cluster expression[83]. These results are consistent with findings from Bechtel *et al*[84] that long-term TGFβ1 exposure induced RASAL1 hypermethylation depends on DNMT1, which is intimately linked to the perpetuation of kidney fibroblast activation and renal fibrosis. More importantly, 5-aza-2-deoxycytidine attenuated folic acid-evoked renal fibrosis by reducing DNMT1–induced methylation of RASAL1[84]. In the heart, whether miRNAs regulate DMNTs in a similar fashion needs to be addressed in the future study.

FUTURE DIRECTIONS IN CARDIAC FIBROSIS

The past two decades have seen a sea of groundbreaking discoveries in epigenetics fueling the research on CVD[85-88]. This mini-review only provides a snapshot

of how research on cardiac fibrosis has benefitted from epigenetic theories and tools. Many of the factors discussed here are enzymes, the activities of which can be manipulated *via* small-molecule compounds for therapeutic interventions. For instance, HDAC inhibitors have been successfully used to treat certain forms of cancer in the clinic[89,90]. The recent elucidation of the human functional genome has re-affirmed the notion that epigenetic regulation is the bedrock of human diseases[91]. In perspective, continued effort in investigating the epigenetic mechanisms underlying cardiac fibrosis will eventually bring cure to this debilitating pathology.

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ORIGINAL ARTICLE

Retrospective Study

Short and long term outcomes of 200 patients supported by continuous-flow left ventricular assist devices

Athanasios Tsiouris, Gaetano Paone, Hassan W Nemeh, Jamil Borgi, Celeste T Williams, David E Lanfear, Jeffrey A Morgan

Athanasios Tsiouris, Jeffrey A Morgan, Section of Cardiac Surgery, Yale University School of Medicine, New Haven, CT 06520, United States

Gaetano Paone, Hassan W Nemeh, Jamil Borgi, Celeste T Williams, David E Lanfear, Division of Cardiac Surgery, Heart and Vascular Institute, Henry Ford Hospital, Detroit, MI 48201, United States

Author contributions: Tsiouris A was responsible for drafting the manuscript, statistical analysis and study design; Paone G, Nemeh HW, Borgi J, Williams CT, Lanfear DE and Morgan JA provided critical analysis of the results and the manuscript and supervised the project.

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Correspondence to: Athanasios Tsiouris, MD, Section of Cardiac Surgery, Yale University School of Medicine, 333 Cedar St, Boardman 204, New Haven, CT 06520, United States. athtsiouris@hotmail.com
Telephone: +1-203-7857627

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Abstract

AIM: To study the institutional experience over 8 years with 200 continuous-flow (CF) - left ventricular assist devices (LVAD).

METHODS: We evaluated our institution's LVAD database and analyzed all patients who received a CF LVAD as a bridge to transplant (BTT) or destination therapy from March 2006 until June 2014. We identified 200 patients, of which 179 were implanted with a HeartMate II device (Thoratec Corp., Pleasanton, CA) and 21 received a Heartware HVAD (HeartWare Inc., Framingham, MA).

RESULTS: The mean age of our LVAD recipients was 59.3 years (range 17-81), 76% (152/200) were males, and 49% were implanted for the indication of BTT. The survival rate for our LVAD patients at 30 d, 6 mo, 12 mo, 2 years, 3 years, and 4 years was 94%, 86%, 78%, 71%, 62% and 45% respectively. The mean duration of LVAD support was 581 d (range 2-2595 d). Gastrointestinal bleeding (was the most common adverse event (43/200, 21%), followed by right ventricular failure (38/200, 19%), stroke (31/200, 15%), re exploration for bleeding (31/200, 15%),

ventilator dependent respiratory failure (19/200, 9%) and pneumonia (15/200, 7%). Our driveline infection rate was 7%. Pump thrombosis occurred in 6% of patients. Device exchanged was needed in 6% of patients. On multivariate analysis, preoperative liver dysfunction, ventilator dependent respiratory failure, tracheostomy and right ventricular failure requiring right ventricular assist device support were significant predictors of post LVAD survival.

CONCLUSION: Short and long term survival for patients on LVAD support are excellent, although outcomes still remain inferior compared to heart transplantation. The incidence of driveline infections, pump thrombosis and pump exchange have declined significantly in recent years.

Key words: Left ventricular assist device; Outcomes; Heart failure; Continuous-flow

Core tip: In this paper, we report our experience over the last 8 years with implanting continuous-flow left ventricular assist devices (LVADs). The aim of this analysis is to identify common occurring complications after LVAD implantation and identify areas for potential improvement in both patient management and selection. This is the largest single institutional LVAD experience that has been published, to the best of our knowledge.

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INTRODUCTION

Continuous-flow left ventricular assist devices (CF LVADs) are now the standard treatment for patients with end stage heart failure refractory to medical management[1-3]. The shortage of heart donors and the overall minimal therapeutic impact of heart transplantation on advanced heart failure have certainly accelerated the recent advances made in LVAD technology. In 2001, the landmark REMATCH trial demonstrated superiority of the pulsatile-flow HeartMate XVE *vs* best medical management, although these devices were still limited by their large size, reduced durability, significant and frequent postoperative complications[4]. Newer generation CF LVAD has by and large overcome most of the limitations of the pulsatile devices. Following the HeartMate Ⅱ (HM Ⅱ) trial[1], continuous flow devices were approved by the FDA, initially for bridge to transplantion (BTT) and subsequently for destination therapy (DT). Increasing clinical implementation and a multidisciplinary approach between cardiac surgeons and cardiologists to postoperative LVAD therapy have in recent years further improved LVAD outcomes. Despite these significant advances, LVAD implantations are still associated with significant morbidity, especially in the early postoperative period[5,6]. Improvements are still required if LVADs are to become a plausible alternative to heart transplantation or a therapeutic option for less sick patients in earlier stages of heart failure. The aim of our study was to investigate our institution's 8-year experience with CF LVADs and to analyze short and long term results with a goal to identify areas of improvement.

MATERIALS AND METHODS

This retrospective study was approved by our health system's Institutional Review Board. We reviewed our institution's LVAD dataset and analyzed all patients who received a CF LVAD as a BTT or DT from March 2006 until June 2014. We identified 200 patients, of which 179 were implanted with a HeartMate II device (Thoratec Corp., Pleasanton, CA) and 21 received a Heartware HVAD (HeartWare Inc., Framingham, MA).

Patient data

Multiple patient comorbidities from our LVAD database were analyzed. Pre and postoperative hemodynamic measurements were also evaluated. Finally we examined post LVAD related complications. We defined ventilator dependent respiratory failure (VDRF) as inability to extubate after 7 d right ventricular (RV) failure was considered for patients who needed a RVAD or who required inotropes in excess of two weeks in order to support the RV. Defining acute renal failure, was based on the RIFLE criteria (two fold increase in creatinine or a decline in glomerular filtration rate by half.

Statistical analysis

Patient data were compared between patients who received LVAD as DT or BTT using chi-squared tests for nominal data and Wilcoxon two-sample tests for continuous variables. Nominal data were reported as count and percent whereas as mean and standard deviations were calculated for continuous variables. For counts that were not large, the fisher exact tests were utilized. Kaplan Meier curves were used to generate estimates of survival and Cox proportional hazards models were used to assess the various covariates effect on survival. A backward stepwise routine was used to generate the most parsimonious model where all variables included were significant. Statistical significance was considered $P < 0.05$. SAS 9.2 was utilized for our analysis.

Table 1 Patient demographics and comorbidities

Variable	Total (n = 200)	BTT (n = 98)	DT (n = 102)	P value
Age (yr)	54.3 ± 12.5	50.1 ± 12.8	58.4 ± 10.7	0.001
Gender				
Female	24% (48/200)	25.5% (25/98)	22.8% (23/102)	
Male	76% (151/200)	74.5% (73/98)	76.5% (78/102)	0.652
Race				
AA	46% (92/200)	39.8% (39/98)	52% (53/102)	0.375
Caucasian	54% (108/200)	54.1% (53/98)	42.4% (47/102)	
Etiology of heart failure				
ICM	52% (104/200)	29% (28/98)	74.5% (76/102)	0.001
NIDCM	48% (96/200)	51% (50/98)	45.1% (46/102)	
BSA	1.97 ± 0.27	1.96 ± 0.27	1.98 ± 0.28	0.667
BMI	28.3 ± 5.5	28.1 ± 4.3	28.5 ± 6.5	0.763
Albumin (g/dL)	4.14 ± 10.03	3.19 ± 0.51	5.06 ± 14.05	0.015
DM	46% (92/200)	38.8% (38/98)	52.9% (54/102)	0.038
HTN	83% (166/200)	79.6% (78/98)	86.2% (88/102)	0.153
CRI	40% (81/200)	29.6% (29/98)	51% (52/102)	0.002
Dialysis	2.5% (5/200)	3.1% (3/98)	1.8% (2/102)	0.680
COPD	15.5% (31/200)	15.3% (15/98)	15.7% (16/102)	0.917
PVD	12% (23/200)	7.1% (7/98)	15.7% (16/102)	0.055
Vented	12% (25/200)	9.2% (9/98)	15.7% (16/102)	0.134
Previous cardiac surgery	32% (63/200)	20.4% (20/98)	42% (43/102)	0.001
Creatinine (mg/dL)	1.42 ± 0.62	1.43 ± 0.58	1.42 ± 0.65	0.869
AST (U/L)	48.3 ± 82.8	58.0 ± 106.8	38.9 ± 45.7	0.212
ALT (U/L)	46.5 ± 78.5	59.8 ± 99.4	33.5 ± 47.3	0.002
CPB time (min)	113.5 ± 46.1	109.5 ± 46.0	117.8 ± 46.1	0.178
XCL time (min)	71 ± 30.6	85.2 ± 33.7	51.7 ± 26.0	0.054
MCS at time of VAD	18% (36/200)	24% (23/98)	13% (13/102)	0.051
On inotropes at time of VAD	75%(150/200)	81% (80/98)	69% (70/102)	0.036
Pre VAD CVP (mmHg)	11.8 ± 6.4	11.6 ± 6.4	12.0 ± 6.4	0.653
Pre VAD PAPs (mmHg)	51.4 ± 14.2	50.5 ± 14.5	52.3 ± 13.8	0.412
Pre VAD PAPd (mmHg)	24.5 ± 9.2	24.4 ± 9.8	24.7 ± 8.5	0.682
Pre Vad CI (L/min per square metre)	1.85 ± 0.51	1.87 ± 0.54	1.83 ± 0.47	0.961
Pre VAD PCWP (mmHg)	23.0 ± 9.6	22.7 ± 9.8	23.4 ± 9.4	0.463
Blood transfusions	23% (46/200)	18% (18/98)	27% (28/102)	0.250
Concomitant cardiac procedure	19% (39/200)	23% (23/98)	15% (16/102)	0.137

BTT: Bridge to transplant; DT: Destination therapy; ICM: Ischemic cardiomyopathy; NIDCM: Non ischemic dilated cardiomyopathy; BSA: Body surface area; BMI: Body mass index; DM: Diabetes mellitus; HTN: Hypertension; CRI: Chronic renal insufficiency; COPD: Chronic obstructive pulmonary disease; PVD: Peripheral vascular disease; AST: Aspartate transaminase; ALT: Alanine aminotransferase; CPB: Cardiopulmonary bypass; XCl: Cross clamp; MCS: Mechanical circulatory support; VAD: Ventricular assist device; CVP: Central venous pressure; PAP: Pulmonary artery systolic pressure; PAPd: Pulmonary artery diastolic pressure; CI: Cardiac index; PCWP: Pulmonary capillary wedge pressure.

RESULTS

Preoperative patient demographics and operative characteristics

The mean age of our LVAD recipients was 59.3 years (range 17-81), 76% (152/200) were males and 24% females (48/200). BTT was the indication for LVAD implantation in 49% of patients (98/200) and DT in 51% (102/200) of patients. Additional patient demographics and comorbidities are presented in Table 1. In terms of operative characteristics, 31% of patients had undergone previous median sternotomy, the average cardiopulmonary bypass time was 113 min, cross clamp time (when used) was 71 min, and 19% of patients underwent a concomitant procedure at the time of LVAD implantation. In our cohort, 18% were on some type of mechanical circulatory support (MCS) at the time of LVAD insertion. Types of pre CF LVAD MCS included intraortic balloon pumps (23/36, 63%), pulsatile flow HeartMate XVE (5/36, 15%), CentriMag devices (5/36, 15%), Impella (2/26, 8%) and AbioMed support (1/36, 3%). BTT patients were significantly younger, had worse pre LVAD liver function and albumin, whereas DT patients were more likely to be diabetic, to have PVD, CRI and to have undergone previous cardiac surgery. Pre-LVAD inotropic support or MCS was more likely in the BTT patients (Table 1).

Duration of support, heart transplant and survival rates

The mean duration of LVAD support was 581 d (range 2-2595 d) (Table 2). A 56-year-old male, who received a CF LVAD for DT, is our longest survivor having been on LVAD therapy for just over 7 years. Overall, 27% of LVAD recipients and 46% of the BTT patient underwent heart transplantation (Table 2). At 2 years, the survival rate for our heart transplant recipients was 95% (52/55) which was significantly superior to the 2 year 71% survival rate for DT patients (P = 0.02). The survival rate at 30 d, 6 mo, 12 mo, 2 years, 3 years and 4 years was 94%, 86%, 78%, 71%, 62% and 45%

Table 2 Postoperative outcomes

Variable	Total (n = 200)	BTT (n = 98)	DT (n = 102)	P value
Postoperative ICU stay (d)	195 10.7 ± 10.4	95 10.2 ± 7.7	100 11.2 ± 12.5	0.833
Overall length of stay (d)	198 21.4 ± 14.3	98 20.8 ± 12.9	100 22.1 ± 15.6	0.517
Readmitted within 30 d	26.5% (53/200)	26.0 (25/96)	27% (28/102)	0.725
Reexploration for bleeding	15% (31/200)	10% (10/98)	5% (6/102)	0.040
DL infection	7% (15/200)	9% (9/98)	5% (6/102)	0.386
Pocket infection	1% (2/200)	1% (1/98)	1% (1/102)	0.493
Pneumonia	7% (15/200)	9% (9/98)	5% (6/102)	0.375
Hemorrhagic stroke	10% (21/200)	9% (9/98)	11% (12/102)	0.432
Emboli stroke	5% (10/200)	6% (6/98)	3% (4/102)	0.493
VDRF	9% (19/200)	10% (10/98)	8% (9/102)	0.774
Tracheostomy	2% (5/200)	1% (1/98)	3% (4/102)	0.369
Dialysis	2% (5/200)	3% (3/98)	1% (2/102)	0.680
GIB	21% (43/200)	17% (17/98)	25% (26/102)	0.289
Reoperation for AI	2% (4/200)	4% (4/98)	0% (0/102)	0.058
RV failure	19% (38/200)	15% (15/98)	22% (23/102)	0.192
RV failure requiring milrinone	13% (26/200)	9% (9/98)	16% (17/102)	0.103
RV failure requiring RVAD	6% (12/200)	6% (6/98)	5% (6/102)	0.803
Heart transplant	27% (55/200)	45% (45/98)	10% (10/102)	0.001
Duration of support (d)	581.0 ± 517.9	554.8 ± 535.0	606.4 ± 502.1	0.253

ICU: Intensive care unit; DL: Driveline; VDRF: Ventilator dependent respiratory failure; GIB: Gastrointestinal bleeding; AI: Aortic insufficiency; RV: Right ventricular; RVAD: Right ventricular assist device.

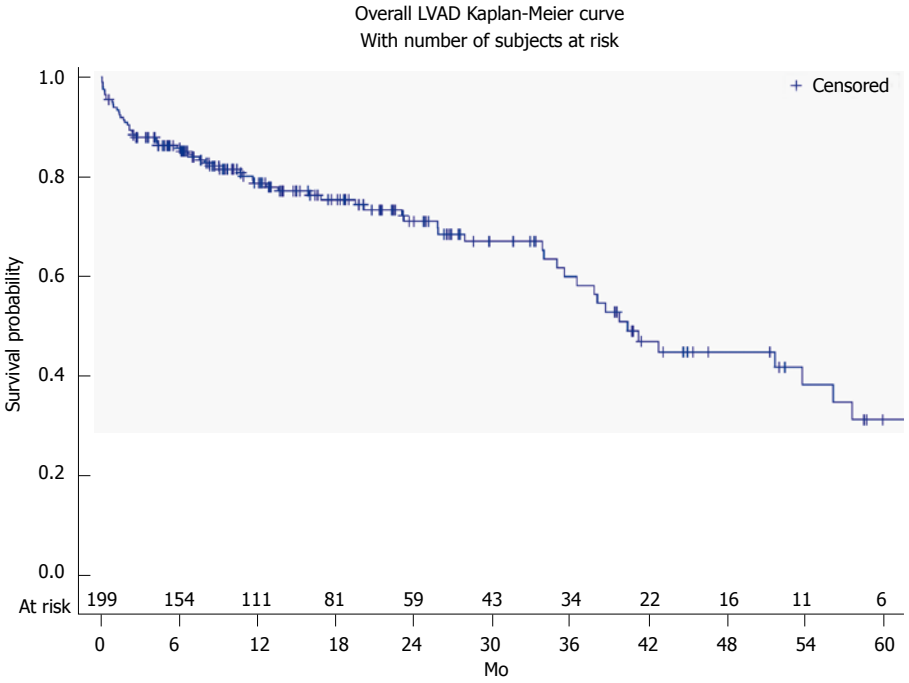

Figure 1 Kaplan-Meier survival curve for all patients receiving continuous-flow left ventricular assist devices. LVAD: Left ventricular assist device.

respectively (Figure 1). Survival rates were similar for BTT and DT patients (P = 0.566). Survival at 1 mo, 6 mo, 1 year, 2 years, 3 years and 4 years for the BTT patients was 93%, 87%, 70%, 70%, 63% and 52% respectively whereas for the DT group survival was 95%, 85%, 78%, 71%, 58% and 40% respectively (Figure 2). Competing outcomes of BTT *vs* DT patients is demonstrated in Table 3.

Causes of death

Since implanting our first CF LVAD in 2006, a total of 63 patients have died. Causes of death included: stroke (20/63, 32% of which 15 /63, 24% were hemorrhagic and 5/63, 8% were ischemic, range 2-654 d postoperatively, median 35 d), sepsis (17/63, 27%, range 5-320 d postoperatively, median 47 d), multi-organ failure (15/63, 24%, range 4-211 d median 35 d), right ventricular failure (6/63, range 2-139 d, median 10 d), refractory arrhythmia (2/63, 3%, at 64 and 128 d after LVAD implantation), bowel perforation (1/63, 1.5%, on postoperative day-11 and day-13), disconnection from the power source (1/63, 1.5%, 14

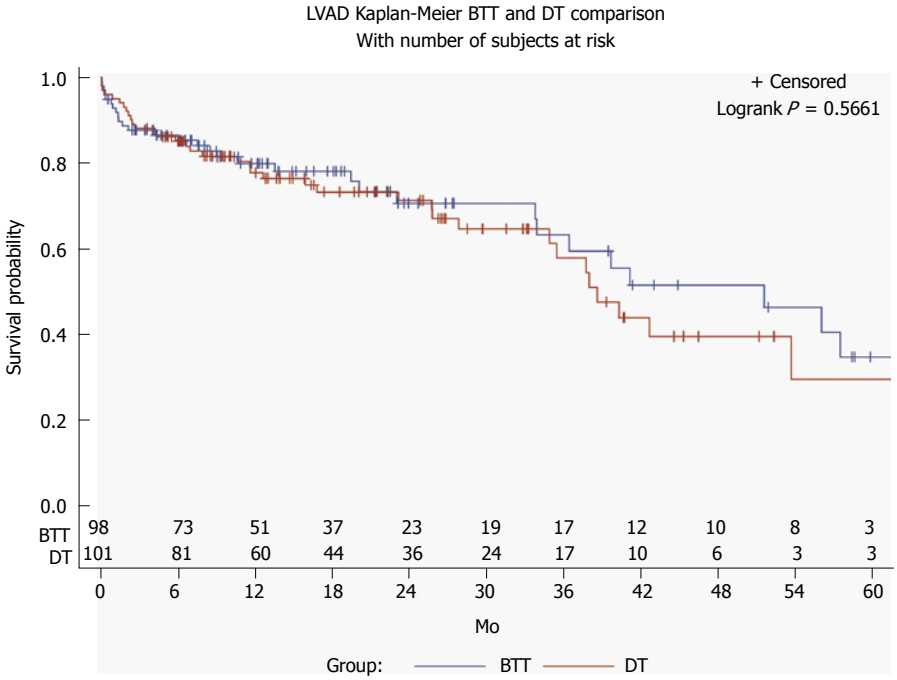

Figure 2 Comparison of Kaplan-Meier survival between bridge to transplan and destination therapy patients. LVAD: Left ventricular assist device; BTT: Bridge to transplan; DT: Destination therapy.

Table 3 Outcomes for bridge to transplan and destination therapy patients

	Variable	Patients (%)
BTT	Died	28.6 (28/98)
	Ongoing	25.5 (25/98)
	Transplant	45.9 (45/98)
DT	Died	34.3 (35/102)
	Ongoing	54.8 (56/102)
	Transplant	9.8 (10/102)

mo after implantation), and pump thrombosis (1/63, 1.5%, 18 mo after implantation).

Postoperative LVAD complications

Post LVAD complications are listed in Table 2. GIB was the most common adverse event (43/200, 21%), followed by RV failure (38/200, 19%), stroke (31/200, 15%), re exploration for bleeding (31/200, 15%), VDRF (19/200, 9%) and pneumonia (15/200, 7%). Our driveline infection rate was 7%. Pump thrombosis occurred in 6% of patients. Device exchanged was needed in 6% of patients, of which 77% (10/13) were for pump thrombosis and 13% (3/13) for severe driveline and pocket infections. No differences were noted between BTT and DT patients in terms of adverse events.

Length of ICU and hospital stay, and early readmissions

The average length of hospital stay (LOS) for our LVAD patients was 21 d, of which 11 d were spent in the intensive care unit (ICU). Readmissions within 30 d of index hospitalization discharge occurred in 27% of patients. No differences were observed between the BTT and DT patients in terms of LOS, ICU stay and readmission (Table 2). The most common cause of 30 d readmission were cardiac related (chest pain, SOB/heart failure, arrhythmia), gastrointestinal bleeding (GIB) (25%), infections 12% (pneumonia, wound/driveline infections, UTI) and stroke 8%.

Hemodymanic measurements pre LVAD and post LVAD at 6 mo

Hemodynamic measurements prior to LVAD implantation and after 6 mo of LVAD therapy are demonstrated in Table 4. Significant improvement was noted for all indices and measurements, which confirmed adequate LV decompression and improvement in RV function.

Predictors of survival

Univariate analysis showed that pre-LVAD renal (HR = 1.56; 95%CI: 1.11-2.21, P = 0.012) and hepatic function (HR = 1.03; 95%CI: 1.01-1.05, P = 0.004), length of ICU stay (HR = 1.34; 95%CI: 1.12-1.61, P = 0.001), the occurrence of VDRF (HR = 4.66; 95%CI: 2.51-8.67, P = 0.001), the need for tracheostomy (HR = 15.18; 95%CI: 5.56-41.4, P = 0.001) and the occurrence of post LVAD RV failure that required RVAD support (HR = 5.81; 95%CI: 2.84-11.9, P = 0.001) were significant predictors of survival. Variables with a P < 0.25 were included in a cox regression model. On multivariate analysis, pre LVAD liver function, VDRF, tracheostomy and implantation of a RVAD for RV failure still predicted survival (Table 5).

DISCUSSION

Continuous flow LVADs have now become an efficient

Table 4 Hemodymanic measurements pre and post left ventricular assist device at 6 mo

Variables	Pre VAD	Post VAD	*P* value
CVP (mmHg)	12 ± 6	8 ± 4.5	0.001
PAPs (mmHg)	53.52 ± 13.76	36.03 ± 11.85	0.001
PAPd (mmHg)	26.15 ± 9.50	16.11 ± 6.24	0.001
CI (L/min per square meter)	1.78 ± 0.39	2.52 ± 0.60	0.001
PCWP (mmHg)	25.09 ± 10.05	11.93 ± 7.84	0.001
LVEDD (mm)	71.70 ± 13.61	57.45 ± 15.3	0.001
LVEF (%)	16 ± 7.90	21 ± 9.00	0.017

VAD: Ventricular assist device; CVP: Central venous pressure; PAP: Pulmonary artery systolic pressure; PAPd: Pulmonary artery diastolic pressure; CI: Cardiac index; PCWP: Pulmonary capillary wedge pressure; LVEDD: Left ventricular end diastolic diameter; LVEF: Left ventricular ejection fraction.

Table 5 Multiple cox proportional hazard models

Variable	HR 95%CI	*P* value	Backwards stepwise model
Albumin	0.64 (0.27, 1.52)	0.310	
Length of stay	0.85 (0.62, 1.17)	0.319	
CPB time	1.05 (0.98, 1.14)	0.175	
CRI	1.13 (0.44, 2.91)	0.804	
PVD	0.95 (0.30, 3.03)	0.931	
Vented	0.93 (0.17, 4.97)	0.929	
Creatinine	0.77 (0.37, 1.63)	0.495	
PreVAD AST	1.03 (1.00, 1.07)	0.072	1.03 (1.01, 1.05) 0.01
PreVAD ALT	1.02 (1.00, 1.05)	0.064	1.02 (1.01, 1.04) 0.02
Blood transfusion	1.19 (0.45, 3.14)	0.732	
ICU stay	0.80 (0.52, 1.24)	0.320	
Reexploration	1.70 (0.50, 5.79)	0.794	
VDRF	4.92 (1.62, 14.93)	0.005	3.05 (1.41, 6.59) 0.005
Tracheostomy	5.53 (0.65, 46.78)	0.116	4.54 (1.35, 15.32) 0.015
RV failure	0.45 (0.09, 2.26)	0.330	
RVAD	8.90 (1.30, 61.06)	0.066	3.64 (1.59, 8.36) 0.002
Age	1.02 (0.95, 1.12)	0.176	
Gender	0.75 (0.66, 1.56)	0.321	
Resternotomy	1.31 (0.83, 4.55)	0.673	
Etiology of heart failure	1.23 (0.59, 4.08)	0.512	

CPB: Cardiopulmonary bypass; CRI: Chronic renal insufficiency; PVD: Peripheral vascular disease; AST: Aspartate transaminase; ALT: Alanine aminotransferase; ICU: Intensive care unit; VDRF: Ventilator dependent respiratory failure; RV: Right ventricular; RVAD: Right ventricular assist device.

treatment for patients with end stage heart failure for the indication of BTT or DT, with excellent short and long term survival, as demonstrated in this study. Our analysis showed that after CF LVAD implantation, survival at 30 d was 94%, at 1 year 78%, at 2 year 71%, and at 4 years 45%. Our longest survivor has been on LVAD therapy for over 7 years. Although these results by far surpass outcomes of patients with advanced heart failure on medical therapy, they are still inferior to heart transplantation which remains the gold standard for treating ESHF[7]. At 2 years the survival rate for our heart transplant recipients was 95% (52/55), which was superior to the 2 year 71% survival rate for DT patients (P = 0.02). Apart from improvement in survival, LVAD patients benefit from improved peripheral perfusion which certainly enhances quality of life. As demonstrated in our hemodynamic and ECHO measurements, 6 mo of LV therapy is associated with adequate LV decompression, significant improvement in RV function and in end organ perfusion. This is achieved with close postoperative surveillance and by obtaining regular echocardiograms to assess for aortic ejection, LV decompression, positions of the inteventricular septum, right ventricular function, and for residual mitral and tricuspid regurgitation[5]. We aim to maintain a flow index (CI) > 2.2 L/min per square metre. We also regularly adjust revolutions per minute (rpm) speed to achieve adequate flow, LV decompression, peripheral perfusion, and end organ function.

Our multivariate analysis demonstrated that preoperative liver dysfunction, and postoperative VDRF, tracheostomy, and RV failure requiring RVAD support were significant predictors of post LVAD mortality. These variables have previously been reported as potential risk factors for early post LVAD death in several published series[8-10]. High preoperative LFTs are an indication of poor end organ perfusion and RV dysfunction, which are certainly expected to increase postoperative mortality. These patients are coagulopathic, which cause postoperative bleeding, tamponade, and makes fluid management more challenging, especially with RV dysfunction which frequently co-exists with abnormal liver function. VDRF and trachesotomy both indicate critical illness and prolonged ICU support which are also expected to be predictors of poor outcome.

Several major centers around the world have also reported excellent survival outcomes, analogous to those reported in our study. A multi-institutional analysis from the United Kingdom and Germany[11] published survival rates of 89% at 30 d, 76% at 1 year and 66% at 2 years, from 139 CF LVAD implantations over a 6-year period. The average duration of support in this study was 514 d. No differences were identified between HeartMate II and HeartWare devices in terms of survival, although there was a trend towards more transfusions in the HeartMate group. These findings match our results when comparing the two types of devices. John *et al*[12] from the University of Minnesota published their single institutional experience with 130 CF LVADs. Overall, 30 d, 6 mo, and 1 year survival was 95.1%, 83.5%, and 78.8%, respectively. Driveline infections (25%), GIB 18% and stroke were the most common adverse events.

Possibly the most common and hazardous adverse events of the old generation pulsatile flow LVADs were resistant pocket/driveline infections and pump thrombosis[4,13,14]. Both these complications resulted in frequent device exchanges. Newer generation devices are more reliable and durable and fortunately these events are less frequent with CF LVADs[15-17], as clearly demonstrated in our study. Device exchange was performed in 6% of our patients, of which 77% (10/13) were for pump thrombosis and 13% (3/13)

for severe driveline and pocket infections. Our overall pump thrombosis rate was 6% (12/200), with 10/12 (83%) of these incidence occurring between 2006-2012 and only two cases of pump thrombosis over the past 3 years. Based on initial reports that suggested that anticoagulation could be less aggressive for Heartmate II devices, we followed a less aggressive anticoagulation policy, which may explain the higher frequency of pump thrombosis during the first six years of our CF-LVAD program. Since 2012, all patients receiving CF LVADs are postoperatively started on Asprin 81 mg and Warfarin with an INR target of 2.0-2.5. In addition we have recently been creating a larger sized pump pockets which reduces the effect of diaphragmatic excursion on the angle of the inflow cannula, thus reducing the incidence of pump thrombosis. Our driveline infection rate was only 7% which is significantly lower than the reported incidence of 20%[15]. It has been over 3 years since we have had a driveline infection. We feel that our success in preventing this challenging complication is linked with a new antibiotic and dressing protocol which was initiated at the end of 2011. The night before surgery patients are given 1.5 g of IV vancomycin, 2 g of IV cefepime, 400 mg IV Fluconazole and 600 mg of IV Rifampin. In penicillin or cephalosporin allergic patients, cefepime is substituted with 2 g of IV Aztreonam. Postoperatively, 4 doses of IV Vancomycin (15 mg/kg) every 12 h, 4 doses of IV Cefepime (2 g) every 12 h (or 2 daily doses of IV Aztreonam) and 2 daily doses of IV Fluconazole (400 mg) and IV Rifampin (600 mg) are administered. In the operating room, the drivelines are covered with Acticoat 3 Flex, which is a silver coated antimicrobial barrier that lasts for 3 d, followed by application of a tegaderm. Chlorhexidine and sterile water is used every 3 d to clean the driveline area, after which a new acticoat dressing is applied[5].

In our series, GIB was the most common adverse event (43/200, 21%), followed by RV failure (38/200, 19%) and stroke (31/200, 15%) rates which are similar to previously published data[1,17-22]. The occurrence of GI bleeding makes postoperative LVAD management more challenging, as temporary discontinuation of anticoagulation is required, which may increase the risk of pump thrombosis and stroke. GI bleeding is also a common cause for early postoperative readmission[23]. The frequent association of GIB with CF LAVDs is presumed to be from the lack of pulsatility which causes AV malformations and angiodysplasia. A similar mechanism, known as Heyde's syndrome[24], has been described in severe aortic stenosis, which also causes AV malformations and GIB. In addition, acquired von Willebrand syndrome has been reported as a potential cause for the development of GIB[25]. This frequent complication can be minimized through close INR monitoring, although recent studies have suggested that prophylactic administration of Octreotide may reduce the incidence of GIB[26,27]. RV failure is also a common LVAD related complication with a complex underlying mechanism. LV decompression causes a leftward shift of the interventricular septum, which reduces its contractility thus impairing RV function[28]. It is also challenging for the RV to keep up with the sudden increase in LV output which further decreases RV function. In addition, subtle changes in the pulmonary microcirculation before and after LVAD implantation also add to RV dysfunction[19,23]. We have previously published the patients who develop post LVAD RV failure and only require inotropic support with Milrinone, have equivalent outcomes to patients without RV failure[29]. It is only when RVAD support is required, does the morbidity and mortality increase, which is clearly demonstrated in our current study. Although certain risk factors predicting RV failure and RVAD support after LVAD implantation have been described, such as renal and liver failure, leucocytosis, high CVP/PCWP ratio, high CVP and decreased right ventricular stroke work index, predicting severe RV failure still remains a challenge[29-31].

Two types of LVADs have been implanted at our institution, HMII and HVAD. Of the 200 LVADs, 179 were HMII and 21 were HVADs. These devices have similarities and divergences. The HMII is an axial flow pump with an electromagnetically suspended rotor. The larger HMII device requires an additional pump pocket formation in the upper abdominal preperitoneal space[32]. The HVAD is a centrifugal flow pump, characterized by a smaller size, which allows for its placement within the pericardial cavity[33]. Although the number of HVADs we implanted was insufficient to generate results for meaningful conclusions, so far we haven't identified a significant difference in the overall mortality rate (32% for HMII *vs* 23% for HVADs, $P = 0.301$) or other complications. There only appeared to be a higher rate of blood transfusions with the HMII (20% *vs* 9%), which possibly corresponds to the need to form a pump pocket. Nevertheless, the higher transfusion rate did not correspond with higher incidences of re-exploration for bleeding and had no significant impact on survival.

An area of controversy and discussion amongst LVAD centers is patient's age as exclusion criteria for LVAD implantation. Several studies[8,10] have shown worse outcomes in older LVAD patients, although this was not observed in our analysis. In our 200 patient cohort, 14 patients were above the age of 70. Our oldest patient was 81 years. Survival at 2 years for patients above 70 was 62%. In addition, age was not found to be an independent predictor of survival. Other reports agree with our findings[34,35]. We feel that in appropriately selected patients, age should not be a contraindication to implantation[36].

Our study was not without limitations. Considering that this was not a prospective, randomize trial, it was subject to limitations inherent to any retrospective analysis. In addition statistical power was limited. Selection bias may also be present, since this is a single institution study. Finally, data on functional status and quality of life were not collected, which is an important target of LVAD therapy.

In conclusion, our single institutional analysis demonstrates superb short and long term outcomes, up to 4 year, with CF LAVDs. Compared to old generation devices, major adverse events such as pump thrombosis and driveline infections and frequent device exchanges, are now less frequent. Nevertheless, certain LVAD-related complications, such as GIB, stroke and RV failure do continue to occur. In addition to identifying new means of power transmission, new LVAD technology aims at reducing these adverse events. Preoperative hepatic and RV dysfunction appear to be predictors of post LVAD survival, which should certainly be taken into account in the patient selection process, whereas other significant variables, such as age, sex, etiology of heart failure, other comorbidities and reoperative cardiac surgery, do not appear to influence short and long term survival.

COMMENTS

Background

As the availability of left ventricular assist device (LVAD) therapy expands, there are still concerns regarding the relatively frequent occurrence of postoperative LVAD complications. Improvements are still required if LVADs are to become a plausible alternative to heart transplantation or a therapeutic option for patients in earlier stages of heart failure. The aim of this study was to review the authors' institutional experience over 8 years with 200 continuous-flow (CF) LVADs.

Research frontiers

To our knowledge this is the largest single institutional CF LVAD report.

Innovations and breakthroughs

Their single institutional analysis demonstrates excellent short and long term outcomes, up to 4 years, with CF LAVDs. Compared to old generation devices, major adverse events such as pump thrombosis and driveline infections and frequent device exchanges, are now less frequent. Nevertheless, certain LVAD-related complications, such as gastrointestinal bleeding, stroke and right ventricular failure do continue to occur.

Applications

Post-operative management of LVADs and appropriate patient selection.

Terminology

CF LVADs: Continuous-flow left ventricular assist device. They are centrifugal or axial flow pumps that replace the function of the failing heart.

Peer-review

The authors present an interesting review of experience with LVAD and analytical results about postoperative prognosis.

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ORIGINAL ARTICLE

Prospective Study

Comparison of echocardiography and device based algorithm for atrio-ventricular delay optimization in heart block patients

Rajesh Vijayvergiya, Ankur Gupta

Rajesh Vijayvergiya, Ankur Gupta, Department of Cardiology, Advanced Cardiac Centre, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India

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Correspondence to: Dr. Rajesh Vijayvergiya, MD, DM, FSCAI, FISES, FACC, Profesor, Department of Cardiology, Advanced Cardiac Centre, Post Graduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India. rajeshvijay999@hotmail.com
Telephone: +91-172-2756512
Fax: +91-172-2744401

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Abstract

AIM: To compare the atrio-ventricular (AV/PV) delay optimization by echocardiography and intra-cardiac electrocardiogram (IEGM) based QuickOpt algorithm in complete heart block (CHB) patients, implanted with a dual chamber pacemaker.

METHODS: We prospectively enrolled 20 patients (age 59.45 ± 18.1 years; male: 65%) with CHB, who were implanted with a dual chamber pacemaker. The left ventricular outflow tract velocity time-integral was measured after AV/PV delay optimization by both echocardiography and QuickOpt algorithm method. Bland-Altman analysis was used for agreement between the two techniques.

RESULTS: The optimal AV and PV delay determined by echocardiography was 155.5 ± 14.68 ms and 122.5 ± 17.73 ms ($P < 0.0001$), respectively and by QuickOpt method was 167.5 ± 16.73 and 117.5 ms ± 9.10 ms ($P < 0.0001$), respectively. A good agreement was observed between optimal AV and PV delay as measured by two methods. However, the correlation of the optimal AV ($r = 0.0689$, $P = 0.77$) and PV ($r = 0.2689$, $P = 0.25$) intervals measured by the two

techniques was poor. The time required for AV/PV optimization was 45.26 ± 1.73 min by echocardiography and 0.44 ± 0.08 min by QuickOpt method (P < 0.0001).

CONCLUSION: The programmer based IEGM method is an automated, quick, easier and reliable alternative to echocardiography for the optimization of AV/PV delay in CHB patients, implanted with a dual chamber pacemaker.

Key words: Atrio-ventricular delay optimization; Complete heart block; Doppler echocardiography; Dual chamber pacemaker; Hemodynamics; QuickOpt algorithm

Core tip: Optimization of sensed and paced atrio-ventricular (AV/PV) delay is required for better hemodynamics in patients with complete heart block (CHB). Aim of the present study was to compare the AV/PV delay optimization by echocardiography and intra-cardiac electrocardiogram (IEGM) based QuickOpt algorithm in patients with CHB. We prospectively enrolled 20 patients of CHB who were implanted with a dual chamber pacemaker. A velocity time-integral of left ventricular outflow tract was measured following AV/PV delay optimization by both echocardiography and QuickOpt algorithm method. An agreement between the two techniques was assessed by Bland-Altman analysis. Optimal AV and PV delay as assessed by echocardiography was 155.5 ± 14.68 ms and 122.5 ± 17.73 ms (P < 0.0001), respectively and by QuickOpt method was 167.5 ± 16.73 ms and 117.5 ± 9.10 ms (P < 0.0001), respectively. The time required for AV/PV optimization was 45.26 ± 1.73 min by echocardiography and 0.44 ± 0.08 min by QuickOpt method (P < 0.0001). In conclusion, automated programmer based IEGM method is a quick, easy and reliable alternative to echocardiography for optimization of AV/PV delay in CHB patients.

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INTRODUCTION

Pacemaker therapy provides a better hemodynamics in addition to pacing support in patients with bradycardia and complete heart block (CHB). A programmed atrio-ventricular (AV) interval is crucial for adequate diastolic filling, optimal cardiac output and prevention of diastolic mitral regurgitation[1]. Optimal AV delay can be assessed by Doppler diastolic flow measurement across mitral valve, or by an invasive left ventricular pressure measurement. An automated intra-cardiac electrogram (IEGM) algorithm known as "QuickOpt" from St Jude Medical, St Paul, MN, United States has the capability to assess the optimal AV delay in implanted patient[2-4]. QuickOpt algorithm is a good alternative to the standard echocardiographic method for optimal AV delay assessment. A good correlation have been demonstrated between optimal AV/PV and inter-ventricular (VV) intervals in patients with cardiac resynchronization therapy (CRT) as assessed by echocardiography and QuickOpt method[3,4]. We have compared echocardiography and QuickOpt method for AV/PV delay optimization in patients of CHB, who were implanted with dual chamber pacemaker.

MATERIALS AND METHODS

It was a prospective, single center, non-randomized, open-label, pilot study. The institute's ethics committee approval was taken prior to initiation of the study. Twenty consecutive patients of CHB who underwent pacemaker implantation (DDDR Mode, Zephyr XL DR 5826, St Jude Medical, United States) from July 2010 to December 2011 were enrolled in the study. Patients with low intrinsic atrial rate of < 40 bpm, NYHA functional class Ⅳ heart failure, permanent or persistent atrial flutter or atrial fibrillation, significant valvular heart disease, pregnancy, age < 18 years, and those enrolled in another study were excluded. All patients underwent optimization of the AV/PV delay by echocardiography and QuickOpt algorithm after at least 8 wk of pacemaker implantation. The order of measurement of the two tests was randomized with the help of a computer generated random number table. A stopwatch was used for time interval measurements for both the optimization methods.

AV delay optimization methods

Echocardiography method: AV delay optimization was performed using two-dimensional Doppler echocardiography on iE33 ultrasound system (Philips Medical Systems, WA, United States). A sweep speed of 100 mm/s was used. Doppler measurements were taken at a delay of 30 s after programming new AV and PV intervals. Optimal AV interval was determined in DVI pacing mode, while optimal PV interval was measured in VDD pacing mode. To pace atria in DVI mode, the atrial rate was increased by 10 beats per minute over the baseline atrial rate. Mitral inflow velocity was measured in the apical four-chamber view. First measurement of mitral inflow duration (both diastolic E and A wave duration) was taken at a long AV delay of > 200 ms. Thereafter, AV interval was decreased by 10 ms each time and simultaneously EA duration was measured, during end expiration. An average of three consecutive beats during expiration was taken for EA measurement. The optimal AV/PV interval by

Table 1 Optimal atrio-ventricular delay (ms) by echocardiography and QuickOpt

	Optimal AV delay (in ms)	Optimal PV delay (in ms)	*P* value
Echocardiography	155.5 ± 14.68 (130-180)	122.5 ± 17.73 (90-150)	< 0.0001
QuickOpt	167.5 ± 16.73 (150-190)	117.5 ± 9.10 (100-140)	< 0.0001

Values are in mean ± 1 SD (range).

echocardiography was the AV/PV interval at which the maximum transmitral inflow duration was documented without the interruption of A wave[5,6]. A velocity time-integral (VTI) of left ventricular outflow tract (LVOT) was measured in an apical five chamber view and an average of three beats was taken. It was measured for each of a programmed AV/PV delay. Measurement by a single echocardiographer (author - Gupta A) ruled out inter-observer bias. Taking measurements of pulsed wave Doppler at fixed points (mitral valve leaflet tips for transmitral inflow duration and 1 cm below the aortic valve for VTI of LVOT) minimized intra-observer bias.

Intra-cardiac electrocardiogram method

Optimal AV interval was measured by intra-cardiac electrocardiogram (IEGM) method using a St Jude Medical programmer (QuickOpt algorithm in Merlin™ Patient Care System Programmer, St Jude Medical, CA, United States)[2,4]. This algorithm calculates the optimal PV delay by measuring the width of atrial IEGM and adding 30 ms if intrinsic atrial depolarization of ⩾ 100 ms and adding 60 ms if intrinsic atrial depolarization is < 100 ms. The off-set factor enables delivery of ventricular pacing after atrial electrical activation and mechanical contraction are completed. An optimal AV delay was calculated as the sum of optimal PV delay and the pacing latency (50 ms). At each AV interval, LVOT VTI was assessed as per the method described above.

Statistical analysis

Continuous variables are expressed as mean and standard deviation, and categorical variables are expressed as counts. Bland-Altman plots was used for agreement between the two optimization techniques[7-9]. These plots depict the mean difference and 95%CI of the differences (mean difference ± 2 SD of difference). A difference of > 20 ms in the AV or PV interval assessed by two optimization techniques was interpreted as poor agreement[2]. For LVOT VTI, a difference of > 2 cm was considered significant for a poor agreement[2]. Correlation between the two techniques was evaluated using linear regression analysis and Pearson's correlation coefficient. A *P* value of < 0.05 was regarded significant. Comparison of LVOT VTI was done using the paired-sample Student's *t* test. Statistical analysis was carried out by Statistical Analysis System SAS 17 and Medcalc Medical Calculator.

Table 2 Left ventricular outflow tract-velocity time integral (left ventricular outflow track velocity time-integral) at optimal AV and PV delay and time required for AV/PV delay optimization

	Echocardiography	QuickOpt	*P* value
LVOT VTI at optimal AV delay (cm)	18.86 ± 4.11 (11.6-27.7)	17.82 ± 4.02 (11.46-27.7)	0.0099
LVOT VTI at optimal PV delay (cm)	19.26 ± 3.01 (13.7-23.9)	18.5 ± 2.92 (13.8-23.9)	0.07
Time required for optimization (min)	45.26 ± 1.73 (41.5-48.1)	0.44 ± 0.08 (0.31-0.57)	< 0.0001

Values are in mean ± 1 SD (range).

RESULTS

Thirty CHB patients had dual chamber pacemaker (Zephyr XL DR 5826 model of St Jude Medical, United States) implantation from July 2010 to December 2011. Twenty eligible patients were included in the study. Ten excluded patients had permanent/persistent atrial flutter or atrial fibrillation (n = 6), slower intrinsic atrial activity of less than 40 bpm (n = 2) or NYHA Class Ⅳ heart failure (n = 2). The mean age of 20 enrolled patients was 59.45 ± 18.1 years; 13 were males and 7 were females. Seventeen patients had degenerative CHB and 3 had congenital CHB. Presenting complaint of syncope or pre-syncope was present in 17 patients. The mean left ventricular ejection fraction was 59.25% ± 7.8%.

Comparison of optimal AV/PV delay measured by echocardiography and QuickOpt algorithm

The optimal AV and PV delay determined by echocardiography was 155.5 ± 14.68 ms and 122.5 ± 17.73 ms, respectively and by QuickOpt was 167.5 ± 16.73 ms and 117.5 ± 9.10 ms, respectively (Table 1). The optimal PV delay was significantly shorter than optimal AV delay by both echocardiography and QuickOpt algorithm (P < 0.0001). Mean time required for optimisation for AV/PV delay was 45.26 ± 1.73 min by echocardiography and 0.44 ± 0.08 min by QuickOpt algorithm, P < 0.0001 (Table 2). There was a good agreement between optimal AV delays as assessed by the two techniques. Only 4-patients had > 20 ms difference in optimal AV interval (Figure 1). However, correlation of the optimal AV intervals assessed by two techniques was poor (Figure 2; r = 0.0689, P = 0.77). There was a good agreement of optimal PV delay with just 4-patients having > 20 ms difference in the optimal PV interval (Figure 3) and a poor correlation between the two techniques (Figure 4; r = 0.2689, P = 0.25).

Comparison of the LVOT VTI achieved at optimal AV/PV delays using the echocardiography and QuickOpt algorithm

Mean LVOT VTI at optimal AV delay was 18.86 ± 4.11 cm by echocardiography and 17.82 ± 4.02 cm by

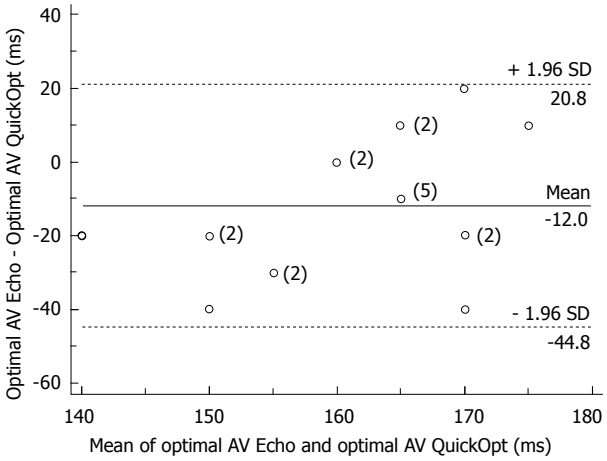

Figure 1 Bland - Altman plot of differences in optimal AV interval measured by echocardiography and QuickOpt.

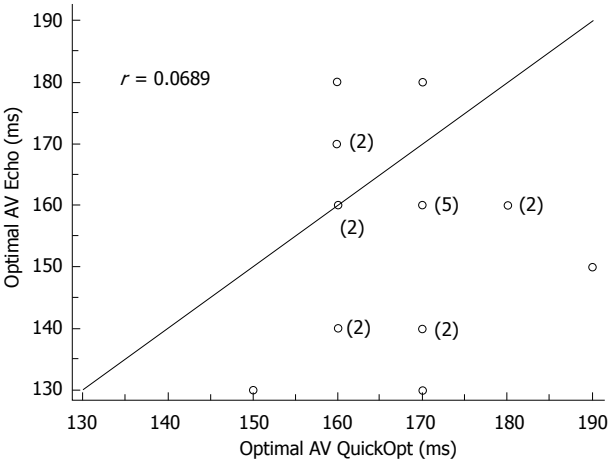

Figure 2 Correlation of optimal AV intervals measured by echocardiography and QuickOpt.

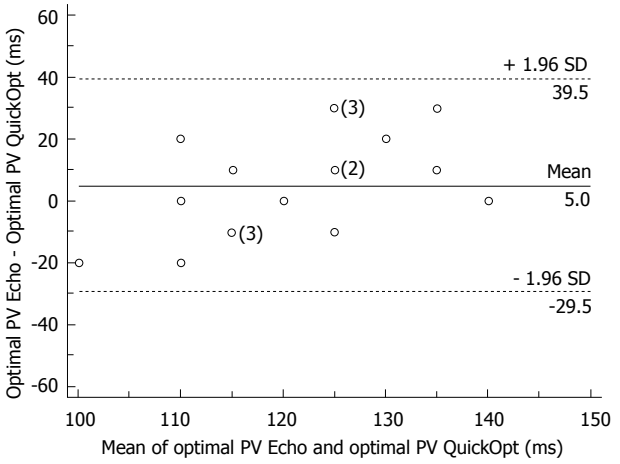

Figure 3 Bland - Altman plot of differences in optimal PV interval measured by echocardiography and QuickOpt.

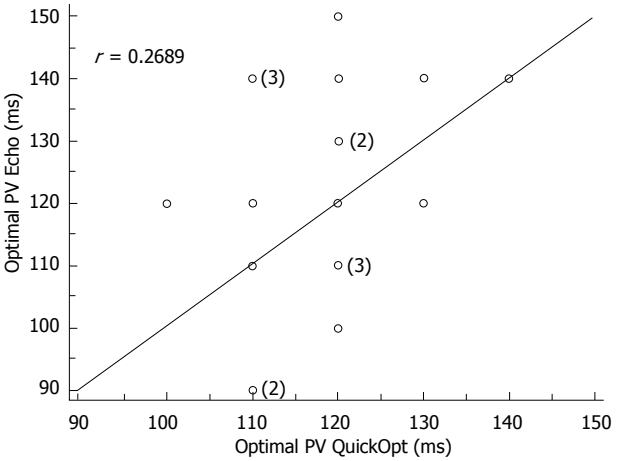

Figure 4 Correlation of optimal PV intervals measured by echocardiography and QuickOpt.

QuickOpt algorithm (P = 0.0099, Table 2), suggesting a better hemodynamic response by echocardiography. Similarly, mean LVOT VTI at optimal PV delay was 19.26 ± 3.01 cm by echocardiography and 18.5 ± 2.92 cm by QuickOpt algorithm (P = 0.07), suggesting a trend towards better hemodynamic response by echocardiography (Table 2). There was a good clinical agreement between LVOT VTI at optimal AV delay assessed by these two techniques, with 4-patients having > 2 cm difference in the LVOT VTI (Figure 5). Also, the correlation of LVOT VTI measured at optimal AV delay was good by two techniques (r = 0.9216, P < 0.0001). Similarly, there was a good agreement between LVOT VTI at optimal PV delay determined by these two techniques, with just 4 of 20 patients having more than 2 cm difference in the LVOT VTI (Figure 6) and a good correlation of LVOT VTI as assessed at optimal PV delay by two techniques (r = 0.8218, P < 0.0001).

DISCUSSION

The present study has demonstrated a good agreement and poor correlation between optimal AV and PV delay as assessed by echocardiography and QuickOpt algorithm in patients with CHB. There was also a good agreement and good correlation of LVOT VTI as determined at optimal AV and PV delay by two techniques. Various studies had shown a good correlation between LVOT VTI as determined by echocardiography and QuickOpt algorithm in patients with heart failure on CRT[3,4]. Gold *et al*[10], demonstrated an excellent correlation between the IEGM method and the maximum achievable invasive LV dP/dt measurement during CRT implantation in both AV and PV modes. Baker *et al*[3], studied AV/PV and VV delay optimization in heart failure patients implanted with a CRT-D or dual chamber ICD. They measured maximum LVOT VTI guided by echocardiography and QuickOpt algorithm. The concordance correlation coefficient between echocardiography and QuickOpt method for AV, PV and VV delays was 97.5%, 96.1%, and 96.6%, respectively (P < 0.05). Kamdar *et al*[2], studied AV and VV delay optimization in CRT patients by echocardiography and QuickOpt method. There was a good correlation of two methods for LVOT VTI

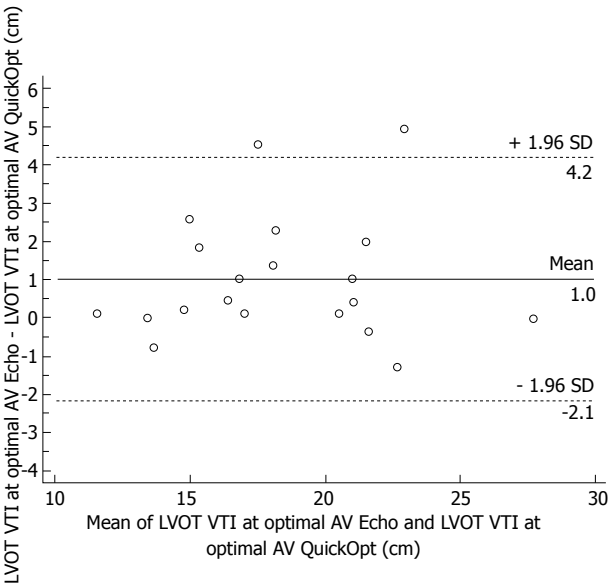

Figure 5 Bland - Altman plot of differences in left ventricular outflow tract velocity time-integral at optimal AV delay measured by echocardiography and QuickOpt. LVOT: Left ventricular outflow tract; VTI: Velocity time-integral.

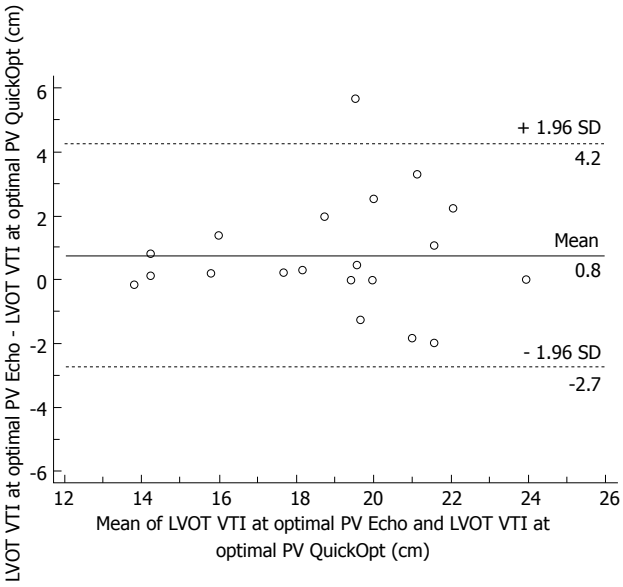

Figure 6 Bland - Altman plot of differences in left ventricular outflow tract velocity time-integral at optimal PV delay measured by echocardiography and QuickOpt. LVOT: Left ventricular outflow tract; VTI: Velocity time-integral.

optimization (R^2 = 0.77, P < 0.001), though it was significantly better ($P \leqslant 0.001$) with echocardiography compared to QuickOpt method. However, an agreement between the two methods was poor, with 15 out of 26 patients had > 20 ms difference in optimal AV interval and 10 out of 26 patients had > 20 ms difference in the optimal VV interval. The frequent optimization study using the QuickOpt method (FREEDOM trial) studied benefits of frequent AV/PV and VV delay optimisation using the QuickOpt algorithm *vs* standard of care (physician guided programming or upto 1 non IEGM based optimization like echocardiography within first 4 wk) in 1647 patients implanted with CRT[4]. This trial observed that QuickOpt optimization was as good as the standard of care programming methods which includes either physician guided or non-IEGM optimization methods by echocardiography. In the present study, there was a poor correlation between optimal AV and PV delay but good correlation between LVOT VTI at optimal AV/PV delay as assessed by echocardiography and QuickOpt algorithm. This is attributed to small changes in hemodynamics (measured by LVOT VTI) caused by large variations in AV/PV intervals.

AV synchrony provides hemodynamic benefit in addition to pacing support in CHB patients. An appropriately timed atrial systole prevents rise in mean atrial pressure, facilitates venous return, coordinates AV valve closure; thus reduces diastolic mitral regurgitation and reduces pulmonary capillary wedge pressure[11,12]. The fact that optimal AV delay results in maximum cardiac output, and small deviations can decrease cardiac output has been demonstrated in various previous studies[1,13]. Various echocardiography studies have reported optimal AV delay of 125-200 ms, and an optimal PV delay of 30-50 ms shorter to optimal AV delay[1,5,6]. Similar mean AV/PV delay was observed in present study. Janosik *et al*[1] studied Doppler derived cardiac output in 24 patients implanted with dual chamber pacemaker. The optimal delay interval during DVI and VDD pacing was 176 ± 44 and 144 ± 48 ms (P < 0.002), respectively. They demonstrated 8% increment in resting cardiac output with optimal AV delay; while same delay with paced P wave (PV delay) did not show maximum cardiac output. Kindermann *et al*[5] documented optimal AV and PV delay in 53 high degree AV block patients as 136 ± 34 ms and 76 ± 40 ms, respectively. They also reported that AV delay optimization results in 19% increase in stroke volume, compared to fixed AV delay. Similarly, Ritter *et al*[6] reported an optimal AV and PV delay of 179 ± 25 ms and 124 ± 18 ms, respectively in 19 CHB patients with dual chamber pacemaker. Ovsyshcher *et al*[14] demonstrated that optimal AV delay is associated with about 30% more cardiac output during DDD pacing, in comparison to VVI pacing.

The present study also documented a good agreement and correlation between LVOT VTI at optimal AV/PV delay by both echocardiography and QuickOpt algorithm. The hemodynamic outcome in term of LVOT VTI was significantly better with echocardiography, in comparison to QuickOpt algorithm. This is possibly because of IEGM based electrical optimization may not be equal to the best mechanical and hemodynamic performance, as achieved by echocardiography. The time required for AV/PV delay optimization in present study was 45.26 ± 1.73 min by echocardiography and 0.44 ± 0.08 min by QuickOpt (P < 0.0001). To best of our knowledge, there is no available published literature about similar comparison between two methods for AV/PV delay optimization in CHB patients. The average time required for VV delay optimization in CRT patients as reported by Hansalia *et al*[15] was significantly lower with

QuickOpt method in comparison to echocardiography (1.5 ± 0.87 min *vs* 41 ± 8.3 min, P = 0.006). Thus, QuickOpt is a cheap, fast, simple, automatic and more practical method of AV delay optimization in "real world" practice which can be performed within a minute during regular clinical follow-up using the device programmer.

The present study has few limitations such as use of non-invasive echocardiography method for hemodynamic assessment, which have inherent bias. A single echocardiographic method of transmitral inflow duration was used for AV delay optimization, instead of using other methods such as impedance cardiography[5], peak endocardial acceleration[6], left ventricular invasive pressure measurement (LV dP/dt_{max})[16], *etc.* A study with larger number of patients is required to validate the results, as the present study was of small sample size. The effects of upright position and exercise on optimal AV delay were not assessed. We only measured hemodynamic response and not the clinical benefit in enrolled patients.

In conclusion, the present study demonstrated that an automated programmer-based IEGM method is quick, easy and reliable alternative to time consuming echocardiography method for AV delay optimization in patients of CHB, implanted with dual chamber pacemaker.

COMMENTS

Background

Optimization of atrio-ventricular (AV/PV) delay is required for better hemodynamics in patients with complete heart block (CHB).

Research frontiers

The present study compared the AV/PV delay optimization by echocardiography and intra-cardiac electrocardiogram based QuickOpt algorithm in CHB patients, subjected to dual chamber pacemaker.

Innovations and breakthroughs

The authors found that QuickOpt algorithm is an automated, easy, quick and reliable alternative to echocardiography for AV/PV delay optimization in CHB patients.

Applications

In a real world practice, AV/PV delay optimization can be performed within a minute using QuickOpt algorithm.

Terminology

IEGM: Intra-cardiac electrogram; CHB: Complete heart block; LVOT VTI: Left ventricular outflow tract velocity time integral; AV/PV: Paced atrio-ventricular/sensed artio-ventricular; CRT: Cardiac resynchronisation therapy.

Peer-review

The authors stated that optimization of sensed and paced atrio-ventricular (AV/PV) delay is required for better hemodynamics in patients with CHB. They studied the AV/PV delay optimization using echocardiography and intra-cardiac electrocardiogram (IEGM) based QuickOpt algorithm in 20 CHB patients. The results revealed a good agreement between optimal AV and PV delay determined by the two methods. Authors concluded that the automated programmer based IEGM method is a quick, easier and reliable alternative to echocardiography for the optimization of AV/PV delay in CHB patients subjected for dual chamber pacemaker. The tables and figures are presented appropriately.

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SYSTEMATIC REVIEWS

Digoxin: A systematic review in atrial fibrillation, congestive heart failure and post myocardial infarction

Sebastiano Virgadamo, Richard Charnigo, Yousef Darrat, Gustavo Morales, Claude S Elayi

Sebastiano Virgadamo, Richard Charnigo, Yousef Darrat, Gustavo Morales, Claude S Elayi, Department of Cardiology, University of Kentucky Gill Heart Institute and Veterans Affairs Medical Center, Lexington, KY 40536, United States

Author contributions: Virgadamo S and Elayi CS designed the research; Virgadamo S performed the research; Charnigo R, Darrat Y and Morales G reviewed the data; Virgadamo S wrote the paper; Charnigo R edited the paper; Darrat Y, Morales G and Elayi CS supervised the paper; all authors approved the final manuscript.

Conflict-of-interest statement: All authors declare that they have no competing interests.

Data sharing statement: The table is available from the corresponding author at samy-claude.elayi@uky.edu.

Correspondence to: Claude S Elayi, MD, Department of Cardiology, University of Kentucky Gill Heart Institute and Veterans Affairs Medical Center, 326 C.T. Wethington Bldg. 900 South Limestone Street, Lexington, KY 40536, United States. samy-claude.elayi@uky.edu
Telephone: +1-859-3235479
Fax: +1-859-3236475

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Abstract

AIM: To review digoxin use in systolic congestive heart failure, atrial fibrillation, and after myocardial infarction.

METHODS: A comprehensive PubMed search was performed using the key words "digoxin and congestive heart failure", "digoxin and atrial fibrillation", "digoxin, atrial fibrillation and systolic congestive heart failure", and "digoxin and myocardial infarction". Only articles written in English were included in this study. We retained studies originating from randomized controlled trials, registries and included at least 500 patients. The studies included patients with atrial fibrillation or heart failure or myocardial infarction and had a significant proportion of patients (at least 5%) on digoxin. A table reviewing the different hazard ratios was developed based on the articles selected. Our primary endpoint was the overall mortality in the patients on digoxin *vs* those without digoxin, among patients with atrial fibrillation and also among patients with atrial fibrillation and systolic heart failure. We reviewed the most recent international guidelines to discuss current recommendations.

RESULTS: A total of 18 studies were found that evaluated digoxin and overall mortality in different clinical settings including systolic congestive heart failure and normal sinus rhythm (n = 5), atrial fibrillation with and without systolic congestive heart failure (n = 9), and myocardial infarction (n = 4). Overall, patients with systolic congestive heart failure with normal sinus rhythm, digoxin appears to have a neutral effect on mortality especially if close digoxin level monitoring is employed. However, most of the observational studies evaluating digoxin use in atrial fibrillation without systolic congestive heart failure showed an increase in overall mortality when taking digoxin. In the studies evaluated in this systematic review, the data among patients with atrial fibrillation and systolic congestive heart failure, as well as post myocardial infarction were more controversial. The extent to which discrepancies among studies are based on statistical methods is currently unclear, as these studies' findings are generated by retrospective analyses that employed

different techniques to address confounding.

CONCLUSION: Based on the potential risks and benefits, as well as the presence of alternative drugs, there is a limited role for digoxin in the management of patients with normal sinus rhythm and congestive heart failure. Based on the retrospective studies reviewed there is a growing volume of data showing increased mortality in those with only atrial fibrillation. The proper role of digoxin is, however, less certain in other subgroups of patients, such as those with both atrial fibrillation and systolic congestive heart failure or after a myocardial infarction. Further studies may provide helpful information for such subgroups of patients.

Key words: Digoxin; Atrial fibrillation; Heart failure; Myocardial infarction; Mortality

Core tip: This systematic review evaluates mortality with the use of digoxin in congestive heart failure (CHF) with sinus rhythm, atrial fibrillation with and without CHF, and post myocardial infarction. In patients with CHF with sinus rhythm, there continues to be a niche for digoxin use as an adjunctive therapy for symptomatic control with the understanding that there is no effect on mortality. The role for digoxin among patients who only have atrial fibrillation seems very limited; however, those with atrial fibrillation and systolic congestive heart failure or post myocardial infarction need further assessment as many questions remain regarding the benefit of digoxin in this population.

Virgadamo S, Charnigo R, Darrat Y, Morales G, Elayi CS. Digoxin: A systematic review in atrial fibrillation, congestive heart failure and post myocardial infarction. *World J Cardiol* 2015; 7(11): 808-816 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i11/808.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i11.808

INTRODUCTION

Digoxin is one of the oldest drugs used today in cardiovascular medicine in the United States and around the globe. It is used frequently to treat heart failure symptoms and to decrease the ventricular rate in atrial fibrillation (AF). Digoxin was one of the first treatments for heart failure management and was shown to decrease hospitalizations without decreasing mortality in patients with sinus rhythm and left ventricular ejection fraction (LVEF) of less than 45%[1]. Nowadays digoxin remains indicated for patients with persistent symptoms despite optimal medical therapy even with the advent of several new classes of cardiovascular medications with proven benefit on symptoms and survival [including beta blockers, angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor blockers and mineralocorticoid antagonists]. In the setting of AF, digoxin is not mentioned in the 2014 guidelines anymore as an option for rate control, except among patients with AF and heart failure; however, concerns arose regarding its safety even in this subgroup of patients[2-4]. No randomized controlled clinical trials have been performed to date to assess the efficacy and safety of digoxin in patients with AF. Most of the current data regarding the safety and efficacy of digoxin are based on observational studies which have had conflicting results. We review the data available regarding the use of digoxin in congestive heart failure (CHF), AF, and after myocardial infarction, as well as the current guidelines indications for digoxin use from the American College of Cardiology/American Heart Association (ACC/AHA) and European Society of Cardiology (ESC).

Digoxin mechanisms of action

Digoxin's primary mechanism of action is through inhibition of sodium-potassium adenosine triphosphatase (ATPase). Its role in heart failure patients is based on its inotropic properties, due to inhibition of sodium-potassium ATPase which leads to increased intracellular calcium concentrations through the sodium-calcium exchanger[5-8]. This causes the cardiac action potential to lengthen which causes lower heart rates as well as increases myocardial contractility due to the increased calcium for sarcomeric excitation-contraction coupling[8]. Digoxin also has neurohormonal effects and causes improved baroreceptor sensitivity, decreases norepinephrine concentration, and decreases activation of the renin-angiotensin system[5,6,9].

From the electrophysiologic standpoint, digoxin has a parasympathetic effect on the sinoatrial node, by decreasing the automaticity as well as on the atrioventricular conduction system by decreasing conduction and increasing the effective refractory periods[6].

MATERIALS AND METHODS

Literature search

A comprehensive PubMed search was performed using the key words "digoxin and congestive heart failure", "digoxin and AF", "digoxin, AF and systolic congestive heart failure", and "digoxin and myocardial infarction". Only articles written in English were included in this study. We retained studies originating from randomized controlled trials, registries and included at least 500 patients. The studies included patients with AF or heart failure or myocardial infarction and had a significant proportion of patients (at least 5%) on digoxin. A table reviewing the different hazard ratios was developed based on the articles selected. Our primary endpoint was the overall mortality in the patients on digoxin *vs* those without digoxin, among patients with AF and also among patients with AF and systolic heart failure. We reviewed the most recent international guidelines to

discuss current recommendations.

RESULTS

Literature review

A total of 18 studies were found that evaluated digoxin and overall mortality in the different clinical settings including systolic heart failure and sinus rhythm (*n* = 5), AF with and without heart failure (*n* = 9), and myocardial infarction (*n* = 4).

Congestive heart failure with sinus rhythm

For over 200 years, digoxin has been used to treat patients with systolic heart failure in normal sinus rhythm, but over the past several decades digoxin has been scrutinized regarding its therapeutic benefit and risk. As studies began to show the benefits of ACEI in reducing mortality, clinicians began to question the role of digoxin. This led physicians to inquire whether discontinuing digoxin from patients' medical regimens had any effect, especially if patients were also taking ACEI, since no long term benefit had been shown with digoxin.

The Randomized Assessment of Digoxin on Inhibitors of Angiotensin-Converting Enzyme (RADIANCE) study randomized 178 patients with New York Heart Association (NYHA) Class Ⅱ-Ⅲ heart failure, LVEF of < 35%, and normal sinus rhythm to evaluate whether removing digoxin had any clinical significance. This study found that stable patients on digoxin, ACEI, and diuretics had an increased risk of clinical decline when digoxin was removed from their medication regimen with a 5.9 estimated relative risk (95%CI: 2.1-17.2) of worsening heart failure compared to those continuing digoxin. In addition, patients' no longer taking digoxin had lower quality-of-life scores, decreased ejection fraction and increased heart rate and body weight[10]. The RADIANCE study established the short term benefit of digoxin in preventing worsening functional decline, exercise capacity and LV ejection fraction in patients with heart failure and normal sinus rhythm[10]. Furthermore, the Prospective Randomized Study of Ventricular Failure and the Efficacy of Digoxin (PROVED) trial also demonstrated the efficacy of digoxin in patients with mild to moderate systolic heart failure on diuretic therapy[11]. Both studies however had a short term follow up (12 wk for RADIANCE and 20 wk for PROVED)[10,11]. It remained unknown whether the results would be similar with longer follow-up. This led the Digitalis Investigation Group to perform a randomized, double-blinded placebo-controlled trial to evaluate the effects of digoxin on mortality and hospitalizations in patients with heart failure and normal sinus rhythm[1].

The DIG study enrolled 6800 patients with LVEF of 45% or less and they were randomized to receive digoxin (3397 patients) or placebo (3403 patients) in addition to diuretics and ACEI. The DIG study failed to demonstrate a beneficial effect of digoxin on overall mortality with 1181 deaths in the digoxin group (34.8%) and 1194 deaths in the placebo group (35.1%) giving an estimated risk ratio (RR) of 0.99 (95%CI: 0.91-1.07, $P = 0.80$)[1]. Also, no difference was seen in cardiovascular deaths with 1016 in the digoxin group (29.9 %) *vs* 1004 in the placebo group (29.5%) with RR = 1.01 (95%CI: 0.93-1.10, $P = 0.78$)[1].

However, there was a trend towards a lower risk of mortality secondary to heart failure with 394 deaths in the digoxin group compared to 449 in the placebo group with a RR of 0.88 (95%CI: 0.77-1.01, $P = 0.06$). Overall, the number of hospitalizations attributed to worsening heart failure was lower in the digoxin group compared to placebo with a RR of 0.72 (95%CI: 0.66-0.79, $P < 0.001$)[1]. When combining death from any cause or hospitalization due worsening heart failure, the digoxin group had fewer events (RR = 0.85; 95%CI: 0.79-0.91, $P < 0.001$). This was also seen when combining heart failure deaths or hospitalizations due to worsening heart failure (1041 *vs* 1291, RR = 0.75; 95%CI: 0.69-0.82, $P < 0.001$). In addition, a subgroup analysis of the prior outcome, digoxin appeared to have the greatest beneficial effect among those at highest risk, especially those with lower ejection fraction, enlarged hearts, and those in NYHA functional class Ⅲ or Ⅳ[1].

A post-hoc analysis evaluated men in the DIG study according to serum digoxin concentrations to assess if drug concentration had an association with mortality and hospitalizations. In this analysis, there was a reduction in all-cause mortality in patients with lower serum digoxin levels (0.5-0.8 ng/mL) with a 6.3% (95%CI: 2.1%-10.5%, $P = 0.005$) absolute lower mortality rate compared with patients receiving placebo. As the serum digoxin concentration increased, the absolute risk in mortality increased to the point that those with levels greater than 1.2 ng/mL had an 11.8% (95%CI: 5.7%-18.0%, $P < 0.001$) higher absolute mortality rate than patients receiving placebo. Similar conclusions persisted even with multivariable adjustments[12].

Finally, a recent meta-analysis by Hood *et al*[13] reviewed 13 randomized controlled trials where patients were randomized to digoxin and focused on mortality, hospitalization, and clinical status. This meta-analysis showed that digoxin had no effect on mortality which was mostly driven by the data from the DIG study. This meta-analysis also found that in the four studies that provided data on hospitalizations for worsening heart failure, digoxin had significantly fewer hospitalizations due to worsening heart failure with an overall relative risk reduction of 23.4% and number needed to treat ranging from 13-17[13].

Current guidelines

The most current ACC/AHA and ESC guidelines recommendation on the use of digoxin in heart failure with reduced ejection fraction and normal sinus rhythm are based on the prior studies. The ACC/AHA guidelines in 2013 (class Ⅱa, level of evidence B) as well as the

ESC guidelines in 2012 (class IIb, level of evidence B) recommended digoxin for symptomatic improvement and improved quality of life as well as to decrease hospitalizations for heart failure exacerbations[14,15]. The guidelines emphasize the importance of initiating goal-directed medical therapy as the primary treatment for heart failure due to its known mortality benefit. However, the guidelines continue to allow physicians discretion regarding digoxin and emphasize the importance of close monitoring for digoxin toxicity[14,15].

Atrial fibrillation with and without congestive heart failure

In the general population, AF is the most common sustained cardiac arrhythmia. For many years, the primary approach to treatment was to maintain normal sinus rhythm with anti-arrhythmic medications and cardioversion, as a rhythm control strategy was thought to decrease morbidity and mortality compared to a rate control strategy. However, after the Atrial Fibrillation Follow-up Investigation of Rhythm Management (AFFIRM) trial as well as other randomized clinical trials, there was a shift in practice towards maintaining rate control in asymptomatic patients, as these trials exposed no significant improvement in mortality with rhythm control[16-22]. Digoxin was one of the four rate control medications used in the AFFIRM trial and remains an option for rate control.

Over the past two decades, controversy regarding the use of digoxin in patients with AF has arisen due to the potential for adverse effects. An initial retrospective analysis of AFFIRM trial data found that digoxin was associated with lower survival[23]. Yet, these findings were attributed to the patients' comorbid conditions which placed them at increased risk of death, rather than to an adverse effect of the medication. This observation was confirmed in another retrospective analysis of AFFIRM trial[24].

Subsequently, a Swedish study evaluated one year mortality among patients admitted to the coronary care unit with AF, CHF, or both in relation to digoxin. This study found that long term use of digoxin was associated with lower survival in patients with AF without CHF, with an adjusted estimated hazard ratio (HR) of 1.42 (95%CI: 1.29-1.56)[25]. However, no significant increase in mortality risk was seen in patients with CHF alone or in combination with AF.

Two retrospective studies re-evaluated the safety of digoxin use from the AFFIRM database by correcting for potential confounders, but they used different methodologies and found apparently conflicting results[4,26]. The first retrospective analysis regarded digoxin as a time dependent covariate in a propensity-adjusted Cox model and found that digoxin was associated with increased all-cause mortality, with a HR of 1.41 (95%CI: 1.19-1.67, $P < 0.001$) as well as increased cardiovascular and arrhythmic mortality[4]. The increased all-cause mortality was also seen in patients with (HR = 1.41, 95%CI: 1.09-1.84, $P = 0.010$) and without (HR = 1.37, 95%CI: 1.05-1.79, $P = 0.019$) heart failure[4].

Shortly after, a second retrospective analysis was published using propensity matching to evaluate digoxin use at baseline. This analysis found no significant difference in all-cause mortality (HR = 1.06, 95%CI: 0.83-1.37, $P = 0.640$) or hospitalizations[26]. The differences in results between the two retrospective analyses appear to arise from the different statistical methods used, with each analysis carrying some potential bias[27]. The study by Whitbeck and colleagues had an indication bias that the authors mitigated using adjustment for covariates and propensity scores[4]. Meanwhile, the second study suffered from crossover bias and a depleted sample size associated with matching[26]. Although the authors' stated conclusions were not in agreement, it is worth noting that there was some overlap in their 95%CI for all-cause mortality and that the overlapping portion (1.19-1.37) is consistent with a clinically significant, deleterious effect of digoxin in this patient population.

The aforementioned analyses of AFFIRM data have been followed by many other studies. In an observational study using the National Health Insurance Research Database in Taiwan, 4781 patients with AF were studied. In this analysis, digoxin was associated with an increased risk of all-cause mortality, with an adjusted HR of 1.21 (95%CI: 1.01-1.44, $P = 0.037$)[28]. During subgroup analysis, digoxin portended worse survival among patients without heart failure but not among those with heart failure[28].

In one of the largest retrospective analyses evaluating newly diagnosed AF, the The Retrospective Evaluation and Assessment of Therapies in Atrial Fibrillation study evaluated 122465 patients in the Veterans Affairs health care system. The study found digoxin to be associated with increased mortality after multivariate adjustments (HR = 1.26, 95%CI: 1.23-1.29, $P < 0.001$) and propensity matching (HR = 1.21, 95%CI: 1.17-1.25, $P < 0.001$)[3]. This conclusion persisted even after accounting for kidney function and history of documented heart failure, heightening the concern that digoxin reduces survival. However, data regarding the degree of left ventricular dysfunction or the NYHA class were not available; it is unknown how accounting for the severity of heart failure would impact this study's findings.

Another large retrospective analysis of the Anticoagulation and Risk Factors In Atrial Fibrillation Cardiovascular Research Network trial evaluated digoxin in patients with new onset AF and no history of CHF. This observational study used patients belonging to the Kaiser Permanente database and living mainly on the west coast of the United States. In this study, digoxin was shown to be associated with a higher risk of death with HR = 1.71 (95%CI: 1.52-1.93, $P < 0.001$)[29]. This conclusion was robust in distinctions between intention-to-treat and as-treated analyses.

A post-hoc analysis of the Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin

K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation (ROCKET AF) evaluating digoxin use and its association with cardiovascular events was performed. The trial enrolled 14171 patients of which 5239 patients were taking digoxin at baseline. In this analysis, baseline digoxin use was associated with an increased all-cause mortality with an adjusted HR of 1.17 (95%CI: 1.04-1.32, $P = 0.009$)[30]. Similar findings persisted when accounting for covariates using a regression model as well as with a time-dependent model. In subgroup analysis, the all-cause increased mortality was observed among patients with and without heart failure, as judged by left ventricular function or NYHA status[30].

In a population based retrospective analysis evaluating digoxin in patients 65 years or older with and without heart failure, an increased risk of all-cause mortality was detected in analyses based on propensity matching and multivariable Cox regression modeling. In this study, the heart failure group had a 14% greater hazard of all-cause mortality with digoxin (adjusted HR = 1.14, 95%CI: 1.10-1.17, $P < 0.001$), similar to the non-heart failure group which had a 17% greater hazard of all-cause mortality with digoxin (adjusted HR = 1.17, 95%CI: 1.14-1.19, $P < 0.001$)[2].

Hazard ratios for total mortality are reported in Table 1 for the main AF studies with digoxin, as well as for patients with or without CHF.

Current guidelines

The 2014 AHA/ACC/Heart Rhythm Society (HRS) guidelines (Class IIa, level of evidence B) and 2010 ESC guidelines (Class IIa, level of evidence C) do not consider digoxin as a first line therapy for rate control in AF; however, digoxin can be considered in combination with a beta blocker and/or nondihydropyridine calcium channel blocker when the ventricular rate is poorly controlled in patients with underlying left ventricular dysfunction[36,37]. Due to controversy and concern regarding increased mortality in many post-hoc analyses, the guidelines continue to stress caution when administering medication and to periodically check digoxin levels, in an attempt to reduce adverse effects especially in the long term setting[36,37].

Digoxin use in myocardial infarction

The AHA/ACC and ESC guidelines agree that in certain clinical situations digoxin use in patients presenting with ST elevation myocardial infarction is effective; moreover, digoxin use has been deemed appropriate for AF rate control in patients presenting with CHF and ongoing ischemia[38,39]. With increased attention toward the risk/benefit tradeoff of digoxin therapy, a recent retrospective analysis evaluated whether patients chronically taking digoxin had increased in-hospital mortality when admitted for acute coronary syndrome. The analysis considered 20331 patients of which 244 were taking digoxin upon admission to the hospital, using multivariate modeling as well as propensity score matching. Neither statistical method showed significantly increased in-hospital mortality[40]. On the other hand, several studies performed in the 1990's evaluated outcomes among patients surviving a myocardial infarction (remotely after the index event) and found increased mortality with digoxin[40-43]. For instance, the retrospective study by Køber *et al*[41] found post-MI patients being treated with digoxin at one year and five years to have 38% and 74% mortality respectively *vs* much lower rates among those not receiving digoxin (8% at one year and 26% at five years), both differences being statistically significant[41]. However, many patients in these older studies were not on current standard therapies including beta blockers.

Current guidelines

Both the AHA/ACC and ESC guidelines address the use of digoxin in the acute management of patients who present with acute ST elevation myocardial infarction. Both sets of guidelines agree that, in patients who present with acute heart failure symptoms due to severe LV dysfunction and AF with rapid ventricular rates and ongoing ischemia, digoxin may be given intravenously to improve rate control without undue concern for negative inotropic effects from beta blockers and calcium channel blockers[38,39]. A potential downside of digoxin in this clinical setting though may be an increase oxygen consumption.

DISCUSSION

This review of the current literature regarding the use of digoxin in CHF with sinus rhythm, AF with and without CHF, and post myocardial infarction highlights the concern regarding mortality risk when using digoxin. In patients with CHF with sinus rhythm, there continues to be a niche for digoxin use as an adjunctive therapy for symptomatic control once goal directed therapy has been optimized, with the understanding that there is no effect on mortality as seen in the DIG study and with a close monitoring of digoxin level.

However, no randomized controlled trial has evaluated the role of digoxin in conjunction with the current mainstay treatment strategy for CHF. It is unknown whether findings from the DIG study or prior studies can be applied to the modern strategy for heart failure[13,44]. Recent observational studies have conflicting findings regarding digoxin when evaluating patients on current optimal heart failure therapy. Although the conflicts might be resolved by a contemporary randomized trial, such a trial may not take place[45-47]. Furthermore, as novel agents like Ivabradin and the new angiotensin receptor neprilysin inhibitor become more prevalent along with left ventricular assist devices, digoxin may become less relevant in this patient population especially that these new therapies have shown to improve survival[48,49].

Questions remain regarding digoxin as a rate control strategy for those with and without heart failure. A

Table 1 Hazard ratio estimates from studies describing the effects of digoxin on total mortality in patients with atrial fibrillation

Study	Subjects	Hazard ratio estimates[1]		
		Overall mortality	AF without CHF	AF with CHF
Shah *et al*[2]	140111	NA	[2]1.17 (95%CI: 1.14-1.19, $P < 0.001$)	[2]1.14 (95%CI: 1.10-1.17, $P < 0.001$)
Turakhia *et al*[3]	122465	[2]1.26 (95%CI: 1.23-1.29, $P < 0.001$)	[2]Significant, details not given	1.29 (95%CI: 1.23-1.36, $P < 0.001$)
TREAT AF		1.21 (95%CI: 1.17-1.25, $P < 0.001$)		1.28 (95%CI: 1.21-1.36, $P < 0.001$)
Hallberg *et al*[25]	38419	NA	[2]1.42 (95%CI: 1.29-1.56, $P < 0.001$)	1.00 (95%CI: 0.94-1.06)
Freeman *et al*[29]	14787	NA	[2]1.71 (95%CI: 1.52-1.93, $P < 0.001$)	NA
ATRIA-CVRN				
Washam *et al*[30]	14171	[2]1.17 (95%CI: 1.04-1.32, $P = 0.0093$)	1.18 (95%CI: 0.94-1.46)	1.24 (95%CI: 0.98-1.57)
ROCKET AF		1.14 (95%CI: 1.01-1.29, $P = 0.0402$)		
Gjesdal *et al*[31]	7329	[2]1.53 (95%CI: 1.22-1.92, $P < 0.001$)	NR	[2]Significant, details not given
SPORTIF III and V				
Chao *et al*[28]	4781	[2]1.21 (95%CI: 1.01-1.44, $P = 0.037$)	[2]1.28 (95%CI: 1.05-1.57)	0.88 (95%CI: 0.62-1.23)
Whitbeck *et al*[4]	4058	[2]1.41 (95%CI: 1.19-1.67, $P < 0.001$)	[2]1.37 (95%CI: 1.05-1.79, $P = 0.019$)	[2]1.41 (95%CI: 1.09-1.84, $P = 0.010$)
AFFIRM				
Friberg *et al*[32]	2824	1.10 (95%CI: 0.94-1.28, $P = 0.23$)	NR	NR
SCAF		1.04 (95%CI: 0.89-1.21)		
Gheorghiade *et al*[26]	1756	1.06 (95%CI: 0.83-1.37, $P = 0.640$)	1.08 (95%CI: 0.8-1.47, $P = 0.609$)	1.08 (95%CI: 0.69-1.69, $P = 0.743$)
AFFIRM				
Pastori *et al*[33]	815	[2]2.22 (95%CI: 1.42-3.48, $P < 0.001$)	NR	NR
Rodriguez-Manero *et al*[34]	777	1.42 (95%CI: 0.77-2.60, $P = 0.2$)	0.94 (95%CI: 0.20-4.41, $P = 0.9$)	1.6 (95%CI: 0.9-2.9, $P = 0.9$)
AFBAR				
Mulder *et al*[35]	608	[2]0.41 (95%CI: 0.19-0.89)	NR	NR
RACE II				

Data also subdivided to those with and without congestive heart failure when applicable. [1]May apply different statistical methods to estimate hazard ratios. [2]Statistically significant. CHF: Congestive heart failure; AF: Atrial fibrillation; NR: Not recorded; NA: Not applicable; SPORTIF: Stroke prevention using oral thrombin inhibitor in atrial fibrillation.

recent meta-analysis reviewing over 300000 patients with AF, CHF or both found that digoxin was associated with an overall 21% increased relative risk of all-cause mortality (HR = 1.21, 95%CI: 1.07-1.38, $P < 0.01$). The meta-analysis also showed increased risk of all-cause mortality during subgroup analyses of patients with AF (HR = 1.29, 95%CI: 1.21-1.39, $P < 0.01$) and CHF (HR = 1.14, 95%CI: 1.06-1.22, $P < 0.01$) even if the hazard ratio was lower than for the other subgroups included in this analysis (*i.e.,* AF without CHF)[50].

To date, no randomized trial has been performed to evaluate the use of digoxin and its associated risk in AF patients. Such a trial might provide clarity about whether digoxin should be indicated in this population but is unlikely to happen considering the generic nature of the drug, the development of new drugs[48,49] and the burden of current healthcare costs. There are also some ethical concerns in enrolling subjects in a trial on a drug where previous studies have shown at best a neutral effect on mortality while many others raise some serious concern on safety. Therefore our understanding of digoxin's adverse effects will likely continue to be driven by retrospective analyses, which have their inherent biases and limitations in trying to evaluate associations corrected for confounders. In many retrospective studies, it is unclear what digoxin dose and/or serum levels patients had during the trials. This may be the driving force for many of the noted adverse outcomes, as prior studies evaluating digoxin in heart failure patients found that those with higher digoxin levels experienced worse outcomes[12]. In the absence of more definitive data from a prospective randomized controlled trial, the widespread adoption of a rate control strategy for AF favoring digoxin as a single first line agent has been appropriately removed from the 2014 ACC/HRS/AHA guidelines; indeed, reasonably safe and inexpensive alternatives such as beta blockers or calcium channel blockers are readily available. The subgroup of patients for which digoxin remains most controversial, in our opinion, consists of those patients with AF and CHF, for whom a benefit for digoxin could potentially extend beyond rate control (*i.e.*, inotropic effect); these patients often have low blood pressure and may be very sensitive to negative inotropic drugs[51]. Another potential clinical situation that may warrant the careful use of digoxin is AF with very low blood pressure when beta blockers and calcium channel blockers cannot be utilized.

Finally, digoxin use in patients following a myocardial infarction requires further investigation, especially immediately post MI. In this particular situation, negative inotropic drugs such as beta blockers and calcium blockers can have a deleterious effect by precipitating or worsening CHF, and digoxin may be used to control AF with rapid ventricular response since it lacks negative inotropic properties. Overall, it seems unreasonable at this point of time with the available data to recommend discontinuing patients that are stable on digoxin or to start new patients on digoxin in some indications, provided that digoxin is used cautiously.

The worrisome signal linking digoxin to increased mortality has been identified by various studies employing different designs and/or statistical methods, even though this signal has not been clearly confirmed

by prospective randomized controlled trial data. It is possible that the increased mortality is due to dosages that were inappropriately high for some patients, but this remains impossible to ascertain from existing data. Based on the potential risks and benefits, as well as the presence of alternative drugs, there is little role for digoxin among patients who only have AF. The proper role of digoxin is, however, less certain in other subgroups of patients, such as those with AF and systolic CHF or at the acute phase of a myocardial infarction. Further studies may provide helpful information for such subgroups of patients.

COMMENTS

Background

Digoxin is one of the oldest drugs used today in cardiovascular medicine around the world, and was one of the first treatments for heart failure management. Currently, this drug is frequently used to treat heart failure symptoms and to decrease the ventricular rate in atrial fibrillation (AF). In regards to heart failure management, digoxin remains indicated for patients with persistent symptoms despite optimal medical therapy. In the setting of AF, digoxin is no longer mentioned in the 2014 guidelines as an option for rate control, except among patients with AF and heart failure; however, concerns arose regarding its safety even in this subgroup of patients. Current data regarding the safety and efficacy of digoxin is based on observational studies with conflicting results as no randomized controlled clinical trials have been performed. Authors' aim is to review the data available regarding the use of digoxin in congestive heart failure (CHF), AF, or after myocardial infarction, as well as the current guidelines for digoxin use from the American College of Cardiology/American Heart Association (ACC/AHA) and European Society of Cardiology (ESC).

Research frontiers

The concern linking digoxin to increased mortality has been shown by various studies; however, this has not been confirmed by prospective randomized controlled trials. If digoxin's role in patients with only AF is limited, its role and safety in certain subgroups of patients such as those with systolic CHF and AF or during the acute phase of a myocardial infarction remain unclear. Further studies may provide helpful.

Innovations and breakthroughs

Digoxin has been used for centuries to treat systolic congestive heart failure, AF and after myocardial infarctions. Authors' goal was to review manuscripts concerning digoxin and mortality in these populations. The authors discussed the current data available and concisely displayed the data in tabular form to summarize the findings. The authors also reviewed the current recommended guidelines from the ACC/AHA and ESC regarding each subgroup when available.

Applications

Given the potential risks and benefits of digoxin, as well as the presence of alternative drugs, there is little role for digoxin among patients who only have AF. The proper role of digoxin is, however, less certain in other subgroups of patients, such as those with AF and systolic CHF or at the acute phase of a myocardial infarction. Further studies may provide helpful information for such subgroups of patients.

Peer-review

In this systematic review, the authors have provided a thorough and critical analysis of the use of digoxin in multiple clinical settings including patients with systolic congestive heart failure, AF or after myocardial infarction.

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CASE REPORT

Catheter-based intervention for symptomatic patient with severe mitral regurgitation and very poor left ventricular systolic function - Safe but no room for complacency

Poay Huan Loh, Christos V Bourantas, Pak Hei Chan, Nikolaj Ihlemann, Fin Gustafsson, Andrew L Clark, Susanna Price, Carlo Di Mario, Neil Moat, Farqad Alamgir, Rodrigo Estevez-Loureiro, Lars Søndergaard, Olaf Franzen

Poay Huan Loh, National University Heart Center Singapore, Singapore 169856, Singapore

Christos V Bourantas, University College London, London W6 8LN, United Kingdom

Pak Hei Chan, Cardiology Division, Department of Medicine, Queen Mary Hospital, the University of Hong Kong, Hong Kong, China

Nikolaj Ihlemann, Fin Gustafsson, Lars Søndergaard, Department of Cardiology, Rigshospitalet University Hospital, DK-2300 Copenhagen, Denmark

Andrew L Clark, Hull and East Yorkshire Hospitals NHS Trust, Cottingham HU6 7RX, United Kingdom

Susanna Price, Carlo Di Mario, Neil Moat, NIHR Cardiovascular BRU, Royal Brompton Hospital and Imperial College, London W6 8LN, United Kingdom

Farqad Alamgir, King Abdulaziz Medical Center, Jeddah 21589, Saudi Arabia

Rodrigo Estevez-Loureiro, Complejo Asistencial Universitario de Leon, 24071 Leon, Spain

Olaf Franzen, Klinik Im Park, 8091 Zurich, Switzerland

Author contributions: Loh PH, Alamgir F, Søndergaard L and Franzen O involved in the conception and design of the report; Loh PH, Bourantas CV and Chan PH collected and analysed the patients' clinical data; all co-authors involved in interpretation of clinical and hemodynamic data for the treatment of patients and preparation of this report; Loh PH, Clark AL, Price S and Franzen O wrote the report; all co-authors read and approved the final report.

Institutional review board statement: This case series is approved by the Institutional Review Board of Rigshospitalet University Hospital (Denmark), Hull and East Yorkshire Hospitals NHS Trust (United Kingdom) and the Royal Brompton Hospital (United Kingdom) where the patients were treated.

Informed consent statement: All patients gave informed consent.

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Correspondence to: Olaf Franzen, MD, Klinik Im Park, HerzGefässZentrum, Seestrasse, 247, 8091 Zurich, Switzerland. conpro.franzen@gmail.com
Telephone: +41-44-2092015
Fax: +41-44-2092028

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Abstract

Many patients with left ventricular systolic dysfunction have concomitant mitral regurgitation (MR). Their symptoms and prognosis worsen with increasing

severity of MR. Percutaneous MitraClip® can be used safely to reduce the severity of MR even in patients with advanced heart failure and is associated with improved symptoms, quality of life and exercise tolerance. However, a few patients with very poor left ventricular systolic function may experience significant haemodynamic disturbance in the peri-procedural period. We present three such patients, highlighting some of the potential problems encountered and discuss their possible pathophysiological mechanisms and safety measures.

Key words: Mitral regurgitation; Mitral valve; Left ventricular systolic dysfunction; Chronic heart failure; MitraClip; Percutaneous

Core tip: We described three patients with severe mitral regurgitation and very poor left ventricular systolic function undergoing percutaneous MitraClip treatment. These patients experienced haemodynamic instability peri-procedurally immediately upon reduction of their mitral regurgitation. These patients shared a few features such as right ventricular dysfunction and pulmonary hypertension which may help to identify those who may develop such peri-procedural haemodynamic compromise. Our cases highlight that although MitraClip is generally a safe procedure, there should not be complacency especially when treating patients with very poor left ventricular function. Extreme caution and vigilance should be exercised when treating such patients.

Loh PH, Bourantas CV, Chan PH, Ihlemann N, Gustafsson F, Clark AL, Price S, Di Mario C, Moat N, Alamgir F, Estevez-Loureiro R, Søndergaard L, Franzen O. Catheter-based intervention for symptomatic patient with severe mitral regurgitation and very poor left ventricular systolic function - Safe but no room for complacency. *World J Cardiol* 2015; 7(11): 817-821 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i11/817.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i11.817

INTRODUCTION

Approximately 35%-50% of patients with left ventricular systolic dysfunction (LVSD) develop significant mitral regurgitation (MR)[1]. As the severity of MR increases, their symptom and prognosis worsen with a 5-year mortality up to 60% in those with severe MR[2]. Whether treating MR in these patients alters the prognosis or progression of LVSD is not known. Percutaneous intervention for mitral regurgitation is now possible and can reduce the severity of MR even in patients with advanced heart failure with associated improvement in symptoms, quality of life and exercise tolerance[3,4]. However, special consideration should be given to patients with very poor left ventricular (LV) systolic function who may have very fragile hemodynamics that can be easily disturbed. We present a series of cases which outline some potential concerns.

CASE REPORT

Case 1

A 72-year-old man presented with NYHA class Ⅲ symptoms of heart failure and was found to have a dilated LV with severe LVSD and moderate-to-severe MR due to ischemic heart disease (IHD) (Table 1). He had previously undergone coronary artery bypass grafting, and had a history of cerebrovascular accident. Echocardiography demonstrated significant right ventricular (RV) impairment and moderately raised pulmonary arterial systolic pressure (PASP) (Table 1).

A centrally placed MitraClip reduced the severity of MR to mild. Although there was no change in blood pressure (BP) which was maintained in the region of 96/50 mmHg, trans-esophageal echocardiogram (TEE) showed worsening of LV systolic function (LV ejection fraction < 10%) and spontaneous echo contrast in the LV. After reversal of general anaesthesia, he had an episode of ventricular fibrillation that was successfully defibrillated with a single biphasic DC shock. Adrenaline and dobutamine support had to be administered for 48 h. At discharge, echocardiography demonstrated mild residual MR with LV ejection fraction (LVEF) returned to 20%. His symptoms improved to NYHA Ⅱ.

Case 2

A 78-year-old lady with severe MR, a dilated LV and severe LVSD secondary to IHD had persistent NYHA class Ⅲ symptoms despite optimal medication and cardiac resynchronisation therapy (CRT) (Table 1). She had moderate aortic stenosis with a valve area of 1.4 cm^2, moderate RV impairment and raised PASP (Table 1).

A centrally placed MitraClip successfully reduced the severity of her MR to mild. However, there was acute deterioration in her LV systolic function on TEE and a reduction in her cardiac output from 3.5 to 3.1 L/min. Her BP dropped from 104/60 to 88/54 mmHg. The MitraClip was re-positioned and deployed more medially which left her with moderate MR but her LV function, cardiac output and BP recovered. At discharge, echocardiography showed residual moderate MR and LVEF 15%. Her symptoms improved to NYHA class Ⅱ which was sustained at 1-mo follow-up.

Case 3

A 68-year-old man with severe LVSD due to idiopathic dilated cardiomyopathy had severe MR and persistent NYHA Ⅲ-Ⅳ breathlessness despite optimal medication and CRT. He had RV dilation with mild RV systolic impairment and raised PASP (Table 1).

Initial placement of the MitraClip reduced the severity of his MR to trivial. However, there was worsening of

Table 1 Echocardiographic characteristics of the patients

	LVEF (%)	LVEDD (mm)	Severity of MR	Cause of MR	Mid RV diameter (mm)	RV systolic dysfunction	PASP (mmHg)
Case 1	20	64	Moderate-to-severe	Secondary	51	Severe	50
Case 2	15	68	Severe	Secondary	42	Moderate	65
Case 3	20	70	Severe	Secondary	45	Mild	65

LVEDD: Left ventricular end-diastolic diameter; LVEF: Left ventricular ejection fraction; MR: Mitral regurgitation; PASP: Pulmonary arterial systolic pressure; RV: Right ventricle.

his LV systolic function on TEE with reduction in cardiac output from 4.9 to 4.3 L/min (Figure 1) and BP from 116/55 to 97/42 mmHg. These gradually improved to baseline after a period of observation and the clip was deployed. Invasive hemodynamic study showed an immediate reduction in his pulmonary arterial systolic pressure from 57 to 46 mmHg and mean left atrial pressure from 32 to 24 mmHg. At discharge, his symptom improved to NYHA II with trivial MR and LVEF of 20% on TTE.

DISCUSSION

These cases suggest that although percutaneous intervention for MR can be safely performed in patients with poor LV systolic function[3,4], significant clinical deterioration can occur in those with very poor LV function. The exact mechanism is unclear but likely to be due to a complex interaction of various cardiac mechanics and haemodynamic factors. In clinical setting, the haemodynamic effects following acute reduction of MR has been poorly understood as most were extrapolated from surgical patients and can be confounded by factors such as cardioplegia and cardiopulmonary bypass. Siegel *et al*[5] evaluated the acute haemodynamic effects of MitraClip therapy in 107 patients and found that successful MitraClip treatment was associated with an increase in cardiac output, cardiac index and forward stroke volume with a reduction in LV end diastolic pressure and calculated peripheral vascular resistance. These haemodynamic changes were thought to reduce the risk of low cardiac output state following the reduction of MR. However, the mean LVEF in their study was approximately 60% and patients with significant LVSD was not represented in their study[5]. In a predominantly secondary MR cohort of 50 patients with overall lower normal range of cardiac index (CI) and mean LVEF approximately 50%, Gaemperli *et al*[6] reported an improvement in CI following MitraClip. However, recent report suggests that afterload mismatch can manifest as early transient worsening of LV function following Mitraclip in patients with poorer LV function (mean LVEF 26%)[7].

It is likely that complex haemodynamic factors may be involved in patients with severe MR and significant LVSD, especially in those with very low

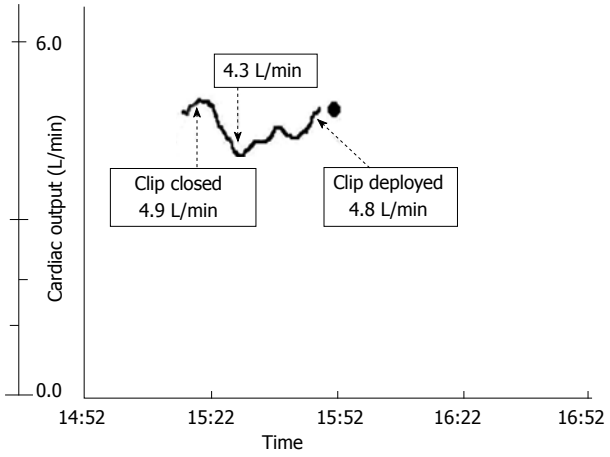

Figure 1 Cardiac output was measured by a Swan-Ganz thermodilution catheter inserted *via* the left femoral vein[8].

LVEF[8,9]. The LV contractility is regulated by the Frank-Starling mechanism, force-frequency relationship and neurohormonal control. The effectiveness of the later two mechanisms is impaired in failing human heart but the Frank-Starling mechanism can be preserved even in end-stage heart failure[10]. In the failing ventricles, the maximum LV performance is achieved at much larger LV volumes than that of the normal ventricles. This shows the importance of the Frank-Starling mechanism as a vital compensatory mechanism to maintain LV systolic contraction in the presence of significant LVSD[11]. In an experimental model using dogs with chronic heart failure, a reduction of LV end-diastolic diameter to normal level by abrupt inferior vena caval occlusion led to further reduction in LV systolic function indicating that the increase in LV preload is crucial to maintain LV systolic function[12]. Therefore, an acute and significant reduction in MR may lead to a decrease in LV end-diastolic volume (preload)[5], compromising the Frank-Starling compensatory mechanism and causes further deterioration in the LV systolic performance. In a way, this may be similar to that observed in the Fontan circulation where preload is known to be the most important determinant of cardiac output especially in the presence of a dilated or impaired ventricle[13].

Another potential factor is the poorly understood "pop-off valve" concept[14]. It was suggested that in patients with poor LV function, there is a need for the ventricle to offload into the low-impedance left atrium. However, the improvement in the outcome of mitral valve surgery in patients with poor LV function and understanding in the importance of chordal preservation has disputed this concept[15-17]. Nevertheless, it may be relevant in patients with very poor LV systolic function experiencing acute and significant reduction in MR. In experimental canine model where an external LV-to-left atrial shunt was created to mimic the effect of MR, acute shunt closure led to an increase in the LV mean systolic pressure and wall stress (afterload)[18,19]. Closure of the shunt was associated with an improvement in the haemodynamic state with reduction in LV end-

diastolic pressure (LVEDP) and increased forward cardiac output. However, in the presence of severe LV systolic dysfunction, LVEDP increased (preload) despite a reduction in LV diastolic filling and the forward flow decreased[18]. The increase in LV systolic and diastolic wall stress leads to an increase in myocardial oxygen consumption; whilst increased LVEDP may reduce coronary blood flow leading to relative subendocardial ischaemia[20]. This represents an experimental model which is not confounded by the effect of cardioplegia and cardiopulmonary bypass and may partly explain the adverse events in our patients. Since percutaneous MitraClip can be performed without cardiopulmonary bypass, the real-time beat-to-beat hemodynamic variables, LV function and MR severity can be assessed. This may help understand the pathophysiology of haemodynamic disturbance following acute MR reduction in patients with significant LVSD.

Our patients shared some common features[7] which may have contributed to the acute adverse events. They had dilated LV with advanced LVSD (LVEF $\leqslant$ 20%), pulmonary hypertension, RV systolic impairment and significant MR with substantial reduction following intervention. A cautious approach in managing the patients with very poor LV systolic function is obligatory, especially in the light of the surgical experience[1].

Every patient with significant LVSD should have individualised management within a multi-disciplinary team approach involving the interventional cardiologists, cardiac surgeons, cardiac anaesthetists and intensive care physicians, heart failure and imaging specialists and other paramedical members. Treatment strategies and options in case of procedural failure should be discussed. The patients should be pre-assessed thoroughly whilst receiving optimal heart failure therapy. TEE is mandatory to determine the anatomical suitability for the procedure and cardiac catheterisation can provide accurate haemodynamic evaluation. The assessment of LV contractile reserve and RV function may be helpful[16].

Pre-procedurally, some patients may need stabilisation of their clinical state with intravenous diuretic and/or nitrate infusion. In patients at risk of significant haemodynamic disturbance during the procedure, pre-procedural infusion of inodilators or insertion of intra-aortic balloon pump (IABP) may be considered with close liaison with all who are involved in the care of the patient including the heart failure and intensive care teams.

During the procedure, LVEF may decrease immediately after the reduction of MR in some patients. Since LVEF represents a combination of forward stroke volume (SV) and regurgitant volume (RVol) of MR, reduction in the RVol may lead to a net increase in SV even if the LVEF reduces[5,9]. Without any significant changes in the heart rate, an increase in cardiac output and systolic blood pressure are signs of positive acute haemodynamic response. Conversely, a reduction in cardiac output suggests serious haemodynamic compromise. Therefore, cardiac output monitoring is important during the peri-procedural period. Surrogate findings such as lower central venous saturation, reduction in systolic blood pressure and the appearance of spontaneous echo contrast in the LV may indicate a decrease in cardiac output. Other information, such as mitral valve opening area, left atrial or pulmonary capillary wedge pressure, left ventricular pressure[9] and if available, contractility derived from a conductance catheter[21] might help assess the situation. Continuous observation of the haemodynamic response following provisional clip deployment but before final release allows the situation to be assessed and as in patient 2, the clip to be repositioned if there is a persistent unfavourable hemodynamic response.

Adverse events may still develop some time after the procedure and so the patients should be monitored continuously until they have fully recovered from the acute effect of the procedure and general anaesthesia.

Patients with poor LV function and significant MR can benefit from percutaneous MitraClip treatment. However, some of them may experience significant acute hemodynamic disturbance peri-procedurally and caution is necessary when treating these patients.

COMMENTS

Case characteristics

Peri-procedural haemodynamic instability during percutaneous MitraClip in three patients.

Clinical diagnosis

Decreased cardiac output and blood pressure on invasive haemodynamic study.

Differential diagnosis

Afterload mismatch, access site bleeding, cardiac tamponde.

Laboratory diagnosis

Invasive hemodynamics and echocardiographic are the main diagnostic tools.

Imaging diagnosis

Trans-esophageal echocardiogram confirmed deteriorating left ventricular (LV) function and often with appearance of spontaneous echo contrast in the LV.

Treatment

Cautious and intensive haemodynamic support and monitoring before MitraClip deployment is effective in most cases; but rare cases may require repositioning of MitraClip to avoid substantial acute reduction of mitral regurgitation.

Related reports

Prior hemodynamic studies mainly limited to patients with better left ventricular ejection fraction. However, similar to this case series, Melisurgo *et al* reported a small group of patients with poor LV function experiencing afterload mismatch peri-procedurally.

Term explanation

"Pop-off valve" concept proposed that mitral regurgitation (MR) allows a degree of afterload reduction in the LV with poor systolic function and this is abolished upon acute reduction in the regurgitant volume. The resultant increase in LV afterload casues afterload mismatch leading to worsening LV function and

haemodynamic instability.

Experiences and lessons

Although MitraClip is beneficial, a minority of patients with very poor LV function and significant MR may experience significant acute hemodynamic disturbance peri-procedurally, especially in those with co-existing right ventricular dysfunction and pulmonary hypertension. Hence caution is necessary when treating these patients.

Peer-review

The article describes a group of patients with very poor LV function and severe secondary MR who may experience significant haemodynamic disturbance during percutaneous MitraClip treatment. Although it is a relatively safe procedure, there is no room for complacency and extreme caution needs to be taken when treating those patients with very poor LV function.

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LETTER TO THE EDITOR

Imaging of pannus formation in patients with mechanical heart valves

Sabahattin Gündüz, Mehmet Özkan, Mahmut Yesin

Sabahattin Gündüz, Mehmet Özkan, Mahmut Yesin, Department of Cardiology, Kosuyolu Kartal Heart Training and Research Hospital, 34846 İstanbul, Turkey

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Correspondence to: Sabahattin Gündüz, MD, Cardiologist, Department of Cardiology, Kosuyolu Kartal Heart Training and Research Hospital, Denizer Cad. No: 2, Cevizli kavşağı, 34846 İstanbul, Turkey. sabahattingunduz@yahoo.com
Telephone: +90-505-5379809
Fax: +90-216-3682527

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Abstract

Patient-prosthesis mismatch (PPM) should be recognized in patients with elevated transprosthetic gradients but without leaflet immobility, since the treatment strategy may differ in either etiology. However, thrombus and/or pannus formation should be excluded before a diagnosis of PPM is made. Particularly, pannus formation may not be diagnosed with 2-dimensional transesophageal echocardiography. Electrocardiographically gated 64-section multidetector computed tomography (MDCT) may be a promising tool in diagnosing or excluding pannus formation. Our report underlines the utility of MDCT in this regard and also emphasizes the importance of recognition of PPM as a differential diagnosis in such patients.

Key words: Multidetector computed tomography; Pannus formation; Patient prosthesis mismatch; Prosthetic heart valves; Transesophageal echocardiography

Core tip: Elevated transprosthetic gradients may be caused by pannus and/or thrombus formation or patient prosthesis mismatch (PPM). The differentiation between these three diagnoses is essential since the treatment strategy may differ in either etiology. Our report emphasizes the usefulness of cardiac multidetector computerized tomography in cases with suspected pannus formation which may not be diagnosed without surgical confirmation. Moreover, we underline the importance of recognizing PPM which may easily be overlooked in patients with elevated transprosthetic gradients. Indeed, pannus, trombus or any other masses as the cause of prosthetic dysfunction should be ruled out for a diagnosis of PPM.

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TO THE EDITOR

We would like to comment on the recent article by Soumoulou *et al*[1] which reports a case of obstructed

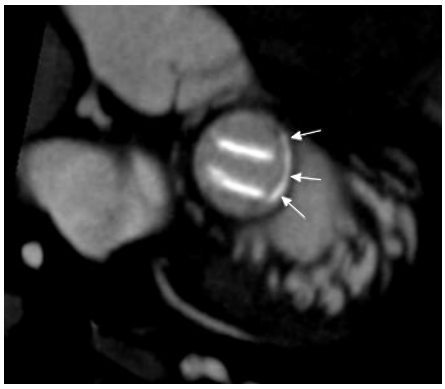

Figure 1 Pannus formation visualized as a high-attenuated periprosthetic mass.

prosthetic aortic valve caused by pannus formation, in which a preoperative definitive diagnosis could not be made by multiple imaging methods. We appreciate the authors since they emphasize the importance of clinical suspicion along with using multimodality imaging in recognizing this infrequent but serious complication of valve replacement surgery. However, two major issues remain to be addressed.

The differential diagnosis of a patient with elevated transprosthetic gradients should include not only pannus formation and thrombosis, but also patient prosthesis mismatch (PPM), since the treatment strategy may differ in either etiology. In the reported case, since there was no identifiable mass on transesophageal echocardiography (TEE) and no limited excursion of prosthetic leaflets, PPM had to be recognized before the decision of re-operation. Because, if the patient had had PPM, improvement of transprosthetic gradients after re-operation woud have been unlikely. Although the prosthetic valve size (#23 St. Jude) is not small and there is no information regarding the patient's body surface area, PPM can not be excluded unless the presence of a periprosthetic mass (pannus or thrombus) is precisely excluded. Real-time 3-dimensional TEE may be a promising tool as previously reported[2]. Although thrombus can be excluded by TEE, pannus may not be diagnosed in most of the cases. We have previously demonstrated that pannus formation may be visualized as a high-attenuated periprosthetic mass (Figure 1) and thrombus can be demonstrated as a low attenuated periprosthetic mass on electrocardiographically gated 64-section multidetector cardiac computed tomography (MDCT)[3-6]. Although the authors mention the use of cardiac computed tomography pre-operatively, there is no information regarding the slice number of the MDCT, use of intravenous contrast agent, electrocardiographic gating during the scan. Hence, without appropriate use of cardiac MDCT, pannus or thrombus may not be visualized. Fortunately, pannus formation was diagnosed peri-operatively in the current case, and the patient was succesfully re-replaced with another mechanical prosthesis.

Clinicians should be cognizant of PPM, when evaluating a patient with elevated transprosthetic gradients but without leaflet blockade. Thrombus can readliy be excluded with TEE but, pannus visualization may require more sophisticated imaging with MDCT in addition to TEE.

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Jin-Lei Wang, Director
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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
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EDITORIAL

Peripheral reflex feedbacks in chronic heart failure: Is it time for a direct treatment?

Alberto Giannoni, Gianluca Mirizzi, Alberto Aimo, Michele Emdin, Claudio Passino

Alberto Giannoni, Gianluca Mirizzi, Michele Emdin, Claudio Passino, Division of Cardiology and Cardiovascular Medicine, Fondazione Toscana Gabriele Monasterio, 56124 Pisa, Italy

Alberto Aimo, Michele Emdin, Claudio Passino, Institute of Life Sciences, Scuola Superiore Sant'Anna, 56126 Pisa, Italy

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Correspondence to: Claudio Passino, MD, Division of Cardiology and Cardiovascular Medicine, Fondazione Toscana Gabriele Monasterio, via Giuseppe Moruzzi 1, 56124 Pisa, Italy. passino@ftgm.it
Telephone: +39-050-3152191
Fax: +39-050-3152109

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Abstract

Despite repeated attempts to develop a unifying hypothesis that explains the clinical syndrome of heart failure (HF), no single conceptual paradigm for HF has withstood the test of time. The last model that has been developed, the neurohormonal model, has the great virtue of highlighting the role of the heart as an endocrine organ, as well as to shed some light on the key role on HF progression of neurohormones and peripheral organs and tissues beyond the heart itself. However, while survival in clinical trials based on neurohormonal antagonist drugs has improved, HF currently remains a lethal condition. At the borders of the neurohormonal model of HF, a partially unexplored path trough the maze of HF pathophysiology is represented by the feedback systems. There are several evidences, from both animal studies and humans reports, that the deregulation of baro-, ergo- and chemo-reflexes in HF patients elicits autonomic imbalance associated with parasympathetic withdrawal and increased adrenergic drive to the heart, thus fundamentally contributing to the evolution of the disease. Hence, on top of guideline-recommended medical therapy, mainly based on neurohormonal antagonisms, all visceral feedbacks have been recently considered in HF patients as additional potential therapeutic targets.

Key words: Baroreflex; Chemoreflex; Ergoreflex; Heart failure; Sympathetic system; Neurohormones

Core tip: At the borders of the neurohormonal model of heart failure (HF), a partially unexplored path trough the maze of HF pathophysiology is represented by the feedback systems. There are several evidences, from both animal studies and humans reports, that the deregulation of baro-, ergo- and chemo-reflexes in HF patients elicits autonomic imbalance associated with parasympathetic withdrawal and increased adrenergic drive to the heart, thus fundamentally contributing to the evolution of the disease. Hence, on top of guideline-recommended medical therapy mainly based on neurohormonal antagonisms, all visceral feedbacks have been recently considered in HF patients as additional

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INTRODUCTION

Heart failure (HF), a pathophysiological state in which the heart is unable to pump blood at a rate commensurate with the metabolizing tissues requirements, or can do so only with elevated filling pressures[1], is currently a real epidemic in western countries, affecting more than 20 million people in the world, with massive socio-sanitary costs[2].

Despite repeated attempts to develop a unifying hypothesis that explains the clinical syndrome of HF, no single conceptual paradigm for HF has withstood the test of time. The last model that has been developed, after the cardiorenal and the cardiocirculatory models focusing respectively on salt-water retention and low cardiac output/peripheral vasoconstriction, is the neurohormonal model[3]. This model has the great virtue of highlighting the role of the heart as an endocrine organ, as well as to shed some light on the key role on HF progression of neurohormones and peripheral organs and tissues beyond the heart itself. However, while survival in clinical trials based on neurohormonal antagonist drugs has improved, HF currently remains a lethal condition, with 50% mortality within 5 years of diagnosis and less than 15% survival after 10 years[2,4].

At the borders of the neurohormonal model, a partially unexplored path trough the maze of HF pathophysiology is represented by the feedback systems (Figure 1). There are indeed several evidences, from both animal studies and humans reports, that the deregulation of baro-, ergo- and chemo-reflexes in HF patients elicits autonomic imbalance associated with parasympathetic withdrawal and increased adrenergic drive to the heart, thus fundamentally contributing to worsening arrhythmias and haemodynamics. Hence, on top of guideline-recommended medical therapy mainly based on neurohormonal antagonisms, all visceral feedbacks have been recently considered in HF patients as additional therapeutic targets: baroreflex activation therapy for baroreceptors[5], physical training for muscle metaboreceptors[6], and carotid body (CB) denervation for chemoreceptors[7].

BAROREFLEX

The baroceptors are mechanoreceptors located in the sinus caroticus and in the aortic arch, where terminal nerve endings are endowed in the wall of these vessels and activated by blood pressure-induced wall stretch. Information deriving from these sites travel along a path constituted by the nerve of Hering, that merges with the fibres of the glossopharingeal nerve; those travelling from the aortic arch take the path of the afferent fibres of the vagus nerve. Inputs hence travel towards the principal centre of integration of information regarding the cardiovascular system, that is the nucleus tractus solitarii in the dorsal area of its medial and lateral divisions. Here signal are processed and integrated with information ascending from the periphery and descending from central nervous system and given back to the heart and peripheral arterial vessels *via* the vagus nerve[8]. The response is a vagally-mediated change in heart rate and a sympathetic modulation of vasomotion, in order to preserve blood pressure stability over time and avoid fluctuations[9]. Altered baroreflex sensitivity (BRS) has been demonstrated to independently contribute to worsen prognosis in HF, mainly by failing to counteract the adrenergic activation with consequent electrical instability and arrhythmic sequelae, in both the pre- and post-betablocker era[10,11].

The baroreflex has been the first neurovegetative feedback to be clinically targeted in HF. BRS activation was first indirectly attempted by vagal nerve stimulation (VNS). After the first safety and tolerability reports on VNS (side effects: Hoarseness, cough and sensation of electrical stimulation) some preliminary studies also showed amelioration of symptoms and indexes of left ventricular (LV) remodelling[12,13]. These observations led to a phase Ⅲ sham-controlled trial. The neural cardiac therapy for HF trial enrolled 87 patients with systolic HF [LV ejection fraction (LVEF) < 35%] who underwent device implantation and randomization to device in ON or OFF modality, but failed to demonstrate any effect of VNS on both primary (LV end systolic diameter) and secondary endpoints (LV end systolic volumes, LVEF, oxygen consumption and natriuretic peptide levels)[14].

Baroreceptor stimulation could also be achieved by directly stimulating carotid sinus by subcutaneously implanted device: This approach is known as baroreflex activation therapy (BAT). The first promising results obtained in an animal model of HF (dog with HF induced by microembolization) in terms of reverse remodelling, improved systolic function and amelioration of neurohormonal profile (reduced adrenergic activity), where secondarily confirmed also in a proof of concept study performed in humans, where an amelioration of symptoms was also observed[15]. Few on-going randomized studies are currently addressing the efficacy and therapeutic potential of baroreflex activation therapy in HF; in particular, the CVRx® Rheos® Diastolic Heart Failure Trial (clinicaltrial.org: NCT00718939) and the Rheos® HOPE4HF Trial (NCT00957073) will address the impact of BAT on diastolic HF (LVEF > 40%), whereas in systolic HF patients, the only ongoing randomized trial is the Barostim HOPE4HF (Hope for Heart Failure) study (NCT01720160).

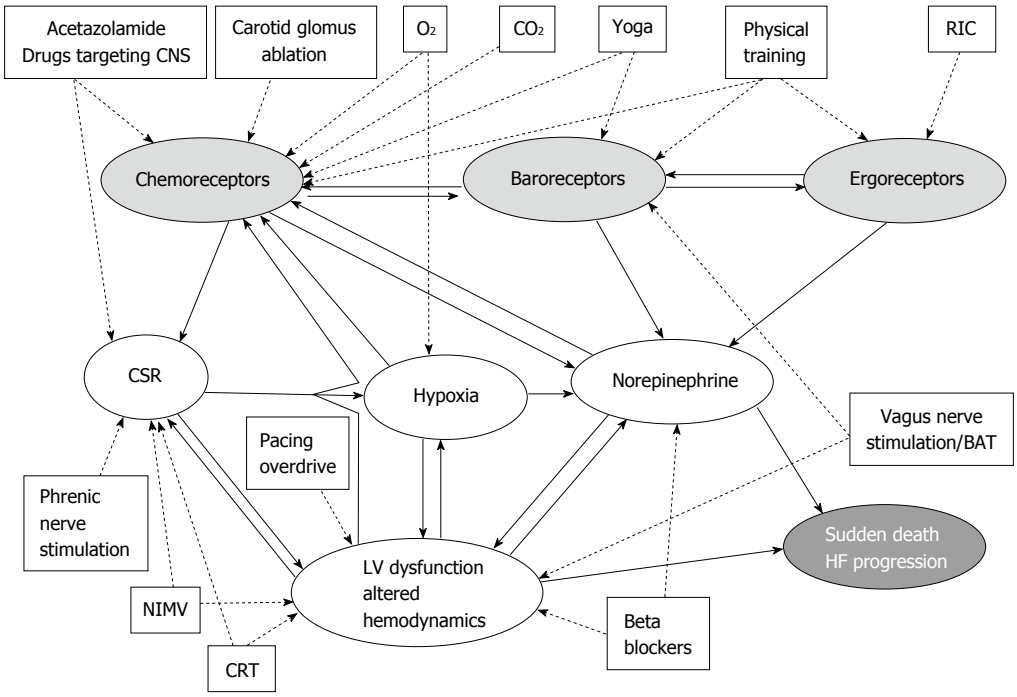

Figure 1 Schematic representation of the reflex feedbacks involved in heart failure. Arrows indicate direct effects/influences. Dotted arrows link established or potential therapeutic interventions with targets. CNS: Central nervous system; RIC: Remote ischemic conditioning; CSR: Cheyne-Stokes respiration; NIMV: Noninvasive mechanical ventilation; LV: Left ventricular; HF: Heart failure; BAT: Baroreceptor activation therapy; CRT: Cardiac resynchronization therapy.

CHEMOREFLEX

The chemoreflex is physiologically in charge of proportionally modulating ventilation in response to a change in the respiratory gases, namely oxygen (O_2) and carbon dioxide (CO_2), in order to keep pH constant for enzymatic processes. Classical physiology indicates two separate chemoreceptor groups: Peripheral chemoreceptors (PC) located in carotid-aortic bodies and sensitive both to hypoxia and hypercapnia/acidosis, and central chemoreceptors (CC) located in different regions of the brainstem, cerebellum, hypothalamus and glia and considered to be sensitive only to hypercapnia/acidosis.

Chemoreceptors seem to act as primary inputs in HF. Several studies indicate that both PC and CC are hyperactive in HF[16-19]. The increased activity of chemoreceptors is commonly considered the main determinants of Cheyne-Stokes respiration[16-19], a detrimental respiratory pattern (with prognostic significance) characterized by alternating cycles of hyperventilation and apneas, with unfavourable oxygen desaturation. Furthermore, PC/CC hypersensitivity also negatively impact on respiration kinetics during exercise with ventilatory inefficiency and dyspnoea on effort in HF patients[18,19]. The hyperactivity of PC/CC, both directly (baseline tonic activity and phasic stimulation during O_2/CO_2 changes)[20] and indirectly, *via* Cheyne-Stokes respiration (CSR) occurrence[21], is also responsible of increased adrenergic drive and arrhythmias in HF patients[17-19]. Finally, increased chemosensitivity to both hypoxia[16] and hypercapnia[19] was found to be an independent prognostic marker in HF.

A partial inhibitory effect on PC was shown in HF patients with both transient hyperoxia, and drugs, such as dihydrocodeine or acetazolamide. In HF patients, dihydrocodeine mediated PC inhibition was only associated with improved exercise performance[22]. In the same setting, acetazolamide[23] and hyperoxia[24] were instead associated with about 50% reduction of CSR severity, translating in the case of hyperoxia also with reduced sympathetic activity. Denervation of the PC chemoreceptors by CB ablation in animals with experimentally induced HF has recently emerged as a very promising option. CB ablation is indeed able to normalize the chemoreflex sensitivity in HF animals, with reduction of both adrenergic activity and disappearance of central apneas[7,25]. This was confirmed also by pharmacologic attenuation of CB activity with an inhibitor of hydrogen sulfide[26]. Interestingly, in a model of HF induced by coronary ligation in rats, CB also reduced the amount of myocardial fibrosis unrelated to myocardial infarction, with positive effect on left ventricular systolic function and, more importantly on short term survival[25]. A single report in a patient with HF has testified the feasibility in humans[27]. Differently from these still preliminary, but intriguing results on PC modulation, currently no studies have tested the possibility to directly act on CC, maybe due to the multiplicity of CC centers in the central nervous system, the complexity of their interlink, and the difficulty to directly and selectively act on these receptors.

ERGOREFLEX

The ergoreflex is the neural mechanism enabling to

modulate ventilation and sympathetic outflow according to the intensity of physical activity[28]. Its components are the metaboreflex, activated by the accumulation of metabolites in the exercising muscles, and the mechanoreflex, responsive to muscle tension during exercise[29-31].

HF patients frequently develop a skeletal myopathy ascribable to deconditioning, reduced perfusion of the muscles, inflammation, and a systemic catabolic state[29,30,32]. In 1994, a "muscle hypothesis" of HF was formulated, suggesting that ergoreceptor contribution to the autonomic, hemodynamic, and respiratory responses to exercise would be enhanced in CHF patients[33]. Two years later, ergoreflex overactivity was first found in HF patients compared with healthy subjects[6]. These results were corroborated by subsequent studies, which correlated increased ergoreceptor sensitivity to lower lean body mass, reduced exercise tolerance, decreased left ventricular function, and worse New York Heart Association functional class[30]. Interestingly, in HF patients with preserved exercise capacity, ergoreflex overactivity has been also associated with increased central and peripheral chemoreceptor sensitivity, and depressed baroreceptor sensitivity[30].

Currently the only acknowledged treatment for modulating ergoreflex overactivity is represented by exercise training. The effects of training on ergoreflex sensitivity have been evaluated mostly in animal models[34]. In humans, six weeks of forearm training were able to markedly reduce metaboreceptor sensitivity, while six weeks of detraining brought the situation back to baseline[29]. A positive effect on muscle structure and function has been after confirmed in other studies, still in HF patients[35,36]. It is reasonable to assume that the positive impact of exercise training on HF patients (in terms of increased exercise tolerance, quality of life, cardiac function, neuro-hormonal activation and overall prognosis)[37-40] partially relies upon reduced ergoreflex overactivity, as confirmed by a recent study[41].

CONCLUSION

The lessons learned from failures (*e.g.*, inotropic drugs) and the successes (*e.g.*, neurohormonal antagonist drugs) in treating HF indicate that the development of innovative treatments for HF should take into account the complex pathophysiology of the disease: In particular, new treatments should target the pathways involved in the evolution of the disease. As outlined above, peripheral reflexes are deeply involved in the pathophysiology of HF and represent a potential target of therapy. Although, some preliminary data in animals and humans are promising, more studies enrolling a large number of patients are clearly needed to reinforce the rationale of treating the peripheral reflex feedbacks and to disclose the prognostic value of these interventions.

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REVIEW

Do neutrophil extracellular traps contribute to the heightened risk of thrombosis in inflammatory diseases?

Ashish N Rao, Nayef M Kazzaz, Jason S Knight

Ashish N Rao, Nayef M Kazzaz, Jason S Knight, Division of Rheumatology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109-5680, United States

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Correspondence to: Jason S Knight, MD, PhD, Division of Rheumatology, Department of Internal Medicine, University of Michigan Medical School, 1150 West Medical Center Drive, SPC 5680, Ann Arbor, MI 48109-5680, United States. jsknight@umich.edu
Telephone: +1-734-9363257
Fax: +1-734-7634151

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Abstract

Thrombotic events, both arterial and venous, are a major health concern worldwide. Further, autoimmune diseases, such as systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, and antiphospholipid syndrome, predispose to thrombosis, and thereby push the risk for these morbid events even higher. In recent years, neutrophils have been identified as important players in both arterial and venous thrombosis. Specifically, chromatin-based structures called neutrophil extracellular traps (NETs) play a key role in activating the coagulation cascade, recruiting platelets, and serving as scaffolding upon which the thrombus can be assembled. At the same time, neutrophils and NETs are emerging as important mediators of pathogenic inflammation in the aforementioned autoimmune diseases. Here, we first review the general role of NETs in thrombosis. We then posit that exaggerated NET release contributes to the prothrombotic diatheses of systemic lupus erythematosus, ANCA-associated vasculitis, and antiphospholipid syndrome.

Key words: Thrombosis; Neutrophil extracellular traps; Lupus; Vasculitis; Antiphospholipid syndrome

Core tip: In order to capture and kill pathogens, neutrophils release webs of chromatin and antimicrobial proteins called neutrophil extracellular traps (NETs). These NETs are also emerging as important players in inflammatory and thrombotic disorders. In this review, we describe the mechanisms by which the various components of NETs promote thrombosis. Further, we highlight emerging evidence that NETs may play a particularly important role when thrombosis occurs in patients with systemic autoimmune diseases such as

lupus, vasculitis, and antiphospholipid syndrome.

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INTRODUCTION

Blood vessel occlusion attributable to thrombosis is a major health concern in both the United States and worldwide. Most United States studies have suggested annual incidence for venous thromboembolism (VTE) on the order of 1/1000. For example, a classic retrospective study reviewed medical records in Minnesota from 1966 through 1990, and found a VTE incidence of 117 per 100000[1]. A more recent community study addressed VTE incidence in Worcester, Massachusetts and found a similar incidence of 104 per 100000[2]. In Norway, incidence of first VTE is at a similar level, estimated at 1.43 per 1000 person years[3].

VTE morbidity is especially problematic in hospitals. For example, a multinational cross-sectional study of the acute inpatient setting noted that VTE, and specifically pulmonary embolism, accounted for 5%-10% of deaths in hospitalized patients[4]. It should also be noted that VTE carries a high risk of not just morbidity, but also death. In the aforementioned Worcester population study, acute all-cause mortality in patients with VTE was 6.6%[2]. Another United States community-based study, found 28-d mortality following VTE to be 11%, with that risk climbing to 25% in patients with cancer-associated thrombosis[5]. The aforementioned Norwegian study found the risk of death to be especially high following pulmonary embolism, specifically 2.1-fold higher than for deep vein thrombosis (DVT)[3].

Similar to VTE, cardiovascular disease (CVD), especially myocardial infarction and stroke, is a major cause of worldwide morbidity and mortality. CVD results from an inflammatory vasculopathy of arteries called atherosclerosis, which places patients at risk for acute arterial occlusions and downstream ischemia. Global data from the late 1990s suggest that on the order of one-third of all deaths worldwide are caused by CVD[6]. It has also been suggested that access to healthcare plays a critical role in the morbidity attributable to events like strokes, with countries in eastern Europe, north Asia, central Africa, and the south Pacific having particularly high levels of disability following such events[7].

While thrombotic events are clearly a major problem in the general population, the risk is further amplified in the setting of many systemic autoimmune diseases. For example, a meta-analysis of VTE risk in such diseases (excluding pregnant and postoperative patients) found an increased risk that was particularly striking in systemic lupus erythematosus (SLE) and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, with odds ratios of 7.29 and 7.97, respectively[8]. Another study of SLE patients found a 7.6% risk of thrombosis over approximately 10 years, which climbs as high as 20.1% in the presence of a particular class of autoantibodies referred to as antiphospholipid antibodies (discussed in more detail below)[9]. When an ANCA-associated vasculitis cohort was followed for six years, there was a 12% prevalence of VTE[10]; interestingly, the incidence was 1.8 per 100 person-years when disease was quiescent, and climbed to 6.71 per 100 during active disease[10].

Patients with systemic autoimmune diseases are also at high risk for CVD. For example, in a prospective cohort of SLE patients, 48% of deaths were attributable to CVD, with risk factors including smoking, endothelial activation, elevated C-reactive protein, and antiphospholipid antibodies[11]. SLE patients may be at particular risk for cerebrovascular events[12], with some studies suggesting that more than 20% of mortality may be attributable to stroke[13]. CVD has similarly been documented at increased levels in ANCA-associated vasculitis, with a rate of acute myocardial infarction that is at least 2.5-times higher than expected based on traditional cardiovascular risk factors[14].

NEUTROPHIL EXTRACELLULAR TRAPS

The neutrophil, as the most abundant leukocyte in circulating blood, plays a critical role in the innate immune system[15-20]. Formed in the bone marrow from myeloid precursors[21], neutrophils are then released into the bloodstream. From there, they can be recruited to sites of inflammation/infection in response to endogenous or pathogen-derived chemoattractants[17,20]. One strategy by which neutrophils target and kill microbes is phagocytosis[22]. Once pathogens are captured in intracellular vacuoles, they are destroyed by reactive oxygen species (oxidative burst)[23] and antimicrobial proteins (degranulation)[24]. Upon the completion of phagocytosis, neutrophils generally undergo apoptosis before being ingested by neighboring macrophages as inflammation resolves[25-27]. For decades, phagocytosis was considered to be the primary mechanism by which neutrophils targeted infections; however, that perception changed with the discovery of neutrophil extracellular traps (NETs) - one of the most interesting and intensively-studied aspects of neutrophil biology in recent years.

NETs target pathogens

NET release (or NETosis), as first described by Brinkmann *et al*[18] in 2004, is an active form of neutrophil death that releases a web of chromatin and antimicrobial proteins into the extracellular space. At the core of NETs are chromatin fibers (about 17 nm in diameter) composed of DNA and histones, positively-charged proteins that normally function in the nucleus to package DNA and regulate gene expression. These fibers are further

lined by granule-derived antimicrobial proteins such as neutrophil elastase, myeloperoxidase (MPO), cathepsin G, proteinase 3 (PR3), defensins, and cathelicidin LL-37. NETs target pathogens by a combination of sequestration (preventing their dissemination in the body) and highly-localized microbicidal activity[18]. Both Gram-negative (*Shigella flexneri*[18], *Klebsiella pneumoniae*[28]) and Gram-positive (*Streptococcus aureus*[29], *Listeria monocytogenes*[30]) bacteria can be targeted by NETs, as can fungi (*Candida albicans*[31], *Aspergillus nidulans*[32], *Aspergillus fumigatus*[33]). NETs have also been shown to be effective in killing particular protozoans and viruses[34-36]. Intriguing recent work has demonstrated that neutrophils are capable of sensing differences in microbe size such that NETs are preferentially released when the neutrophil is confronted by larger pathogens and microbial clusters that cannot be engulfed by phagocytosis[37].

It is also interesting to note that certain microbes have evolved mechanisms for evading NETs. For example, surface modification may dampen neutrophil activation and NET binding[38-40]. Also, pathogen-derived nucleases are well established as destabilizers of NETs[41-43]. That NETs form an important arm of antimicrobial innate immunity is exemplified by the fact that defects in NET generation, or experimental NET depletion, increase susceptibility to various kinds of infections in mice and humans[28,44-49].

Mechanisms of NET release

NET release can be triggered by a variety of stimuli including microbes, pharmacological agents (phorbol 12-myristate 13-acetate and calcium ionophore[50]), inflammatory cytokines (interleukin 8[51], tumor necrosis factor α[52]), growth factors (granulocyte colony-stimulating factor[53]), activated endothelial cells[54], activated platelets[55], and immune complexes[56]. Following this initial trigger, various pathways intersect to facilitate the extrusion of NETs. For example, some think of NETosis as a variant of autophagy since netting neutrophils display characteristics of autophagy including the formation of autophagosomes[57]. Indeed, inhibition of autophagy-associated signaling prevents NETosis in some contexts[58]. Generation of reactive oxygen species (ROS) by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex has also been considered by many as an absolute prerequisite to NET formation[48,59,60]. Mechanistically, protein kinase C activation[61] and RAF/MEK/ERK signaling[62] lead to phosphorylation of gp91^{phox}[63], p67^{phox}[64], and p47^{phox}[65], which results in assembly of the functional NADPH oxidase complex for ROS generation. However, recent evidence has also shown that activation of SK3 potassium channels, mediated by calcium influx, may lead to an alternative, NADPH oxidase-independent mechanism of NETosis[66].

Once activated, neutrophils preparing for NETosis flatten and adhere tightly to the substratum. ROS are generated and cytoplasmic granules disintegrate releasing their contents into the cytoplasm. Neutrophil elastase then migrates to the nucleus, where it degrades linker histone H1 and processes core histones, thereby promoting chromatin relaxation[28]. This is followed by the translocation to the nucleus of MPO, which also binds chromatin and promotes decondensation, albeit by an unknown mechanism[28]. In addition, the relaxation of chromatin is further promoted by post-translational modification of histone arginine residues to neutral citrullines by the enzyme peptidylarginine deiminase 4 (PAD4)[46,67-69]. Following dissolution of the nuclear membrane, the plasma membrane ruptures casting NETs into the extracellular space[48].

It should be noted that the above description is of what is sometimes called "suicidal" NETosis. However, NETs can also be released in more rapid fashion, in a manner that does not lead to neutrophil death. This concept of "vital" NETosis, which especially occurs in the context of the direct interaction between neutrophils and microorganisms, has been described in detail in a recent review article[70].

HOW DO NETS PROMOTE THROMBOSIS?

Thrombosis results from dysregulation of normally-protective hemostatic systems, with the end result being a clot in the vessel lumen and obstruction of blood flow. If the occlusion is not resolved, it can have marked consequences including infarction, embolization, and even death. Blood coagulation can be initiated by two classic pathways. The first, historically termed "extrinsic", starts with the release of thrombogenic tissue factor from endothelium and leukocytes, while the second "intrinsic" pathway is initiated by the activation of circulating clotting factors on negatively-charged surfaces. Both of these pathways converge at a common point (factor Ⅹ) with the subsequent activation of the protease factor Ⅱ (also called thrombin). Thrombin then converts fibrinogen into insoluble fibrin, which is indispensable for clot formation[71]. Platelet activation (associated with the release of procoagulant polyphosphates among other bioactive molecules) and platelet aggregation (to form a platelet plug) are also important processes in normal hemostasis, as well as pathologic thrombosis[72,73]. These pathways are further regulated by natural anticoagulants like tissue factor pathway inhibitor, antithrombin, thrombomodulin, and protein C, which act on various targets to limit thrombin generation[74].

NETs are now known to be an integral component of thrombi, and actually essential for thrombosis in many contexts (Figure 1). NETs serve as structural scaffolding for entrapment and aggregation of platelets and erythrocytes[75]. Additionally, negatively-charged NETs bind plasma proteins like fibrinogen, fibronectin, and von Willebrand factor (VWF), thereby stabilizing the clot[75]. In animal models, it has been shown that dismantling NETs by deoxyribonuclease (DNase) treatment or knocking out PAD4 (an enzyme essential for NET formation) diminishes thrombosis[76-79]. Mechanistically, interesting studies, using both *in vitro* and *in vivo* systems, have

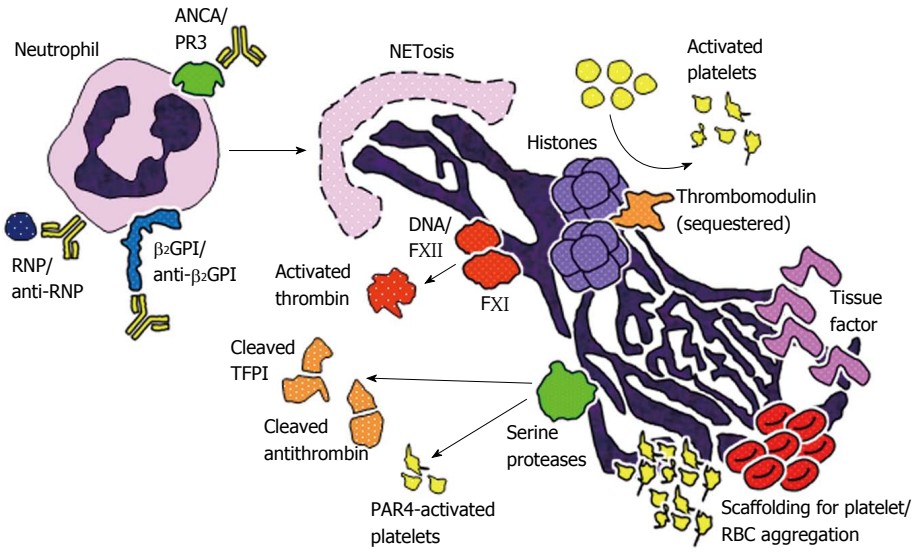

Figure 1 Schematic representation of potential mechanisms by which neutrophil extracellular traps may promote thrombosis in systemic autoimmune diseases. First, a number of stimuli may promote NETosis in systemic autoimmune diseases including ribonucleoprotein (RNP)/anti-RNP complexes in systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody (ANCA) engagement with surface proteinase 3 (PR3) in vasculitis, and the interaction of anti-beta-2 glycoprotein I (β_2GPI) with surface β_2GPI in antiphospholipid syndrome. The DNA component of NETs activates factor XII (FXII), initiating a cascade (along with factor XI) that ultimately leads to the formation of thrombin. Histones in NETs activate platelets and sequester certain anticoagulant molecules like thrombomodulin and protein C. Neutrophil serine proteases present in NETs, such as neutrophil elastase and cathespin G, cleave the anticoagulant molecules tissue factor pathway inhibitor (TFPI) and antithrombin, and also activate platelets through various pathways including protease-activated receptor 4. NETs also may present procoagulant tissue factor in some contexts. Finally, NETs serve as scaffolding for the assembly and aggregation of platelets and red blood cells (RBCs). NET: Neutrophil extracellular trap.

Table 1 Neutrophil extracellular trap-associated molecules that may play a role in promoting thrombosis

NET component	Role in thrombosis
DNA backbone	Negatively charged surface capable of activating factor XII[80]
	Coassembly of TFPI and serine proteases at thrombus[86]
Histones	Platelet activation[83,84]
	Prevent activation of natural anticoagulant, protein C[85]
Elastase	Cleavage of TFPI[86]
	Cleavage of antithrombin[87]
	Strips proteoglycan layer of arterial media to expose VWF[88]
Cathepsin G	Cleavage of TFPI[86]
	Platelet activation[89,90]
Tissue factor	Platelet activation[91,104]
	Thrombin generation by extrinsic pathway of coagulation[91,104]

TFPI: Tissue factor pathway inhibitor; VWF: Von Willebrand factor; NET: Neutrophil extracellular trap.

shown that several NET components are capable of contributing to coagulation and thrombus formation (Table 1).

DNA backbone

Coagulation factor XII, a plasma serine protease capable of activating factor XI and prekallikrein, is recognized as the traditional initiator of the intrinsic pathway. factor XII is well known to be activated by negatively-charged surfaces both *in vitro* and *in vivo*, and it turns out that the anionic backbone of NETs (*i.e.,* DNA) is a capable activator of factor XII[80]. It has consequently been shown that factor XIIa (the activated form) can contribute to thrombus formation by both factor XI-dependent and independent mechanisms[81,82].

Histones

As mentioned above, histones are positively-charged proteins that normally function to package DNA in the nucleus; they are also the most abundant proteins in NETs. Histones trigger platelet activation and thrombin generation in a dose-dependent manner[83,84]. Indeed, upon treatment with histones, platelets exhibit several activation-associated characteristics such as aggregation, exposure of phosphatidylserines, and surface expression of P-selectin[83,84]. The ability of histones to activate platelets seems to be at least partially dependent on signaling through platelet Toll-like receptor 2 (TLR2) and TLR4[83,84], with a further contribution from several notable intracellular pathways including ERK, Akt, p38, and nuclear factor-κB. Importantly, when histones complex with DNA (as is observed in NETs), their ability to promote platelet activation and thrombin generation is further amplified[84]. Intersecting with coagulation pathways, histone-activated platelets release polyphosphates, which potently promote thrombin activation[84]. Independent of platelets, it has also been suggested that histones contribute to the activation of thrombin by sequestering thrombomodulin and protein C (a natural anticoagulant), and thereby preventing thrombomodulin-dependent activation of protein C[85]. These varied experiments (primarily done with purified components *in vitro*) have been supported by work in animal models, where infusion of histones promotes DVT formation in mice in the context of inferior vena cava

flow restriction[76].

Serine proteases: Neutrophil elastase and cathepsin G

Granule-derived serine proteases, which are among the most abundant non-histone proteins in NETs, potentially engage with blood coagulation in a number of ways. For example, mice deficient in neutrophil elastase and cathepsin G exhibit defects in tissue factor activation, fibrin formation, and thrombus stabilization[86]; in this system, at least one function of the proteases is to degrade an antagonist of coagulation, tissue factor pathway inhibitor (TFPI). Interestingly, the DNA component of NETs is required for the coassembly of TFPI and the proteases, thereby inactivating TFPI at the point of thrombosis[86].

Other mechanisms have also been described. Neutrophil elastase promotes the proteolytic cleavage of the anticoagulant antithrombin[87]. Elastase (in cooperation with matrix metalloproteinase 9) also degrades the proteoglycan network of the arterial media, thereby exposing collagen for VWF binding and platelet adhesion[88]. Further, cathespin G can promote a procoagulant state by cleaving and activating platelet protease activated receptor 4 signaling, thereby enhancing thrombus formation and fibrin deposition under flow conditions[89,90].

Tissue factor

In 2012, von Brühl *et al*[80] showed that the combination of intravascular NET formation and tissue factor are essential for development of thrombi in a mouse model of DVT. The NETs were not only decorated with tissue factor, but also with protein disulfide isomerase, which can activate it. In this system, the tissue factor was felt to originate especially from monocytes, before migrating to, and activating on, the NETs[80]. However, in neutrophils isolated from patients with sepsis, neutrophils themselves seem to be the source of tissue factor, utilizing the machinery of autophagy to deliver tissue factor to the NETs[91]; indeed, in this context, tissue factor-bearing NETs can stimulate both thrombin generation and platelet activation *ex vivo*.

NETS AND THROMBOTIC EVENTS

Arterial and venous thrombotic events, despite certain common risk factors, are pathophysiologically-distinct processes[92,93]. For example, arterial thrombosis is particularly dependent on platelets since, under the high shear stress of arterial flow, platelets are effective at adhering to the vessel wall[94]. Rupture of atherosclerotic plaques (as in CVD) leads to marked platelet activation and aggregation, and ultimately to the development of platelet-rich "white" clots. In contrast, an important factor in venous thrombosis is a reduction in blood flow (stasis) with the development of red blood cell-rich "red" thrombi that result from the local accumulation and activation of circulating coagulation factors[95]. Interestingly, as the components of NETs are capable of activating both platelets and the coagulation cascade, NETs may be a unifying link/risk factor for the two processes. This notion has been validated in the animal models and clinical studies that are highlighted below.

Venous thrombosis

In one clinical study, 150 patients with symptomatic DVT were compared to controls who had clinical suspicion for DVT, but negative objective testing[96]. As compared to controls, patients with DVT had higher levels of both circulating nucleosomes and activated neutrophils, with elevated levels of either suggesting an approximately three-fold risk of DVT[96]. Another group obtained venous thromboembolism specimens from 11 patients and classified these into various stages of thrombus organization based on morphological characteristics[97]. Immunochemical staining suggested that NETs were especially present in organizing venous thrombi, indicating that they play an important role in thrombus maturation[97].

Experimentally, restriction (stenosis) of blood flow in the iliac vein of baboons[75] or the inferior vena cava of mice[76,80], results in elevation of plasma DNA levels and development of NET-containing venous thrombi. Further, in this model, infusion of histones increases both thrombus size and plasma levels of VWF, with the latter potentially contributing to platelet activation and recruitment[76]. Importantly, neutrophil depletion results in comparatively smaller thrombi[80], as does treatment with DNase[76]. Thrombus formation is also abrogated in PAD4-knockout mice, which are deficient in NET production[79]. In the PAD4 knockouts, thrombosis could be rescued by infusion of wild-type neutrophils[79], arguing that PAD4's role in thrombosis is at the level of neutrophils (and presumably NETosis).

Cardiovascular disease and arterial thrombosis

Correlation studies have hinted at a relationship between DNA, NETosis, and atherosclerotic/atherothrombotic disease[98]. In a cohort of 282 patients with well-characterized coronary artery disease, severity of disease was predicted by levels of circulating cell-free DNA as well as a number of NET markers (nucleosomes, citrullinated histone H4, and MPO-DNA complexes)[98]. Further, these markers also correlated with evidence of active coagulation (soluble CD163 and thrombin-antithrombin complexes)[98]. In mice, NETs can be detected in close association with plaques in the carotid lumen of atherosclerosis-prone ApoE(-/-) mice[99], while the PAD4 inhibitor Cl-amidine (which also blocks NETosis) prevents NET formation and decreases atherosclerotic lesion area in this model[77]. Mechanistically, the cathelicidin-derived proteins LL-37 (human) and CRAMP (mouse), which are abundant in NETs, seem to promote atherosclerosis[100,101]. For example, Döring *et al*[102] demonstrated that CRAMP-DNA complexes stimulate plasmacytoid dendritic cells (pDCs) to produce type I interferons that promote plaque growth, a phenotype that could be reversed by either CRAMP

deficiency or degradation of the DNA backbone of NETs.

Regarding arterial thrombosis, coronary thrombi can be rich in NETs as detected by immunochemical staining[103]; the authors of this study were particularly interested in the role of neutrophil interleukin-17A/F, and indeed both cytokines were present in not only neutrophils, but NETs themselves[103]. It has also been suggested that NETs present in the thrombi of acute myocardial infarctions expose tissue factor, which is functional in activating both thrombin generation and platelets when studied *ex vivo*[104]. However, that functionality was lost with digestion of the DNA backbone of NETs[104]. Finally, in a mouse model of arterial wall injury by ferric chloride, NET nucleosomes, as well as neutrophil serine proteases (elastase and cathepsin G), are essential for thrombus formation[86].

SLE

SLE is a systemic autoimmune disease that preferentially affects women. While the etiology of SLE is not fully understood, it is widely accepted that a hallmark of SLE is the near universal detection of an "antinuclear" autoimmune response. In particular, autoantibodies form to double-stranded DNA and to ribonucleoprotein (RNP) complexes. These autoantibodies participate in immune complex formation, with subsequent deposition in organs such as the kidneys (where they cause glomerulonephritis). Given the key roles of both autoantibodies and immune complexes in SLE pathogenesis, the majority of research over the years has understandably focused on abnormalities in the adaptive immune system, with particular attention paid to B cells, T cells, and antigen-presenting cells. However, in recent years, increasing attention has been paid to mediators of the innate immune response, especially neutrophils, which release NETs[105], and pDCs, which manufacture large quantities of type I interferons[106].

Regarding NETs, some patients with SLE have a deficiency in circulating DNase function, and therefore an impaired ability to degrade NETs in plasma[107,108]. This DNase defect fluctuates, and has been shown to correlate with both glomerulonephritis and hypocomplementemia[109]. Not surprisingly, the levels of circulating NETs themselves have also been shown to correlate with nephritis[110].

While impaired degradation surely plays a role in the increased levels of circulating NETs[110], the situation is further exacerbated by the increased propensity of SLE neutrophils to undergo NETosis[111-113]. In some cases this is likely a result of stimulation by circulating autoantibodies, such as anti-RNP and anti-LL-37, which are common in SLE patients[111,112,114]. In other cases, enhanced NETosis may be attributable to environmental factors, like low vitamin D levels[115], or increased susceptibility to infection resulting from treatment with immunosuppressive drugs. Accelerated NETosis may also stem from inherent differences in SLE neutrophils, as evidenced by their lower density (sometimes referred to as low-density granulocytes) and their proinflammatory phenotype[113]. Further, SLE NETs may be especially potent stimulators of the immune system. For example, they contain LL-37, which stimulates both pDCs and macrophages[112,116]. The immunostimulatory potential of SLE NETs may also be further amplified by acetylated histones and demethylated DNA[117,118].

As is discussed above, the risk of thrombotic events, both arterial and venous, is significant in SLE patients[119,120]. From an arterial perspective, the relative risk for myocardial infarction and stroke are both increased (10- and 7-fold, respectively) relative to that seen in the general population[121]. Similarly, the risk of DVT and pulmonary embolism is increased at least 10-fold in SLE[122]. Other venous complications, such as retinal vein occlusion[123], also stand out as more common. Further, it should be noted that with improved treatment of organ-threatening SLE manifestations such as kidney disease, 50% of SLE patients now die of some type of cardiovascular disease[11].

NETs, endothelial damage, and thrombosis in SLE

An important intersection between NETs and the vasculature involves the ability of SLE NETs to engage TLRs and thereby promote the formation of type Ⅰ interferons by pDCs[111-113]. Type Ⅰ interferons then play a multifaceted role in endothelial dysfunction, accelerating foam cell formation and impairing endothelial progenitor numbers and function[124,125]. Further, given the abundance of neutrophils in circulation (especially relative to rare cells like pDCs), it is noteworthy that netting neutrophils may themselves be a source of type Ⅰ interferons in SLE[113,126].

In addition to promoting the production of potentially anti-vascular cytokines like type Ⅰ interferons, NETs may also play a direct role in endothelial damage in SLE[113]. For example, SLE NETs contain matrix metalloproteinase-9, which activates endothelial matrix metalloproteinase-2, and thereby triggers endothelial cell death[127]. Endothelial damage may be further compounded in SLE by the NET- and MPO-mediated oxidation of high-density lipoprotein (HDL), which causes HDL to lose its normally vasculoprotective properties[128].

The best evidence for a role of NETs in not just the vascular damage, but also the prothrombotic diathesis of SLE, comes from mouse models of the disease. Indeed, NETs play an important role in pathogenesis of some[78,129], but not all[130], SLE models. In the NZM2328 model, NET release can be prevented by treatment with an inhibitor of PAD4 that prevents histone citrullination and consequently NETosis[78]. Over time, PAD inhibition protects against endothelial damage as measured by an acetylcholine-dependent vascular relaxation assay[78]. NZM2328 mice are also prothrombotic at baseline, rapidly forming carotid thrombi after photochemical injury of the endothelium. These carotid thrombi are rich in neutrophils and NETs, and can be prevented by treatment with either DNase or a PAD inhibitor[125]. These findings are reminiscent of work in models of

atherosclerosis, where NETs are important in not just vascular damage[100,102], but also thrombosis[77]. Whether prevention of NETosis can protect against thrombotic disease in patients with SLE remains to be determined, although it is noteworthy that antimalarial drugs like chloroquine both block NETosis[128], and track with a reduced risk of thrombosis in patients[131].

ANCA-ASSOCIATED VASCULITIS

ANCA-associated vasculitis describes a group of closely-related relapsing-remitting diseases, characterized by (1) small-vessel inflammation that especially targets the lungs and kidneys; and (2) autoantibodies against the neutrophil granule proteins MPO and PR3. The two best characterized syndromes are microscopic polyangiitis, in which patients are typically positive for anti-MPO, and granulomatosis with polyangiitis (Wegener's), which classically has anti-PR3 positivity. Neutrophils/NETs and ANCA likely interact in two important ways: (1) NETs contain both MPO and PR3 and may thereby stimulate the autoimmune response to these antigens; and (2) ANCA can interact with neutrophils to promote NET release, with NETs then contributing to vascular and organ damage.

Consistent with NETs playing a role in ANCA induction, netting neutrophils are more efficient than apoptotic neutrophils in loading murine myeloid dendritic cells with MPO and PR3[132]. This efficiency is dependent on the DNA backbone of NETs, as it can be almost completely abrogated with DNase[132]. NET-loaded dendritic cells induce glomerulonephritis in mice[132], while myeloid dendritic cells can be detected interacting with netting neutrophils in skin samples from patients with microscopic polyangiitis[132]. The ability of NETs to induce ANCA has also been observed anecdotally in patients, for example, in the setting of infectious endocarditis apparently driving both anti-PR3 formation and glomerulonephritis[133].

Mechanisms of ANCA-mediated NET release

Mechanistically, ANCA likely promote NETosis by engaging granule proteins that have migrated to the cell surface in primed neutrophils[134]. Indeed, one study found that ANCA are more potent than SLE IgG in this regard, and further that ANCA-associated NETosis correlates well with vasculitic disease activity[135]. The mechanism of NET induction by a nontraditional ANCA, anti-lysosomal membrane protein-2 (LAMP-2), has recently been investigated in detail. It appears that anti-LAMP-2 directs neutrophils away from apoptosis and toward NETosis by activating the vacuolization typically seen in autophagy[136]. Whether autophagy machinery is also required for NETosis mediated by traditional ANCA (anti-PR3 and anti-MPO) remains to be elucidated.

When NETs form in ANCA patients, they are relatively resistant to degradation by plasma DNase, an effect that is not explained by a direct effect of ANCA on DNase itself[135]. Along similar lines, the anti-thyroid drug propylthiouracil (PTU) is a recognized inducer of ANCA production in humans; in an animal model, PTU leads to the formation of NETs that are particularly resistant to DNase-mediated degradation, thereby exacerbating both pulmonary capillaritis and glomerulonephritis[137]. It was recently shown that ANCA-induced NETs appear to be relatively potent activators of the alternative complement cascade[138], and can also promote both platelet activation and conversion of pentameric C-reactive protein (CRP) into prothrombotic monomeric CRP[139].

ANCA-mediated NETs and thrombosis

NETs have been found in close proximity to inflamed glomeruli in vasculitic kidneys[134], as well as in vasculitic skin lesions[140], arguing that NETs play a role in tissue toxicity. It has also been suggested that NETs play a particular role in ANCA-associated thrombotic events, especially venous. For example, thrombi obtained from ANCA vasculitis patients are particularly rich in both NETs[141] and histone citrullination[142].

An intriguing mechanistic role has also been suggested for tissue factor[143]. Specifically, Kambas *et al*[143] demonstrated tissue factor-positive NETs in sera, bronchoalveolar lavage fluids, and renal biopsies of ANCA vasculitis patients. Further, tissue factor-positive NETs and microparticles correlated with higher disease activity (similar to thrombosis), and could be induced when control neutrophils were treated with ANCA *in vitro*[143]. How unique these phenotypes are to ANCA-associated NETs, as compared to NETs that form in other infectious and inflammatory diseases, remains to be determined.

ANTIPHOSPHOLIPID SYNDROME

Antiphospholipid syndrome (APS), an autoimmune disease of unknown etiology, is among the most common acquired causes of both thrombosis and pregnancy loss in the United States. About half of APS cases are diagnosed in patients with lupus, and the remainder as a standalone syndrome called primary APS[144]. Primary APS manifests not just with thrombosis and pregnancy loss, but also with additional features including livedo reticularis, thrombocytopenia, chorea, leg ulcers, cognitive dysfunction, seizures, alveolar hemorrhage, and nephropathy[145]. This heterogeneity of manifestations clearly points to APS as a truly systemic autoimmune disease on the spectrum of lupus, rheumatoid arthritis, and small-vessel vasculitis.

Pathophysiology of APS

Despite the name of the syndrome (anti-phospholipid), the best understood antigen in APS is not a phospholipid, but rather a lipid-binding protein that circulates at high levels in blood (100-200 μg/mL) called beta-2 glycoprotein I (β_2GPI). Autoantibodies to β_2GPI activate various types of cells *in vitro*[146-149], and promote both thrombosis and pregnancy loss when injected into mice[150,151]. Currently, three assays are used to diagnose APS clinically. These include tests for (1) anti-cardiolipin

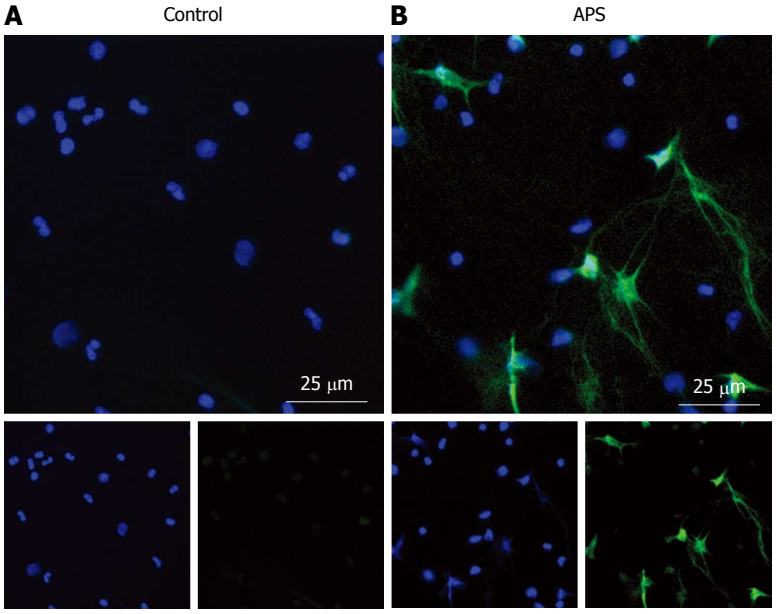

Figure 2 Antiphospholipid syndrome neutrophils are prone to "spontaneous" neutrophil extracellular trap release. Freshly-isolated neutrophils from a healthy control (A) or antiphospholipid syndrome (APS) patient (B) were seeded onto poly-lysine-coated coverslips and incubated in serum-free media for 2 h. Samples were then fixed with paraformaldehyde and stained with Hoechst 33342 (DNA = blue) and anti-neutrophil elastase (Abcam, green). Cells were not specifically permeabilized and neutrophil elastase staining is therefore primarily extracellular. These representative micrographs show more neutrophil extracellular trap release in the APS neutrophils, as determined by overlapping DNA and neutrophil elastase staining.

antibodies; (2) anti-β2GPI antibodies; and (3) a group of coagulation assays collectively referred to as "lupus anticoagulant" - functional testing that takes advantage of the fact that antiphospholipid antibodies paradoxically prolong phospholipid-dependent clotting assays *in vitro*. It is interesting to note that ELISAs for anti-cardiolipin are often actually detecting anti-β2GPI, with the reactivity to cardiolipin mediated by β2GPI protein present in the patient's serum. Antibodies to thrombin may also sometimes cause APS, although testing has not been standardized, and anti-thrombin is therefore not routinely assessed in clinical practice. In summary, this group of antibodies is (despite the inaccuracy) referred to as antiphospholipid antibodies, with anti-β2GPI being the best characterized and the most likely to be pathogenic.

While antiphospholipid antibodies are recognized to be pathogenic, the origin of these antibodies, and the reason that lupus patients are especially at risk for their development, are not well understood. Further, there are currently no targeted treatments for APS. Instead, therapy focuses on masking the prothrombotic effects of antiphospholipid antibodies with anticoagulant medications like warfarin and heparin. These drugs often need to be taken for life, and at the same time predispose to catastrophic bleeding complications[152]. While anticoagulants are somewhat effective in preventing APS-associated blood clotting, they often have no bearing on the neurologic and renal complications of APS, which can progress to organ failure[145].

Heightened NET release in APS

Our group has recently made a number of important observations about APS neutrophils[153]. First, NETs circulate at high levels in the plasma of APS patients, even between thrombotic episodes[153]. Indeed, freshly isolated neutrophils from APS patients are primed to undergo spontaneous NETosis when cultured *ex vivo* (Figure 2). Mechanistically, anti-β2GPI IgG promotes NETosis by engaging β2GPI protein on the neutrophil surface; this process is independent of the Fc receptor, but does require ROS production and TLR4 signaling[153]. Further, and pointing to disease relevance, anti-β2GPI-stimulated NETs promote thrombin generation *in vitro*[153]. In addition to our work, Leffler *et al*[154] have shown that some patients with APS have a defect in DNase-mediated NET degradation. This potentially sets up a vicious prothrombotic cycle, in which the threshold for NETosis is reduced in APS neutrophils, followed by the exaggerated persistence of the NETs that do form. A final interesting point is that antiphospholipid antibodies seem to engage not just neutrophils[153], but NETs themselves[154]. This observation deserves further exploration as to its potential role in APS pathogenesis.

CONCLUSION

While NETs have yet to be assigned a clear function in normal hemostasis, their roles in venous thrombosis, atherosclerosis, and arterial occlusions continue to be defined. It is notable that many systemic autoimmune diseases are not only associated with increased NETosis and decreased NET clearance, but also demonstrate an increased risk of both arterial and venous events. We therefore find it quite plausible that NETs contribute to the prothrombotic nature of diseases like SLE, ANCA-associated vasculitis, and APS. As is detailed above, there are also hints that these sterile inflammatory NETs

may differ structurally from NETs released during infection (for example, by being enriched in tissue factor or being more resistant to degradation), although further study in this area is clearly needed. More work in disease-specific experimental models will also be required before clinical interventions can be considered. In summary, there is a need to continue to explore the association between thrombosis and inflammatory disease-associated NETosis, in order to better understand whether treatment algorithms can be developed that will allow us to prevent, rather than simply treat, life-threatening thrombotic episodes in these at-risk patients.

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REVIEW

Diagnostic and prognostic value of circulating microRNAs in heart failure with preserved and reduced ejection fraction

Christian Schulte, Dirk Westermann, Stefan Blankenberg, Tanja Zeller

Christian Schulte, Dirk Westermann, Stefan Blankenberg, Tanja Zeller, Department of General and Interventional Cardiology, University Heart Center Hamburg Eppendorf, 20246 Hamburg, Germany

Dirk Westermann, Stefan Blankenberg, Tanja Zeller, German Center for Cardiovascular Research (DZHK), Partner site Hamburg/Luebeck/Kiel, 20246 Hamburg, Germany

Author contributions: Schulte C, Westermann D, Blankenberg S, Zeller T designed the topic and structure of the review; Schulte C wrote the manuscript; Schulte C, Zeller T performed literature research, created figures and tables.

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Correspondence to: Tanja Zeller, PhD, Professor for Genomics and Systems Biology, Department of General and Interventional Cardiology, University Heart Center Hamburg Eppendorf, Building N27, Martinistr 52, 20246 Hamburg, Germany. t.zeller@uke.de
Telephone: +49-40-741056575
Fax: +49-40-740040194

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Abstract

microRNAs (miRNAs) are powerful regulators of post-transcriptional gene expression and play an important role in pathophysiological processes. Circulating miRNAs can be quantified in body liquids and are promising biomarkers in numerous diseases. In cardiovascular disease miRNAs have been proven to be reliable diagnostic biomarkers for different disease entities. In cardiac fibrosis (CF) and heart failure (HF) dysregulated circulating miRNAs have been identified, indicating their promising applicability as diagnostic biomarkers. Some miRNAs were successfully tested in risk stratification of HF implementing their potential use as prognostic biomarkers. In this respect miRNAs might soon be implemented in diagnostic clinical routine. In the young field of miRNA based research advances have been made in identifying miRNAs as potential targets for the treatment of experimental CF and HF. Promising study results suggest their potential future application as therapeutic agents in treatment of cardiovascular disease. This article summarizes the current state of the various aspects of miRNA research in the field of CF and HF with reduced ejection fraction as well as preserved ejection fraction. The review provides an overview of the application of circulating miRNAs as biomarkers in CF and HF and current approaches to therapeutically utilize miRNAs in this field of cardiovascular disease.

Key words: MicroRNA; Heart failure; Cardiac fibrosis; Biomarker; Diagnostic; Prognostic; Heart failure with reduced ejection fraction; Heart failure with preserved ejection fraction

Core tip: Recent study results suggest microRNAs (miRNAs) as promising biomarkers in the diagnosis of heart failure (HF) with reduced ejection fraction (HFrEF) and with preserve ejection fraction (HFpEF). The therapeutic application of antagomirs and mirmimics in

heart failure is still in its infancy but promising experimental results are reported. This review provides an overview of miRNAs as diagnostic and prognostic biomarkers in HF and gives details on the utilization of miRNAs in the differentiated diagnosis of HFpEF and HFrEF. The manuscript evaluates the therapeutic applicability of miRNAs in HF and thus provides valuable information for researchers dealing with miRNAs in HF.

Schulte C, Westermann D, Blankenberg S, Zeller T. Diagnostic and prognostic value of circulating microRNAs in heart failure with preserved and reduced ejection fraction. *World J Cardiol* 2015; 7(12): 843-860 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i12/843.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i12.843

INTRODUCTION

The term "microRNA" (miRNA) was established in 1993 when researchers started to study the function of small RNAs[1]. Lin-4 was the first miRNA described[2] and after its discovery scientists began to recognize miRNAs' importance as regulators in gene expression. Ever since miRNAs have not only been assessed for their promising regulatory role in various diseases, but also their diagnostic potential in risk prediction as well as their use as circulating biomarkers[3-5]. Furthermore, promising data have depicted miRNAs as gene specific therapeutic targets in disease modeling[6].

Numerous studies have analyzed miRNAs with respect to their utilization as disease-specific biomarkers. In cardiovascular disease, miRNAs have successfully been proven to be quantitatively modified in particular disease entities such as myocardial fibrosis and heart failure (HF)[7-12].

In this article we will review the value of miRNAs in cardiac fibrosis (CF) and HF. We will discuss the current knowledge about their role in two different entities of the disease - HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF). In particular, we will provide an overview about the use of miRNAs as diagnostic and prognostic biomarkers in CF and HF as well as potential therapeutic agents.

miRNA: GENERALLY SPEAKING

miRNAs are non-coding RNAs with a length of 19-25 nucleotides[13,14]. By binding to the 3'-untranslated region of target messenger RNAs (mRNAs), miRNA either initiate translational repression or degradation of mRNAs thereby regulating gene expression at the post-translational stage[15,16] (Figure 1). Every single miRNA has target sites in hundreds of different genes[17]. At the same time computational prediction of target mRNAs suggests that more than 60% of all mammalian protein-coding genes are conserved targets of miRNAs[18]. At the time of writing this review 2588 mature homo sapiens miRNAs were listed in "miRBase" (mirbase.org).

miRNA quantification showed organ- and cell-specific expression patterns of certain miRNAs[19] while quantification measures have shown concentration-dependent effects in pathologically altered organs[20]. *In-vitro* findings suggest groups of miRNAs being specifically up and down-regulated and polymorphisms in the miRNA regulatory pathway - so called miRSNPs - have been found to be associated with different types of disease[21-24]. miRNAs fulfill several criteria of an ideal biomarker: stability in the circulation, tissue- and pathology-specific regulation as well as high sensitivity and specificity. These characteristics predestine miRNAs as biomarkers. In fact, there is evidence that miRNAs' applicability as circulating biomarkers for certain diseases might even exceed that of protein-based biomarkers[25,26]. The field of miRNA research has paved the way for the development of new means of biomarker-based risk stratification for cardiovascular events. In this regard promising data have been collected in large-scale prospective clinical studies[27].

HF

The discovery of miRNAs as promising new biomarkers in cardiovascular disease has ignited great expectations and especially in the field of HF, the last years have witnessed great success. The syndrome of HF ranges among the leading causes of death and morbidity worldwide[28] with mortality rates of up to 50% in patients with new onset HF[29]. HF can be classified by the contraction of the left ventricle into HFrEF and HFpEF. While HFrEF is defined by a reduced left ventricular ejection fraction (LVEF), HFpEF describes HF patients with normal or only mildly reduced LVEF (over 50%)[30]. Approximately half of all HF patients present with preserved LVEF[31,32] illustrating its clinical importance, while morbidity and mortality are suggested to be equally distributed.

HF, CARDIAC HYPERTROPHY AND FIBROSIS

There are three major causes of HF: hypertensive heart disease, ischemic heart disease and idiopathic dilated cardiomyopathy. Hypertension initiates molecular pathways that lead to increased cardiomyocyte size and protein synthesis as well as augmented sarcomer organization[33,34]. Persisting hypertrophy is associated with an unfavorable outcome and can result in HF and sudden death[35,36]. Independently from the underlying pathology failing hearts remodel in regard to extracellular matrix and myocyte size. This leads to augmented hypertrophy and death of cardiomyocytes followed by tissue fibrosis and scarring[37]. CF results in increased myocardial stiffness affecting systolic as well as diastolic left ventricular function[37,38]. The initial molecular steps in the development of HF can hardly

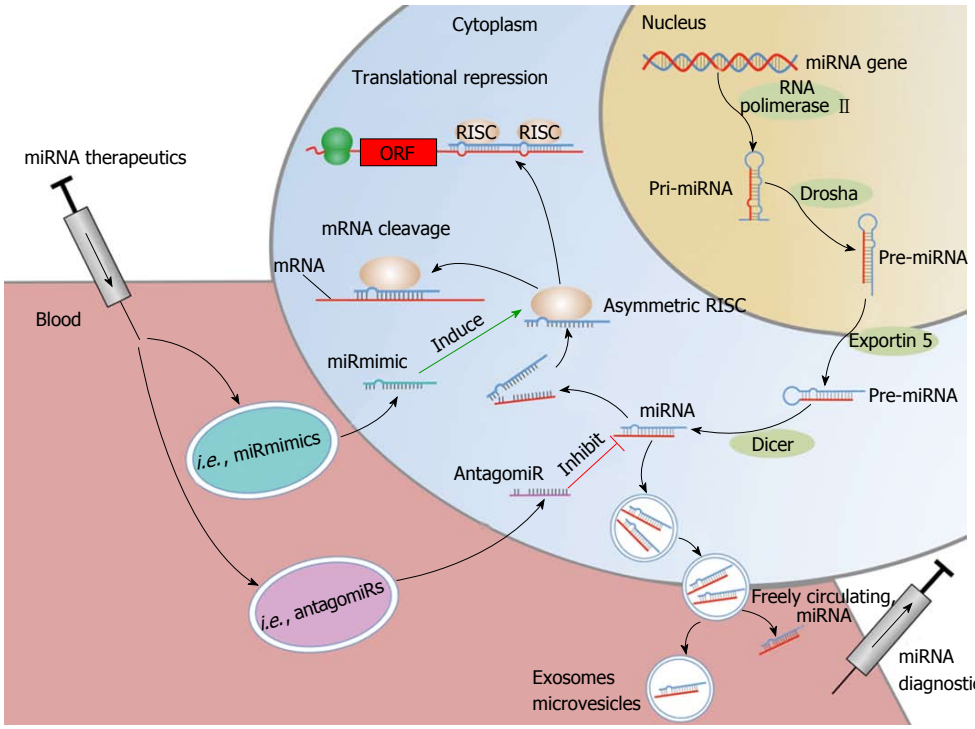

Figure 1 microRNA synthesis and mode of function. Pri-miRNAs are generated in the nucleus by RNA polymerase Ⅱ. The endonuclease "Drosha" catalyzes the transformation of pri-miRNA into pre-miRNA, which is transported into the cytoplasm by Exportin 5. Subsequently, the mature miRNA is generated by the endonuclease "Dicer". The mature miRNA is incorporated into the RISC complex; in this form leading to degradation of target mRNAs and/or inhibition of translation. Mature miRNAs can be released into the circulation, incorporated into vesicles such as MVB, exosomes, microvesicles or as freely circulating miRNAs. miRNA therapeutics such as miRmimics and antagomirs can be administered directly into the blood flow or applied by oral uptake. Antagomirs specifically bind to and silence endogenous miRNAs, leading to reduced RISC activation and mRNA degradation. miRmimics specifically bind to target mRNAs to increase their degradation. mRNA: Messenger RNA; miRNA: microRNA; ORF: Open reading frame; RISC: RNA-induced silencing complex; MVB: Multivescular bodies.

be analyzed using imaging techniques and protein biomarkers may be involved at later stages only[39]. In this respect the diagnosis of HF is most often been made at an advanced stage of the disease when symptoms and physical confinement have already developed. The fact that so-called early HFpEF usually is clinically apparent merely under exercise conditions complicates an early diagnosis[40-42]. This determines the clinical need for markers identifying the disease at the earliest possible stage.

miRNAS IN CARDIAC HYPERTROPHY AND FIBROSIS

Given that cardiac remodeling and fibrosis are significant factors in the development of ventricular wall stiffness with compromised ventricular contractility and compliance, the expression of miRNAs is directly linked to the development of HF - with preserved and with reduced ejection fraction. miRNAs were identified to play a major role in the transcriptional and translational changes in gene expression with respect to cardiac hypertrophy and fibrosis[36,43]. The regulatory involvement of miRNAs in the development of cardiac hypertrophy and fibrosis ultimately suggests their causal roll in HF.

In a mouse model of aortic constriction induced cardiac hypertrophy van Rooij *et al*[36] described altered levels of several miRNAs in murine cardiomyocytes while Sayed *et al*[44] found a set of more than 50 miRNAs dysregulated in a similar setting with induced hypertrophy. Especially miR-1 was identified as significantly down-regulated compared to sham operated controls, probably mediated *via* an inhibition of the translation of calmodulin-encoding mRNAs[45]. Similar results were reported by Carè *et al*[46]. They reported down-regulated levels of miR-1 and miR-133 in cardiomyocytes of hypertrophic murine as well as human hearts[46]. Furthermore, in an *in-vitro* model the authors found a causal relationship between adeno-virus induced elevation of miR-1 and miR-133 levels and an inhibition of cardiac hypertrophy. Supporting data were reported from an *in-vitro* study involving neonatal rat cardiomyocytes[47]. Recently miR-150 has been described as a regulator in cardiac hypertrophy[48]. In a mouse model the authors induced cardiac hypertrophy by aortic banding and found miR-150 levels down-regulated compared to sham operated animals[48]. Several more *in-vitro* and mouse model studies reported altered miRNA levels in cardiac hypertrophy on the one hand and induction of cardiac hypertrophy by artificial alterations of specific miRNAs on the other[49-52]. Especially miR-1[44,53], miR-21[54,55], miR-133[53,54], miR-195[36,56], miR-208[57-59] were proven to be involved in the regulation of cardiac hypertrophy. Alterations of miRNA levels were also

reported in induced hypertrophy of cardiomyocytes in engineered heart tissue[60].

The heart's initial hypertrophic response to volume overload or increased afterload as well as pathological conditions after myocardial infarction (MI) is followed by the process of remodeling which leads to CF. A major regulatory roll of miRNAs in the process of cardiac remodeling and fibrosis was suggested when a key enzymatic step towards miRNA activation catalyzed by the enzyme Dicer was blocked in knock out mice[61]. The authors found biventricular enlargement, myocyte hypertrophy and pronounced CF[61]. miRNAs involved in regulatory pathways of hypertrophy such as miR-208 have also been found in cardiac remodeling[57]. van Rooij *et al*[57] found mutant mice overexpressing miR-208 to not develop cardiomyocyte fibrosis despite being exposed to an increased afterload. Opposite, miR-208a has been identified as a regulator of endoglin expression and increases myocardial fibrosis in volume overloaded hearts[62]. Comparable results were reported in a model of cultured rat myoblasts[59].

miR-133a was reported to be down-regulated in a mouse model of aortic constriction-induced hypertrophy[63]. Expression of miR-133a prevented this down-regulation while the authors found less myocardial fibrosis along with improved diastolic function of the analyzed mouse hearts[63]. Similar results were reported from analyses of CF in canines. Shan *et al*[64] described reduced miR-133 and miR-590 levels in canine hearts after nicotine-induced CF mediated by up-regulation of transforming growth factor β (TGF-β) 1 and TGF-β RII. By transfection of miR-133 or miR-590 into cultured atrial fibroblasts it was possible to reduce fibroblast activity as well as collagen production while this effect was reversible by administration of antisense oligonucleotides against miR-133 or miR-590[64]. Confirming results of reducing CF by miR-133 induction were recently reported in a mouse model of aortic banding-induced hypertrophy[65]. Besides miR-133 also miR-30 was reported to control pro-fibrotic proteins and thus regulate changes in the extracellular matrix of the myocardium[66]. The decrease of miR-133 and miR-30 in a gene-modulated rat model of pathological cardiac hypertrophy was found to be linked with an up-regulation of collagen synthesis and CF[66].

In a transgenic mouse model miR-21 was discovered as a key regulator of signaling pathways in cardiac fibroblasts controlling the extent of cardiac hypertrophy and interstitial fibrosis[55]. A different working group was able to show that elevated miR-21 expression was highly related to CF[67]. These findings are in line with the observation that miR-21 is involved in the regulation pathway of cardiac fibroblasts in infarcted mouse hearts[68]. Cardin *et al*[69] were able to suppress atrial fibrosis in miR-21 knock out mice after induced MI. Also, the authors succeeded in depressing post infarction fibrosis by means of anti-miR-21 reduction of miR-21 availability. More recent studies validate these findings of miR-21 promoting cardiac remodeling and fibrosis[67,70,71]. On the other hand silencing of miR-21 by means of antagomirs resulted in cardiomyocyte necrosis and apoptosis[72,73] indicating the integration of this miRNA in cardiac remodeling.

MI is frequently followed by ventricular remodeling processes and fibrotic structural changes in the infarcted areas. This process eventually leads to HF. A miRNA involved in post-MI CF is miR-24. In a mouse model of induced MI Wang *et al*[74] reported miR-24 down-regulated and found a simultaneous increase of extracellular matrix remodeling. *In-vivo* lentivirus-based intramyocardial elevation of miR-24 levels caused attenuation of fibrosis in the infarct border zone[74]. The authors described TGF-β to mediate the miR-24 modulated effect and concluded miR-24 to be a potential target for the treatment of post-MI remodeling.

miR-29 is involved in fibrotic processes in different types of body tissue[75,76]. It controls a variety of pro-fibrotic genes such as collagens, fibrillins, laminins, integrins and elastin[77]. Furthermore, miR-29 negatively regulates a number of anti-apoptotic genes, including *Tcl-1*, *Mcl-1*, *YY1*, *p85a*, *CDC42* and *DNMT3*[77-81]. In a mouse model of induced MI miR-29 was down-regulated[82]. Further analyses revealed miR-29 being predominantly expressed in cardiac fibroblasts and the *in-vivo* inhibition of miR-29 resulted in an induction of collagen mRNA expression[82]. Raising miR-29 concentrations using Mirmimics lead to a down-regulation of collagen mRNA expression[82]. Affirmative results were recently reported by Yang *et al*[83]. With respect to these results miR-29 can be attributed a key roll in regulation of tissue fibrosis and CF in particular.

Study results like these gave rise to projects analysing the roll of miRNAs in manifest HF.

miRNAS IN HF

In the process of HF development different intracellular signaling pathways are activated including an up-regulation of structural fetal genes, such as β-myosin heavy chain (β-MHC) and down-regulation of adult structural genes, such as α-MHC[84-86]. miRNAs are involved in regulatory processes of activating fetal genes that are known to be up-regulated in failing hearts[87]. The involvement of miR-208 in α- and β-MHC regulation was reported by van Rooij *et al*[57]. *Via* this mechanism miR-208 regulates cardiomyocytes growth under stress conditions as reported by the authors in a transgenic mouse model[57]. The same working group had previously found a set of miRNAs up-regulated not only in a mouse model of induced hypertrophy but also in failing human hearts[36]. They found an increased expression of miR-24, miR-125b, miR-195, miR-199a and miR-214 in both mice and human hearts and postulated that these miRNAs are part of a molecular signature of adverse cardiac remodeling[36].

Besides the development of cardiac hypertrophy and fibrosis as described above a knockout of the key enzyme Dicer, essential for intracellular miRNA

processing, leads to dilatative cardiomyopathy (DCM) and HF[88]. The authors found reduced Dicer expression in human failing hearts and reported a significant increase of Dicer expression in hearts of patients with improved cardiac function after implantation of left ventricular assist device (LVAD) for HF[88]. These results depict the importance of miRNAs in the regulation of pathophysiologic processes involved in the development of HF and have lead to considerations of clinical implications of miRNAs dysregulated in cardiomyopathies and HF in particular. Matkovich *et al*[89] drove the Dicer-related findings by Chen *et al*[88] (mentioned above) a step further and analyzed a miRNA expression profile of cardiac tissue from HF patients with and without LVAD-based recovery compared to healthy controls. The authors found 28 miRNAs up-regulated in failing hearts compared to healthy controls and 20 of these miRNAs returned to near normal levels in the LVAD-treated group with significant improvement of left ventricular performance[89]. While these results link defined miRNAs to clinically apparent HF and suggest their potential in treatment monitoring, a more distinct analysis of miRNAs in different types of cardiomyopathies was performed by Ikeda *et al*[90]. In left ventricular biopsy samples of 67 humans with ischemic cardiomyopathy (ICM), DCM, aortic stenosis and healthy controls they analyzed miRNA expression[90]. Using a genome-wide miRNA expression profiling they detected 87 miRNAs and found their expression profiles significantly altered in the three heart diseases compared to healthy controls. While seven miRNAs were altered in the same direction in all three disease entities, the global pattern of miRNA expression was distinct in different types of HF[90]. miR-19 appeared as the most strongly down-regulated miRNA in DCM and AS but not in ICM, while miR-1 was down-regulated in all three diseases. miR-214 - considered pro-hypertrophic[36] - was most strongly up-regulated. Surprisingly, miR-133 and miR-208 levels, which are associated with myocardial hypertrophy[46,57-59,63] and fibrosis[59,62,64], were unchanged. These reported data suggest miRNAs to be specifically dysregulated in different types of HF pathology. In this regard an interesting study was recently reported by Leptidis *et al*[91] who performed miRNA deep sequencing analyses in myocardial biopsies of end stage DCM, hypertrophic cardiomyopathy (HCM) and healthy controls to analyze the human heart's miRNOME with respect to these two different HF pathologies. They were able to identify a set of ten miRNAs (miR-23b, miR-30d, miR-125a, miR-143, miR-145, miR-193, miR-197, miR-342, miR-365, miR-455) that is differentially expressed in HCM and DCM compared to healthy controls and had not been linked to HF previously[91]. The authors were able to confirm previously described dysregulated levels of miR-133a, miR-1, miR-21, miR-214, miR-212, miR-29, miR-129, miR199a in HCM, while miR-119 and miR-214 expression was reported only to be altered in DCM[91]. miR-145 was identified as a new regulator of pathologic left-ventricular remodeling. Satoh *et al*[92] who analyzed miRNA expression in cardiac tissue from myocardial biopsies of patients with DCM reported higher levels of miR-208, miR-208b and miR-499 than in healthy controls. Follow-up revealed baseline miR-208 levels to be strong predictors of clinical outcome[92] indicating a potential utilization of miRNAs in risk prediction of HF.

Based on these results, the potential applicability of miRNAs as distinct biomarkers for the diagnosis of HF and for different entities of the disease seems possible. miRNAs seem to be promising biomarkers in risk prediction of HF patients. For obvious reasons, though, the availability of heart tissue is limited and therefore different sources of biomaterial for miRNA analysis are needed. In this respect body fluids appear to present an ideal origin to non-invasively win such biomaterial.

CIRCULATING miRNAS

Besides regulating gene expression and phenotypic control in the cell of origin[93] and mediating metabolism on an intracellular level[94] miRNAs are also secreted from the producing cell and capable of transmitting their silencing signals to different cells[95]. miRNAs have been detected in numerous body fluids such as serum and plasma as well as saliva and urine[25,26] and can be found in pericardial fluid of HF patients[96]. Consequently, miRNAs have been tested to function as detectable extracellular messengers in cell-to-cell communication[97]. Their structure prevents miRNAs from early degradation in circulating blood[25,98-102] and their ideal biomarker characteristics including size, abundance and tissue specificity suggest circulating miRNAs as blood-based biomarkers for tissue injury[12,103-105].

CIRCULATING miRNAS IN THE DIAGNOSIS OF HF

The ability to detect and measure miRNAs in a minimal-invasive way has led to their evaluation as potential circulating biomarkers for cardiovascular disease[3,106]. The promising results of disease-specific cellular miRNA dysregulation in HF and their suitable characteristics with regard to circulating biomarker diagnostics have led to their evaluation as blood based biomarkers in HF. In a rat model of induced left ventricular hypertrophy with consecutive development of HF the authors reported significantly elevated plasma levels of miR-16, miR-20b, miR-93, miR-106b, miR-223 and miR-423-5p[107]. These results were in line with earlier findings of Tijsen *et al*[108] who were amongst the first researchers to evaluate circulating miRNAs as diagnostic biomarkers in HF in a clinical approach. The authors reported that besides 6 miRNAs (miR-18b[a], miR-129-5p, miR-1254, miR-675, oncomir HS_202.1 miR-622), that were moderately elevated in plasma of 30 HF patients compared to 20 dyspnea patients and 20 healthy controls, miR-423-5p was found to be a significant predictor of HF diagnosis in a multivariate logistic regression model[108]. Despite the

small sample size and suboptimal matching of baseline characteristics[109] these were promising initial results that were confirmed by Goren *et al*[110] in a similar study setup. The authors were one of the first groups to perform a screening of circulating miRNAs on a larger scale in cardiovascular disease and HF. They screened 186 miRNAs in serum of 30 HF patients compared to 30 healthy controls and were able to detect four miRNAs (miR-423-5p, miR-320a, miR-22, miR-92b) that were up-regulated in the serum of HF patients compared to the control group[110]. Furthermore, the authors succeeded in generating a score out of these miRNAs that discriminates HF patients from healthy controls. The group was able to describe a significant association between the miRNA score and several established prognostic HF parameters such as NT-proBNP, a wide QRS complex and left ventricular (LV) dilatation underlining the significance of these results not only with respect to diagnostic but also to prognostic applicability of circulating miRNAs[110]. At that time the analyzed combination of miRNAs miR-320 and miR-423-5p had previously been associated with HF[86,111,112].

Ellis *et al*[113] analyzed miRNA plasma levels of 44 HF patients compared to 32 Chronic obstructive pulmonary disease (COPD) patients, 59 patients with breathlessness for other diagnoses and 15 healthy controls after an initial miRNA screening phase. Not only were seven miRNAs (miR-103, miR-142-3p, miR-342-3p, miR-199a, miR-23a, miR-27b, miR-324-5p) associated with the diagnosis of HF in regression and receiver operating characteristics (ROC) analysis, plasma levels of four miRNAs (miR-103, miR-142-3p, miR-30b and miR-342-3p) were able to distinguish between HF and exacerbation of COPD, other causes of dyspnea and controls[113]. Although miR-423-5p could not be identified as a predictor of HF diagnosis, the addition of miR-423-5p to NT-proBNP significantly improved the area under the operating receiver curve (AUC) for predicting the diagnosis HF[113]. These findings confirm previous results of the potential applicability of miR-423-5p as circulating biomarker in HF diagnosis.

In a larger clinical trial serum miRNA levels of 81 HF patients were compared to 60 non-HF patients and 15 healthy subjects[114]. The authors reported a set of 24 miRNAs significantly down-regulated in the HF group compared to controls. miR-26b-5p, miR-145-5p, miR-92a-3p, miR-30e-5p and miR-29a-3p inversely correlated with NT-proBNP and directly correlated with EF, while ROC analysis to predict differentiation of HF patients from non-HF cases revealed strong AUC values between 0.84 and 0.91, suggesting these miRNAs to be potentially strong circulating biomarkers in the diagnosis of HF[114].

Recently, Wong *et al*[115] identified miR-1233, miR-183-3p, miR-190a, miR-193b-3p, miR-193b-5p, miR-211-5p, miR-494 and miR-671-5p to be able to distinguish HF from healthy controls in plasma levels of 60 HF patients and 30 healthy subjects.

Circulating blood cells and endothelial cells contain higher miRNA concentrations than serum and plasma[116]. Thus, further approaches in quantification efforts of circulating miRNAs with respect to HF were aimed at analyzing their concentration in circulating blood cells such as peripheral blood mononuclear cells (PBMCs). Gupta *et al*[117] analyzed miRNA concentrations in PBMCs of 44 DCM HF patients compared to 48 healthy controls. Real time polymerase chain reaction (RT-PCR) revealed miR-548c and miR-548i significantly down-regulated in PBMCs of DCM patients, while miR-138 was up-regulated in PBMCs of those patients. ROC analysis showed an AUC of 0.85 for miR-548c with respect to its discriminatory power to distinguish DCM from controls[117].

Frequently, HF consecutively develops after ischemic events such as MI. Corsten *et al*[10] analyzed whole blood samples, plasma and urine of 32 acute myocardial infarction (AMI) patients compared to 36 non-AMI controls and reported plasma miR-208b and miR-499 to correlate with cardiac injury markers and, hence, to correlate with myocardial damage. miR-499 was significantly up-regulated in a subgroup of patients with acute HF. Another group that analyzed a predefined set of circulating miRNAs in plasma of 12 post-MI patients compared to 12 healthy controls was able to find levels of miR-1, miR-21, miR-29a, miR-133a and miR-208 altered in the time course after MI[118]. These miRNAs had previously been described to affect myocardial growth, hypertrophy, fibrosis and viability[118] implying that the same miRNAs that have been shown to be associated with these pathophysiological processes preceding HF can be found dysregulated in plasma of patients with cardiovascular disease.

Independently from the pathophysiological cause miRNAs have been reported to complement biomarker-based prediction of outcome in HF. In a study including 20 clinically stable and 22 decompensated HF patients as well as 15 healthy controls the authors performed a microarray-based miRNA profiling and reported a large number of miRNAs to be quantitatively dysregulated in HF patients compared to controls[119]. More importantly, Cox regression identified miR-182 to be able to predict cardiovascular mortality. Remarkably, the prognostic value of miR-182 was identified to be superior to NT-proBNP as well as high-sensitive C-reactive protein by ROC analysis[119]. Table 1 gives an overview of cellular and circulating miRNAs dysregulated in heart failure.

The above studies suggest blood-based circulating miRNAs as potential strong tools in the diagnosis and risk evaluation of HF. On the other hand, most trials included rather small sample sizes and most identified miRNAs were not confirmed in repetitive studies.

miRNA SIGNATURES IN HF

Combining two or more biomarkers as a defined set for diagnostic purposes can enhance discriminatory power compared to the use of single biomarkers. In the field of miRNA biomarker research the assessment of sets (so-called signatures) of miRNAs might deliver superior

Table 1 Systematic overview of microRNAs dysregulated in heart failure

miRNA	Study type	Bio-material	Group/size	Detection method	Effect	Value as biomarker	Ref.
Single miRNAs							
Let-7	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Up-regulated in DCM and ICM	Diagnostic	[90]
miR-1	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Down-regulated in DCM	Diagnostic	[90]
miR-15b	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Up-regulated in DCM	Diagnostic	[90]
miR-16	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
miR-17-5p	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Down-regulated in DCM	Diagnostic	[90]
miR-18b	Clinical	Plasma	HF n = 30 Dyspnea n = 20 HC n = 20	qRT-PCR	Up-regulated in HF	Diagnostic	[108]
miR-20b	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
miR-21[1]	Clinical	Tissue	LVAD-patients	Micro-array	Up-regulated in HF	Experimental	[89]
miR-22	Clinical	Serum	HFrEF n = 30 HC n = 30	qRT-PCR	Up-regulated in HFrEF	Diagnostic/ prognostic	[110]
miR-23a	Clinical	Tissue	LVAD-patients	Micro-array	Up-regulated in HF	Experimental	[89]
miR-24	Experimental	Tissue	Mice, human hearts	Micro-array	Up-regulated in HF, CH	Experimental	[36]
miR-26b-5p	Clinical	Plasma	HF n = 81 HC n = 15	qRT-PCR	Down-regulated	Diagnostic	[114]
miR-28	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Down-regulated in DCM	Diagnostic	[90]
miR-29a-3p	Clinical	Plasma	HF n = 81 HC n = 15	qRT-PCR	Down-regulated	Diagnostic	[114]
miR-30b	Clinical	Plasma	HF n = 44 COPD n = 32 Dyspnea n = 59 HC n = 15	RT-PCR	Down-regulated in HF	Diagnostic	[113]
miR-30e-5p	Clinical	Plasma	HF n = 81 HC n = 15	qRT-PCR	Down-regulated	Diagnostic	[114]
miR-92b	Clinical	Serum	HFrEF n = 30 HC n = 30	qRT-PCR	Up-regulated in HFrEF	Diagnostic/ prognostic	[110]
miR-92a-3p	Clinical	Plasma	HF n = 81 HC n = 15	qRT-PCR	Down-regulated	Diagnostic	[114]
miR-93	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
miR-103	Clinical	Plasma	HF n = 44 COPD n = 32 Dyspnea n = 59 HC n = 15	RT-PCR	Down-regulated in HF	Diagnostic	[113]
miR-106a	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Down-regulated in DCM	Diagnostic	[90]
miR-106b	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
miR-125b	Experimental	Tissue	Mice, human hearts	Micro-array	Up-regulated in HF, CH	Experimental	[36]
miR-126	Clinical	Plasma	HF n = 10 HC n = 17	qRT-PCR	Down-regulated in HF	Diagnostic	[150]
miR-133	Clinical	Tissue	LVAD-patients	Micro-array	Up-regulated in HF	Experimental	[89]
miR-138	Clinical	PBMC	DCM n = 44 HC n = 48	qRT-PCR	Up-regulated in DCM	Diagnostic	[117]
miR-142-3p	Clinical	Plasma	HF n = 44 COPD n = 32	RT-PCR	Down-regulated in HF	Diagnostic	[113]

			Dyspnea n = 59 HC n = 15				
	Clinical	Plasma	HFpEF n = 8 Stable DCM n = 10 Decompensated DCM n = 13 HC n = 8	qRT-PCR	Down-regulated in stable and decompensated DCM	Diagnostic	[121]
miR-145-5p	Clinical	Plasma	HF n = 81 HC n = 15	qRT-PCR	Down-regulated	Diagnostic	[114]
miR-182	Clinical	Serum	HF n = 42 HC n = 15	Micro-array	Up-regulated in HF	Prognostic	[119]
miR-183-3p[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]
miR-190a	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]
miR-193b-3p[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]
miR-193b-5p[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]
miR-195	Experimental Clinical	Tissue Tissue	Mice, human hearts ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	Micro-array qRT-PCR	Up-reglated in HF, CH Down-regulated in DCM and ICM	Experimental Diagnostic	[36] [90]
miR-199a	Experimental Clinical	Tissue Tissue	Mice, human hearts ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	Micro-array qRT-PCR	Up-reglated in HF, CH Down-regulated in DCM and ICM	Experimental Diagnostic	[36] [90]
miR-208	Experimental Clinical	Tissue Tissue	Mice DCM n = 82 HC n = 21	Micro-array qRT-PCR	Up-regulated in HF, CF, CH Up-regulated in DCM	Experimental Diagnostic/ prognostic	[57] [92]
miR-208b	Clinical	Tissue	DCM n = 82 HC n = 21	qRT-PCR	Up-regulated in DCM	Diagnostic	[92]
miR-211-5p[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]
miR-214 miR-222	Experimental Clinical	Tissue Tissue	Mice, human hearts ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	Micro-array qRT-PCR	Up-reglated in HF, CH Down-regulated in DCM and ICM	Experimental Diagnostic	[36] [90]
miR-223	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
miR-320a	Clinical	Serum	HFrEF n = 30 HC n = 30	qRT-PCR	Up-regulated in HFrEF	Diagnostic/ prognostic	[110]
miR-342-3p	Clinical	Plasma	HF n = 44 COPD n = 32 Dyspnea n = 59 HC n = 15	RT-PCR	Down-regulated in HF	Diagnostic	[113]
miR-422b	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Down-regulated in DCM and ICM	Diagnostic	[90]
miR-423-5p	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
	Clinical	Plasma	HF n = 30 Dyspnea n = 20 HC n = 20	qRT-PCR	Up-regulated in HF	Diagnostic/ prognostic	[108]
	Clinical	Serum	HFrEF n = 30 HC n = 30	qRT-PCR	Up-regulated in HFrEF	Diagnostic/ prognostic	[110]
	Clinical	Plasma	HF n = 44 COPD n = 32 Dyspnea n = 59 HC n = 15	RT-PCR	Down-regulated in HF	Prognostic when combined with NT-proBNP	[113]
miR-494[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]

miR-499	Clinical	Tissue	DCM n = 82 HC n = 21	qRT-PCR	Up-regulated in DCM	Diagnostic	[92]
	Clinical	Plasma	Acute HF n = 33 HC n = 34	qRT-PCR	Up-regulation in acute HF	Diagnostic	[10]
miR-548c	Clinical	PBMC	DCM n = 44 HC n = 48	qRT-PCR	Down-regulated in DCM	Diagnostic	[117]
miR-548i	Clinical	PBMC	DCM n = 44 HC n = 48	qRT-PCR	Down-regulated in DCM	Diagnostic	[117]
miR-671-5p[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Up-regulated in HF	Diagnostic	[115]
miR-675	Clinical	Plasma	HF n = 30 Dyspnea n = 20 HC n = 20	qRT-PCR	Up-regulated in HF	Diagnostic	[108]
miR-1233[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Up-regulated in HF	Diagnostic	[115]
miRNA signatures							
miR-520d-5p miR-558 miR-122* miR-200b* miR-622 miR-519e* miR-1231 miR-1228*	Clinical	Whole blood	HFrEF n = 53 HC n = 39	qRT-PCR	Dysregulated in HF - superior to single miRNAs	Diagnostic	[120]

[1]See also Table 2; *Most microRNAs have two mature products, one derives from the 5′ arm of the miRNAs hairpin and the other from the 3′ arm of the hairpin; the latter is marked "*". miRNA: microRNA; HF: Heart failure; CF: Cardiac fibrosis; CH: Cardiac hypertrophy; HC: Healthy control; ICM: Ischemic cardiomyopathy; DCM: Dilated cardiomyopathy; AS: Aortic stenosis; COPD: Chronic obstructive pulmonary disease; MI: Myocardial infarction; HFpEF: Heart failure with preserved ejection fraction; HFrEF: Heart failure with reduced ejection fraction; PBMC: Peripheral blood mononuclear cell; LVAD: Left ventricular assist device; qRT-PCR: Quantitative real time polymerase chain reaction.

results compared with the application of single miRNAs. In order to assess circulating miRNAs as biomarkers for HFrEF, Vogel *et al*[120] performed miRNA quantification measures in whole blood samples of 53 HFrEF patients with non-ischemic HF compared to 39 healthy controls. In a two-step screening-validation study the authors found a signature of eight miRNAs (miR-520d-5p, miR-558, miR-122*, miR-200b*, miR-622, miR-519e*, miR-1231 and miR-1228*) which reliably predicted the diagnosis of HFrEF with an AUC of 0.81[120] (Table 1). Compared to the most powerful single miRNAs miR-558, miR-122[a], and miR-520d-5p (AUC between 0.7 and 0.71) this miRNA signature further improved discrimination of HFrEF patients from controls[120] confirming the idea to improve sensitivity and specificity when utilizing combinations of more than one miRNA.

miRNAS IN THE DIFFERENTIATED DIAGNOSIS OF HF WITH PRESERVED EJECTION FRACTION

Pathophysiologically, HFpEF is the clinical manifestation of LV diastolic dysfunction as a major differentiating factor from HFrEF. Therefore, diastolic dysfunction appears as a useful parameter in the early diagnosis of HFpEF.

Initial results proving the involvement of miR-21 in cardiac remodeling and fibrosis[55,67-73] (see above) suggested its roll in HFrEF. These findings were taken up by Dong *et al*[67] in order to analyze this miRNA with respect to HFpEF. The authors created a rat model of aortic constriction-induced HFpEF. HFpEF was diagnosed *via* echocardiographic parameters and quantitative RT-PCR (qRT-PCR) analyses showed higher cellular miR-21 levels in HFpEF rats compared to healthy controls[67]. These results confirm the former pathophysiologic miR-21 findings and indicate their potential to be transferrable to a functional level in HFpEF. In order to assess whether circulating miRNAs as opposed to cellular miRNAs can be utilized as biomarkers in the detection of HFpEF and in a differentiated diagnosis compared to HFrEF, results were published recently by Nair *et al*[121]. They analyzed miRNA plasma levels of HF patients with diastolic dysfunction. The authors found miR-454, miR-500 (both down-regulated) and miR-1246 (up-regulated) significantly dysregulated in diastolic dysfunction indicating that circulating miRNAs can serve as biomarkers for diastolic dysfunction[121]. This suggests itself to further considerations for miRNA-based diagnostics to differentiate HFrEF from HFpEF. Wong *et al*[115] performed a miRNA quantification of whole blood and plasma samples in 39 HFrEF and 19 HFpEF patients as well as 28 healthy controls and identified 344 miRNAs dysregulated between the three groups. Of these, 90 serum derived miRNAs were identified that showed high correlation with or an AUC > 0.7 for LVEF. Again a selection of 32 miRNAs with considerably high detection levels was made. These analytical steps allowed for a qualitative selection of promising miRNAs and those, that can easily be detected in serum. Further analyses of these 32 miRNAs in plasma of an

independent cohort of 30 HFrEF and 30 HFpEF patients as well as 30 controls identified 12 miRNAs that could segregate HFrEF and HFpEF from non-HF controls as well as HFrEF from HFpEF[115]. In detail, miR-125a-5p, miR-183-3p, miR-193b-3p, miR-211-5p, miR-494, miR-638 and miR-671-5p differed significantly between HFrEF and controls while miR-1233, miR-183-3p, miR-190a, miR-193b-3p, miR-193b-5p and miR-545-5p showed significant differences in expression between HFpEF and controls[115]. miR-125a-5p (up-regulated in HFrEF - normal in HFpEF), miR-190a (down-regulated in HFpEF - normal in HFrEF), miR-550a-5p (directionally opposite expression pattern between HFrEF and HFpEF) and miR-638 (down-regulated in HFrEF - normal in HFpEF) were revealed to distinguish between HFrEF and HFpEF. Clinically relevant, the combinatory use of NT-proBNP with miR-125a-5p improved the AUC value to differentiate HFrEF from HFpEF from 0.83 for NT-proBNP alone to 0.91 for the combinatory use and thus significantly increased NT-proBNP's discriminative diagnostic abilities[115]. Another aspect that was addressed in this study was the application of panels of miRNAs. The authors reported that miRNA panels had comparable performance to NT-proBNP with respect to the discrimination of HFrEF from HFpEF while single miRNAs tended to perform slightly inferior to NT-proBNP[115].

In order to identify a miRNA signature helping to differentiate HFpEF from HFrEF Watson *et al*[122] performed miRNA quantification analyses in sera of 90 HFpEF patients compared to 90 HFrEF patients and 90 healthy controls. The diagnosis of HFrEF and HFpEF was made echocardiographically. In an initial miRNA screening in serum samples of 15 individuals per group five candidate miRNAs (miR-30c, miR-146a, miR-221, miR-328 and miR-375) were identified as differentially expressed between the three groups and validated in an independent study cohort of 225 individuals[122]. The authors performed AUC analyses to differentiate HFpEF from HFrEF and reported an equal predictive value of any of the single miRNAs compared with the use of brain natriuretic peptide (BNP)[122]. Importantly, combinations of two or more of miR-146a, miR-221, miR-328 and miR-375 with BNP significantly improved the predictive power to differentiate HFpEF from HFrEF as compared with BNP alone in the AUC model[122]. The latter two very recent studies were the first to investigate circulating miRNAs as promising new biomarkers to differentiate HFpEF from HFrEF. They similarly provide evidence that combinatory utilization of miRNAs can improve discriminative power compared to single miRNAs. Critically evaluated, though, there was no overlap in the identified miRNAs in these studies that were able to distinguish between HFrEF and HFpEF. Schmitter *et al*[123] discussed potential explanations for these differences. The authors regarded several explanations relevant in this respect. First, methodological variances such as the choice of body liquid, detection methods and the importance to perform microarray screenings prior to qRT-PCR analyses were identified to be contributors to a lack of comparability. Also, pre-analytical variations like sample storage, degree of hemolysis, extraction efficiency and standardization methods are mentioned as important contributors to a reduced comparability[123]. The authors furthermore define the need for more large-scale studies with well-defined control- and validation cohorts limiting the influence of different HF etiologies, concomitant diseases, and treatments. Another important factor to be considered when interpreting miRNA quantification results is the influence of confounding medications and classical cardiovascular risk factors[123].

Taken together the very recent results in miRNA-based diagnostics of HFpEF and HFrEF are highly promising but urgently need verification in large-scale studies with harmonized methods and well-defined study samples.

Table 2 gives an overview of miRNAs in the diagnosis of HFpEF and HFrEF.

miRNAS IN DISEASE TREATMENT

Molecular diagnostics and therapeutics represent an important contributor to improve outcome for HF[123]. In contrast to traditional treatments, gene therapy is capable of modifying the genetic structure of the cell and can modulate the disease phenotype[124]. In this respect miRNAs are promising new players in the development of molecular therapeutics in cardiovascular disease and HF in particular. The regulation of selected miRNAs highly involved in cardiac remodeling could be a key factor in influencing the development of HF by controlling hypertrophy and fibrosis.

The concept of miRNA related disease treatment bases on the idea to specifically influence miRNA levels by raising or suppressing miRNA levels. Several different approaches have successfully been tested[125]. The major method to raise miRNA levels is the miRNA mimic technology (miR-mimic), which operates *via* miRNA substitution by artificially generated double-stranded miRNA-like RNA fragments[126]. They "mimic" endogenous miRNAs and bind - unlike endogenous miRNAs - gene-specifically to their target mRNA[126]. On the other hand, so called antagomirs can be used to suppress miRNA levels[127]. Antagomirs are chemically engineered oligonucleotides that competitively bind to and thus inhibit the mature target miRNA[127,128]. This mechanism leads to an up-regulation of specific mRNAs and gene expression[129]. Furthermore, miRNA sponges (also referred to as "target mimicry") are competitive inhibitors that contain binding sites for a miRNA family and thus inactivate miRNAs of that particular family[130-132]. As opposed to antagomirs, sponges are specific only to the seed region of a miRNA and thus can interfere with a whole family of miRNAs[133]. Masking (also called "target occupiers") describes a mechanism to prevent specific miRNAs from binding to their very binding site[134]. Consequently, fewer miRNAs remain to bind

Table 2 microRNAs in the diagnosis and differentiation of heart failure with preserved ejection fraction and heart failure with reduced ejection fraction

miRNA	Study type	Bio-material	Groups/size	Detection method	Effect	Value as biomarker	Ref.
miR-21[1]	Experimental	Tissue	Mice - HFpEF *vs* HC	qRT-PCR	Up-regulated in HFpEF	Diagnostic	[67]
miR-30c	Clinical	Serum	HFpEF n = 90 HFrEF n = 90 HC n = 90	qRT-PCR	AUC analyses	Differentiating HFrEF from HFpEF	[122]
miR-125a-5p	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Up-regulated in HFrEF, unchanged in HFpEF	Diagnostic in HFrEF + differentiating HFrEF from HFpEF	[115]
miR-146a	Clinical	Serum	HFpEF n = 90 HFrEF n = 90 HC n = 90	qRT-PCR	AUC analyses	Differentiating HFrEF from HFpEF	[122]
miR-183-3p[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFrEF and HFpEF	Diagnostic	[115]
miR-190a*	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFpEF, unchanged in HFrEF	Diagnostic in HFpEF + differentiating HFrEF from HFpEF	[115]
miR-193b-3p[1]	clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFrEF and HFpEF	Diagnostic	[115]
miR-193b-5p[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFpEF	Diagnostic	[115]
miR-211-5p[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFrEF	Diagnostic	[115]
miR-221	Clinical	Serum	HFpEF n = 90 HFrEF n = 90 HC n = 90	qRT-PCR	AUC analyses	Differentiating HFrEF from HFpEF	[122]
miR-328	Clinical	Serum	HFpEF n = 90 HFrEF n = 90 HC n = 90	qRT-PCR	AUC analyses	Differentiating HFrEF from HFpEF	[122]
miR-375	Clinical	Serum	HFpEF n = 90 HFrEF n = 90 HC n = 90	qRT-PCR	AUC analyses	Differentiating HFrEF from HFpEF	[122]
miR-454	Clinical	Plasma	HFpEF n = 8 Stable DCM n = 10 Decompensated DCM n = 13 HC n = 8	qRT-PCR	Down-regulated in HFpEF	Diagnostic	[121]
miR-494[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFrEF	Diagnostic	[115]
miR-500	Clinical	Plasma	HFpEF n = 8 Stable DCM n = 10 Decompensated DCM n = 13 HC n = 8	qRT-PCR	Down-regulated in HFpEF	Diagnostic	[121]
miR-545-5p	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Up-regulated in HFpEF	Diagnostic	[115]
miR-550a-5p	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30	qRT-PCR	Up-regulated in HFrEF Down-regulation in HFpEF	Differentiating HFrEF from HFpEF	[115]
miR-638	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFrEF, unchanged in HFpEF	Diagnostic in HFrEF + differentiating HFrEF from HFpEF	[115]
miR-671-5p[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Up-regulated in HFrEF	Diagnostic	[115]
miR-1233[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Up-regulated in HFpEF	Diagnostic	[115]
miR-1246	Clinical	Plasma	HFpEF n = 8 Stable DCM n = 10	qRT-PCR	Down-regulated in HFpEF	Diagnostic	[121]

	Decompensated DCM n = 13 HC n = 8

[1]See also Table 1. HC: Healthy control; miRNA: microRNAs; DCM: Dilated cardiomyopathy; HFpEF: Heart failure with preserved ejection fraction; HFrEF: Heart failure with reduced ejection fraction; qRT-PCR: Quantitative real time polymerase chain reaction; AUC: Area under the operating receiver curve.

to the target and their effect is lessened. Erasers are oligonucleotides complementary to a specific miRNA. By binding to the miRNA the eraser inhibits its endogenous function[127].

miRNA therapeutics in HF

Up to now there are no clinical trials published dealing with miRNA therapeutics in humans. Therefore, the following results relate to *in-vitro* studies and animal models. Initial studies in the field of miRNA therapeutics in HF were designed to identify differential regulations of miRNAs in HF. Sucharov *et al*[135] extracted miRNAs from 6 nonfailing, 5 idiopathic dilated cardiomyopathy (IDC) and 5 ischemic dilated cardiomyopathy (ISC) patients. The authors were able to find a set of miRNAs dysregulated in both IDC and ISC. In order to further evaluate the function of increased or decreased expression of those miRNAs the group introduced virus-delivered mirmimics as well as antagomirs against miR-92, miR-100 and miR-133b into neonatal rat ventricular myocytes and was able to cause dramatic down-regulation or up-regulation of the particular miRNAs[135]. Pathophysiologically, an up-regulation of miR-100 resulted in repression of adult genes α*MyHC* and *SERCA* while fetal genes *ANF* and β*MyHC* were up-regulated. These observations suggest the involvement of miR-100 in the specific regulation of gene expression involved in the repression of adult isoforms. The study shows that an artificial dysregulation of miR-100 is able to affect HF associated gene expression.

Raising miRNA levels as a therapeutic approach in HF

An initial project studying miRNA-associated therapeutic aspects in HF with respect to specifically raising miRNA levels was performed by Karakikes *et al*[136]. The authors addressed previous findings that proved miR-1 to be a key regulator of cardiac hypertrophy[44-47,90,137] and analyzed whether the restauration of miR-1 expression has protective effects on maladaptive cardiac remodeling. They established a hypertrophy and ventricular dilatation model in rats by ascending aortic banding before they raised miR-1 expression *in-vivo* by systemically administered adeno-associated virus-mediated gene transfer[136]. The authors were able to detect improved systolic as well as diastolic LV function in the miR-1 restauration group as measured by echocardiography and catheter-based pressure-volume loop analyses[136].

A similar approach was pursued by Pan *et al*[138] who induced an adenovirus-mediated overexpression of miR-101a in rats with chronic MI and were able to find a significant improvement of cardiac performance in those subjects treated with miR-101a overexpression. Recently, these results were confirmed in a rat model of induced MI[139]. The authors found decreased miR-101a levels at the site of the infarction and were able to verify this observation in cultured cardiac fibroblasts exposed to hypoxia and linked this effect to a TGF-β-modulated fibrotic effect. An administration of miR-101a mirmimics reduced the expression of TGF-β[139] indicating that miR-101a mimicry might negatively regulate fibrosis in ischemic cardiac tissue. These findings point out a potential applicability of mirmimics in the field of HF therapy and ignited studies further evaluating this aspect. In a recent study the authors succeeded in modulating myocardial fibrosis and apoptosis in a hypertrophic mouse model by regulating miR-455 levels[140]. Tail vein injection of viral delivered miR-455 resulted in aggravated cardiac hypertrophy on the one hand but also reduced myocardial fibrosis and inhibited apoptosis suggesting that this treatment can prevent maladaptive ventricular remodeling[140].

A different approach was addressed by Dakhlallah *et al*[141]. The authors used mirmimics to raise miR-133a levels in mesenchymal stem cells (MSC) and implanted these into ischemic rat hearts. Compared to non-miR-133a treated MSCs these rat hearts were shown to have increased cardiac function, decreased fibrosis and presented with improved cell engraftment due to better survival of miR-133a treated MSCs[141]. These study results indicate a potential roll of miRNAs in HF treatment with respect to an improvement of bioengineering of stem cells and are an example of the broad potential applicability of miRNAs in the field of HF treatment.

Lowering miRNA levels as a therapeutic approach in HF treatment

Reduced miR-29 levels were observed to be associated with a decrease of cardiac remodeling in mice[142]. The authors used an ischemia/reperfusion model in mice to analyze the effect of miR-29 on post-infarction remodeling. They found that an antisense inhibition of miR-29 implemented by an antagomir against miR-29 inhibited post infarction/reperfusion apoptosis and necrosis and led to a reduction of cardiac remodeling[142]. In a rat model of aortic constriction-induced HFpEF revealing higher cellular miR-21 levels in HFpEF rats compared to sham operated animals[67] (see above), Dong *et al*[67] performed further analyses after administering a miR-21 antagonist. The authors were able to find a reduction of fibrosis in those rats' cardiac tissues that were transfected with anti-miR-21 and attributed this effect to a reduction of Bcl-2 expression - an anti-apoptotic

factor involved in the apoptosis of cardiac fibroblasts[67]. The same miRNA was analyzed in a transgenic mouse model of cardiac failure. Thum *et al*[55] were able to show that *in-vivo* silencing of miR-21 by a systemically applied specific antagomir inhibits interstitial fibrosis by a reduction of mitogen-activated protein kinase activity when applied to pressure-overload-induced cardiac dysfunction in mice.

In order to assess the therapeutic potential of miR-652 in another mouse model with established pathological hypertrophy and cardiac dysfunction due to induced pressure overload Bernardo *et al*[143] first proved miR-652 expression to be elevated in pressure overloaded hearts compared to healthy controls. The authors then systemically administered antimiR-652 and found the expression of miR-652 effectively silenced in heart tissue of treated mice. The authors were able to show that antimiR-652 treated mice had better cardiac function and improved cardiac diameters compared to controls[143].

The first discovered miRNA Let-7 recently was found to be a potential therapeutic target in the treatment of deteriorated cardiac function after MI[144]. After induced MI in mice Let-7 was inhibited with a specific systemically applied antagomir. Molecularly, the expression of pluripotency-associated genes *Oct4* and *Sox2* was increased in cardiac fibroblasts *in vitro* and *in vivo*. Let-7 antagomir treated mice showed preserved LVEF and improved cardiac output compared to controls[144].

Addressing the hallmark of phathological hypertrophy and HF - the reactivation of fetal cardiac genes, in which miR-208 is highly involved, study results were reported in a model of antagomir-based silencing of miR-208. Montgomery *et al*[145] were able to silence miR-208 in a rat model of diastolic HF (Dahl salt-sensitive rats) by means of systemically administered locked nucleic acid-modified antimirs. On the one hand the authors found pathological myosin switching and cardiac remodeling lessened in antagomir-208 treated animals. More important from a clinical point of view was the observation that in diastolic HF therapeutic silencing of miR-208 resulted in lessened HF symptoms, a reduction of cardiac remodeling and an improved cardiac function as well as longer survival compared to control animals[145].

Current treatment strategies in HF are predominantly focused on HFrEF and no distinct therapy is established with respect to HFpEF[146-149]. The current non-specific therapy of HFpEF is limited and requires development and improvement of more distinct diagnostic and therapeutic options. Molecular diagnostics and therapeutics might provide the foundation for differential therapeutic approaches with regards to HFpEF and HFrEF.

CONCLUSION

Numerous studies have proven miRNAs to be key regulators and moderators in the development of HF and its pathophysiological precursors hypertrophy and fibrosis. Their molecular construction and integration in cellular and intercellular transport mechanisms define miRNAs as ideal circulating biomarkers for diagnostic and prognostic purposes while they can easily be collected and analyzed. Therefore, the application of miRNAs as circulating biomarkers represents a promising tool to complement established protein-based biomarkers of HF such as NPs on the one hand or novel stand-alone biomarkers in the diagnosis and prognosis of HF. In the differential diagnostics of HFrEF and HFpEF miRNAs can reliably differentiate between these two disease entities, although this has to be confirmed in larger samples. This is especially interesting considering the fact that the diagnosis of HFpEF at an early stage might significantly improve secondary prevention and established biomarkers of HF still lack precision in the differentiated diagnosis of HFpEF. Nevertheless, looking at the large number of studies only few of them confirmed previous findings with identical results and still different miRNAs are identified to be linked to HF presumably reflecting the complex interaction of miRNAs and their target sites. In this respect analysis of combinations of several miRNAs - miRNA signatures - represent a promising way to increase diagnostic and prognostic accuracy. An important aspect that should get attention when performing miRNA analyses is the comparability and standardization of analytical methods and the need for well-defined study samples.

Over the past years several different possibilities have been identified to alter levels of circulating miRNAs by systemically administering agents such as mirmimics or antagomirs. This therapeutic approach has been reported to significantly reduce hypertrophy and CF and improve LV function in animal models. It represents a promising approach to complement existing therapeutic options in the treatment of HF. Nevertheless, results of *in-vitro* and *in-vivo* models have not yet led to an application in clinical studies. A successful implementation of those insights in clinical trials represents the next step towards realizing this idea.

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REVIEW

Myocardial perfusion echocardiography and coronary microvascular dysfunction

Giuseppe Barletta, Maria Riccarda Del Bene

Giuseppe Barletta, Maria Riccarda Del Bene, Cardio-Thoracic and Vascular Department, Azienda Ospedaliero-Universitaria Careggi, Largo Brambilla, 50134 Florence, Italy

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Correspondence to: Giuseppe Barletta, MD, FESC, Cardio-Thoracic and Vascular Department, Azienda Ospedaliero-Universitaria Careggi, Largo Brambilla, 50134 Florence, Italy. barlettagius@tiscali.it
Telephone: +39-333-3298907
Fax: +39-55-7947578

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Abstract

Our understanding of coronary syndromes has evolved in the last two decades out of the obstructive atherosclerosis of epicardial coronary arteries paradigm to include anatomo-functional abnormalities of coronary microcirculation. No current diagnostic technique allows direct visualization of coronary microcirculation, but functional assessments of this circulation are possible. This represents a challenge in cardiology. Myocardial contrast echocardiography (MCE) was a breakthrough in echocardiography several years ago that claimed the capability to detect myocardial perfusion abnormalities and quantify coronary blood flow. Research demonstrated that the integration of quantitative MCE and fractional flow reserve improved the definition of ischemic burden and the relative contribution of collaterals in non-critical coronary stenosis. MCE identified no-reflow and low-flow within and around myocardial infarction, respectively, and predicted the potential functional recovery of stunned myocardium using appropriate interventions. MCE exhibited diagnostic performances that were comparable to positron emission tomography in microvascular reserve and microvascular dysfunction in angina patients. Overall, MCE improved echocardiographic evaluations of ischemic heart disease in daily clinical practice, but the approval of regulatory authorities is lacking.

Key words: Contrast echocardiography; Myocardial perfusion; Myocardial ischemia; Microvascular angina; Coronary flow

Core tip: Diagnostic work-up of coronary heart disease is evolving to include evaluations of the coronary microcirculation in addition to the imaging of obstructive atherosclerosis of coronary arteries and its eventual effects. Functional assessments of coronary microvasculature have become the challenge. Myocardial contrast echocardiography (MCE) emerged as a promising tool several years ago to detect myocardial perfusion abnormalities and quantify coronary blood flow. MCE compared favorably with other expensive techniques, and it accurately evaluated coronary microvascular

reserve and dysfunction in research studies. However, its daily use in clinical practice is not established. Therefore, the future of this technique is questionable.

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INTRODUCTION

The past paradigm of ischemic heart disease was the relationship between myocardial ischemia and obstructive atherosclerosis of the epicardial coronary arteries[1]. Coronary angiography was the gold standard to evaluate the severity and extent of coronary artery disease (CAD). Clinical data in the last two decades challenged this paradigm and shifted the attention to the possible role of anatomical and functional abnormalities of the coronary microcirculation throughout the clinical spectrum of myocardial ischemia.

Functional assessments of coronary microvasculature have become the challenge. Direct visualization of the coronary microcirculation is not possible with any currently available technique, but descriptions of its function are possible. The thrombolysis in myocardial infarction (TIMI) frame count is a qualitative method to evaluate coronary blood flow[2], and intracoronary thermodilution and intracoronary Doppler wire, which are based on thermal dilution curves and the Doppler principle, respectively, measure myocardial blood flow (MBF). Transthoracic Doppler echocardiography is a noninvasive technique that is widely used to measure coronary blood flow reserve primarily in the left anterior descending coronary (LAD) artery territory[3].

The well-documented diagnostic accuracy of single-photon emission computer tomography (SPECT) myocardial perfusion imaging for CAD[4] promoted its widespread clinical use[5]. Myocardial perfusion is generally evaluated in a qualitative or semi-quantitative manner[6] that suffers from several limitations: Attenuation and Compton scatter effects; the plateau effect of ^{99m}Tc uptake, which limits the detection of further increase in flow, and the limited spatial resolution[7]. Recent technical and methodological advances, such as dynamic SPECT using a SPECT/computed tomography camera[8-10], allow measurements of absolute MBF and its reserve, but the approximately 12-mSv exposure to the patient limits its clinical use[11].

Positron-emission tomography (PET) allows the calculation of blood flow per unit of mass, which quantifies microvascular function[12]. Contrast-enhanced cardiac magnetic resonance (CMR) imaging is the other technique that accurately quantifies MBF[13,14].

Myocardial contrast echocardiography (MCE) is a bedside and relatively low-cost tool to detect myocardial perfusion abnormalities and quantify regional and global coronary blood flow. The clinical use or MCE is limited despite growing evidence to support its reliability[15].

This review discusses research results and the established settings in which MCE use may support clinical decision making.

MCE PROTOCOLS

Ultrasound contrast agents

The following contrast agents are commercially available: Optison (Amersham Health AS, Oslo, Norway), Definity (Bristol-Myers Squibb Billerica, Massachusetts), and Sonovue (Bracco, Milan, Italy). These agents consist of a shell of albumin, lipids or galactose filled with a gas to form microspheres smaller than 10 μm. The shell allows the low diffusible and low solubility gas the resistance to intravascular pressure and the ability to share erythrocyte rheology in the intravascular compartment, including the transpulmonary passage. Therefore, microspheres reach the left heart cavities and opacify the left ventricle and myocardium.

Physical principles

Microspheres are strong ultrasound scatterers. Microsphere behavior in an ultrasound field depends on the energy of the ultrasound source. Very low-energy ultrasound induces linear oscillations of the microspheres, and its fundamental frequency is reflected. Non-linear oscillations of microspheres emerge with increasing incident ultrasound energy when compression and rarefaction waves of variable magnitude are produced and generate harmonics, *i.e.*, ultrasounds of higher-order frequencies than the fundamental frequency. High-intensity ultrasound is used in medical imaging and disrupts microbubbles (Figure 1). In contrast, enhanced ultrasound imaging uses low-energy ultrasound in which the myocardium primarily exhibits linear responses and generates few harmonic frequencies, as opposed to the contrast agent. Selective reception and amplification of harmonic echoes allows sensitive detection of the contrast with good signal-to-noise ratio.

Imaging modalities

High-intensity ultrasounds (mechanical index-MeI > 0.3) in standard echocardiographic imaging destroy microbubbles. The acquisition of one ECG-triggered systolic frame every several cardiac cycles improves the contrast effect by reducing microbubble destruction, and it allows time for the replenishment of myocardial microvasculature with contrast for each subsequent triggered frame. Low-MeI real-time contrast echocardiography greatly improved left ventricular and myocardial opacification compared to low-frame rate high-MeI intermittent contrast imaging. Contrast enhancement in pulse inversion Doppler technique is obtained by the transmission of two pulses of the same amplitude and inverted phase: The subtraction of reflected ultrasounds of a linear scatterer (the myocardium) generates

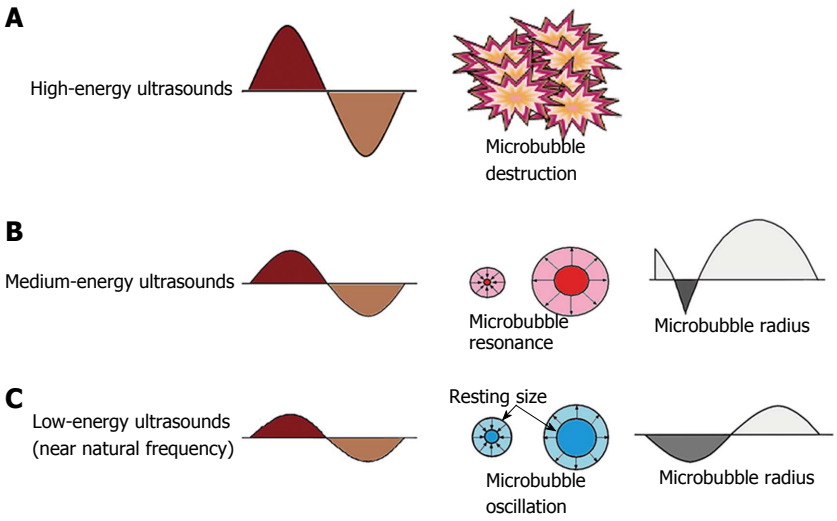

Figure 1 Behavior of microbubbles in an ultrasonic field. An acoustic wave generated by an ultrasound system consists of alternating high and low pressures: The positive pressure compresses the microbubble, and the negative pressure expands it. High-energy ultrasound (within the energy levels used for diagnostic echocardiographic imaging) destroys microbubbles (A); Intermediate energy ultrasound triggers asymmetrical nonlinear oscillations of microbubbles so that the magnitude of compression and rarefaction waves are not the same with each oscillation, and frequencies other than (*e.g.*, multiple of) the intrinsic fundamental frequency are generated (B); Low-energy ultrasound causes microbubbles to oscillate linearly, which reflects ultrasound at their intrinsic fundamental frequency (C).

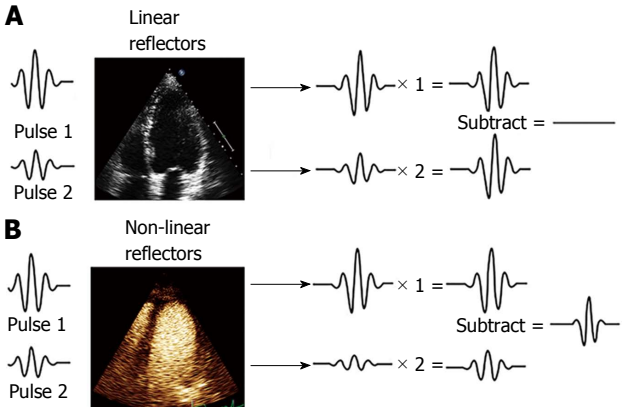

Figure 2 Pulse inversion harmonic imaging signal processing: Effects on linear and non-linear reflectors. This technique consists of the transmission of a first pulse and a second inverted replica of the first pulse. Any linear target, such as blood in conventional echocardiography, responds equally to positive and negative pressures (A) and reflects back to the transducer equal but opposite echoes, which will be canceled (blood displayed in black in 2-dimensional echocardiography); B: Pulse 1 and pulse 2 excite microbubbles generating fundamental and higher order harmonic responses with different phases that constructively add.

no signal, and the summated signals result from nonlinear scatterers (microspheres) (Figure 2). Other multi-pulse techniques were developed to improve the signal-noise ratio. The amplitude of one pulse can be increased (Power modulation, Philips, Andover, Massachusetts) (Figure 3), or amplitude and phase can be modulated (Cadence™ contrast pulse sequencing, Siemens Acuson Sequoia; Mountain View, California) to generate ultraharmonic oscillations. Both techniques exhibit excellent spatial resolution to assess myocardial contrast in real time.

Qualitative assessment

Low flow constant contrast infusion produces homogeneous opacification of the myocardial wall in patients without significant stenosis of epicardial coronary arteries because new microbubbles uniformly replaced those that are destroyed by a flash of high-intensity ultrasound. In contrast, the rate of contrast replenishment is reduced at rest in myocardial regions where microcirculation is damaged by previous infarction or during stress in regions supplied by a significantly stenosed epicardial vessel. Myocardial flow increase during pharmacological or physical stress enhances regional differences of myocardial opacification and allows the quantification of regional perfusion as normal, reduced, or severely reduced.

Quantitative assessment

The basis for myocardial flow quantification in MCE studies is the complete intravascular compartmentalization of microbubbles, which allows reaching a steady state concentration of microbubbles during continuous infusion. Replenishment of myocardial microvasculature after complete destruction of microbubbles using a flash of high-intensity ultrasounds may be assessed as a time-intensity curve. Intensity values "y" fitted to a monoexponential function: $y = A \times (1-e^{-\beta t})$ gives a measure of mean myocardial microbubble velocity (β), whereas the microvascular cross-sectional area is obtained from the plateau value of the replenishment curve (A). The product of A and β represents MBF.

Vogel *et al*[16] proposed the use of ratio of myocardial video intensity to the adjacent left ventricular cavity as an adjustment for the inhomogeneous contrast enhancement of the myocardium due to attenuation or other technical factors. An excellent correlation was found between absolute MBF (mL/min per gram of myocardium) measured with MCE and the values obtained using PET.

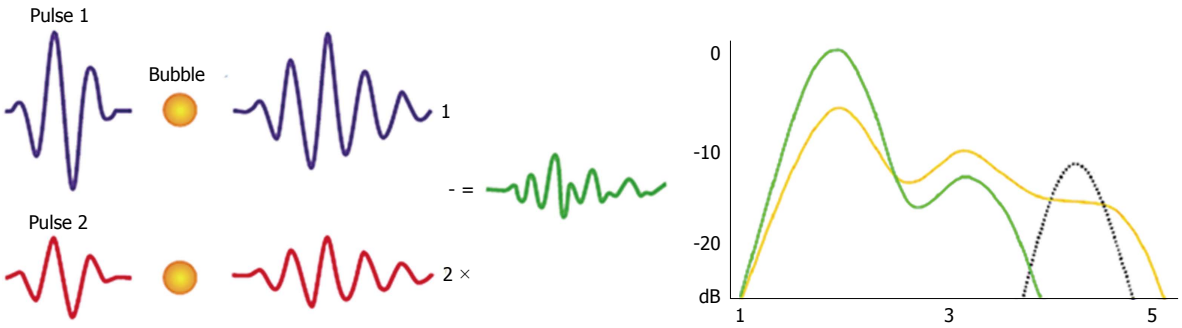

Figure 3 Power modulation imaging. This technique changes the amplitude of each successive pulse in a group of transmitted pulses and detects the differential nonlinear responses generated from two different excitations. Microbubbles' response to multipulse cancellation technique produces ultra-harmonic oscillations that are detected in the field of higher frequencies.

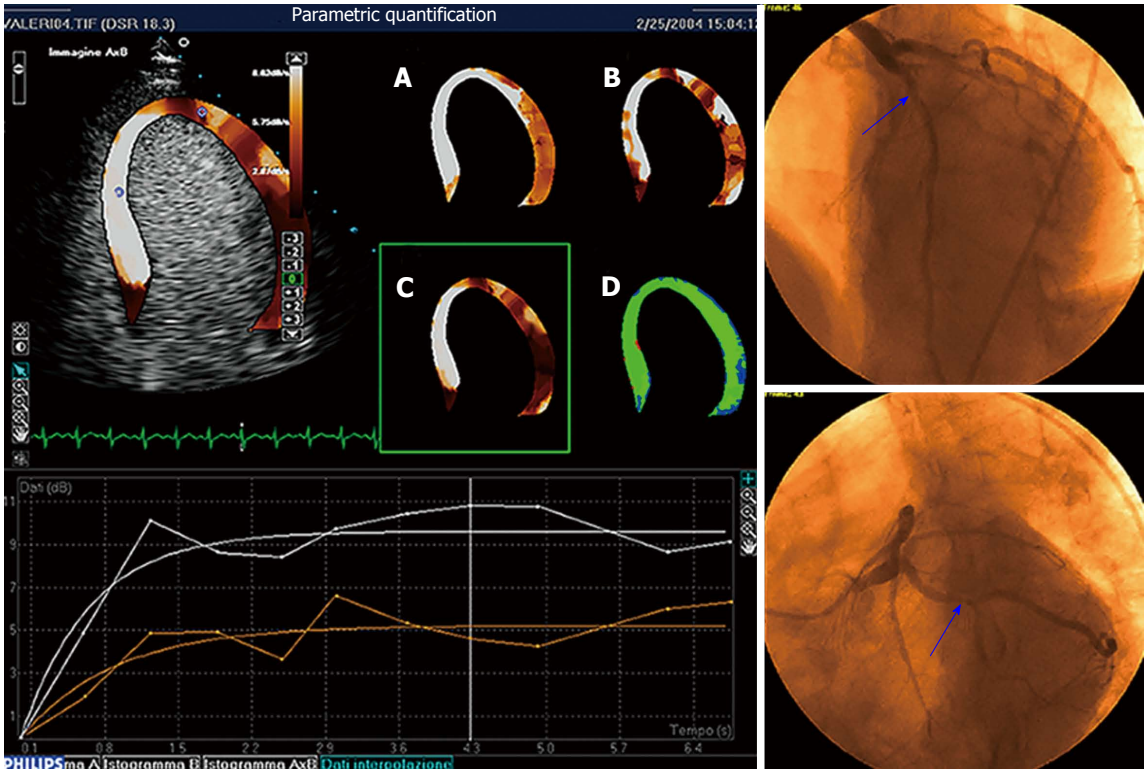

Figure 4 Parametric quantification of myocardial perfusion. Critical stenosis of the circumflex artery is present at coronary angiography (right panels). Parametric images of peak dipyridamole stress echo reported in panels A through D represent, respectively, the plateau value A of the contrast replenishment curve, the slope β of the replenishment curve, the A × β value, and the goodness of fitting. The A × β value parametric imaging is also displayed superimposed onto the apical four-chamber view in the top left panel. Replenishment time-course curves (sampling and interpolation) relative to the septum (white curves) and the apico-lateral wall (red curves) are reported in the graph at the bottom. Flow is reduced in the territory perfused by circumflex artery.

A fast and easy method to quantify MCE is represented by parametric images (Figure 4), which furnished visual information on the maximal intensity of contrast (A), rate of replenishment (β) and quality of acquisition[17]. Four, two and three chamber apical acquisitions of MCE create feasible computations of MBF.

CORONARY ANATOMY AND REGULATION

A detailed description of coronary anatomy and regulation is beyond the scope of this review. However, some features must be described to appreciate the potential of MCE. Figure 5 shows the complexity of coronary anatomy. Coronary circulation comprises the large epicardial conduit vessels and resistance vessels. Resistance to flow is very low in conduit vessels and progressively increases as resistance vessel diameter decreases to the arteriolar bed (from 300 to 100 μm). The so-called "coronary driving pressure", *i.e.*, the pressure gradient between the aortic root and the right atrium, is the main determinant of blood flow across the myocardium. The coronary driving pressure under normal conditions reduces little, if any, along the epicardial conduit vessels, but it declines progressively along the microvasculature, particularly in 300-100 μm

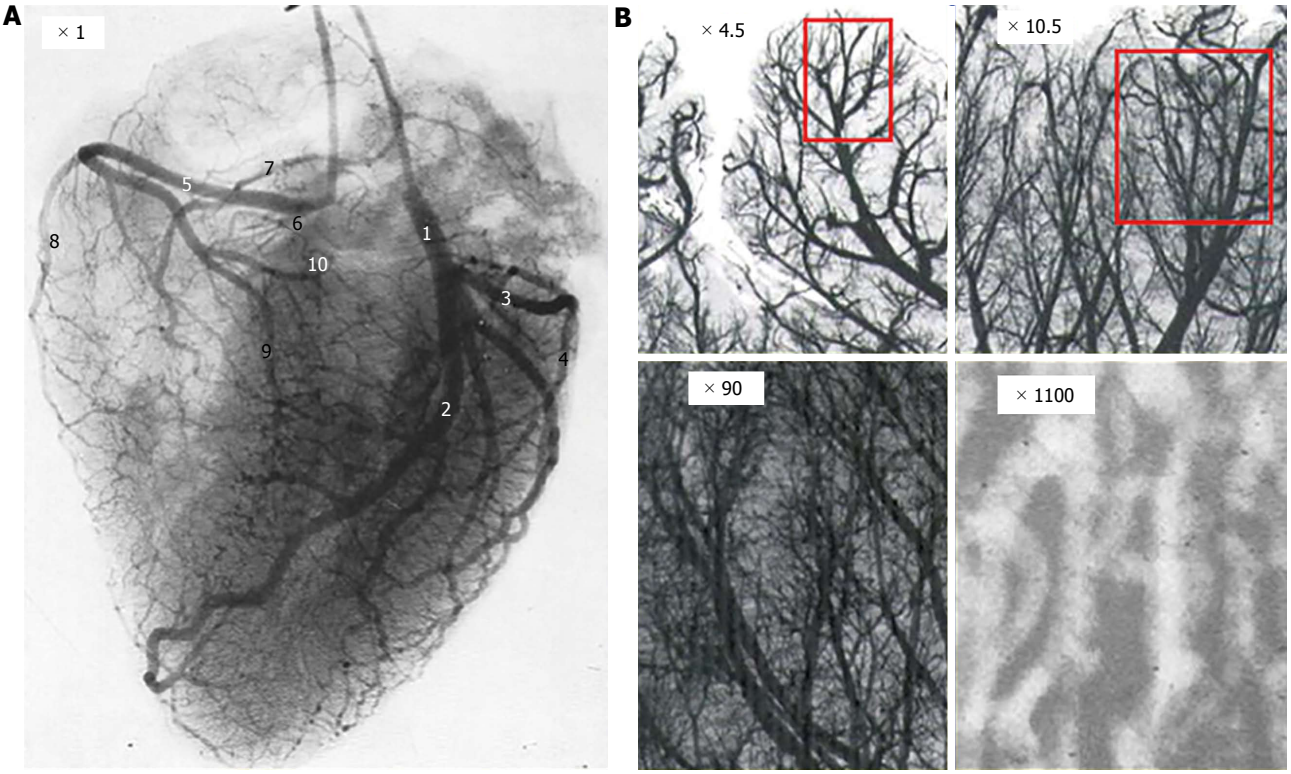

Figure 5 Coronary artery tree arborization. A: Angiographic still frame of left and right coronary arteries of an isolated human heart after injection of radiopaque dye; B: Progressive magnification of a region of interest showing the coronary tree fractal anatomy.

diameter arterioles, until reaching the 20-30 mmHg level. Extravascular resistive forces, which are related to the development of left ventricular systolic pressure, inotropism of the myocardium, and heart rate are additional determinants of the resistance to flow across the myocardium.

Coronary circulation has two main characteristics. First, oxygen extraction at rest is approximately 75%, but coronary venous oxygen saturation during strenuous exercise may decrease to approximately 10%. The increase in flow needed to meet the 4- to 5-fold increase in oxygen demand is accomplished by vasodilation of resistance coronary arteries, which is mediated by mechanisms that are intrinsic to the vascular wall and metabolic and neuro-humoral factors[18]. Second, coronary flow is pulsatile, *i.e.*, it is high in diastole and low in systole.

Coronary arterial pressure and myocardial oxygen consumption are the major determinants of coronary flow, but coronary autoregulation allows the flow to be relatively independent of the driving pressure at fixed oxygen consumption level, whereas at any given coronary arterial pressure a metabolic adaptation of coronary flow to oxygen requests may occur[19].

The hemodynamic impact of a focal diameter reduction of epicardial vessels is modulated by the vasodilation of resistance coronary arteries. Maximal flow reduces at maximal vasodilation because coronary perfusion pressure decreases with the square of flow through the stenosis. This law of coronary hemodynamics is the basis for dynamic assessment of coronary stenosis by pharmacological or physical stress that can elicit a reversible perfusion defect and eventually a transient regional asynergy of contraction.

Experimental canine models, in which external constriction was applied to normal coronary arteries, led to the concept of "critical" coronary stenosis. In these studies, an 85% diameter narrowing caused a fall in resting coronary flow, but a 50% diameter narrowing reduced maximum coronary flow. In surgical decision-making, the angiographic criterion of > 70% coronary stenosis was appropriate to define eligibility for revascularization.

However, this paradigm failed to describe the correlation between coronary flow reserve (CFR) and percent coronary stenosis in human studies[20], and it does not consistently identify patient groups whose prognosis can be improved by primary coronary interventions. A further confirmation of the dissociation between anatomic and functional severity of stenosis came from the poor correlation between quantitative percent stenosis on invasive or computed tomography coronary angiograms and fractional flow reserve (FFR) using pressure flow wire[21]. FFR is a validated, reproducible measurement of relative CFR. The reference model for FFR is the hyperemic pressure-flow relation with the assumptions that a discrete stenosis induces proportional changes of perfusion pressure and flow, and downstream of a stenosis minimal coronary resistance equals that of a normally perfused region[22]. In practice, FFR derives from pressure measurements upstream and downstream of a given coronary stenosis at maximal

pharmacological arteriolar vasodilation. FFR was proposed as the best available technique to guide clinical decision making (whether mechanical revascularization or medical therapy), and FFR-guided PCI is the way to improved prognoses with respect to PCI based on angiographic severity[23-26].

Absolute CFR equals the ratio of maximal to baseline flow for any given arterial distribution with or without a stenosis or diffuse narrowing[27]. Relative CFR equals the ratio of maximal stress flow in the diseased artery to maximal flow in non-diseased arterial segments either in the same or adjacent arterial distribution. CFR in healthy volunteers with no risk factors is up to 4.5 ± 0.7. Values for mild disease in patients with coronary risk factors average 2.7 ± 0.6[28]. CFR < 2.0 is the proposed threshold for inducible ischemia[29]. FFR pressure ratio for a single discrete stenosis in the absence of diffuse disease also equals relative CFR using flow or flow velocity measurements. However, flow-based CFR and pressure-based FFR may not give evidence of comparable stenosis severity in approximately 40% of lesions[30]. Diffuse narrowing may reduce CFR significantly, with only a minimal fall in segmental pressure gradients or FFR. In situations of mixed diffuse and segmental disease, which are common in the clinical setting, noninvasive absolute maximal perfusion and CFR together likely define the severity of each coronary stenosis. Gould[31] provides the following example: "A 38% diffuse diameter narrowing in the absence of arterial remodeling would reduce CFR to 1.4 whereas the same diffuse disease plus a discrete angiographic 60% stenosis without remodeling would reduce CFR to 1.0, both without significant fall in FFR". Therefore, FFR is not a direct measure of low-flow ischemia, and it does not reflect absolute flow or absolute CFR, which are the determinants of ischemia. Further, FFR and CFR results may be inconsistent because microvascular resistance during hyperemia causes FFR and CFR to change in opposite directions. Higher hyperemic resistance means reduced maximal flow and higher distal pressure, *i.e.*, reduced CFR and increased FFR. Vice versa, low hyperemic microvascular resistance for equal stenosis means higher maximal flow and increased pressure gradient through the stenosis, which may result in low FFR.

Therefore, the microvascular compartment must be considered in an integrated view of coronary perfusion because its functional anatomy is complex. The branching of vessels in the myocardial wall, the different structure of subendocardium and subepicardium vessel wall, and the incremental vascular capacity across the myocardial wall depth describe this complexity, which is the substrate for more important functional differences that are physiologically intended to compensate the effects of myocardial contraction on subendocardial perfusion[32]. More intensely in early systole, the development of intraventricular pressure squeezes intramural vessels and reduces intramural blood volume, which causes coronary venous flow to increase and arterial flow to decrease[33].

The diagnosis of coronary microvascular disease likely occurs when CFR is significantly reduced in the absence of segmental perfusion defects. Coronary microvascular endothelial dysfunction may be assessed noninvasively by evaluating CFR with perfusion imaging in combination with cold pressor stress or demonstrating improvements in resting perfusion heterogeneity using a vasodilator stress. These methods may help correctly evaluate the physiological meaning of anatomical coronary stenosis when FFR and CFR results are inconclusive.

PHARMACOLOGICAL STRESS TESTING

Two possible pharmacological methods of challenging coronary circulation evolved over the years: (1) pharmacological interaction with adenosine receptors of vascular smooth muscle cells (dipyridamole, adenosine, and regadenoson[34,35]); and (2) pharmacological inotropic stimulation (dobutamine[36]).

Adenosine vasodilator stress induces maximal increases in blood flow (three- to five-fold in healthy subjects) by reducing vascular resistance. The hyperemic flow is dependent on systemic pressure and the residual resistance at the microcirculation level *via* relaxation of smooth muscle cells, which uncouples coronary flow (supply) and myocardial work (demand). The mismatch between coronary flow and myocardial demand may induce the stealing phenomenon[34]. However, hyperemia induced by adenosine is mediated by endothelium receptors and neuronal-mediated mechanisms, which was elegantly demonstrated in two studies using L-nitroarginine methyl ester (L-NAME). Intracoronary L-NAME attenuated the hyperemic dilation in healthy volunteers *via* inhibition of endothelial nitric oxide synthase[37], but systemic L-NAME counterbalanced CFR increase *via* the neuronal response[38]. This effect may explain the limited accuracy of the ratio of hyperemic to rest MBF that exists in pathological conditions, such as hypertension, which is characterized by elevated flow at rest.

Dobutamine stressor is an alternative to physical stress. This synthetic sympathomimetic amine stimulates β- and α-adrenoreceptors, increases myocardial oxygen consumption (inotropic effect) and MBF[39-41]. Thickening of subendocardial myocardial layers primarily contributes to resting wall thickening, but catecholamines also stimulate a thickening of subepicardial layers. This effect is useful to detect myocardial viability, and it explains why patients with resting wall motion (WM) abnormalities consequent to subendocardial infarction still exhibit improved contractility during dobutamine infusion. This pharmacological effect is beneficial for the diagnosis of viability, but it may mask the subendocardial ischemia in patients with normal resting WM and significant coronary stenosis[42].

The cold pressor test deserves some attention for its capacity to interact with the sympathetic system[43-45].

Sympathetic stimulation using cold exposure induces a sharp rise in heart rate and systolic arterial pressure

and a norepinephrine release in the coronary circulation from adrenergic nerve terminals. This increase in rate-pressure product induces a similar increase in MBF, and norepinephrine vasoconstriction is balanced by endothelium-related vasodilation of epicardial coronary vessels and myocardial microcirculation. This mechanism is related to flow augmentation, and it exhibits a similar extent as pharmacologically induced hyperemia.

Therefore, the cold pressor test produces a similar increase in rate-pressure product and MBF. The effects on sympathetic endothelium modulation and endothelium-related flow augmentation suggest the utility of this stressor in evaluating microvascular function.

CORONARY ARTERY STENOSIS DETECTION

The ischemic cascade concept, whereby abnormal perfusion precedes abnormal mechanical function during increased demand-induced ischemia, was demonstrated in an experimental setting. The spatial extent of perfusion abnormality is greater than the contraction abnormality, and the mismatch between perfusion and function is more evident in single-vessel *vs* multi-vessel disease[46].

Pharmacological and physical stress echocardiography is a mainstay of the noninvasive assessment of CAD. Technological advances and the use of contrast agents provided echocardiography the ability to increase and match the perfusion information to the classic evaluation of WM abnormalities that are induced by adrenergic or adenosinic mechanisms[47].

The use of echocardiographic contrast agents improved the accuracy of stress echocardiography[48-50]. Shah *et al*[48] demonstrated the usefulness of myocardial contrast stress echocardiography in 193 patients (88%) in a prospective clinical study, and it provided an incremental benefit over WM analysis in 25% of patients and greater confidence with WM evaluations in 62% of patients.

Thomas *et al*[51] randomized 1776 patients to real-time myocardial contrast (RTMCE) dobutamine, physical stress echocardiography or standard stress echocardiography in which contrast was used for opacification of the left ventricle only (non-RTMCE). Myocardial perfusion tests exhibited a higher positivity (22% for RTMCE *vs* 15% with non-RTMCE, $P = 0.0002$) with similar positive values to predict > 50% diameter stenosis using quantitative coronary angiography (67% for non-RTMCE, 73% for RTMCE). The higher positivity of RTMCE was related to the detection of subendocardial wall thickening abnormalities that were missed in non-RTMCE studies that only examined transmural wall thickening[51] (see the representative case in Figure 6).

At variance with a previous study[51], a large multicenter study[7] demonstrated a higher sensitivity of myocardial contrast stress echocardiography *vs* SPECT (75.2% *vs* 49.1%, $P < 0.0001$) for the detection of $\geqslant$ 70% or $\geqslant$ 50% stenosis, but the specificity was lower (52.4% *vs* 80.6%, $P < 0.0001$). Sensitivity for the detection of $\geqslant$ 70% single-vessel stenosis was higher for MCE (72.5% *vs* 42.7%, $P < 0.0001$) and the detection of proximal vessel disease (80% *vs* 58%, $P = 0.005$), but a cautionary note must be placed relative to fair inter-reader agreement ($k = 0.37$ for MCE, 0.34 for SPECT in the mentioned study)[15]. The sensitivity of MCE was greater for the detection of LAD and multivessel disease[15].

Dobutamine-atropine stress RTMCE and CMR exhibited comparable diagnostic accuracies for significant CAD detection due to the incremental values of myocardial perfusion imaging over WM analysis for RTMCE and CMR[52].

Myocardial contrast stress echocardiography also facilitates the measurement of the CFR-LAD using transthoracic Doppler, and CFR-LAD exhibited incremental value for WM analysis[53-55].

The prognostication potential of myocardial contrast stress echocardiography requires further research. MCE detects stress-induced perfusion defects that are uncoupled to WM abnormalities and identifies a sub-group of patients who are at a higher risk of coronary events among patients without inducible WM abnormalities at stress echocardiography, who are by definition at low risk[49,56-60].

Porter *et al*[60] studied 2014 patients with intermediate to high pre-test probability of CAD who were randomized to dobutamine or exercise stress RTMCE or conventional stress echocardiography (CSE) and followed prospectively for a median of 2.6 years. They demonstrated that patients with abnormal RTMCE studies had higher death rates, nonfatal myocardial infarction (MI), or subsequent revascularization rates than patients with abnormal CSE studies. No difference emerged in primary end-point rates following normal CSE or RTMCE studies. Notably, patients with perfusion defects and WM abnormalities and patients with perfusion defects only exhibited similar rates death/nonfatal MI (7.2% and 6.5%, respectively)[60].

Gaibazzi *et al*[61] confirmed these data in 718 patients in a multicenter cohort study who were followed for 16 mo after high-dose dipyridamole MCE with measurements of LAD flow reserve, and patients who underwent revascularization after the diagnostic test were censored[61].

NO REFLOW

The "no reflow" phenomenon, first described by Ito *et al*[62] and subsequently confirmed by several investigators in MCE studies[63-67], refers to the situation when myocardial tissue perfusion is not restored despite a grade 3 TIMI flow on coronary angiography after primary coronary intervention for acute MI. The no reflow phenomenon influences the eventual infarct size and affects up to one third of patients who undergo reperfusion coronary interventions. This phenomenon

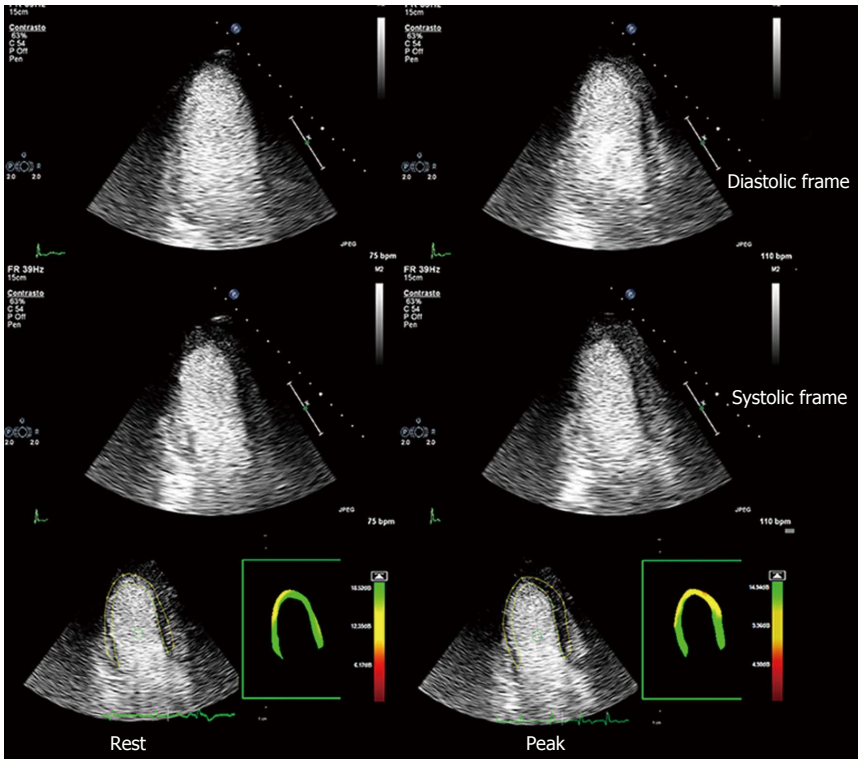

Figure 6 Myocardial contrast stress echocardiography. Apical three-chamber view of a patient with previous by-pass graft (left internal mammary artery graft onto left anterior descending coronary artery). Baseline diastolic and systolic frames on the left, top and intermediate rows, respectively; peak stress diastolic and systolic frames on the right, top and intermediate rows, respectively. Baseline and peak stress myocardial perfusion parametric quantification are displayed, respectively, in the bottom left and right panels. At baseline, akinesis of the infero-apical region is evident, which is concordant with a transmural defect of perfusion of the same region. At peak stress, no new wall motion abnormalities are detected, whereas parametric quantification of myocardial perfusion shows a large transmural defect of perfusion of all the apical regions and a subendocardial defect of perfusion of middle and basal anterior septum. A critical stenosis of distal mammary graft anastomosis was found on coronary angiography.

results from microvascular obstruction caused by the embolization of thrombus and plaque debris during balloon angioplasty and stent deployment in the setting of short duration myocardial ischemia (< 45 min). Other mechanisms are major determinants in prolonged ischemia, in which the no reflow correlates to microvascular damage. There may be areas of low reflow all around the no reflow area, whose salvage may be the potential target of treatment options. The presence of collaterals and the dynamics of the target vessel occlusion, whether it was an abrupt event or a chronic intermittent reduction of flow, influence the low reflow area.

Addressing the no reflow phenomenon may affect therapeutic intervention outcome in acute coronary syndromes. Several studies demonstrated that thrombus aspirations and occlusive protection devices may increase microvascular perfusion, and platelet inhibitors may reduce the no reflow area and infarct size. Pharmacological interventions (nicorandil, verapamil, adenosine) may also increase the chance to reduce myocardial infarct size[68-71].

ISOLATED MICROVASCULAR DYSFUNCTION

Abnormalities of coronary microcirculation beyond myocardial ischemia caused by atherosclerosis of the epicardial coronary arteries are an alternative cause of, or may contribute to, myocardial ischemia in several conditions. Coronary microvascular disease (MVD) is a unique cause of symptoms in several patients with angina. This condition is known as microvascular angina (MVA)[72], and it is better defined as primary MVA to distinguish it from secondary MVA, which occurs in the setting of specific diseases (Table 1)[73].

The clinical presentation of MVA covers the entire spectrum of coronary syndromes, from chronic to unstable angina and acute syndromes.

Stable primary MVA is characterized by angina episodes that are exclusively or predominantly related to effort, and it can be identified with the clinical entity that is generally known as cardiac syndrome X[74]. No cardiac or systemic diseases should be detectable by definition. However, patients with uncomplicated hypertension or diabetes mellitus are often classified as syndrome X patients because these pathological conditions confer a risk for MVD similar to atherosclerosis obstructive CAD[74].

Functional abnormalities of resistive coronary vessels were documented in numerous studies on MVD. Blunted endothelium-dependent vasodilation due to impaired nitric oxide release is the most commonly proposed mechanism for MVD in stable MVA patients,

Table 1 Pathogenetic classification of cardiac microvascular dysfunction

MVD in the absence of myocardial and obstructive coronary artery diseases
MVD in the presence of myocardial disease
MVD in the presence of obstructive coronary artery disease
MVD caused by coronary recanalization interventions

MVD: Microvascular dysfunction.

and it is based on a reduced coronary blow flow (CBF) response to acetylcholine[75]. A reduced CBF response to endothelium-independent vasodilators, such as adenosine, dipyridamole, and papaverine, was repeatedly reported[76-78], which suggests an important role of primary impaired relaxation of small vessels. Other studies demonstrated enhanced vasoconstrictor activity in coronary microcirculation in several patients with stable MVA. Ergonovine injection, mental stress, and hyperventilation resulted in impairments of CBF[79]. Tests to diagnose MVD in the clinical setting should explore the vasodilation and vasoconstriction responses of coronary microcirculation. Vasodilator tests are the first choice in patients with stable MVA, but the response to vasoconstrictor stimuli should be assessed when the former tests are normal or inconclusive. Transthoracic Doppler echocardiographic evaluation of CBF may be used as a first-line method to identify MVD in the LAD territory of patients with normal coronary arteries with suspected MVA. Contrast stress echocardiography may represent the frontier method to detect MVD in the entire myocardial circulation[80].

Unstable primary MVA should be suspected in patients with non-ST-segment elevation acute coronary syndrome and normal coronary arteries on angiography. *De novo* abnormalities on standard ECG in these patients (*e.g.*, ST-segment depression, negative T waves), their gradual normalization, and mild elevation of serum markers of myocardial damage (troponins) indicate a cardiac ischemic origin of symptoms. Diagnosis requires the exclusion of epicardial coronary spasm and transient coronary thrombosis as the cause of angina together with evidence of MVD.

Multiple studies evaluated the prognosis in MVD and demonstrated more cardiac events in patients with reduced CFR. However, there is no consensus in the literature on the best prognostic CFR cutoff (range 1.5-2.5). Murthy *et al*[81] demonstrated a 5.6-fold increased risk of cardiac death in patients with suspected CAD and CFR < 1.5.

Two forms of unstable MVA are described, microvascular variant angina and stress-related cardiomyopathy. Mohri *et al*[82] described the first form in Japanese patients with angina attacks at rest (less frequently with associated effort angina) in the presence of normal coronary arteries. Intracoronary acetylcholine reproduced angina and ST-segment changes in these patients. The absence of vasospasm of epicardial coronary vessels suggested diffuse coronary microvascular spasm.

Stress-related cardiomyopathy (also known as apical ballooning syndrome or takotsubo disease) is generally triggered by sudden emotional or even physically intense stress[83]. Acute chest pain may be associated with abrupt heart failure or cardiogenic shock. The clinical picture includes normal epicardial coronary arteries, depressed left ventricular function, left ventricular ballooning at angiography or echocardiography due to apical and mid-ventricular akinesia with preserved contraction of basal segments, relatively minor elevations of troponins and creatine kinase-MB and a favorable clinical course with recovery of all abnormalities in 1 to 3 mo. The disease is considered adrenergic-mediated because of the cause-effect relationship with stress. Findings that support this hypothesis include increased catecholamine levels, histological signs of catecholamine-mediated cardiotoxicity in endomyocardial biopsy specimens[84], and the unique distribution of cardiac WM abnormalities, which may reflect the variable distribution of adrenergic innervation in the myocardium[84]. Sustained intense coronary microvascular constriction or spasm induced by excessive adrenergic stimulation that results in myocardial ischemia and stunning may be an alternative pathological mechanism, at least in some patients[85]. Some reports demonstrated abnormal myocardial perfusion in the affected myocardial segments[86], and MVD was documented by evidence of reduced CBF responses to vasodilator stimuli in the acute phase[87]. MVD subsided in several weeks, which paralleled clinical improvement[87], but subclinical microvascular dysfunction persisted long after the acute phase. This persistence was demonstrated by the abnormal response of coronary flow to the cold pressor test and left ventricular regional contraction on contrast echocardiography[88]. Our follow-up study of patients with takotsubo syndrome demonstrated transient WM abnormalities and reduced CFR without regional myocardial perfusion abnormalities in response to the cold pressor test, which suggests the persistence of microvascular dysfunction (Figure 7).

CONCLUSION

Second-generation echocardiographic contrast agents received regulatory authorities' approval for clinical use in left ventricular opacification studies with the only limitation of patients with intracardiac shunts or pulmonary hypertension. In contrast, MCE did not receive approval for clinical use, and the technique remains an option for research. MCE is a demanding technique in the technical skills that are required of sonographers and physicians and the investment of software of analysis systems. The reimbursement issue may represent an adjunctive drawback. Reimbursement for MCE studies is approximately $60 in the United States, but reimbursement covers only the cost of the drug in most European countries. Analysis of cost/effectiveness and cost saving based on the procedural

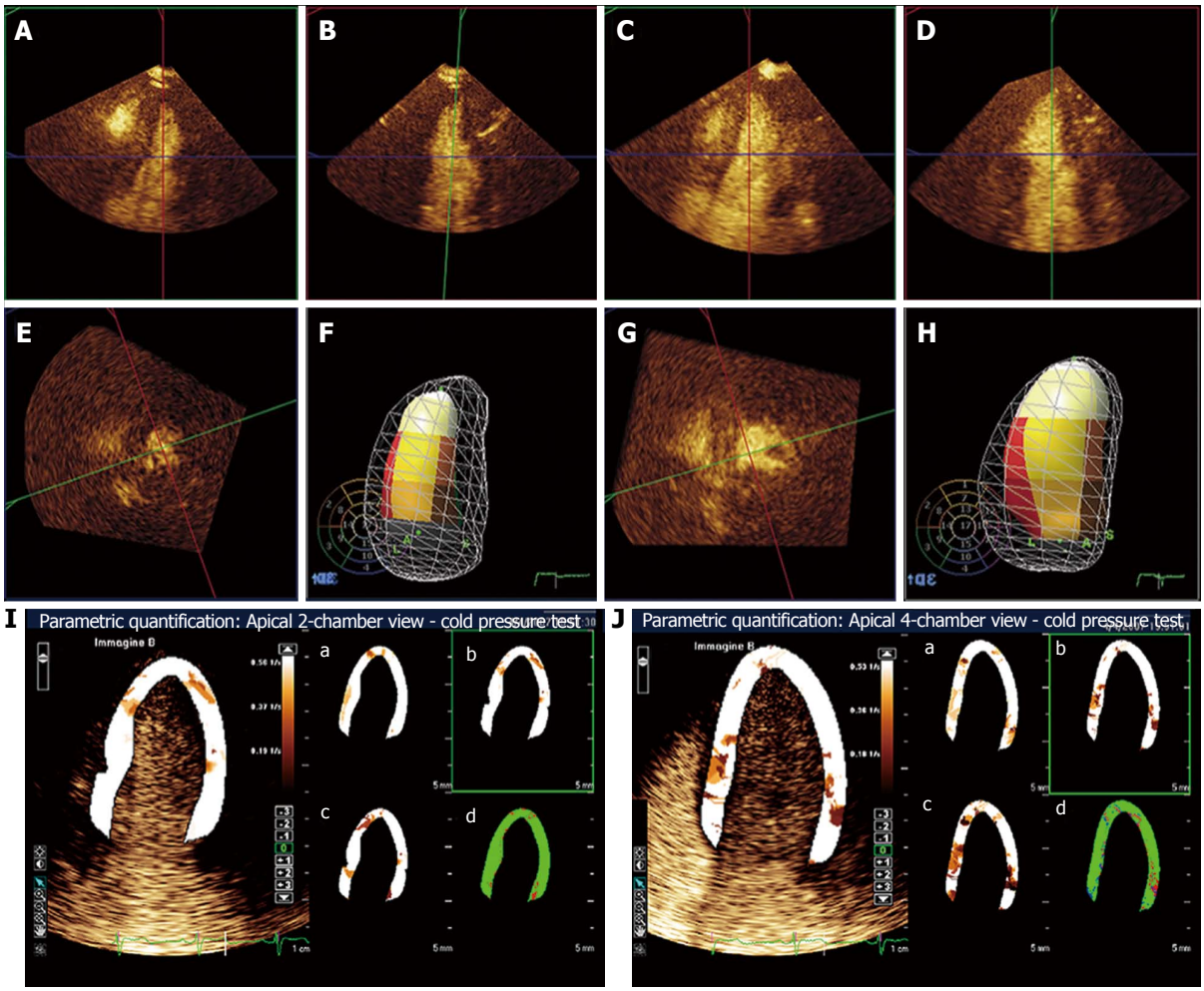

Figure 7 Real-time 3-dimensional myocardial contrast echocardiography during follow-up using cold pressor test in a patient who recovered from apical ballooning syndrome. A, B and E: Reconstructed 4-chamber (A), 2-chamber (B) and short-axis (E) end-systolic frames at baseline; F: 3-dimensional systolic volume rendering as left ventricular cast inside the diastolic mesh volume rendering. The American Society of Echocardiography 17-segment model of the left ventricle is reproduced as a bulls-eye in the background, and superimposed color-coded onto the left ventricular cast; C, D and G: Reconstructed 4-chamber (C), 2-chamber (D) and short-axis (G) end-systolic frames using the cold pressor test; H: The diastolic and systolic 3-dimensional casts as in panel 1d. Note that wall motion is normal at baseline, whereas apical akinesia develops during the cold pressor test; I and J: Apical 2-chamber and 4-chamber, respectively, parametric myocardial contrast echocardiography quantification using the cold pressor test; the slope β of the replenishment curve is superimposed onto the left ventricular wall in 2-chamber and 4-chamber views, respectively. Perfusion parameters A, β and A × β are superimposed onto the same left ventricular wall as in panels I and J, respectively, in panels a, b and c; panels d represents the goodness of fit. Parametric images demonstrate homogeneous perfusion during the cold pressor test. The coronary flow reserve in this patient was 1.10 (normal range 2.77 ± 0.70).

and downstream investigation costs, as the one conducted by the Medical Services Advisory Committee of Australia on the use of second-generation contrast agents in patients with suboptimal echocardiograms[89], is lacking for MCE. However, the diagnostic potentials of MCE, which covers the entire spectrum of cardiac circulation physiopathology and clinical presentation of coronary syndromes, may reduce overall costs.

Research has established that the application fields for MCE represent the clinical perspective to pursue this technique in the future daily workflow of echo laboratories. The integration of FFR with quantitative MCE offers the opportunity to demonstrate the effects of anatomical diffuse non-critical coronary stenosis and furnishes an integrated vision of the extent of ischemic burden that is comprehensive of collateral flow contribution. MCE in the setting of acute coronary syndromes identifies complex anatomo-functional features, such as no reflow and low flow, within and around the infarct area, respectively, which foresees the potential for functional recovery of stunned myocardium to guide therapeutic interventions. In an era when typical angina is less frequently associated with significant coronary stenosis[90] and acute coronary syndromes are dissociated from intracoronary thrombosis or significant coronary stenosis in up to 10% of cases, MCE is a relatively low-expensive, bedside technique to examine microvascular reserve and identify patients with MVD.

In conclusion, current research provides good evidence that MCE improves comprehensive echocardiographic evaluations of ischemic heart disease. The approval of regulatory authorities and the availability of quantitative operator-independent analysis software will hopefully prompt physicians and sonographers to

implement MCE into the daily work flow of echo laboratories.

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MINIREVIEWS

Mechanical valve obstruction: Review of diagnostic and treatment strategies

Jason Salamon, Jerson Munoz-Mendoza, Jared J Liebelt, Cynthia C Taub

Jason Salamon, Jerson Munoz-Mendoza, Cynthia C Taub, Department of Cardiology, Montefiore Medical Center, Bronx, NY 10467, United States

Jared J Liebelt, Department of Medicine, Jacobi Medical Center, Albert Einstein College of Medicine, Bronx, NY 10461, United States

Author contributions: Salamon J was involved in researching, writing, editing and compiling this paper; Munoz-Mendoza J was involved in researching, writing, editing and compiling this paper; Liebelt JJ was involved in researching, writing, editing and compiling this paper; Taub CC was involved in researching, writing, editing and compiling this paper.

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Correspondence to: Jason Salamon, MD, Department of Cardiology, Montefiore Medical Center, 211 E 210th Street, Bronx, NY 10467,
United States. jason.salamon@med.einstein.yu.edu
Telephone: +1-718-9202117

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Abstract

Prosthetic valve obstruction (PVO) is a rare but feared complication of mechanical valve replacement. Diagnostic evaluation should focus on differentiating prosthetic valve thrombosis (PVT) from pannus formation, as their treatment options differ. History of sub-optimal anticoagulation and post-op time course to development of PVO are useful clinical characteristics in differentiating thrombus from pannus formation. Treatment of PVT is influenced by the patient's symptoms, valve location, degree of obstruction and thrombus size and may include thrombolysis or surgical intervention. Alternatively, pannus formation requires surgical intervention. The purpose of this article is to review the pathophysiology, epidemiology, diagnostic approach and treatment options for aortic and mitral valve PVO.

Key words: Prosthetic valve thrombosis; Pannus overgrowth; Thrombolysis; Prosthetic valve obstruction; Echocardiography

Core tip: Prosthetic valve obstruction (PVO), while rare, is a dreaded complication of mechanical valve replacement. Careful clinical and multiple non-invasive imaging modalities are necessary to assess suspected PVO and evaluate for pannus overgrowth or valve thrombosis. Unlike pannus overgrowth, prosthetic valve thrombosis is more common, occurs earlier in the post-op period, is frequently related to inadequate anticoagulation, and can often be treated through non-invasive thrombolysis. While the current understanding of pannus overgrowth remains elusive, future clarification of its pathophysiology may allow for the development of non-invasive therapeutic options.

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INTRODUCTION

A 60-year-old male underwent 1-vessel coronary artery bypass graft and a 31 mm bileaflet St. Jude's mechanical mitral valve (MV) replacement for newly diagnosed ischemic cardiomyopathy and functional mitral regurgitation. His post-op course was uneventful and he reported self-compliance with all his medications. Three months after his surgery he was admitted for shortness of breath and was found to be hypotensive with jugular venous distention, warm extremities with pitting edema bilaterally, and a new 3/6 holosystolic murmur with a 2/4 diastolic rumble- both radiating to the axilla. His international normalized ratio (INR) was 1.3.

Transthoracic echocardiography (TTE) revealed an unchanged ejection fraction and a fixed closed mitral leaflet disc with a transmitral Doppler mean gradient of 13 mmHg. His calculated MV area was 0.41 cm^2 (*via* continuity equation), maximum MV E wave velocity of 1.7 m/s and new severe right ventricle dilatation, dysfunction, and tricuspid regurgitation were also present. Transesophageal echocardiography (TEE) confirmed a fixed mitral leaflet (Figure 1), and a soft thrombus in left atrial appendage. A small soft non-mobile mass (5-6 mm) adjacent to the sewing ring on the fixed leaflet was identified. Follow up TTE and cine fluoroscopy (CF) confirmed residual immobility of the posterior occluded prosthetic leaflet.

He was treated with intravenous furosemide with symptomatic improvement. Tissue plasminogen activator was administered (10 mg bolus centrally through Swan Ganz catheter followed by a 90 mg infusion peripherally over 5 h). Follow up TTE transmitral gradient *via* Doppler interrogation demonstrated a significant decrease to 4 mmHg. A decision was made to pursue redo-mitral valve replacement with a 31 mm St. Jude's porcine bioprosthesis since the valve remained in the closed position. Gross sample revealed residual organized thrombus on the mitral valve disc (Figure 2). Three-month follow-up TEE showed no change in transmitral gradient.

Pathophysiology

Prosthetic valve replacement whether mechanical or bioprosthetic carries an inherent risk for serious, sometimes devastating complications. Obstruction of prosthetic valves can result from thrombus, pannus overgrowth, vegitations or combination of thrombus and pannus formation.

Prosthetic valve thrombosis: Prosthetic valve obstruction (PVO) is a rare but dreaded post-surgical complication, with the most common cause being prosthetic valve thrombosis (PVT). PVT occurs more commonly in mechanical, as compared to biologic prostheses, likely related to the underlying pathophysiology of thrombus development[1,2].

Post-surgical endothelization after prosthetic valve surgery occurs over weeks to months. During this time, the exposed and healing endothelium may serve as a nidus for clot formation. Typically, an initial small thrombus may develop and act as a further substrate for additional layering of new thrombus[3]. In addition, the post op course of a newly placed mechanical valve results in the development of turbulent flow and stasis which is an additional contributor to thrombus development. This relative stasis and aberrant flow helps explain why tricuspid valve thrombosis is 20 times as common as left sided thrombosis, and MV thrombosis is more common than aortic valve (AV) thrombosis[3]. Similarly, increased prosthetic surface area has been correlated to a greater formation of both thrombi and pannus[4].

The intrinsic prothrombotic milieu post valve replacement requires strict anticoagulation to avoid complications. Thus, multiple investigators have observed a significantly higher incidence of thrombotic complications among patients with subtherapeutic anticoagulation, which has been validated as the best clinical tool to differentiate pannus from thrombus, as discussed below[1,5,6].

Pannus overgrowth: Although less common than thrombus formation, pannus may develop over prosthetic valves. A biologic reaction to the prosthesis material with unknown mechanism is thought to cause fibroelastic and collagen overgrowth, with subsequent infiltration of endothelial cells, myofibroblasts, and chronic inflammatory cells resulting in fibrinous ingrowth around the prosthetic valve annulus[1,4,7].

The precise trigger for pannus formation remains unclear at this time, further limiting the ability to prevent and treat this phenomenon. Peripheral blood samples of patients with pannus formation have elevated levels of the proliferation and cell differentiation signaling protein transforming growth factor-beta (TGFβ) when compared to a control cohort (87.7 ng/mL *vs* 73.7 ng/mL, $P < 0.05$)[7]. A careful immunohistochemical analysis of these patients' surgical valve specimens revealed endothelial cells, myofibroblasts and macrophages-each with a cell specific expression profile at the left ventricular pre-annular septum. While the profile differed based on cell type, two of the three cells had increased expression of TGFβ, with all three having increased expression of TGFβ receptor 1. Thus, it would appear that aortic valve pannus originates from the healing process occurring at the junction of the neointima, which is mediated by TGFβ.

It should be noted that many investigators have identified mechanical valve obstructions with both elements of pannus and thrombus. It is likely that pannus serves as a nidus for thrombus, with pannus formation being the underlying cause[1]. The prevalence of these concomitant factors has been reported to be between 12%-75% of all PVO[1,3,4].

Epidemiology

The overall incidence of PVO ranges from 0.4%-6.0%

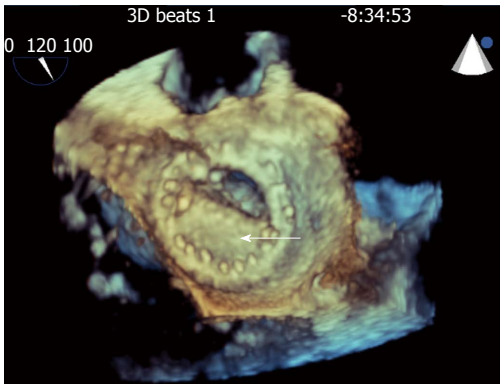

Figure 1 Still frames of 3-dimensional transesophogeal echocardiographic rendering of the mechanical bi-leaflet mitral valve as visualized from the left atrial perspective during diastole showing fixed mitral leaflet (arrow).

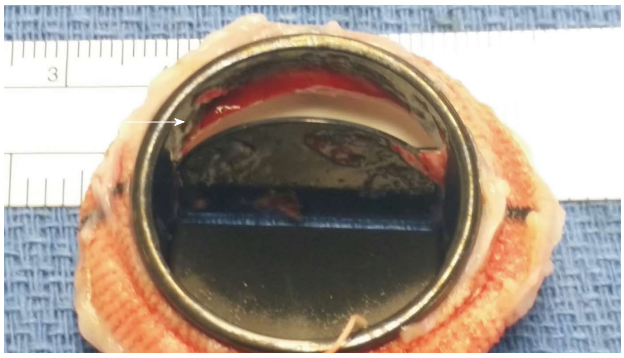

Figure 2 Gross sample of explanted mechanical mitral valve revealing the transesophogeal echocardiography finding residual organized thrombus, apparent on the mitral valve disc (arrow).

annually with the difference in rates depending on the type and location of prosthetic valve replacement[1,8-10]. This may be underestimated as routine post-op screening for PVO is not typically performed, unless patients become symptomatic. For instance, in observing 680 consecutive patients who underwent prosthetic valve surgery, Laplace *et al*[11] observed 64 patients (9.4%) with evidence of significant valve thrombosis starting as early as 9 d post-op, a significantly higher rate as compared to those who present with symptoms.

An observational study by Deviri *et al*[1] found thrombus associated obstruction in 78% of cases (both MV and AV), pannus formation in 10.7% cases, and combination of thrombus and pannus for the remaining 11.6% cases. Overall, the time from valve replacement to obstruction ranged from 6 wk to 13 years (median 4 years)[1]. Alternatively, a report by Vitale *et al*[4] found pannus in 31%, thrombus in 24%, and both pannus and thrombus in 45% of MV PVO. When comparing mitral to aortic valve complications, aortic valves appear to have a higher incidence of pannus, while mitral valves more commonly have PVO from thrombus[1,5,8,12].

The annual incidence of PVT ranges between 0.03%-5.7%[1,3,10,13,14]. PVT can occur in mechanical or bioprosthetic valves and can result in non-obstruction to complete obstruction[9-12]. PVT is more common in mechanical compared to bioprosthetic valves, with the immediate post-op period being the time of highest risk. Although PVT can occur any time after valve replacement, 24% occur within one year postoperatively with subsequent decreases in incidence with each year that follows[1,3]. Compared to pannus formation, thrombotic valvular dysfunction appears to occur at an earlier time with larger masses on imaging[4,5,8,15]. As mentioned above, larger valves, valves exposed to decreased flow (*i.e.*, mitral *vs* aortic, tricuspid *vs* left sided valves) and subtherapeutic anticoagulation status have been shown to be significant risk factors for PVT development[3,6,16].

Diagnosis

While a patient's clinical presentation may suggest a possible prosthetic valve complication, diagnosis of PVO, and differentiating its etiology, requires direct visualization of the valve by various imaging modalities.

Valvular obstruction should be considered when an unexpected rise in trans-valvular gradient is observed on Doppler echocardiography. Non-invasive visualization utilizing modalities such as TTE, TEE, and CF are necessary to accurately diagnose and guide treatment strategies. Since the etiology of obstruction may guide choice of therapy, the differentiation of thrombus from pannus is an essential but often challenging task. Initial diagnostic evaluation should commence with TTE in order to assess valve motion, degree of obstruction, and clot burden but also exclude non-acquired obstruction like patient prosthetic mismatch (PPM).

Echocardiography: TTE with color Doppler is regarded as the initial step for diagnosis of PVO and is required to determine hemodynamic severity and impact on valve function[5,12,17]. Sudden increases in transvalvular gradients from baseline are indicative of valvular obstruction. However, it is important to consider other causes of increased prosthetic valve gradients such as high cardiac output states, pressure recovery [in AV replacement (AVR)], regurgitation, and PPM. Furthermore, TTE may be limited by prosthetic reverberation artifacts. In this scenario, the use of spectral Doppler may detect a stuck valve due to aberration of opening and closing spikes. More importantly, image optimization despite these limitations, can be attempted through the use of 3-dimensional (3D) TEE allowing a more precise and realistic visualization[18]. Girard *et al*[12] found that TTE correctly identify the pathological mechanism of mechanical AVR obstruction in only 10% of cases but 63% of bioprosthetic AVR. While TTE is usually inadequate for valvular leaflet investigation and often not sensitive enough to identify thrombi as compared to pannus, it is an essential screening modality and may accurately identify obstructive masses in > 80% of cases[5].

MV Doppler echocardiographic evaluation should focus on measuring the mean transmitral gradient and pressure half time (PHT), in addition to the use of continuity equation to calculate valve area[19,20]. PHT

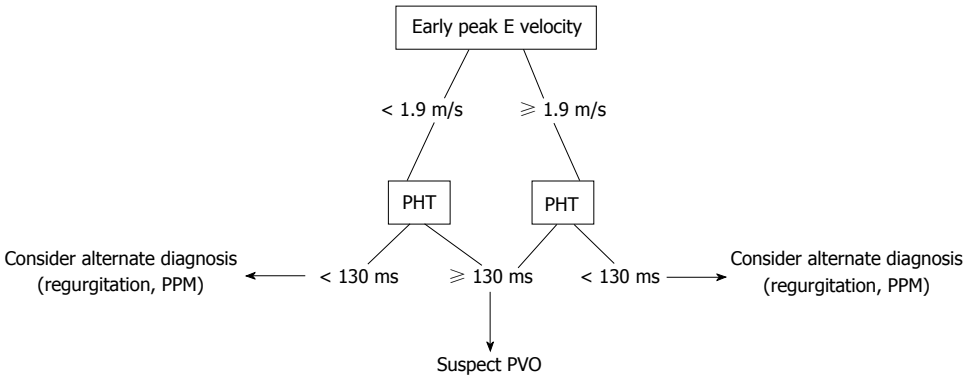

Figure 3 Proposed echocardiographic evaluation for suspected prosthetic mitral valve obstruction. PHT: Pressure half-time; PVO: Prosthetic valve obstruction; PPM: Prosthetic patient mismatch.

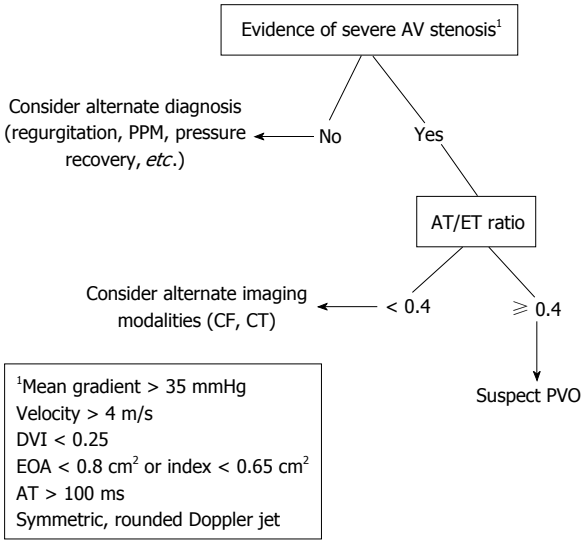

Figure 4 Proposed non-invasive evaluation for suspected prosthetic aortic valve obstruction. AT: Acceleration time; ET: Ejection time; PPM: Prosthetic-patient mismatch; CF: Cine fluoroscopy; CT: Computed tomography; PVO: Prosthetic valve obstruction; EOA: Effective orifice area; DVI: Doppler velocity index; AV: Aortic valve.

⩾ 130 ms has been shown to identify MV PVO in 99% of patients, however, its sensitivity is limited due to its relationship with atrial and ventricular compliance in addition to heart rate[2,21]. While peak early E velocity (PEV) ⩾ 1.9 m/s has also been shown to be a useful screening tool for PVO (OR = 3.51; 95%CI: 1.62-7.57; for every 10-unit increments of peak E velocity in cm/s), even with a peak E < 1.9 m/s, the presence of HT ⩾ 130 ms still correlates well with PVO. In addition, PHT ⩾ 130 ms is especially helpful in differentiating PVO from prosthetic valve dysfunction and regurgitation. Thus, while PEV may suggest PVO, PHT ⩾ 130 ms is necessary to indicate PVO, irrespective of PEV (Figure 3)[21].

Aortic valve PVO investigation by echocardiography should begin with a Doppler peak and mean transvalvular gradients. These acquired values should then be compared to known brand and size specific published values[2]. Severe prosthetic AV stenosis is suggested (assuming a normal stroke volume) with the presence of a rounded symmetric Doppler jet, a peak velocity ⩾ 4 m/s, mean gradient ⩾ 35 mm/Hg, Doppler velocity index < 0.25, effective orifice area < 0.8 cm^2 (or an indexed area to surface body area of < 0.65 cm^2), and an acceleration time ⩾ 100 ms. A ratio of acceleration time to ejection time of ⩾ 0.4 has been demonstrated as a reliable angle-independent variable that is consistent with PVO (Figure 4)[22]. In addition, careful assessment should be made for any abnormal echo densities or valve motions. Other non-valvular parameters should also be closely measured and compared to prior studies, including left ventricular size, function, and hypertrophy[2].

If there is a clinical suspicion for PVO but TTE Doppler is equivocal then stress TTE can be considered for further evaluation. While there is limited data regarding strict ranges and diagnostic cutoffs for PVO on stress TTE, a mean transmitral gradient rise of ⩾ 15 mmHg (or ⩾ 18 mmHg with AV prostheses) with stress has been suggested as a reliable marker to suggest PVO, even if the resting mean gradient is normal[2].

Use of TEE remains the gold standard for diagnosis of PVO and is required to determine the etiology of PVO as well as identifying candidates for thrombolytic therapy *vs* surgical intervention[12,23,24]. There is an additional benefit in using 3D echocardiography to more precisely visualize and evaluate the anatomy of both aortic and mitral prosthetic valves. As compared to standard 2-dimensional echo, 3D echocardiography allows a more detailed and accurate assessment of valve leaflets, prosthetic rings and struts. However, AV visualization still remains relatively difficult to image, as compared to the MV, given its distance from the transducer and its oblique angle of incidence as related to the ultrasound beam. Additionally, 3D TEE has been shown to have a high correlation with surgical findings, especially in regards to MV pathology[25-27].

Characteristics on TTE and TEE that differentiate pannus from thrombus include a larger size (2.8 cm *vs* 1.7 cm) and a soft mass-like appearance, as compared to pannus. A quantitative evaluation of mass characteristics can be done by comparison to myocardium

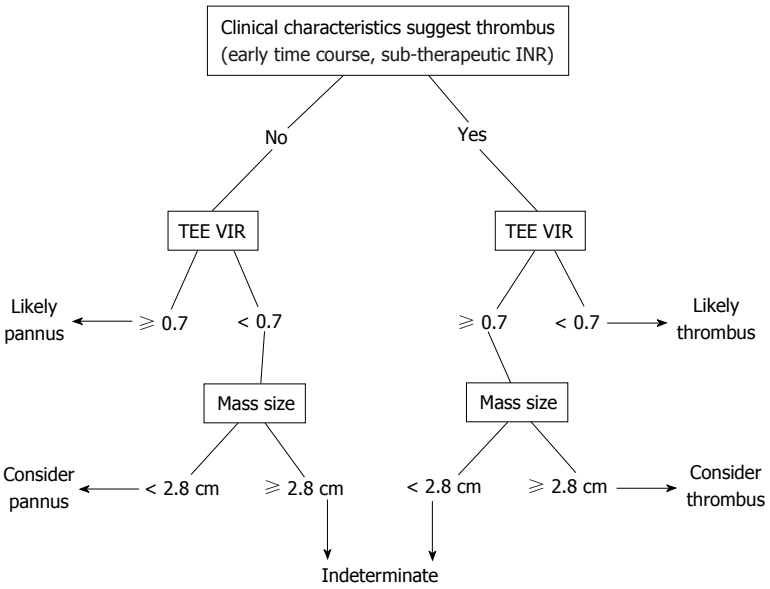

Figure 5 Proposed non-invasive evaluation for differentiating thrombus from pannus as underlying cause of prosthetic valve obstruction. TEE: Transesophogeal echocardiography; VIR: Video intensity ratio; INR: International normalized ratio.

using a video intensity ratio (VIR), with a VIR < 0.7 as being similar to myocardium (VIR; video intensity of the mass in relation to the prosthetic material). A VIR < 0.7 has a positive predictive value (PPV) 87% and negative predictive value (NPV) 89% and specificity of 80% with a sensitivity of 93%, slightly better than identification of soft mass alone (NPV 80%, PPV 86%, sensitivity 86%, specificity 80%). Interestingly, a clinical history of inadequate anticoagulation alone had a specificity of 92% and a sensitivity of 79% for thrombus. When it was combined with imaging findings thought to be thrombus-specific on TEE (either soft mass-like or VIR < 0.7), sensitivity and specificity remained the same at 93% and 80% respectively. Furthermore, in the mitral position, unlike pannus formation, thrombi on TEE characteristically extend into the left atrium and appendage (Figure 5)[5].

In addition to its diagnostic role, TEE, unlike TTE, has been shown to contribute to risk stratification for embolic phenomenon which in turn may assist to guide therapeutic decision making. A thrombus area of < 0.85 cm^2 on TEE has the lowest risk of systemic embolization[17]. As discussed below, fibrinolytic therapy has emerged as a therapeutic option in PVO.

Cine-fluoroscopy: While CF was historically the original imaging technique to evaluate for PVO, it has remained a useful tool to this day. CF allows for direct visualization of the radiopaque valve disks and allows comparison of the opening and closing angles of leaflets to normal or baseline angles, something that has limited usage with tissue valves[2,28]. Abnormal tilting of the ring base may suggest valve dehiscence, which can be confirmed with the injection of contrast dye. Valve obstruction is suggested with incomplete seating of the valves moving parts or impaired excursion[2].

TEE may appear to provide more robust information as compared to CF due to its ability to evaluate valve motion, structure, and hemodynamic parameters. However, CF is an essential complimentary examination to TEE, especially when TTE is insufficient at determining the difference between PVO and PPM[24]. In a comparison study between TTE and CF in the evaluation for PVT, sensitivity and specificity were 75% and 64% for TTE, 87% and 78% for CF[24]. Positive and negative predictive values for TTE and CF were 57%/78% and 80%/91% respectively. When used together, CF and TTE correctly diagnosed PVT in 85% of cases with TEE only required in 15% of cases.

Computed tomography scan: While computed tomography (CT) may appear to have a limited diagnostic role in the evaluation for PVO due to its incomplete evaluation of valve motion and hemodynamics, it may afford superiority over echocardiography when imaging pannus, especially in the atrial position[29]. At this time, there are no comprehensive comparative studies of echocardiography and CT in evaluating PVO. While CT may not be primarily indicated in the evaluation of PVO, it should be considered as an adjunct to TEE and CF, especially if the results are inconclusive[2].

Treatment

Treatment options for PVO include either a medical or surgical approach. In general, medical treatments are favored as an initial therapy, as the mortality of repeat valve surgery can be extremely high, depending on patient specific factors. However, pannus, due to its highly fibrotic makeup does not respond to medical therapy. When indicated, thrombolysis affords a non-invasive approach to clot dissolution and valve restoration. Thrombolysis should be considered based on the level of obstruction, ejection fraction (in aortic obstruction) and symptomatic burden (*i.e.*, NYHA class

Ⅲ-Ⅳ)[1,6]. Thrombolysis, when used appropriately, has shown complete resolution of valvular obstruction in 71%-82% of patients with 17% showing a partial hemodynamic resolution. If unsuccessful, a second dose of thrombolysis shows an additive effect and further hemodynamic benefit[30-32]. Thrombolysis may be more effective in aortic valves as compared to mitral valves, however, at this time the data is limited[32]. A multicenter registry has demonstrated complications from PVT thrombolysis treatment in 18% of patients, with death occurring at a rate 6%. Specifically, prior history of stroke and increased thrombus area (for every 1 cm^2 $\geqslant$ 0.8 cm^2, defined by TEE) are independent predictors of complications to thrombolysis for PVT[17].

It remains to be seen if medical therapies can be applied to treatment of pannus obstruction. While a monoclonal antibody targeting TGFβ may seem sensible, it would likely require intervention at the very early stages of pannus formation in order to be fully effective. However, early identification remains difficult and, to date, such an approach has yet to be attempted.

The 2006 ACC/AHA guidelines for management of valvular disease[23] suggest prioritizing surgery over medical therapy in select situations. With left sided valvular obstruction, surgery is considered a first line treatment for valve dysfunction if a patient has significant symptoms (NYHA Ⅲ-Ⅳ), or a large clot burden. The ACCP has suggested a cutoff thrombus area of $\geqslant$ 0.8 cm^2[10]. Patients at high surgical risk should first be considered for thrombolysis administration. Right sided valvular dysfunction should first be considered for thrombolysis, even if NYHA class is Ⅲ-Ⅳ. Importantly, after treatment, whether medical or surgical, ACC/AHA recommend a new higher chronic INR goal of 3.5 for aortic valve and 4 for mitral valve. Patients that undergo treatment for PVO (especially fibrinolysis, as discussed below) should undergo serial Doppler echocardiography to ensure there is no change in transvalvular gradients that may suggest rethrombosis[2]. The use of novel oral anti-coagulants has yet to be studied in this population and cannot be recommended at this time.

CONCLUSION

PVO, while rare, is a dreaded complication of mechanical valve replacement. Careful clinical and multiple non-invasive imaging modalities are necessary to definitively evaluate a patient with suspected PVO. As compared to pannus formation, PVT is more common, occurs earlier in the post-op period, is commonly related to inadequate anti-coagulation, and in many patients can be treated by thrombolysis. While the pathophysiology of pannus formation remains elusive, a better understanding of pannus may allow for the development of non-invasive therapeutic options.

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MINIREVIEWS

Space radiation and cardiovascular disease risk

Marjan Boerma, Gregory A Nelson, Vijayalakshmi Sridharan, Xiao-Wen Mao, Igor Koturbash, Martin Hauer-Jensen

Marjan Boerma, Vijayalakshmi Sridharan, Martin Hauer-Jensen, Division of Radiation Health, Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR 72205, United States

Gregory A Nelson, Xiao-Wen Mao, Department of Basic Sciences, Loma Linda University, Loma Linda, CA 92354, United States

Gregory A Nelson, Xiao-Wen Mao, Department of Radiation Medicine, Loma Linda University, Loma Linda, CA 92354, United States

Igor Koturbash, Department of Environmental and Occupational Health, University of Arkansas for Medical Sciences, Little Rock, AR 72205, United States

Martin Hauer-Jensen, Central Arkansas Veterans Healthcare System, Surgical Service, Little Rock, AR 72205, United States

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Correspondence to: Marjan Boerma, PhD, Division of Radiation Health, Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, 4301 West Markham, Slot 522-10, Little Rock, AR 72205, United States. mboerma@uams.edu
Telephone: +1-501-6866599
Fax: +1-501-6866057

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Abstract

Future long-distance space missions will be associated with significant exposures to ionizing radiation, and the health risks of these radiation exposures during manned missions need to be assessed. Recent Earth-based epidemiological studies in survivors of atomic bombs and after occupational and medical low dose radiation exposures have indicated that the cardiovascular system may be more sensitive to ionizing radiation than was previously thought. This has raised the concern of a cardiovascular disease risk from exposure to space radiation during long-distance space travel. Ground-based studies with animal and cell culture models play an important role in estimating health risks from space radiation exposure. Charged particle space radiation has dense ionization characteristics and may induce unique biological responses, appropriate simulation of the space radiation environment and careful consideration of the choice of the experimental model are critical. Recent studies have addressed cardiovascular effects of space radiation using such models and provided first results that aid in estimating cardiovascular disease risk, and several other studies are ongoing. Moreover, astronauts could potentially be administered pharmacological countermeasures against adverse effects of space radiation, and research is focused on the development of such compounds. Because the cardiovascular response to space radiation has not yet been clearly defined, the identification of potential pharmacological countermeasures against cardiovascular effects is still in its infancy.

Key words: Space radiation; Cardiovascular disease risk; Experimental models; Countermeasures; Ionizing radiation

Core tip: This review article provides an overview of studies in experimental models that have begun to shed light on the potential risks of damage in heart and blood vessels after exposure to space radiation.

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INTRODUCTION

Participants of future long-distance space missions will be exposed to significant doses of ionizing radiation in space, and the health risks of these exposures need to be assessed. Because the cardiovascular system has recently been shown to be more sensitive to ionizing radiation than was previously thought, there is current concern that exposure to radiation during long-distance space travel may be associated with a cardiovascular disease risk. This review article provides an overview of studies in experimental models of ionizing radiation exposure relevant to that found in space that have started to shed light on the potential risks for heart and blood vessels.

Characteristics of ionizing radiation

Exposure of living cells and tissues to ionizing radiation, forms of radiation that can remove electrons from the atoms in these cells or tissues, may result in molecular damage, which can eventually lead to early and late injury. Exposure of cells or tissues to ionizing radiation causes DNA damage, which has long been considered as the primary cause of cellular injury and cell death. However, additional mechanisms are now recognized as important in normal tissue radiation injury[1]. Doses of ionizing radiation are indicated in Gray (1 Gy equals 1 Joule of absorbed energy per kilogram of mass, *e.g.*, tissue). Because equal doses of different types of ionizing radiation may not have equal biological effects, one can express radiation exposure as equivalent dose in Sieverts (Sv), which is the absorbed dose multiplied by a unit-less radiation weighting factor and accounts for difference in the biological response[2].

Ionizing radiation can take many forms, including electromagnetic waves and high energy charged particles; the latter deposit their energy along densely ionizing cylindrical tracks. These forms of radiation can be distinguished, among other characteristics, by the amount of energy the radiation transfers to the target material per unit of track length, or linear energy transfer (LET)[2]. Ionizing radiation in the form of electromagnetic waves, such as X-rays or γ-radiation, are considered forms of low-LET radiation and deposit their energy uniformly in target volumes, while high energy charged particles release their energy along dense tracks of ionization and are considered high-LET radiation. Space travel is associated with low-dose-rate exposure to high-LET radiation if the form of galactic cosmic rays (GCR) and occasional high dose rate solar particle events (SPEs)[3].

IONIZING RADIATION AND THE CARDIOVASCULAR SYSTEM

Ionizing radiation has long been known to cause injury in heart and blood vessels. These effects first became apparent from follow-up of patients after radiation therapy, which delivers high doses of low-LET radiation locally to the tumor but in some cases also exposes normal (non-cancer) tissues such as the heart and blood vessels[4-8]. Several previously published review articles[9-11] have provided a comprehensive overview of the effects of low-LET radiation on the cardiovascular system. In short, manifestations of radiation-induced heart disease as a result of exposure to high doses of ionizing radiation include accelerated atherosclerosis, myocardial fibrosis, and cardiac conduction and valve abnormalities. Most deleterious effects in heart and blood vessels are observed years to decades after exposure to ionizing radiation. Therefore, long post-radiation follow-up is required for a full assessment of cardiovascular risk. Mechanisms by which ionizing radiation has its effects in the cardiovascular system are not yet fully known.

Recent reports of health assessments in atomic bomb survivors[12-15] have shown an increased incidence of cardiovascular disease, including ischemic heart disease and stroke, in people several decades after exposure to doses of γ-radiation as low as 2 Gy. Moreover, other epidemiological studies in occupational exposure and low-dose exposure due to medical treatments indicate that cardiovascular disease may occur after lower doses of ionizing radiation than was previously thought[16-20]. The main cardiovascular effects seen in atomic bomb survivors include hypertension and ischemic heart disease, suggesting that after low-dose radiation exposure a vascular component may play a central role in the cardiovascular disease risk.

These recent reports on health effects from exposure to low doses of low-LET radiation have raised the concern about potential risk of cardiovascular disease from exposure to ionizing radiation during space travel[21]. However, care should be taken when the results of terrestrial radiation exposures such as those from atomic bombs are used to support the potential for a cardiovascular disease risk from space radiation, since certain conditions such as dose rate are different

between atomic bomb events and radiation exposure in space. The remainder of this review is focused on studies in experimental models that have aimed to shed light on the cardiovascular risk of exposure to space radiation.

SPACE RADIATION

Characteristics of space radiation

While astronauts in the International Space Station are somewhat protected from exposure to space radiation due to the earth's magnetosphere, future long-distance space travel (beyond low-Earth orbit) will be accompanied by exposure to higher cumulative doses of space radiation, and short-term and long-term health risks need to be assessed[22,23].

GCR and solar emissions are dominated by protons and iron, silicon, oxygen, and carbon that are highly energetic. The greatest particle abundance is found for particles with energies ranging from hundreds of MeV per nucleon (MeV/n) up to about 1 GeV/n[24]. Practical levels of current shielding materials cannot easily protect against these particles[25]. Chronic exposure occurs at a dose rate of 1.3 mGy/d, or the dose equivalent of 4.8 mSv/d, when assuming the radiation weighting factors of the International Commission on Radiological Protection Publication 60 outside the earth's magnetosphere[26,27]. The exposure is characterized by the traversal of most cells in the body by one or more protons and electrons per day, with infrequent traversals (days to weeks) by ions of higher atomic number (Z).

SPEs consist predominantly of protons, and exposure to the largest SPEs occurs at dose rates up to 0.5 Sv/h over hours to a few days[28]. Energies of SPE protons are less than those for GCR and therefore have shorter ranges in material, which may enable effective shielding inside a spacecraft but not inside a thin spacesuit. These higher dose rate exposures may put an astronaut at risk for acute radiation effects, sometimes collectively called acute radiation sickness[29]. Both SPEs and GCR may also cause long-term degenerative disease in various tissues, including the heart and blood vessels.

Experimental data obtained from animal and cell culture models play an important role in estimating health risks from exposure to space radiation. Appropriate simulation of the space radiation environment, including the long-term low-dose rate exposures to various charged particles and the appropriate energy of these particles, and the choice of the most relevant animal or cell culture model are challenging but key to providing relevant estimates of health risks[30-32]. The concern of adverse cardiovascular effects of exposure to space radiation is relatively new, and studies on the cardiovascular effects in animal models of space radiation exposure are not yet abundant. An overview of existing studies on heart and blood vessels is given below. Since much of this work is ongoing, we have had to occasionally refer to proceeding abstracts, but hope to find the results in peer-reviewed publications in the near future.

Cardiac response in animal models of charged particle exposure

Studies in animal models of charged particle exposure have shown cardiovascular effects at doses lower than those required to cause cardiovascular changes if low-LET radiation is used. This may not be surprising, since high-LET radiation typically causes more damage per unit of absorbed dose. Among studies with charged particles, some previous research has focused on the cardiac response to fission spectrum neutrons in animal models[33-36]. More recently, studies were designed to provide answers about the cardiovascular risk from exposure to high-LET radiation in space. Exposure of male C75Bl/6NT mice at 8-10 mo of age to protons (1 GeV, 0.5 Gy) or iron ions (1 GeV/n, 0.15 Gy) induced cardiac infiltration of CD68-positive cells (monocytes and macrophages), increased DNA oxidation, myocardial fibrosis, and modified cardiac function, both at baseline and in response to myocardial infarction, in a radiation-type specific manner[37-39]. Exposure of male CBA/CaJ mice at 10-12 wk of age to silicone ions (300 MeV/n) at doses between 0.1 and 0.5 Gy caused prolonged apoptosis and increased expression of the common pro-inflammatory cytokines interleukin (IL)-1β, IL-6, or tumor necrosis factor-α in the heart[40]. Low doses of high-LET radiation have been shown to cause long-term alterations in DNA methylation in various organ systems *in vivo* and cells in culture[41-43]. Similarly, we recently found changes in cardiac DNA methylation in male C57BL/6J mice exposed at 10 wk of age to protons (150 MeV, 0.1 Gy) or iron ions (600 MeV/n, 0.5 Gy) (Figure 1), suggesting that epigenetic alterations may contribute to the cardiac radiation response[44]. Analysis of the response in individual cardiac cell types is also ongoing[45].

Vascular response in animal models of charged particle exposure

Whole-body exposure of rats to iron ions at doses of 0.5 and 1 Gy induced long-term indications of endothelial dysfunction and increased aortic stiffness[46]. It is difficult to assess the effects of ionizing radiation on atherosclerosis when using regular rodent models, due to the low prevalence of atherosclerosis in these animals. Targeted exposure of the atherosclerotic-prone apolipoprotein E-deficient ($Apo^{-/-}$) mouse model to iron ions (600 MeV/n) at doses of 2 and 5 Gy caused accelerated atherosclerosis in the exposed parts of the aorta[47]. Additional studies with lower doses of particle irradiation may provide a more comprehensive estimate of cardiovascular risk in this mouse model. Studies on adhesiveness of endothelium in charged particle-exposed animal models are also underway[48].

The microvasculature also plays an important role in normal organ function, degenerative tissue effects, and tissue injury from ionizing radiation[49,50]. Exposure of 10-wk old male C57BL/6 mice to iron ions (600 MeV/n) at doses between 0.5 and 2 Gy caused a long-term loss of endothelial cells in the hippocampus[51].

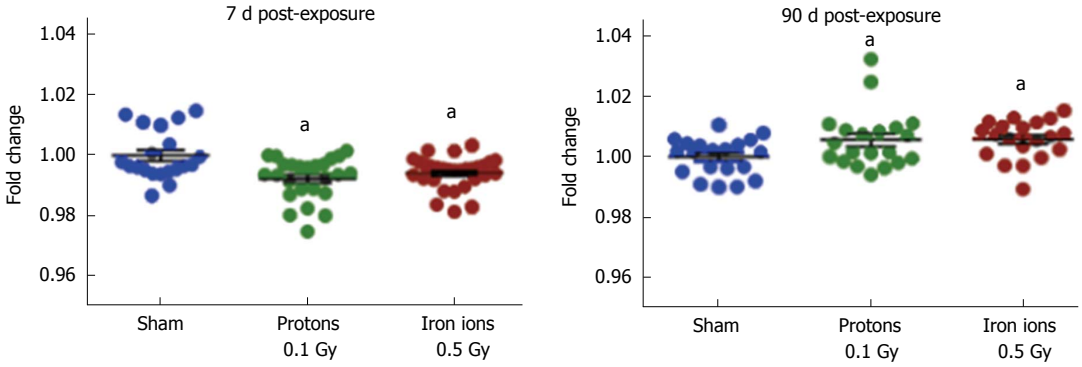

Figure 1 Methylation of genomic DNA isolated from hearts of male C57Bl/6 mice at 7 d and 90 d after exposure to protons (150 MeV, 0.1 Gy) or iron ions (600 MeV/n, 0.5 Gy). DNA methylation of the open reading frame 1 of long interspersed nuclear element-1, a transposable element that comprises about 20% of the mouse genome, as assessed by pyrosequencing and indicated as fold change compared to sham-irradiated controls. Each group contained 4-5 animals. Horizontal lines indicate average ± standard error of the mean. [a]$P < 0.05$ *vs* the sham-irradiated control group.

More research is required to assess the effects of space radiation on the microvasculature.

Studies on charged particle exposure in cells culture models

Endothelial cells are considered to play a central role in the cardiovascular response to ionizing radiation. Endothelial dysfunction, which is characterized by a pro-inflammatory and profibrogenic phenotype of endothelial cells, is a critical contributor to the patho-physiological manifestations of radiation injury[52-54]. Experimental models of exposure to low-LET radiation have shown that ionizing radiation can cause prolonged endothelial dysfunction, thereby sustaining a detrimental tissue environment that leads to chronic inflammation and adverse remodeling[55,56].

Because of the central role of endothelial cells in the radiation response, studies are addressing the effects of space radiation on endothelial cells in cultures[57]. Various tissue-relevant cell culture models are being used[58]. For instance, in three-dimensional culture models of human endothelial cells, protons (1 GeV) and iron ions (1 GeV/n) at doses up to 3 Gy caused alterations in vasculogenesis and endothelial cell death in a radiation-type specific manner[59,60]. These results raise the concern of damage of the human vasculature from exposure to charged particles *in vivo*.

Potential countermeasures against the cardiovascular response to space radiation

Astronauts could potentially be administered pharmacological countermeasures against adverse effects of space radiation, when the countermeasure is safe, stable during long-term space flight, and has a relatively light weight. Therefore, research is focused on the development of countermeasures against various biological effects of space radiation[29]. Interestingly, pharmacological countermeasures are being developed for low-LET radiation in exposure scenarios on earth and may point to potential countermeasures against adverse effects of space radiation. Neupogen [filgrastim, recombinant human granulocyte colony stimulating factor (G-CSF)], for instance, was recently approved by the American Food and Drug Administration as a countermeasure against acute injury from accidental radiation exposure. G-CSF has also been shown to protect animal models against acute injury from exposure to SPE-like protons[61].

Because the cardiovascular response to space radiation has not yet been clearly defined, the identification of potential pharmacological countermeasures against cardiovascular effects is still in its infancy. Nonetheless, similar to the acute response scenario, potential countermeasures against cardiovascular effects of terrestrial radiation exposure, albeit not yet approved for clinical use, may be pursued in space radiation models. For example, the angiotensin converting enzyme (ACE) inhibitor captopril has been shown to reduce cardiac injury in animal models of localized irradiation of the heart[62,63]. In addition, the vitamin E analog γ-tocotrienol is one of the most potent dietary countermeasures to radiation injury currently known. It is safe and nontoxic and has no known drug interactions. It is commercially available, requires no specific storage conditions, and is currently in advanced stages of development for terrestrial applications in radiation protection[64,65]. In addition, γ-tocotrienol has several beneficial effects in the cardiovascular system. It is a potent inhibitor of the cholesterol biosynthesis pathway, thereby reducing the isoprenylation of Rho proteins that modify a wide range of cellular functions, including stress fiber formation, hypertrophy, regulation of NOS, and production of cytokines and growth factors[66]. Indeed, γ-tocotrienol reduces vascular oxidative stress and protects against vascular radiation injury at least in part *via* HMG-CoA reductase inhibition[67,68]. The protective properties of agents such as ACE inhibitors or γ-tocotrienol against cardiovascular effects of space radiation need to be assessed.

CONCLUSION

The cardiovascular system may be more sensitive

to ionizing radiation than was previously thought, which raises the concern of a cardiovascular risk from exposure to ionizing radiation during long-distance space missions. Animal and cell culture models have started to shed light on risk of cardiovascular complications from exposure to charged particle irradiation. Additional studies, including those that employ low radiation doses/dose rates and mixed particle fields to mimic GCR are required to aid in assessing the cardiovascular risk of space radiation.

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MINIREVIEWS

Thrombus aspiration during primary percutaneous coronary intervention for acute myocardial infarction: A review of clinical evidence and guidelines

Muhammad Muzaffar Mahmood, Jonathan Watt, Javed M Ahmed

Muhammad Muzaffar Mahmood, Jonathan Watt, Javed M Ahmed, Cardiology Department, Freeman Hospital, Newcastle-upon-Tyne NE7 7DN, United Kingdom

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Correspondence to: Dr. Javed M Ahmed, Consultant Cardiologist, Cardiology Department, Freeman Hospital, Freeman Road, Newcastle-upon-Tyne NE7 7DN, United Kingdom. javed.ahmed@nuth.nhs.uk
Telephone: +44-191-2137145
Fax: +44-191-2231175

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Abstract

Acute ST segment elevation myocardial infarction (STEMI) is characterized by complete thrombotic occlusion of a major coronary artery. Early recanalization of the infarct-related artery is most efficiently delivered by primary percutaneous coronary intervention (PPCI), however this does not always restore normal myocardial perfusion, mainly due to distal embolization of the thrombus and microvascular obstruction. Early evidence for manual thrombus aspiration during PPCI was promising and this was once considered an important aspect of the procedure, especially in patients with a high thrombus burden. However, a large body of evidence from recent major randomized controlled trials (notably TASTE and TOTAL) does not support the routine use of manual thrombus aspiration in patients with STEMI undergoing PPCI.

Key words: Primary percutaneous coronary intervention; Clinical evidence; Stroke; Acute myocardial infarction; Thrombus aspiration

Core tip: The role of manual thrombus aspiration during primary percutaneous coronary intervention (PPCI) for acute ST segment elevation myocardial infarction (STEMI) has been a matter of significant research and intense debate recently. The rationale for manual thrombus aspiration during PPCI is the removal of intracoronary thrombus, thus avoiding the complication of downstream embolization leading to impaired myocardial perfusion. In this review article, we present the data from early clinical trials and meta-analyses of thrombus aspiration during PPCI, and the more recent evidence from larger multi-center randomized controlled trials that have had a major influence on clinical practice. We highlight the relevant major society guidelines for thrombus aspiration during PPCI and provide the reader with an overview of this technology and its role in contemporary management of STEMI.

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INTRODUCTION

The common pathophysiological mechanism of acute coronary syndrome is sudden disruption of a coronary arterial plaque due to rupture, fissuring or superficial erosion leading to obstructive intracoronary thrombosis. Other less frequent mechanisms include acute plaque expansion, embolism, spontaneous dissection or coronary inflammation[1]. Acute ST segment elevation myocardial infarction (STEMI) is characterized by complete thrombotic occlusion of a coronary artery, with a great potential to cause a large myocardial infarction if not treated promptly. The primary therapeutic goal in STEMI, therefore, is early restoration of normal coronary blood flow, most efficiently delivered by primary percutaneous coronary intervention (PPCI) in combination with adjunctive pharmacological treatment. PPCI aims to achieve myocardial salvage, electrical stability and preserve left ventricular function, improving both early and late outcomes after STEMI.

However, restoration of epicardial coronary artery patency does not always equate with normal myocardial reperfusion. The hallmarks of reperfusion failure despite achieving arterial patency are microvascular obstruction and the no-reflow phenomenon. A high burden of intracoronary thrombus and subsequent distal embolization during PCI are possible major contributors to these events. Myocardial reperfusion failure clinically manifests as persistent ST segment elevation, poor myocardial blush grade (MBG) and low thrombolysis in myocardial infarction (TIMI) flow grade[2].

Earlier investigations revealed that angiographically distal embolization occurred in around 15% of patients undergoing PPCI[3]. Distal embolization was associated with impaired myocardial reperfusion, larger infarct size and an unfavorable prognosis. Further evidence of distal embolization and its impact on myocardial reperfusion is provided by intravascular ultrasound analysis (IVUS). In a study of 35 patients undergoing PCI for myocardial infarction, Kotani *et al*[4] applied volumetric IVUS analysis before and after PPCI to assess the plaque reduction as evidence of distal embolization. Plaque reduction following PPCI was associated with impaired myocardial reperfusion. "The enhanced myocardial efficacy and recovery by aspiration of liberated debris (EMERALD)" trial investigators, while investigating a distal balloon occlusion and aspiration system, demonstrated that visible debris was retrieved in 73% of the patients undergoing PPCI[5]. Avoidance of distal embolization is hence a considerable therapeutic challenge during STEMI.

PHARMACOLOGICAL AND MECHANICAL MEANS OF REDUCING THROMBUS

Pharmacological agents (especially glycoprotein Ⅱb/Ⅲa inhibitors), mechanical thrombectomy devices, embolic protection devices and manual aspiration thrombectomy catheters have been investigated over the past couple of decades as adjunctive therapies during PPCI with the aim of reducing thrombus burden and subsequent distal embolization. Glycoprotein Ⅱb/Ⅲa inhibitors inhibit the final common pathway of platelet activation and are a useful adjunct to PPCI, albeit with an increased risk of bleeding. While theoretically attractive, the clinical value of mechanical thrombectomy and embolic protection devices during PPCI is unproven, after several negative trials. Manual thrombus aspiration (thrombectomy) during PPCI is the focus of this review article.

A major technical advantage of a manual thrombus aspiration device is its simplicity, consisting of a monorail catheter containing a central lumen that connects one or more large holes at the distal end to an aspiration syringe at the proximal end. The commonly used aspiration devices in clinical practice are Export® (Medtronic, MN, United States), Eliminate™ (Terumo), Pronto™ (Vascular solutions, MN, United States) Diver™ CE (Invatec, Italy), QuickCat (Spectranetics Inc, United States) and Hunter® (IHT Cordynamic, Barcelona, Spain). All these devices are formed on the same principle and convincing clinical advantage of one particular device over the other is lacking.

CLINICAL EVIDENCE

Randomized controlled trials

A number of studies, including randomized clinical trials and subsequent meta-analyses have evaluated the clinical efficacy of routine manual thrombus aspiration during PPCI. In the initial "randomized evaluation of the effect of mechanical reduction of distal embolization by thrombus-aspiration in primary and rescue angioplasty (REMEDIA)" trial, 100 patients with STEMI were randomized to PPCI with or without manual thrombus aspiration (Diver™ CE). More patients in the manual thrombus aspiration group achieved MBG 2 or more and ST segment resolution (STR) of 70% or more (46% *vs* 25%)[6]. In "Thrombectomy with Export Catheter in Infarct-Related Artery During Primary Percutaneous Coronary Intervention" (EXPIRA) trial, 175 patients with STEMI were randomized to PCI with or without manual thrombus aspiration. The primary end points of MBG 2 or more (88% *vs* 60%) and STR of 70% or more (64% *vs* 39%) occurred more often in PCI with thrombus aspiration group compared with standard PCI. Patients in the aspiration group had less microvascular obstruction and smaller infarcts[7]. After 24 mo, major adverse cardiac events were 4.5% *vs* 13.7% and cardiac death was 0% *vs* 6.8%, respectively, in patients with PCI with manual thrombus aspiration compared with standard

PCI[8].

INFUSE-AMI was a multicenter, single-blind trial of 452 patients presenting within 4 h of anterior STEMI undergoing PPCI with bivalirudin who were randomized in a 2 × 2 factorial design to bolus intracoronary abciximab *vs* no abciximab and to manual thrombus aspiration (using the Export® catheter) *vs* no thrombectomy. To maximize the likelihood of demonstrating a reduction in infarct size, enrollment was limited to the patients with proximal or mid LAD occlusion and baseline TIMI 0-2 flow. There was no significant difference in the infarct size at 30 d, the primary end point, in the thrombus aspiration *vs* no aspiration arm (17% *vs* 17.3% respectively, $P = 0.51$) as assessed by cardiac magnetic resonance imaging[9].

Thrombus aspiration during primary percutaneous coronary intervention trial

The first large randomized controlled trial (RCT) evaluating use of manual thrombus aspiration (Export® catheter) during PPCI was "thrombus aspiration during primary percutaneous coronary intervention (TAPAS)". In this single-center all-comers RCT, 1071 patients with STEMI were randomized, to either thrombus aspiration during PCI or standard PCI alone, prior to coronary angiography. The primary end-point was the post-procedural frequency of a MBG of 0 or 1. All patients received standard pharmacological therapy including the glycoprotein Ⅱb/Ⅲa inhibitor abciximab, unless contraindicated. Ninety-two percent patients underwent stent implantation in both groups. A MBG of 0 or 1 occurred less frequently in the thrombus aspiration group compared with the conventional PCI group (17% *vs* 26%, $P < 0.001$). Complete ST-segment resolution was more frequent in the manual thrombus aspiration group (56% *vs* 44%, $P < 0.001$). Atherothrombotic material was retrieved in 73% of the patients in thrombus aspiration group. Clinical outcomes at 30 d, including the rate of death and major adverse cardiac events, were significantly related to the MBG and ST-segment resolution. Rates of target vessel revascularization were similar between the two groups[10]. A 1-year follow-up study showed reduced rates of cardiac death (3.6% *vs* 6.7%) and cardiac death or non-fatal reinfarction (5.6% *vs* 9.9%) in the thrombus aspiration group[11]. The benefit of manual thrombus aspiration was irrespective of vessel size, infarct-related coronary artery or visible thrombus on the angiogram. A total ischemic time of less than 180 min was associated with a trend towards increased benefit ($P = 0.09$). Angiographically proven acute stent thrombosis (< 24 h) occurred with a similar frequency between both groups (0.2%) but subacute (1-30 d) and late stent thrombosis (> 30-365 d) was observed less frequently in the thrombus aspiration cohort (RR = 0.5, 95%CI: 0.19-1.32). The findings of TAPAS form the basis for major society guidelines recommending manual thrombus aspiration as an adjunct for PPCI. The trial, however, was criticized for being underpowered for clinical events and susceptibility to selection bias (single center study).

Thrombus aspiration in STEMI in Scandinavia and the Trial of Routine Aspiration Thrombectomy with PCI vs PCI alone in patients with STEMI trials

TASTE trial: The above inconsistent results were followed by the two major randomized controlled trials in the field, thrombus aspiration in STEMI in Scandinavia (TASTE) and the Trial of Routine Aspiration Thrombectomy with PCI *vs* PCI alone in patients with STEMI (TOTAL). TASTE was a multi-center (29 PCI centers in Sweden, 1 each in Iceland and Denmark), randomized study that utilized the platform of population-based "Swedish coronary angiography and angioplasty registry". A total of 7244 STEMI patients were randomized to PCI with manual thrombus aspiration or standard PCI alone[12]. The primary end point of all-cause mortality at 30 d was not different between the two groups (2.8% for thrombus aspiration with PCI *vs* 3% for PCI alone, $P = 0.63$). The majority of patients in TASTE had a low thrombus burden (thrombus grade 0-3). Bailout thrombus aspiration was performed in 4.9% patients assigned to PCI alone. The 30-d rates of secondary end-points (hospitalization for recurrent myocardial infarction, target-vessel revascularization, target-lesion revascularization, stent thrombosis and the composite of all-cause mortality or recurrent myocardial infarction) were not statistically different. The rate of stroke or neurological complication was identical (0.5%) in each group. The incidence of stent thrombosis, although statistically not significant, was lower (0.2% *vs* 0.5%, $P = 0.06$, HR = 0.47, 95%CI: 0.20-1.02) in the thrombus aspiration group. Similarly, hospital length of stay, incidence of heart failure or left ventricular dysfunction were all unaffected by manual thrombus aspiration. The failure to influence the primary end-point was consistent across all subgroups, including patients with diabetes, previous myocardial infarction, smokers and various measures of ischemic time. Outcomes in TASTE were similar irrespective of the infarct-related coronary artery, intra-arterial culprit segment (proximal *vs* non-proximal), TIMI flow grade before PPCI, use of glycoprotein Ⅱb/Ⅲa drugs and importantly thrombus burden. All-cause mortality at 1 year was a pre-specified secondary end-point of the study, which later reported no benefit of thrombus aspiration across all the major subgroups[13]. There were concerns that TASTE was underpowered to detect a difference in its primary end-point and also for its registry-based design (it was the first major trial ever to use this concept) with no separate, dedicated data monitoring and adjudicating set-up.

TOTAL trial: The most recent and so far the largest trial evaluating the benefit of manual thrombus aspiration in PPCI is TOTAL. This multi-center, prospective, randomized controlled trial assigned 10732 patients with acute STEMI to routine upfront manual aspiration thrombectomy *vs* PCI alone[14]. Almost 80% patients

had a high thrombus burden as assessed by TIMI thrombus grade 4 or 5. The primary outcome (composite of death from cardiovascular causes, recurrent myocardial infarction, cardiogenic shock, or New York Heart Association class Ⅳ heart failure within 180 d) was similar between the two groups (6.9% in the thrombus aspiration group *vs* 7% in the PCI alone group). The key safety outcome of stroke within 30 d occurred more frequently in the thrombectomy group compared to PCI alone group (0.7% *vs* 0.3%, *P* = 0.02). Within 180 d, stroke had occurred in 1% of patients with thrombectomy *vs* 0.5% in those without. The incidence of definite stent thrombosis within 180 d was similar between both groups (1.3% for thrombectomy *vs* 1.4%, *P* = 0.72). Bailout manual thrombus aspiration was performed in 7.1% patients originally assigned to PCI alone. As noted in TASTE, the negative primary trial outcome was consistent across all pre-specified subgroups, including those with high thrombus burden, initial TIMI flow, time of symptom onset and anterior *vs* non-anterior myocardial infarction. The strength of the trial was the study design and the large study population. Concerns were raised towards potential selection bias of a lower-risk population (in view of lower than expected event rates for the primary outcome) and bailout thrombus aspiration in PCI alone group[15] The finding of increased incidence of stroke in the thrombectomy group is potentially significant, however, the absolute number of stroke events was small. The trial was also underpowered to detect a difference in stroke. It is possible that the higher risk of stroke in the thrombus aspiration group was not directly related to the thrombectomy procedure, supported by the observation that the increased stroke risk was not confined to the periprocedural period.

Observational studies: In a single-center retrospective analysis of 2567 consecutive STEMI patients treated with PPCI, aspiration thrombectomy (*n* = 1095, using Export catheter in 93%) was associated with improved post-procedure TIMI 3 flow as well as reduced in-hospital (adjusted OR = 0.51, 95%CI: 0.29-0.93, *P* = 0.027) and long-term (adjusted HR = 0.69, 95%CI: 0.48-0.96, *P* = 0.028) mortality rates (4.5% *vs* 9.0%), over a mean follow-up of 9.9 mo. The study identified that the mortality benefit of thrombus aspiration was driven by results in patients with a total ischemic time of less than 180 min[16]. However, critics of the study called the extent of mortality reduction excessive and implausible[17].

In a retrospective observational cohort study of 10929 STEMI patients treated with PPCI at 8 centers across London, United Kingdom, manual aspiration thrombectomy (32.7%, *n* = 3572) was associated with a higher procedural success rate (90.9% *vs* 89.2%; *P* = 0.005) and lower in-hospital major adverse cardiac event rates (4.4% *vs* 5.5%; *P* = 0.012). However, no significant differences in the primary outcome of all-cause mortality were evident between patients with or without manual thrombus aspiration (14.8% *vs* 15.3% respectively; *P* = 0.737) during the median follow-up of 3 years[18].

Meta-analyses: A pooled analysis of 2686 patients enrolled in 11 thrombectomy trials (7 trials using manual aspiration devices such as TAPAS and EXPIRA and 4 non-manual devices trials) similarly concluded that thrombectomy (especially manual aspiration thrombectomy) significantly improves clinical outcomes, including lower all-cause mortality, in STEMI patients undergoing PPCI[19]. However, the suggestion of improved clinical outcome with thrombectomy was questioned by a meta-analysis of 21 trials (including 16 with manual thrombus aspiration devices) involving 4299 PPCI treated STEMI patients which concluded that adjunctive thrombectomy, despite improving the early markers of myocardial reperfusion, does not significantly affect 30-d mortality, reinfarction or stroke[20]. A meta-analysis of 21 trials involving 4514 patients (50% randomized to thrombectomy, either manual or mechanical) concluded that while both types of thrombectomy did improve myocardial perfusion, a trend towards short-term mortality benefit was evident only with manual aspiration. The meta-analysis also observed a trend towards higher risk of stroke with thrombectomy (*P* = 0.06)[21]. Another meta-analysis of PPCI-treated STEMI patients included data from 25 trials, including 18 trials with manual aspiration thrombectomy; this study suggested that use of manual thrombus aspiration, but not mechanical thrombectomy, was associated with reduced major adverse cardiovascular events, including mortality, at 6 to 12 mo. A trend towards a higher risk of stroke was noted with mechanical thrombectomy[22].

Unlike the previous meta-analyses, two recent meta-analyses have included data from the large TASTE trial however both were performed before the publication of the largest and most reliable trial investigating the use of manual thrombus aspiration in PPCI (TOTAL). A recent meta-analysis of 26 PPCI randomized trials in 11943 patients (thrombus aspiration *n* = 5969, PCI alone *n* = 5974) and a weighted maximum follow-up duration of 10.4 mo concluded that the routine unselected use of adjunctive thrombus aspiration during PPCI does not significantly reduce all-cause mortality (polled RR = 0.88; 95%CI: 0.74-1.04; *P* = 0.124), reinfarction, target-vessel revascularization or definite stent thrombosis. Although thrombus aspiration was noted to be associated with reductions in failure to achieve TIMI 3 flow, MBG 3, incomplete ST-segment resolution and distal emobolization, these effects were less obvious among the larger, higher quality recent trials. The risk of stroke was noted to be similar between both groups[23]. In another recent meta-analysis of 16 randomized trials in PPCI including 10518 patients (thrombus aspiration *n* = 5256, PCI alone *n* = 5262), routine use of manual thrombus aspiration compared to PCI alone did not reduce the rate of all-cause mortality (6.6% *vs* 7.4% respectively, *P* = 0.149), reinfarction, target vessel

revascularization/target lesion revascularization and stent thrombosis. The rate of stroke was similar between the two groups (0.5% *vs* 0.5%, P = 0.819). Thrombus aspiration was associated with improved rates of post-procedural TIMI 3 flow, MBG 2-3 and ST-segment resolution[24].

GUIDELINES

The 2014 ESC/EACTS guidelines on myocardial revascularization suggest that while routine use of manual thrombus aspiration is not essential in patients undergoing PPCI for STEMI, selected use may be useful to improve TIMI 3 flow or prevent stent thrombosis. Thrombus aspiration in selected patients during PPCI has a class Ⅱb indication (level of evidence A). These guidelines take into account the evidence including the TASTE trial but predate the publication of TOTAL trial, so far the largest trial addressing this question[25].

The 2013 ACCF/AHA Guideline for the Management of ST-Elevation Myocardial Infarction consider manual thrombus aspiration reasonable in patients undergoing PPCI. Thrombus aspiration in a PPCI setting is a class Ⅱa indication in these guidelines (level of evidence B). These guidelines predate the publication of TASTE and TOTAL trials[26].

CONCLUSION

The success of PPCI for STEMI is marred by suboptimal myocardial reperfusion, despite achieving epicardial coronary patency, mainly secondary to distal embolization of the thrombus and microvascular obstruction. Early evidence for manual thrombus aspiration during STEMI was promising and this was once considered an important aspect of PPCI, especially in patients with a high thrombus burden. However, recent clinical evidence from major randomized controlled trials (notably TASTE and TOTAL) does not support the routine use of manual thrombus aspiration in patients with STEMI undergoing PPCI.

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MINIREVIEWS

Inequalities in care in patients with acute myocardial infarction

Shabnam Rashid, Alexander Simms, Phillip Batin, John Kurian, Chris P Gale

Shabnam Rashid, Alexander Simms, Department of Cardiology, Leeds General Infirmary, West Yorkshire LS1 3EX, United Kingdom

Phillip Batin, Department of Cardiology, Pinderfields General Hospital, West Yorkshire WF1 4DG, United Kingdom

John Kurian, Department of Cardiology, Bradford Royal Infirmary, West Yorkshire BD9 6RJ, United Kingdom

Chris P Gale, Department of Cardiology, University of Leeds, Leeds LS2 9JT, United Kingdom

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Correspondence to: Shabnam Rashid, MBChB, BSc, Department of Cardiology, Leeds General Infirmary, Great George Street, Leeds, West Yorkshire LS1 3EX, United Kingdom. shabnamrashid@doctors.org.uk
Telephone: +44-793-9976651
Fax: +44-113-3925751

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Abstract

Coronary heart disease is the single largest cause of death in developed countries. Guidelines exist for the management of acute myocardial infarction (AMI), yet despite these, significant inequalities exist in the care of these patients. The elderly, deprived socioeconomic groups, females and non-caucasians are the patient populations where practice tends to deviate more frequently from the evidence base. Elderly patients often had higher mortality rates after having an AMI compared to younger patients. They also tended to present with symptoms that were not entirely consistent with an AMI, thus partially contributing to the inequalities in care that is seen between younger and older patients. Furthermore the lack of guidelines in the elderly age group presenting with AMI can often make decision making challenging and may account for the discrepancies in care that are prevalent between younger and older patients. Other patients such as those from a lower socioeconomic group, *i.e.*, low income and less than high school education often had poorer health and reduced life expectancy compared to patients from a higher socioeconomic group after an AMI. Lower socioeconomic status was also seen to be contributing to racial and geographical variation is the care in AMI patients. Females with an AMI were treated less aggressively and had poorer outcomes when compared to males. However even when females were treated in the same way they continued to have higher in hospital mortality which suggests that gender may well account for differences in outcomes. The purpose of this review is to identify the inequalities in care for patients who present with an AMI and explore potential reasons for why these occur. Greater attention to the management and a better understanding of the root causes of these inequalities in care may help to reduce morbidity and mortality rates associated with AMI.

Key words: Coronary artery disease; Dual antiplatelet

therapy; Inequalities; Guidelines; Myocardial infarction

Core tip: Coronary heart disease is the leading cause of death in developed countries. Guidelines exist for the management of acute myocardial infarction (AMI), yet despite these, significant inequalities exist in patient care. The elderly, deprived socioeconomic groups, females and non-Caucasians are the patient populations where practice tends to deviate from the evidence base. The purpose of this review article is to identify the inequalities in those who present with an AMI and explore potential reasons for this. Greater attention to the management and a better understanding of the root causes of these inequalities may help to reduce morbidity and mortality rates.

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INTRODUCTION

Coronary heart disease is the leading cause of death in the world and accounted for 7.4 million deaths in 2012[1]. In Europe the mortality per annum from coronary heart disease is 20%[2] and 25% in the United States[3]. Guidelines exist to aid with the management of patients who present with acute myocardial infarction (AMI)[4-7] yet inequalities in the management and outcomes of these patients are clearly apparent.

The World Health Organisation defines inequality in health care as avoidable inequalities in health between groups of people within countries and between countries[8]. Factors that drive care inequality can be defined as biological for instance age and gender, socioeconomic, race/ethnicity or geographical. In this review article, we describe the inequalities in care for patients presenting with AMI and explore the potential reasons for inequity in AMI care.

Age differences

Life expectancy is increasing in the United Kingdom and much of the developed world. From 2003 to 2010 life expectancy increased from 76.5 to 78.1 years for males and 80.9 to 82.1 years for females. AMI accounted for the majority of deaths in the United Kingdom in 2012 especially in those individuals over the age of 85 years[9]. Age is a risk factor for AMI and poorer prognosis thereafter, and with the rising life expectancy this will lead to a greater number of patients presenting with AMI with potentially greater morbidity and mortality[9-11].

Data from the Myocardial Ischemia National Audit Project (MINAP), a multicentre clinical registry for patients who have been hospitalised with a myocardial infarction in England and Wales demonstrated that in-hospital mortality in 2010 following a myocardial infarction was 20.4% in those $\geqslant$ 85 years compared to 0.9% in those < 55 years old[10,11]. In part, greater frailty and co-morbidity in older patients explain this variation in outcome. However, the provision of evidenced based care to older patients was significantly lower than that provided to younger patients following an AMI. For instance, MINAP data showed that up to 75% of patients $\geqslant$ 85 years presenting with a ST segment myocardial infarction (STEMI) were less likely to receive thrombolysis or primary percutaneous coronary intervention (PPCI) compared to those < 55 years[11,12]. Furthermore, although evidence suggests benefit in the use of anti-platelets and statins in the elderly following an AMI[13], the use of such therapies are not equivocal across age groups. For example, in-hospital use of aspirin for those < 65 years was 95% *vs* 87% in those $\geqslant$ 85 years of age[14,15]. Thus, strategies targeting improved adherence to evidenced based treatments in the elderly may narrow the inequality in outcomes.

Nevertheless, outcomes after an AMI have improved over time across all age groups. This is partly due to better therapeutic options and treatments, such as, PPCI for STEMI that has a better safety profile in elderly patients compared to thrombolytic therapy[16]. In 2004 PPCI rates for all elderly STEMI patients was 2.0% *vs* 36.1% in 2009. During the same time period, 11.5% and 25.5% of elderly patients had coronary angiography following AMI. The rate of prescription of secondary medications increased in all age groups but a greater rate of change was observed in those $\geqslant$ 85 years compared to those < 55 years old from 2003 to 2010: 28% to 89% *vs* 56% to 97%, respectively[12].

In addition to higher mortality following an AMI, elderly patients have more frequent complications and greater risk of physical de-conditioning compared to younger patients[17]. Indeed, the latter can be improved by interventions such as cardiac rehabilitation (CR) programmes, yet the overall rates of participation in CR are relatively low[18,19] especially in the elderly[20,21]. Greater awareness by the caring Physician of the benefits of CR might help improve care and outcomes post AMI as recommendation by a Physician was a strong predictor of CR uptake[21].

The better evidenced based care provided to younger patients following an AMI might explain the continued variation in outcome compared to older counterparts. Understanding the reasons for this inequality is paramount if care and outcomes are to be improved. It is likely that the reasons for this variation are multifactorial. For instance, elderly patients have a greater degree of co-morbidity such as anaemia, cerebrovascular disease and dementia which potentially provides a further barrier to the use of AMI therapies[14,22]. For instance, older patients with dementia had lower rates of evidenced based treatment than elderly patients without dementia, 44% *vs* 62%, respectively. Such variation may occur as

patients with dementia often need prompting to ensure compliance with medications. Non-compliance with dual antiplatelet therapy after percutaneous coronary intervention (PCI) may lead to significant implications such as a further AMI. Advanced dementia may signify to the caring Physicians that evidence based therapies have less long term benefit and subsequently have a higher threshold to prescribe such treatments. Other factors such as frailty may preclude invasive treatment[23] as there is the perception that the patient is likely to gain more harm than benefit from advanced treatments. Clinicians may be reluctant to prescribe dual antiplatelet therapy or list patients for cardiac catheterisation in the presence of anaemia[24], more commonly seen in the elderly. Such clinical concerns highlight the potential risk of therapies and may encourage decisions that are risk adverse, aiming to do no harm in the first instance.

There is little evidence base to help guide care decisions in elderly patients with AMI. For instance, advanced age is often an exclusion criteria in coronary heart disease trials[25]. Only 9% of all trials included those $\geqslant$ 75 years and 2% included those $\geqslant$ 85 years of age. The elderly patients included in these trials had less risk factors for coronary heart disease, fewer co-morbidities, better kidney function and haemodynamics on presentation to hospital compared to similar aged patients that are seen in real life practice[14]. This highlights a lack of clarity in the evidence base for managing older patients and underpins further the uncertainty in treating patients in the more conventional way seen in younger patients with an AMI.

Additionally, elderly patients are more likely to present with atypical symptoms of an AMI. For example, only 40% of those $\geqslant$ 85 years with an AMI had chest pain compared to 77% of those < 65 years. Indeed, they were more likely to present with dyspnoea (49%) but less commonly with nausea and vomiting (24%) or syncope (19%)[14]. Due to the atypical presentation, these patients are often misdiagnosed and therefore not receive timely recommended therapies which maybe contributing to the greater morbidity and mortality[26]. Furthermore, 40% of patients with an AMI $\geqslant$ 85 years of age did not have diagnostic electrocardiogram changes compatible with AMI compared to 25% of patients < 65 years[14]. Even when the diagnosis of AMI has been established, those > 80 years were less likely to be admitted under cardiology care compared to patients < 65 years of age (39.1% *vs* 64%, respectively)[27], despite evidence to suggest care by a cardiologist improved outcomes[25].

Guidelines recommend using risk scoring systems to identify those individuals at high risk after an AMI[5-7,28,29] and who may potentially benefit from invasive and aggressive therapies. Clinicians often subjectively assess individuals risk by taking into account other co morbidities that are not incorporated into clinical risk scores[30]. There is poor correlation between the perceived risk judged by physicians and actual validated risk scores[31]. Equally age is a major driver of heightened risk in risk scores, such as, the Global Registry of Acute Coronary Events (GRACE) and thus most elderly patients would be in a high risk group post AMI[32]. This suggest that physician decisions are potentially influenced by other factors not represented in the risk scores, such as perceived frailty, and highlights the difficult in driving optimum AMI care in the elderly.

With the aging population, it is likely that the number of elderly patients presenting with AMI will increase. This will have a significant impact on morbidity and mortality as well as health care resources. Several societies including the American College of Cardiology, American Heart Association and the European Society of Cardiology guidelines on non STEMI (NSTEMI) advocate the use of intensive and early interventional therapies in high risk groups[5-7], such as the elderly, who are likely to achieve better outcomes if therapies for AMI are advocated, although this is confounded by the lack of a strong evidence base. Thus, uncertainty of risk *vs* benefit of AMI therapies in elderly patients makes decisions around the use of effective therapies difficult. In part, unmodifiable risk factors associated with age drive the disparity in outcomes post AMI between young and old. However, whilst inequalities in care exist across age groups there is still potential to narrow the gap in adverse outcomes by improved provision of evidence based care to older patients post AMI.

Socioeconomic factors

The socioeconomic status of patients can be defined according to the patient's occupation, income wealth, education or where they live[33]. Lower socioeconomic status, *i.e.*, low income, less than high school education, is a key determinant of inequality in care and results in these individuals experiencing poor health and reduced life expectancy. Studies from Sweden, Finland, Canada and the United States have found that prognosis is worse after an AMI in patients from a lower socioeconomic status group[34-36]. In a study conducted in Finland, a higher number of patients with lower socioeconomic status had AMI's and lower prescription of secondary prevention medication compared to the higher socioeconomic group. These patients often presented later to hospital with chest pain[21,36] and higher numbers were treated in urban hospitals compared to their counterparts who were treated in specialist hospitals[36]. This may have been because specialist centres were situated in more affluent areas. In addition poorer patients were likely to refuse invasive procedures[37]. Mortality rates were also significantly higher in patients with a low income and basic education. Males with a low income had a 28 d mortality rate of 49.5% compared with 14.5% of those with higher income. In males who had a basic level of education 28 d mortality rate was 80.3% and 19.7% in those with higher education. A similar trend was also seen in females[36]. This suggests that low income and potentially a lack of understanding of physical health contributes to these findings.

In the United States, patients of a lower socioe-

conomic status were less likely to proceed to a coronary angiogram within 24 h of a STEMI compared to those with a higher socioeconomic status (69.5% *vs* 73.7%) or within 48 h of a NSTEMI (47.6% *vs* 51.8%). Probable reasons included, those from a lower socioeconomic group were less educated about their co-morbidities, did not have consistent medical records and were unable to obtain anti-platelets therapies reliably. Therefore physicians often spent longer trying to establish if there were any contraindications to anti-platelet therapy. In contrast, individuals from a higher socioeconomic status had higher expectations for early treatment to be instituted when presenting with chest pain. They also tended to receive more frequently drug eluting stents, possibly because their level of insurance covered the cost of the procedure. However, the perception of the operating physician, that a patient was of a higher socioeconomic status, was independently related to higher drug eluting stent use than level of insurance as this was not checked prior to procedure in all patients[38].

Poor health was further contributed by factors such as occupational stress, social isolation and depression which are seen more frequently in the lower socioeconomic groups[35]. On the other hand, some therapies were equally provided across socioeconomic groups. For example, there were no significant differences in patients referred for coronary artery bypass grafting (CABG) post AMI in the United States. It was speculated that patients of a lower socioeconomic status were likely to have severe and more complex coronary artery disease making them appropriate for CABG rather than PCI. This would also resolve the problem of compliance with dual antiplatelet therapy which is required post PCI[38]. On the other hand, if the lower socioeconomic group had coronary disease more suitable for CABG, one would expect the referral rate for CABG to exceed the higher socioeconomic group. However, rates of CABG referral between the two groups were comparable. This might suggest that either those of lower socioeconomic class were under-referred for CABG or those in higher socioeconomic were over-referred for CABG.

Intriguingly, MINAP data from 2003 to 2007 in England and Wales, suggested that there was no socioeconomic differences in the management of patients with AMI[39]. This is likely explained by differences in the healthcare systems with the United States being predominantly paid for through medical insurance whilst in the United Kingdom, a nationally funded service offers universal access to care at the point of need.

Racial and ethnic factors

Within racial and ethnic groups there is variation in the way AMI patients are treated. In the United States, the national registry of myocardial infarction from 1994-2002 showed that black (not well defined as to whether these were Afro-Caribbean patients or black United States patients) patients received less coronary reperfusion therapy and coronary angiography compared to Caucasian patients[37,40,41]. Compared to Caucasians, Afro-Caribbean females were less aggressively treated and had higher in hospital mortality[40]. Reasons for such differences were unclear, but may be related to socioeconomic factors rather than race alone.

Data from hospital discharges in the state of Pennsylvania between 2003 and 2004 showed that 46% of Caucasian patients underwent PPCI compared to 40% of African Americans because more than often African American patients presented later to hospitals at which point the benefit of PPCI had elapsed[37].

In Singapore and Malaysia, ethnic variations in the treatment of patients with an AMI were prevalent. Ethnicity is defined as groups of people who identify with each other based on social and cultural experience. Malays had the least invasive treatment and had the highest mortality rate after an AMI compared to the Indians and the Chinese[42,43]. The level of education and household income may have contributed to these differences. In 2000, less than 5% of Malays in Singapore progressed to higher education compared to nearly 20% of Chinese and Indians[43]. Education and income both act together to enhance health and reduce the need for health care. Provision of education may serve as a key strategy to reduce disparities in AMI care.

In the United Kingdom there is a paucity of data regarding AMI care between different racial groups.

Gender differences

Studies in the United States, Switzerland, United Kingdom and France demonstrate gender differences in AMI treatment. PCI rates were lower in females compared to males (14.2% *vs* 24.4% respectively)[44]. Females that presented with an AMI were generally older than men with greater co-morbidities and presented later to hospital[45,46]. The latter point may drive some of the variation seen with PCI rates as late presenters would derive less benefit. Furthermore, females with an AMI more frequently had non obstructive coronary atheroma therefore precluding the need for any interventional therapy[44]. There is also evidence to suggest that females are less inclined to consent to coronary angiography compared to males[47].

Similar findings were seen in England, with females compared to males, less likely to be given thrombolytic therapy (37% *vs* 46%), aspirin (83% *vs* 90%), have angiography, exercise testing or revascularisation. However when adjusted for age these inequalities were less apparent but poor outcome was statistically higher in females than males yet, despite females being higher risk partly due to age and co-morbidity they were treated less aggressively than males[46,48,49]. However, it is difficult to know if this now represents contemporary practice as this data precedes 2000.

Even when females were treated in the same way as males there was still higher in hospital mortality despite correction by age, co-morbidities, haemodynamic status and time to treatment. Mortality rates in females were 2.3 times higher in comparison to males (7.9% *vs* 2.3% respectively). Furthermore hypotension and shock was

more prevalent in females despite the degree of left ventricular systolic impairment being the same in the male group. This suggests that gender in itself may account for the differences in outcomes[50]. Inequalities in care between the genders are not fully understood and like elderly patients, females have historically been under represented in clinical trials.

Geographic variation

GRACE looked at the management of patients with AMI from 95 hospitals from 14 countries including Europe, North and South America, Australia and New Zealand. Aspirin and ACE inhibitor use was similar across all regions with over 91% receiving Aspirin on admission. There was geographical variation in the discharge use of statin, ranging from 26% to 57%. This was due to the uncertainty about the benefit of statins acutely. Furthermore the United States appeared to use more Glycoprotein Ⅱb/Ⅲa inhibitors compared to other countries, explained by United States GRACE centres having more direct access to coronary angiography facilities[51]. Post AMI 30 d and 1 year mortality varied in 458 hospitals across 24 countries, 5.0% to 13.9% and 4.9% to 14.8%, respectively. However, patient level factors, such as socioeconomic status accounted for most of this variation (96% to 99%) whilst hospital level factors at most accounted for 4% of variation in post AMI outcome[48]. Similar findings were reported in other studies[52,53].

Old practice data from 1998 comparing AMI treatments in the United States and the United Kingdom revealed that coronary angiography was performed in 61% *vs* 22% of cases respectively. United States patients were more likely to receive coronary revascularisation, 69% *vs* 41%, respectively, although the extent of coronary disease was similar between the two patient groups. The greater availability in coronary angiography and revascularisation in the United States may have accounted for these findings at the time. There were no significant differences in the primary end points of recurrent angina, myocardial infarction and death in the United States (29%) compared to the United Kingdom (25%)[54]. It is not clear how differences in the two healthcare systems and how they are funded affected the variation in care provided to AMI patients.

Geographical variation in AMI care is likely driven by several factors including the economical strength of countries or the way healthcare systems are funded, which makes comparisons difficult.

CONCLUSION

Inequalities in the treatment of an AMI are described with regards to age, socioeconomic factors, race, gender and geographical location. Age is known to be a risk factor for an AMI and with the aging population more patients are predicted to have an AMI, resulting in a significant impact on morbidity, mortality and healthcare resources. The inequality in care between younger and older patients suggests that older patients may still gain a survival benefit by equalising the disparity in care by simple measures such as ensuring guideline recommended care being provided to more elderly patients. There is an increasing need for further research to guide optimum care of elderly patients post AMI. Clinicians taking a more proactive role in the treatment of these patients may further narrow the gap between the young and the old. A similar model in the care of elderly patients following orthopaedic surgery has been successful with the evolution of the Ortho-geriatrician. Females had the highest mortality and given that they make up 50% of the global population it is imperative that treatment is equalised. Further research is required to help understand the inequality of care that exists amongst females and ultimately guide further AMI management.

Further discrepancies were seen between the higher and lower socioeconomic groups with the latter experiencing poor healthcare. Furthermore, Lower socioeconomic status probably accounted for geographical and racial variation. Socioeconomic status is strongly linked to education which also potentially allows the understanding and prevention of illness, control of risk factors and compliance to medications as well as a determinant of higher income. This would therefore suggest that education is a fundamental component but outside the influence of the medical sphere.

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MINIREVIEWS

Predicting mortality in patients with acute heart failure: Role of risk scores

Andrea Passantino, Francesco Monitillo, Massimo Iacoviello, Domenico Scrutinio

Andrea Passantino, Domenico Scrutinio, Division of Cardiology and Cardiac Rehabilitation, "S. Maugeri" Foundation, IRCCS, Institute of Cassano Murge, 70020 Bari, Italy

Francesco Monitillo, Massimo Iacoviello, Cardiology Unit and Cardiothoracic Department, University Hospital, Policlinico Consorziale, 70124 Bari, Italy

Author contributions: Passantino A and Monitillo F performed the literature search, wrote the text and drew tables; Iacoviello M and Scrutinio D reviewed the text and made critical corrections and revisions until the submitted version was achieved.

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Correspondence to: Andrea Passantino, MD, Division of Cardiology and Cardiac Rehabilitation, "S. Maugeri" Foundation, IRCCS, Institute of Cassano Murge, km 2 Strada per Mercadante, 70020 Bari, Italy. andrea.passantino@fsm.it
Telephone: +39-08-07814293
Fax: +39-08-07814280

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Abstract

Acute heart failure is a leading cause of hospitalization and death, and it is an increasing burden on health care systems. The correct risk stratification of patients could improve clinical outcome and resources allocation, avoiding the overtreatment of low-risk subjects or the early, inappropriate discharge of high-risk patients. Many clinical scores have been derived and validated for in-hospital and post-discharge survival; predictive models include demographic, clinical, hemodynamic and laboratory variables. Data sets are derived from public registries, clinical trials, and retrospective data. Most models show a good capacity to discriminate patients who reach major clinical end-points, with C-indices generally higher than 0.70, but their applicability in real-world populations has been seldom evaluated. No study has evaluated if the use of risk score-based stratification might improve patient outcome. Some variables (age, blood pressure, sodium concentration, renal function) recur in most scores and should always be considered when evaluating the risk of an individual patient hospitalized for acute heart failure. Future studies will evaluate the emerging role of plasma biomarkers.

Key words: Acute heart failure; Prognosis; Scoring; Risk stratification; Outcome

Core tip: We present a review of the most relevant scores developed for the risk stratification of patients hospitalized for acute heart failure. For each score, the strengths, weaknesses, statistical pertinence and applicability in a real-world situation are evaluated. Furthermore, we revisit the general criteria and statistical metrics that should be considered in the design and analysis of prognostic studies.

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INTRODUCTION

Acute heart failure (AHF) is a complex and heterogeneous clinical syndrome defined as the rapid onset or change in symptoms and signs of heart failure requiring immediate medical attention and urgent therapy[1]. It is a leading reason for hospitalization and is burdened by high short-(intra-hospital) and long-term (6 to 12 mo) mortality.

Most often, the first triage of patients with AHF is performed in the emergency department (ED), where these patients present to receive initial care. Then, on the basis of the clinical profile and risk stratification, patients are discharged, admitted to a medical ward or cardiac division, or transferred to an intensive care unit. At the end of hospitalization, a structured follow-up is planned to reduce the risk of early rehospitalization (a major issue in health care system) and improve long-term survival.

Therefore, the risk stratification of patients with AHF is a pivotal medical task aimed to improve the outcome of patients with AHF and the efficiency of the health care delivery system. Physicians involved in the care of acute heart failure patients should be able to evaluate the risk profile especially in two critical turning points: (1) at the time of hospital admission, for choosing the best hospital setting according to the risk profile and for identifying low-risk patients who can be safely discharged to home, thus pursuing both the best outcome of patients and the correct allocation of resources; and (2) at the time of discharge, for planning disease management of patients for a given risk profile, and for the selection of patients suitable for more advanced therapies.

Physicians always determine an initial prognosis by integrating the patient's characteristics, clinical signs and laboratory tests. The prediction is inherently multivariable; however, the relative weight that a doctor assigns to each variable, which relies on his clinical judgment, previous experiences, personal beliefs and, eventually, on his current mood, could be inaccurate and misleading.

Even the most skilled physician might incorrectly estimate the risk of death in heart failure patients or be uncertain about prognosis[2]. Furthermore, the precision of risk estimate based on clinical judgment might be reduced by the urgency of making a critical decision in the case of more severe clinical scenarios[3-5]. An incorrect prognosis might generate a mismatch between intensity of care and the risk profile of the patient.

Risk score are multivariable predictive models in which relative weights are assigned to each variable in order to calculate the probability that a specific event (death, rehospitalization) will occur in the future. They are tools that help doctors estimate prognosis in a more unbiased way, translating the result of prognostic studies in clinical practice.

Beyond the benefit to an individual patient, the research of valid prognostic models is fundamental for public health policy, for comparative effectiveness and health service research, for quality of care outcome assessment, for health technology assessment of therapies and laboratory tests, and for studying new approaches, mechanisms and targets for clinical trials[6].

Methodological issue and critical points of risk stratification of AHF patients

A risk model is the final output of prognostic research, which is a three-step course that calls for (1) development studies aimed to identify relevant predictors entering the model and their relative weights. In this phase the performance of the models estimated by evaluating the calibration and the discrimination, and the model is adjusted for overfitting; an internal validation should be performed by bootstrapping techniques in the same population from which the model is derived; (2) external validation studies, in which the model is validated in new populations; and (3) impact studies designed to evaluate if the decision making for a single patient, driven by the risk status assigned according to the predictive model, could improve clinical outcome[7].

Correct statistical metrics should be used for reporting prognostic studies. To measure the ability of a model to discriminate patients for a binary outcome, the C-statistic (equivalent to the area under the receiver operating characteristics curve) is calculated[8]; it ranges from 0.50 (no discrimination) to 1 (perfect discrimination).

Calibration measures the correlation between observed and predicted events, and it is generally assessed with the Hosmer-Lemeshow statistic[9]. Recently, the standardization of reporting of a multivariable prediction model has been proposed[10]. Many reasons make the development of a prognostic score in the setting of acute heart failure a challenging task.

The validity of a risk score depends on the population from which it is derived and on the choice of the variables; AHF syndromes include different clinical scenarios: (1) decompensated HF and worsening chronic heart failure; (2) pulmonary oedema; (3) cardiogenic shock; (4) hypertensive HF; and (5) right HF[11]. Moreover, each class could undergo a further classification; for example, worsening chronic heart failure patients could have preserved or reduced ejection fraction as well. It is unlikely that the same prognostic model could fit miscellaneous clinical patterns, as each of one is endowed with peculiar physiopathological aspects.

Another relevant issue is the source of the data set from which the model is derived. Community-based settings and clinical trial populations are often very divergent; the latter generally includes younger people with a lower rate of co-morbidities that might have a relevant role in driving prognosis, especially in older

Table 1 Main risk scores in acute heart failure

Risk score	Data source	Publication year	Sample size (derivation)	Sample size (validation)	Validation	Model development	Endpoint
In-hospital mortality							
ADHERE[15]	Registry	2005	33046	32229	External	Classification trees	In-hospital mortality
AHFI[16]	Statewide databases	2005	33533	8384	External	Classification trees	In-hospital mortality and complications
OPTIMIZE-HF[19]	Registry	2008	37548	181830	Internal/external	Logistic regression model	In-hospital mortality
GWTG-HF[20]	Registry	2010	27850	11933	Internal/external	Logistic regression model	In-hospital mortality
EHMRG[21]	Population-based cohort	2012	7433	5158	Internal/external	Logistic regression model	7 d mortality
PROTECT[23]	Clinical trial	2012	2015	1435	Internal/external (clinical trial population)	Cox proportional hazards model	Composite endpoint of death, worsening heart failure or heart failure rehospitalization
Post-discharge mortality							
EFFECT[24]	Community	2003	2624	1407	Internal/external	Logistic regression model	30 d mortality/1 yr mortality
OPTIME-CHF[25]	Clinical trial	2004	949	-	Internal	Cox proportional hazards model	60 d mortality
OPTIMIZE-HF[26]	Registry	2008	4402	949/433	Internal/external (clinical trial population)	Cox proportional hazards model	60-90 d post-discharge mortality
APACHE-HF[27]	Community (single centre)	2014	824	-	-	Cox proportional hazards model	90 d mortality
ELAN[28]	Pooled data of seven cohorts	2014	1301	325	External (clinical trial population)	Cox proportional hazards model	180 d mortality
ADHF/NT-proBNP[29]	Community	2013	453	371	External	Logistic regression model	1 yr mortality
ESCAPE[30]	Clinical trial	2010	433	471	Internal/external (clinical trial population)	Cox proportional hazards model	6 mo mortality

ADHERE: Acute decompensated heart failure national registry; AHFI: Acute heart failure index; OPTIMIZE-HF: Organized program to initiate lifesaving treatment in hospitalized patients with heart failure; GWTG-HF: Get with the guidelines-heart failure; EHMRG: Emergency heart failure mortality risk grade; PROTECT: Placebo-controlled randomized study of the selective A1 adenosine receptor antagonist rolofylline for patients hospitalized with acute decompensated heart failure and volume overload to assess treatment effect on congestion and renal function; EFFECT: Enhanced feedback for effective cardiac treatment; OPTIME-CHF: Outcomes of a prospective trial of intravenous milrinone for exacerbations of chronic heart failure; APACHE-HF: Acute physiology and chronic health evaluation in heart failure; ADHF/NT-proBNP: Acutely decompensated heart failure n-terminal pro-brain natriuretic peptide; ESCAPE: Evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness; ELAN: European collaboration on acute decompensated heart failure.

populations. The external validity of a model derived from clinical trial is, at minimum, controversial.

Another critical point is the choice of variables used to calculate the score. A huge number of determinants of survival for AHF have been studied; many variables have been associated with prognosis in univariate and multivariate analysis, including clinical characteristics, hemodynamic markers, serum biomarkers, and medication use[12]. If a stepwise selection is used, then the availability of so many variables could lead to the inclusion of too many parameters in the model, causing overfitting, with the model generating random error or noise and resulting in a spurious prognostic association.

A model that has been overfit will have a poor predictive performance in other populations. Parsimony in the number of parameters and developing the simplest model with the highest accuracy are proper ways to improve the applicability of the model to other populations[13]. In AHF syndrome, clinical, laboratory and hemodynamic variables might suddenly change during the clinical course. Some variables could be associated with short-term improvement but worse long-term survival (for example, use of inotropic drugs); therefore, the timing of data collection and the timeline for the endpoint survey are pivotal.

RISK SCORES

Several prognostic models combining different variables have been developed to predict in-hospital mortality and to estimate outcomes between 30 d up to 6 mo post-discharge. Table 1 summarizes methodological characteristics of the risk scores, Table 2 the variables entering different models, and Table 3 the models' performances.

In-hospital risk models

Acute decompensated heart failure national

Table 2 Variables used in the risk score models

Risk score	Variables
ADHERE[15]	BUN, creatinine, SBP
AHFI[16]	Gender, CAD, diabetes, lung disease, SBP, HR, respiratory rate, temperature, blood urea nitrogen, sodium, potassium, white blood cell count, acute myocardial infarction o myocardial ischemia at ECG, pulmonary congestion or pleural effusion on radiographic examination
OPTIMIZE-HF[19]	Creatinine, sodium, age, HR, liver disease, previous CVA/TIA, peripheral vascular disease, race, left ventricular systolic dysfunction, COPD, SBP, previous HF hospitalization
GWTG-HF[20]	Older age, low SBP, elevated heart rate, presence of COPD, and non-black race
EHMRG[21]	HR, creatinine, systolic blood pressure initial oxygen saturation, serum troponin
PROTECT[23]	BUN, respiratory rate, HR, albumin, cholesterol, diabetes, previous HF hospitalization
EFFECT[24]	Age, SBP, BUN, sodium concentration, cerebrovascular disease, dementia, COPD, hepatic cirrhosis, cancer, hemoglobin
OPTIME-CHF[25]	Age, BUN, SBP, sodium, NYHA class
OPTIMIZE–HF[26]	Age, weight, SBP, serum creatinine, history of liver disease, history of depression history of reactive airway disease
APACHE-HF[27]	Mean blood pressure, HR, serum sodium, serum potassium, creatinine, haematocrit, Glasgow coma scale, age
ELAN[28]	NT-proBNP at discharge, NT-proBNP reduction, age, peripheral oedema, SBP, sodium, serum urea, NYHA class
ADHF/NT-proBNP risk score[29]	COPD, SBP, eGFR, serum sodium, hemoglobin, NT-proBNP; left ventricular ejection fraction, tricuspid regurgitation
ESCAPE[30]	Age, BUN, six-minute walk test, sodium, CPR/mechanical ventilation, diuretic dose at discharge, no-blocker at discharge, BNP

BUN: Blood urea nitrogen; SBP: Systolic blood pressure; CAD: Coronary heart disease; HR: Heart rate; CVA: Cerebral vascular accident; COPD: Chronic obstructive pulmonary disease; CPR: Cardiopulmonary resuscitation; eGFR: Estimated glomerular filtration rate; NT-proBNP: N-terminal brain natriuretic peptide; ADHERE: Acute decompensated heart failure national registry; AHFI: Acute heart failure index; OPTIME-CHF: Outcomes of a prospective trial of intravenous milrinone for exacerbations of chronic heart failure; EHMRG: Emergency heart failure mortality risk grade; EFFECT: Enhanced feedback for effective cardiac treatment; OPTIMIZE-HF: Organized program to initiate lifesaving treatment in hospitalized patients with heart failure; PROTECT: Placebo-controlled randomized study of the selective A1 adenosine receptor antagonist rolofylline for patients hospitalized with acute decompensated heart failure and volume overload to assess treatment effect on congestion and renal function; GWTG-HF: Get with the guidelines-heart failure; APACHE-HF: Acute physiology and chronic health evaluation in heart failure; ADHF/NT-proBNP: Acutely decompensated heart failure n-terminal pro-brain natriuretic peptide; ESCAPE: Evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness; ECG: Electrocardiogram; TIA: Transient ischemic attack; ELAN: European collaboration on acute decompensated heart failure.

Table 3 Performances of risk scores

Risk score	Calibration	C-statistic (derivation cohort)	C-statistic (validation cohort)	Low-risk group mortality (%)	High-risk group mortality (%)
In-hospital mortality					
ADHERE[15]	NV	0.75	0.75	2.1	21.9
AHFI[16]	NV	NA	0.59	0.3	NA
OPTIMIZE-HF[19]	NV[1]	0.75	0.746	NA	NA
GWTG-HF[20]	Calibrated	0.75	0.75	0.4	9.7
EHMRG[21]	Calibrated	0.80	0.803	0.3	8.2
PROTECT[23]	Calibrated	0.67	0.67	4.8[1]	28.7[2]
Post-discharge mortality					
EFFECT[24]	Calibrated	0.80 (30 d) 0.77 (1 yr)	0.79 (30 d) 0.76 (1 yr)	0.4 (30 d) 7.8 (1 yr)	59 (30 d) 78.8 (1 yr)
OPTIME-CHF[25]	NV[1]	0.77	0.76	2	30
OPTIMIZE-HF[26]	NV	0.72	NA	NA	NA
APACHE-HF[27]	NV	0.78			20[2]
ELAN[28]	NV	0.77	NA	3.6	51.1
ADHF/NT-proBNP risk score[29]	Calibrated	0.84	0.77	3.7	89.5
ESCAPE[30]	NV[1]	0.76	0.65[3]	7	100

[1]A graphic plot of the predicted *vs* observed probability of outcome was reported; [2]Relative risk of death in the high-risk group *vs* the low-risk group; [3]In the validation cohort, the model did not include brain natriuretic peptide and diuretic dose. NA: Not available; NV: Calibration was not verified by statistical tests; ADHERE: Acute decompensated heart failure national registry; AHFI: Acute heart failure index; OPTIMIZE-HF: Organized program to initiate lifesaving treatment in hospitalized patients with heart failure; GWTG-HF: Get with the guidelines-heart failure; OPTIME-CHF: Outcomes of a prospective trial of intravenous milrinone for exacerbations of chronic heart failure; EHMRG: Emergency heart failure mortality risk grade; EFFECT: Enhanced feedback for effective cardiac treatment; BNP: Brain natriuretic peptide; ADHF/NT-proBNP: Acutely decompensated heart failure n-terminal pro-brain natriuretic peptide; ESCAPE: Evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness; PROTECT: Placebo-controlled randomized study of the selective A1 adenosine receptor antagonist rolofylline for patients hospitalized with acute decompensated heart failure and volume overload to assess treatment effect on congestion and renal function; ELAN: European collaboration on acute decompensated heart failure.

registry: The "acute decompensated heart failure national registry" (ADHERE) provides a risk stratification model to predict in-hospital mortality in patients admitted with acutely decompensated heart failure[14]. The authors analysed the clinical, demographic and biochemical data of 33046 patients from the Acute Decompensated Heart Failure National Registry in order to develop a risk stratification model. The model

was prospectively tested using data from 32229 hospitalizations, which comprised the validation cohort. Statistical analysis revealed that blood urea nitrogen (BUN) level of 43 mg/dL or higher was the best single predictor for mortality. The second best predictor was admission systolic blood pressure (SBP) < 115 mmHg. Serum creatinine levels of 2.75 mg/dL or higher provided additional prognostic value in patients with BUN levels ⩾ 43 mg/dL and SBP ⩽ 115 mmHg. The authors employed the CART method to derive a risk tree identifying acutely decompensated heart failure (ADHF) patients at low, intermediate and high risk for in-hospital mortality in the validation cohort. Heart rate and age did not improve the risk stratification of patients in the final algorithm.

Finally, ROC curves were used to assess the accuracy of the models. The study provided a useful and validated tool for mortality risk stratification by employing signs and laboratory data evaluated on hospital admission. The combination of two different markers of renal function confirms the established link between the heart and kidney and thus the association between clinical outcomes and markers of renal function[15]. Mortality in the low- and high-risk group was 2.1% and 22%, respectively.

The ADHERE algorithm was derived from a real-world population, the model was adequately validated in an additional cohort of patients, and it meets parsimony criteria requiring only three variables, which are easily measured at the time of hospital admission. A major criticism of the ADHERE algorithm is that the registry entries reflect individual hospitalizations, and repeated hospitalizations of the same patient are entered as separated records. This is a clear violation of the fundamental research principle of independence of experimental units, which limits the internal validity of the study. Another limit is the overly high mortality of the low-risk group in comparison with other models. However, the ADHERE algorithm might allow for immediate and simple triage at admission in the emergency department, not requiring complex calculations.

AHF index: Auble *et al*[16] analysed 33533 patients admitted from the ED with a diagnosis of heart failure. The authors derived a prediction rule to identify patients at low-risk of in-hospital death and serious medical complications. The proposed prediction rule resulted from a combination of demographic, biochemical and non-invasive diagnostic tools.

The performance of this algorithm, named the AHF index, was further examined, and the index was validated in an independent group of 8383 patients admitted to the ED with heart failure, with respect to inpatient mortality, serious medical complications before hospital discharge, and 30 d mortality. The mortality rates in the low-risk group were significantly higher in the validation cohort compared to the two derivation cohorts (0.7%-1.7% *vs* 0.3%)[17,18].

Organized program to initiate lifesaving treatment in hospitalized patients with HF: Beginning with an analysis of a national hospital-based registry and quality improvement program [organized program to initiate lifesaving treatment in hospitalized patients with HF (OPTIMIZE-HF) registry], predictors of in-hospital mortality were identified, and a practical risk-prediction tool of in-hospital mortality that is applicable in routine clinical practice for patients hospitalized for heart failure was derived. The identification of the most important predictors from the multivariate logistic regression analysis allowed the development of a point scoring system to predict in-hospital mortality. The ability of the logistic regression model to discriminate mortality was tested by a classification and regression tree (CART) analysis. The model combined multiple variables, and the final risk-prediction normogram included age, heart rate, SBP, serum creatinine, serum sodium, primary cause of admission (heart failure or other), and left ventricular systolic dysfunction. For each value of each variable, a score associated with the probability of in-hospital mortality is calculated. The model had a good performance, with a C-statistic of 0.75; however, no validation of the score has been reported[19].

Get with the guidelines-HF: Another useful risk model has been provided by the American Heart Association's "get with the guidelines-heart failure" programme. The score combines clinical variables to predict in-hospital mortality. The programme involved 39783 patients, with a derivation sample of 27850 and a validation sample of 11933 patients, and can be applied to heart failure patients, with both preserved and reduced left ventricular ejection fraction. The proposed score combined 7 clinical factors routinely collected at the time of admission. The 7 predictor variables (older age, low SBP, elevated heart rate, presence of chronic obstructive pulmonary disease, and non-black race) were identified in the multivariate model. The estimation of in-hospital mortality can be carried out by summing points assigned to each predictor, with a total score ranging from 0 to 100. The inclusion of race among the predictors might limit the application of the model in different countries. The risk score had good discrimination: C-index was 0.75 in both derivation and validation data set.

In-hospital mortality in the lower and higher risk group was 0.4% and 9.7%, respectively. The model was thought to be helpful in patient triage and in the use of evidence-based therapy in the highest-risk patients, reducing resource allocation in those at low risk[20].

Emergency heart failure mortality risk grade: Lee DS *et al*[21] proposed a multivariate risk index for 7-d mortality using initial vital signs, clinical and presenting features and readily available laboratory tests, with the aim of predicting acute mortality and guiding acute clinical decision making for patients with HF who present to the ED. The derivation cohort was comprised of 7433

patients, and the validation cohort was comprised of 5158 patients. The authors developed the "emergency heart failure mortality risk grade" (EHMRG), which comprises multiplicative and additive variables with an available online calculator. The EHMRG encompassed all patients presenting to the ED, regardless of whether they were hospitalized or discharged, providing a useful tool to guide hospitalization-*vs*-discharge decisions based on prognosis. A higher heart rate and creatinine concentration, a lower SBP and oxygen saturation, and non-normal serum troponin levels were associated with an increased mortality risk and were entered into the score. The area under the receiver-operating characteristic curves of the model was 0.805 for the derivation data set and 0.826 for the validation data set. Despite the fact that left ventricular ejection fraction and natriuretic peptide analysis have been validated as predictive variables in both acute and chronic heart failure, they were not included in the model because they are not frequently assessed in the ED.

Placebo-controlled randomized study of the selective A1 adenosine receptor antagonist rolofylline for patients hospitalized with acute decompensated heart failure and volume overload to assess treatment effect on congestion and renal function: The international "placebo-controlled randomized study of the selective A1 adenosine receptor antagonist rolofylline for patients hospitalized with acute decompensated heart failure and volume overload to assess treatment effect on congestion and renal function" (PROTECT) trial enrolled 2033 patients hospitalized with AHF and mild or moderate impairment of renal function[22]. Of the 2033 patients, 2015 had complete data for the analysis, and a risk score was developed for predicting the composite endpoint (death, worsening heart failure, rehospitalization for HF) at 7 d; points assigned to each predictor were summed, for a total point score ranging from 0 to 100 points. All variables employed were collected within 24 h of admission. The strongest predictor of the outcome was higher BUN concentration. Other predictors of an adverse outcome were lower values of serum albumin, serum cholesterol, and SBP, as well as higher heart and respiratory rates. The variables employed in the model demonstrate the role of metabolic status, neurohormonal activation and reduced cardiac performance in influencing patient outcomes. The model underwent an external validation in a study population of another clinical trial; the C-index in the derivation and validation population was 0.67.

The study population of the derivation data set was enrolled in the trial with strict inclusion and exclusion criteria: Patients taking inotropic agents and those with severe pulmonary disease, recent ischemia or preserved ejection fraction were not included; therefore, the applicability of the PROTECT risk score to a wide range of community-based populations is limited[23].

Post-discharge risk models

In addition to prediction of in-hospital mortality, attempts to assess short-, medium- and long-term prognosis, as well as the risk of events, in patients hospitalized for AHF, has led to the proposal of different risk models.

Enhanced feedback for effective cardiac treatment: The "enhanced feedback for effective cardiac treatment" study analysed multiple variables available at the time of hospital presentation of more than 4000 patients hospitalized for heart failure. The authors identified predictors of mortality, and they developed and validated a model that could predict all-cause 30 d and 1 year mortality. Age, lower SBP, higher respiratory rate, higher BUN level, hyponatremia, and co-morbidities were independent predictors of mortality at both 30 d and 1 year. Very low risk scores (< 60) identified patients with a mortality rate of 0.4% at 30 d and 7.8% at 1 year. Patients with very high-risk scores (> 150) had a mortality rate of 59% at 30 d and 78.8% at 1 year. The authors suggested the importance of assessing selected variables during the first hours of hospital presentation in order to help the physician to identify patients with a high risk of events and optimize patient management[24].

Outcomes of a prospective trial of intravenous milrinone for exacerbations of chronic heart failure: The data from the "outcomes of a prospective trial of intravenous milrinone for exacerbations of chronic heart failure" (OPTIME-CHF) study were analysed to develop a model predicting the post-discharge outcome of inpatients hospitalized for acute decompensated heart failure[25].

A multivariate model allowed the assessment of variables predictive of mortality or the composite endpoint of death and re-hospitalization at 60 d. Age, lower SBP, New York Heart Association class Ⅳ symptoms, elevated BUN, and decreased sodium were predictors of death at 60 d. The C-index for mortality at 60 d was 0.77. As for other models derived from clinical trials, the populations used to derive the models represent only a subgroup of AHF patients; the study populations have consisted entirely of patients with reduced ejection fraction, whereas patients with renal dysfunction or who required inotropes were excluded from the studies.

OPTIMIZE-HF: O'Connor et *al*[26] developed a clinical model predictive of short-term clinical outcome in patients discharged after hospitalization for HF. The authors employed logistic regression analysis that initially included 45 potential variables and finally identified 8 significant risk factors to predict the risk of mortality within 60 d after discharge, with a C-index of 0.72. Co-morbidities (liver disease, depression, reactive airway disease) have a major role in the score.

In addition to the risk score, the study confirmed the

importance of evidence-based therapies prescribed at discharge; β-blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blocker and lipid-lowering therapies were associated with decreased mortality and rehospitalization.

Acute physiology and chronic health evaluation in HF: This score, constructed by Okazaki *et al*[27] includes all factors significantly predictive of survival after discharge and assigns one point for each factor. The parameters considered in the scoring system are the mean blood pressure, pulse, sodium, potassium, creatinine, haematocrit, age and glasgow coma scale; these parameters exhibited a high sensitivity and specificity and an adequate area under the curve. The score was able to predict all-cause death or readmission due to heart failure at 90 d. The study did not include NYHA class, left ventricular ejection fraction, BUN, hemoglobin and brain natriuretic peptide (BNP), which has been found to be predictive of prognosis in previous studies. Acute physiology and chronic health evaluation in HF has other major limitations: It was derived from a single centre population, all patients were admitted to an intensive care unit for respiratory or circulatory support, and the score has not been validated.

European collaboration on acute decompensated-HF: The data from seven cohorts of prospective studies of patients admitted due to acutely decompensated heart failure were pooled by Salah *et al*[28] to develop a predictive discharge score based on different predictors of mortality, including the absolute value at discharge and percentage reduction of NT-proBNP. The European collaboration on acute decompensated-HF score assigned one point for each factor but 3 points for n-terminal (NT)-proBNP values at discharge ranging from 5001 to 15000 pg/mL and 4 points for values > 15000 pg/mL. The score showed that the absolute values of NT-proBNP at discharge and the percentage reduction during hospitalization, combined with other established risk markers, might improve the risk stratification for adverse events within 180 d after discharge.

ADHF/NT-proBNP risk score: Confirming the relevance of natriuretic peptide measurements in patients with acutely decompensated heart failure, Scrutinio *et al*[29] studied the improvement in the risk reclassification of patients with AHF by adding NT-proBNP to other common clinical variables. The authors proposed the ADHF/NT-proBNP risk score, with a possible total score ranging from 0 to 22. The score proved to be effective in predicting one-year mortality in patients hospitalized for acutely decompensated heart failure, providing clinicians with a validated and easy-to-use predictive tool in daily clinical practice. Adding NT-proBNP to the reference model did not improve discrimination, but resulted in significant risk reclassification.

Evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness: The evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness (ESCAPE) trial enrolled 433 patients hospitalized with ADHF, and it analysed the relationship between clinical factors at discharge and 6 mo mortality. The aim of the analysis was to create a score, potentially useful to identify patients at high and low risk of recurrent events. Among the variables analysed, a high discharge BNP level showed the strongest association with death. The proposed score included 8 variables, with 1 point possible for each variable, except for BUN and BNP, for which additional points were assigned for the highest value, with a maximum 13 possible points. The C-index for 6 mo mortality was 0.78 in the derivation data set, but it was reduced to 0.65 in the validation population[30].

CLINICAL APPLICATIONS AND FUTURE DIRECTIONS

The great number of validated prognostic models, each combining different variables, suggests how difficult it is to estimate risk in patients with AHF. Nevertheless, efforts to develop risk models are justified by the evidence that the risk of in-hospital mortality, early post-discharge mortality, and re-hospitalization remains high[31]. Approximately 12%-15% of patients hospitalized for AHF die within 12 wk, and 30% of these patients die within 12 mo of admission[32].

The accurate estimation of risk is essential for proper in-hospital and post-discharge treatment plans and outpatient follow-up. Nevertheless, despite all the proposed prognostic models, the clinical application remains challenging, and clinical scores are not considered part of the standard of care[33].

A major limit of the risk scores approach is that these tools evaluate a "class risk", that is to say, the risk of a cohort of patients sharing common characteristics. In addition, the scores' applicability in evaluating the risk of an individual patient remains elusive. Lemeshow demonstrated that valid predictive models might produce markedly different prognosis for an individual[34], suggesting that they should not be used for individual patient decision making. Due to the great number of prognostic variables, the discordance between prognosis for an individual by different scores might be substantial.

Risk stratification by scoring methods should support rather than replace medical judgment in the clinical decision making process concerning the single patient. Physicians involved in the care of patients with AHF should be familiar with a number of risk scores and should choose the most suitable on the basis of the patient's profile according to the characteristics of the derivation population of the score.

Beyond the evaluation of an individual patient, risk scores are useful tools for managing the process of care, defining diagnostic and therapeutic pathway,

and identifying possible subjects to include in a clinical trial. In patients with chronic advanced heart failure, the Heart Failure Survival Score was able to identify medium- and high-risk patients who benefit from heart transplantation in comparison with a low-risk group in which heart transplantation was not associated with a survival benefit[35]. Currently, no study has evaluated if allocation of patients, driven by risk status according to a predictive model, could improve the clinical outcome in acute heart failure, and currently, no pharmacological intervention has been able to reduce mortality in AHF. Appropriate risk stratification could allow targeting of patients who could benefit from established or new therapies.

Even if the phenotypic heterogeneity of AHF patients makes difficult to find a risk model suitable for all patients, some parameters recur in most of the models. Age, low blood pressure, reduced cardiac performance, low sodium renal concentration due to neurohormonal activation, and decreased renal function are included in most risk models.

Notably, baseline renal dysfunction is a relevant predictor of short and long-term outcome in AHF patients. Worsening renal function, which occurs in 20%-30% of patients hospitalized for AHF, is associated with a poor outcome[36], and the possible role of new therapies for AHF in patients with worsening renal function has recently been investigated[37].

Regarding biomarkers, the role of natriuretic peptides is well-known, and it has a significant prognostic value at both baseline and discharge. Nevertheless, new plasma biomarkers are continuously being identified and validated but have yet to enter in clinical practice[38-41]. In the MOCA trial, biomarkers such as sST2, MR-proADM, natriuretic peptides and CRP provided incremental value for risk stratification of ADHF patients when added to a clinical variables-based model. Further studies are needed to determine if a multi-marker strategy could improve the prognosis and outcome of acute heart failure patients[42].

How to choose a risk score? To choose a risk score, statistical and methodological pertinence should be evaluated. Models have a high grade of evidence when they are derived from large community or registry populations, when they have been validated in an external population, and when they show good discrimination (c-statistic > 0.70) in both derivation and validation cohorts; eventually, adequate calibration is crucial.

Clinicians should be suspicious of risk models derived from clinical trials and that were not validated in an external population and that were not calibrated. Risk models in which in-hospital mortality is the outcome must be used at the time of hospital admission. Obviously, when patients with AHF are admitted to the emergency department, risk stratification based on models with few easily measurable variables is preferred. Risk models that evaluate long-term mortality are useful during hospitalization and at discharge to plan the follow-up or to select patients for advanced therapies.

CONCLUSION

Scores for the risk stratification of AHF patients are useful tools that might support, not replace, clinical judgment and supply a rational approach for prognostication of the individual patient. Further studies are necessary to evaluate if the outcome of patients with acute heart failure can be improved with the use of these tools.

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MINIREVIEWS

Current status of high on-treatment platelet reactivity in patients with coronary or peripheral arterial disease: Mechanisms, evaluation and clinical implications

Stavros Spiliopoulos, Georgios Pastromas

Stavros Spiliopoulos, Georgios Pastromas, Department of Interventional Radiology, Patras University Hospital, 26504 Rio, Greece

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Correspondence to: Stavros Spiliopoulos, MD, PhD, EBIR, Department of Interventional Radiology, Patras University Hospital, Ippokratous Avenue, 26504 Rio, Greece. stavspiliop@upatras.gr
Telephone: +30-261-3603219

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Abstract

Antiplatelet therapy with aspirin or clopidogrel or both is the standard care for patients with proven coronary or peripheral arterial disease, especially those undergoing endovascular revascularization procedures. However, despite the administration of the antiplatelet regiments, some patients still experience recurrent cardiovascular ischemic events. So far, it is well documented by several studies that *in vitro* response of platelets may be extremely variable. Poor antiplatelet effect of clopidogrel or high on-treatment platelet reactivity (HTPR) is under investigation by numerous recent studies. This review article focuses on methods used for the *ex vivo* evaluation of HTPR, as well as on the possible underlying mechanisms and the clinical consequences of this entity. Alternative therapeutic options and future directions are also addressed.

Key words: Coronary disease; Clopidogrel; Aspirin; High on treatment platelet reactivity; Peripheral arterial disease; Antiplatelet therapy; Ticagrelor; Prasugrel

Core tip: Recent data related high on-treatment platelet reactivity (HTPR) with adverse clinical outcomes, such as stent thrombosis and repeat procedures, following coronary or peripheral endovascular revascularization procedures. Notably, the incidence of patients suffering from peripheral arterial disease demonstrating inadequate response to clopidogrel is around 50%, which is much higher than the approximately 30% reported for patients suffering from coronary artery disease. Novel more potent antiplatelet P2Y12 agents seem to overcome the phenomenon of HTPR decreasing ischemic events with the cost of increased bleeding risk. Until today no major trial demonstrated clinical improvement for patients undergoing platelet function test-guided individualized antiplatelet therapy. Prescription of new antithrombotic agents aims in avoiding major cardiovascular adverse events, as well as sustaining vessel patency following revascularization. Therefore, improving antiplatelet therapy, considering the risk/benefit ratio, is imperative

especially in HTPR patients. Further large-scale studies are awaited to elucidate the role of individualized therapy.

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INTRODUCTION

Thrombus generation resulting from platelet activation and aggregation is the established main process involved in atherosclerotic vascular disease, including coronary artery disease (CAD) and peripheral arterial disease (PAD)[1,2].

Therefore, antiplatelet therapy has been the cornerstone therapy in patients with documented arterial disease, especially in those undergoing coronary or peripheral percutaneous endovascular procedures[3-5]. However, a variable amount of these patients continue to experience recurrent ischemic events[6,7]. This clinical phenomenon has been correlated with various parameters among which poor antiplatelet effect of clopidogrel or aspirin, described by consensus as high on-treatment platelet reactivity (HTPR) initially identified in patients with CAD[8,9]. HTPR in patients with PAD, especially those undergoing percutaneous peripheral angioplasty (PTA) has recently been documented by several studies[10,11].

This review focuses on the clinical significance of HTPR, the possible mechanisms and the common tests used to measure the phenomenon, as well as future perspectives of novel antiplatelet agents and platelet function-guided antiplatelet therapy.

HTPR DEFINITION

Despite the fact that the optimal method to define HTPR has not been clarified in the literature the clinical challenge of inter-individual variability of the inhibitory effect of antiplatelet agents on platelet function, initially named non-responsiveness or resistance, should be definitely considered as failure of the antiplatelet drug to inhibit its target of action[12]. HTPR has been strongly associated with an increased incidence of major adverse cardiovascular events (MACEs) especially for patients on clopidogrel. Clopidogrel is an adenosine diphosphate (ADP)-receptor antagonist that obstructs platelet activation and aggregation by irreversibly binding to both ADP receptors (P2Y1 and P2Y12)[13]. Therefore, the basic principles of assessing HTPR are to quantify the activity of the target receptor after administration of the antiplatelet agent by using a laboratory method and to determine consensus HTPR cut-off values for various assessment methods[14].

MEASURING HTPR

Numerous tests are available for measuring HTPR. Light transmission aggregometry is the most well established laboratory method for the determination of HTPR. It evaluates the response of the platelet to ADP agonist as an increase in light transmittance measuring as maximal platelet aggregation. However, because it is time and labor intensive, today it is seldom used for monitoring response to clopidogrel[15]. Many other platelet function assays are now available but the most common platelet function tests (PFTs) used in everyday clinical practice are the flow cytometric vasodilator-stimulated phosphoprotein phosphorylation (VASP) analysis and the VerifyNow P2Y12 assay[16].

The VASP assay uses flow cytometry to measure inhibition of VASP phosphorylation by ADP *via* the P2Y12 receptor. The ratio of VASP phosphorylation is indicative for the receptors' activity and reported as platelet reactivity index (PRI). Several studies reported high correlation between high PRI values and recurrent stent thrombosis after percutaneous coronary intervention (PCI)[17]. However, the specific method has been gently criticized for its lack of standardization and therefore the inability of establishing a universal PRI cut-off value[18,19].

The most widely used method of routinely monitoring platelet function is the VerifyNow bedside assay. It is a very practical, rapid and well-standardized point-of-care test that measures platelet-induced aggregation to fibrinogen-coated beads in whole blood in response to an ADP induced stimulus[20,21]. Results are expressed as P2Y12 reaction units (PRU) reflecting P2Y12 mediated platelet reactivity. Published studies using this instrument have demonstrated the relationship between HTPR values and long-term cardiovascular events after PCI[14,22].

Several additional PFTs are also available but rarely used in clinical research: PFA-100, Impedance Aggregometry (Multiplate Analyzer) and whole blood thromboelastography[23-25]. Wisman *et al*[26] in a recent meta-analysis of 59 studies using 15 different tests stated that HTPR was associated with a significant 2.8 times higher risk of MACE. Based on all the available evidence and according to the most recent expert consensus paper issued by the Working Group on Thrombosis of the European Society of Cardiology, the recommended assays for monitoring P2Y12 platelet inhibition are the VerifyNow P2Y12 assay, the Multiplate device with the ADP kit and the VASP assay[27].

HTPR CUT-OFF VALUES

In order to overcome the lack of universally defined cut-off values for the various PFTs for HTPR, Bonello *et al*[14] based on numerous studies using receiver operating characteristic (ROC) established consensus values for HTPR for every major platelet function test: (1) > 46% maximal for a 5-μmol/L ADP-induced aggregation; (2) > 50% PRI using the Platelet VASP test; and (3)

Table 1 Common platelet function assays

Test	LTA	VASP	VerifyNow
Function	Increase in light transmittance	Flow cytometric measurement of VASP phosphorylation	Measurement of platelet-induced aggregation to fibrinogen-coated beads
Receptor	P2Y1 and P2Y12	P2Y12	P2Y12
Results	MPA	PRI	PRU
Cut-off value	> 46%	> 50%	230-240

LTA: Light transmission aggregometry; VASP: Vasodilator-stimulated phosphoprotein phosphorylation; MPA: Maximal platelet aggregation; PRI: Platelet reactivity index; PRU: P2Y12 reaction units.

230-240 P2Y12 reaction units PRU by the VerifyNow P2Y12 assay (Table 1).

However, the majority of the data for this consensus were extrapolated from the coronary studies, given the lack of data from PAD patients. The PRECLOP study, a prospective single-center trial was the first study suggesting the optimal HTPR cut-off value exclusively in patients with PAD using the VerifyNow test[28]. ROC analysis performed in this trial revealed an identical to CAD patients' cut-off value (PRU $\geqslant$ 234; area under the curve 0.883; 95%CI: 0.811-0.954; P = 0.0001).

HTPR MECHANISMS

The antiplatelet effect of clopidogrel is based on the inhibition of platelet aggregation by irreversibly binding to the P2Y12-ADP receptor. It is basically an inactive prodrug that undergoes two consecutive oxidations by the hepatic cytochromes P450 (CYP) to create an active metabolite. This accounts for 15% of the drug metabolism[29]. Multiple potential factors for HTPR have been proposed mainly correlated with distorted activity of cytochrome P450 isoenzymes[30,31].

Genetic factors

Several studies initially documented that poor response to clopidogrel may be greatly heritable[32]. Specifically, genetic polymorphisms to the hepatic CYP450 enzymes, especially to CYP2C19 that is involved in both steps of clopidogrel's biotransformation might disturb the metabolism and therefore the effect of the drug[33]. It has been described that carriers of at least one low function CYP2C19 allele experience a reduction of the active metabolite in plasma up to 32.4% in comparison to healthy gene carriers[34]. The most notorious *2 allele follows an autosomal co-dominant inheritance[35]. Therefore, the highest risk profile group links with those who are homozygous for *2 allele[36]. Latest clinical trials have also suggested that alternative alleles (CYP2C19*3 and *4), as well as polymorphisms in alternative CYP450 enzymes (CYP2C9 and CYP2B6), may also induce HTPR[37]. Another genetic factor responsible for low response to clopidogrel is the ABCB1 gene polymorphisms responsible for reduced enteric absorption of the drug[33]. Notably, the Food and Drug Administration issued a boxed warning on clopidogrel stating that the clinical antiplatelet effectiveness is reduced for poor metabolizers, indicating that genetic tests are available to identify poor metabolizers and highlighting their emerging role in clopidogrel treatment decisions. Nonetheless, genotype accounts for approximately 2% to 12% of inter-individual variability of response to clopidogrel and various demographic and clinical factors largely contribute to the phenomenon[27].

Clinical factors

Beside the genetic background, a major issue in the field of HTPR has been the interaction with other concomitant drugs that are also metabolized by the CYPP450 system. Proton-pump inhibitors, especially omeprazole, were the first class of drugs to be investigated for possible interference with clopidogrel metabolism in early studies. Initial data outlined high incidence of HTPR in patients with CAD after PCI[38]. However, a large randomized control trial investigating clopidogrel with or without concomitant use of omeprazole following PCI revealed no significant difference in terms of MACEs between the two groups[39]. Drug-drug interactions between antiplatelet agents and calcium-channel blockers or statins were also originally reported[40,41] but additional studies demonstrated conflicting findings[42,43]. As a result according to updated guidelines there is no contraindication for the concomitant use of the above mentioned drugs with clopidogrel[27].

On the other hand, clinical entities such as chronic kidney disease (CKD) and diabetes mellitus (DM) seems to be associated with HTPR according to recent studies[44,45]. CKD, an established cardiovascular risk factor, has been recognized as an independent factor of HTPR in patients with CAD[46], while several studies also revealed poor response to clopidogrel and high incidence of stent thrombosis in diabetic patients after PCI, especially those requiring insulin therapy[47]. The possible causes include various pharmacokinetic processes such as the increased platelet turnover and the up-regulation of P2Y12 pathway in these patients[48,49]. Finally, body mass index (BMI) may be another contributing factor to attenuated platelet inhibition. Limited studies reported that overweight patients (BMI > 25 kg/m^2) while on clopidogrel demonstrated reduced antiplatelet effect[50]. However, available data are scarce and further data from larger trials are awaited.

HTPR IN CAD

Numerous studies have demonstrated that the insuffi-

cient response to clopidogrel may lead to adverse clinical outcomes, such as stent thrombosis (acute or subacute) and myocardial infarction. Moreover, recent meta-analysis including thousands of patients treated with PCI either for ST-elevation myocardial infarction (STEMI) or non-STEMI using several platelet function tests reported the correlation between high on-clopidogrel platelet reactivity and MACE, while the incidence of CAD patients detected with HTPR is approximately 35%[26,51].

Müller *et al*[52] published one of the first studies associating poor response to clopidogrel among patients experiencing MACEs after stent implantation in 2003. Successively, Gurbel *et al*[53], in a thorough analysis of the CREST study identified HTPR as a risk factor for stent thrombosis. Subsequently, the possible correlation of the phenomenon with stent thrombosis was investigated by numerous studies[54-56]. The ARMYDA-PRO study (Antiplatelet therapy for Reduction of Myocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) was the first study investigating HTPR by using the user-friendly point-of-care assay VerifyNow and overcoming technical limitations of previous traditional platelet function methods[57]. The authors supported the concept of bedside monitoring platelet inhibition in clinical practice by proving a strong correlation between HTPR and MACEs at 30-d follow-up after PCI. These findings were amplified by latter similar studies with longer follow-up periods. Price *et al*[58] documented HTPR as a risk factor for cardiac death and stent thrombosis after drug eluting stent (DES) implantation at 6 mo follow-up. The authors also noted the perspective of modifying the antiplatelet regimen.

Of note, all the previously mentioned studies reported the correlation between high on-clopidogrel platelet reactivity and MACE, while until today there are no data indicating an analogous correlation between response to aspirin and stent thrombosis or adverse clinical events[27]. Notably, in 2014 the French VERIFRENCHY, multi-center, prospective trial published data regarding the prognostic value of testing antiplatelet response to clopidogrel and aspirin with the VerifyNow assay, in an intermediate-risk population (1.001 patients) undergoing elective stent implantation due to stable coronary disease or non-ST-segment elevation acute coronary syndrome. Overall 36.0% and 8.6% of the patients demonstrated HTPR to clopidogrel or aspirin, respectively. According to one year results, although ischemic events were numerically more in patients with high on-clopidogrel platelet reactivity (composite endpoint 3.9% of *vs* 2.3% and definite or probable stent thrombosis: 1.1% *vs* 0.3%) results did not reach statistical significance, while there was no difference in rates of major bleeding. In patients receiving aspirin there was also no significant difference in ischemic endpoints[59]. These results indicate that either HTPR may not affect clinical outcomes or that it is difficult to statistically prove the role of HTPR in populations at low to intermediate risk of stent thrombosis, due to the low number of ischemic events.

HTPR in PAD

Contrary to CAD, there is a lack of high quality evidence demonstrating the possible correlation between HTPR and adverse clinical events in patients with PAD undergoing peripheral endovascular procedures. The MIRROR single-blinded, single-center, randomized controlled trial was the first to report the existence of low response to clopidogrel in patients undergoing PTA[11]. The authors alongside with the clinical superiority of dual antiplatelet therapy following femoropopliteal angioplasty or stenting, also reported a 30% HTPR rate, similar to that identified in coronary studies. Subsequently, Pastromas *et al*[60] in a retrospective audit of 113 patients treated with clopidogrel after angioplasty or stenting, noticed an even higher HTPR incidence rate (approximately 54%). The authors speculated that this difference was mainly driven by high comorbidity rates and advanced arterial disease characteristic in critical limb ischemia (CLI) cohorts. The specific study also originally associated HTPR with significantly higher re-intervention rates. In American College of Cardiology (ACC) 2012, Kliger *et al*[61] presented the results from a study investigating responsiveness in patients undergoing PCI or PTA, which also detected a higher HTPR incidence in PAD patients.

Following these initial results, Spiliopoulos *et al*[28] further investigated the phenomenon in the PRECLOP study (NCT01744613) and established the optimal cut-off value for HTPR in PAD patients using the VerifyNow assay (PRU $\geqslant$ 234). In total 100 patients were screened with the VerifyNow assay and were stratified according to PRU values in four quartiles. The study's primary endpoint was the 1-year composite of cardiovascular death, major amputation and re-intervention events. Results revealed patients with HTPR demonstrated a less than 40% event-free survival at 1-year, while an approximately 90% event-free survival at 1 year was noted in patients with an adequate response to clopidogrel. Moreover, high on-clopidogrel platelet reactivity was identified as an independent predictor of increased events (mainly repeat revascularization procedures; HR = 16.9; 95%CI: 5-55; $P = 0.0001$). The incidence of HTPR was 51%, considerably higher than that reported in CAD trials, and was again correlated to CLI, DM and chronic kidney disease[28].

High on-aspirin platelet reactivity has been also investigated by several authors and its incidence has be reported to range between 4%-40%, a variability attributed to the multiplicity of methods used and the small sample studied. Moreover, Karnabatidis *et al*[62] and Spiliopoulos *et al*[63] reported that nearly 12% of PAD patients on dual antiplatelet therapy, demonstrated HTPR for both clopidogrel and aspirin. However, the clinical implication of low response to aspirin remains controversial and more data are needed.

NOVEL ANTIPLATELET AGENTS

Recently, novel and stronger antiplatelet agents, such as prasugrel and ticagrelor, have been introduced in

everyday clinical practice in patients suffering from acute coronary syndrome (ACS) undergoing PCI[64,65].

Prasugrel, a third generation thienopyridine agent is also a prodrug that requires metabolism before its active metabolite will bind to ADP receptor and inhibits platelet aggregation. The PRINCIPLE-TIMI 44 trial proved that prasugrel promotes platelet inhibition more rapidly and effectively in comparison with clopidogrel, showing that the degree of inhibition of platelet aggregation achieved with prasugrel within 30 min after treatment is comparable to the peak effect of clopidogrel 6 h after administration[66].

The first trial dedicated to the clinical outcomes of prasugrel was the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction (TRITON-TIMI) 38[67]. Over 13000 patients with ACS receiving prasugrel or clopidogrel scheduled for endovascular treatment were enrolled in this large multi center trial. The results demonstrated a significant reduction in the rate of periprocedural myocardial infarction and stent thrombosis. However, high incidence of major bleeding in some patients of the subgroup receiving prasugrel was noted. The authors concluded that prasugrel reduces the rate of recurrent ischemic events compared with clopidogrel, but with a significantly higher bleeding risk. Based on these results, the 2011 updated ACC/American Heart Association guidelines do not recommend the use of prasugrel in patients > 75 years old, or weight < 60 kg (with a recommended decreased dose of 5 mg), history of stroke or pathologic active bleed[68]. Moreover, Bonello *et al*[69] investigating the clinical effect of prasugrel in CAD patients after PCI identified a persistent high rate of HTPR (approximately 25%) correlated with high incidence of MACEs at 30 d follow-up.

Ticagrelor, in contrast to clopidogrel, binds reversibly to the P2Y12 receptor and therefore prevents binding of ADP. Another major advantage of this novel P2Y12 antagonist is that it does not require metabolic activation, in order to exert its effect.

The DISPERSE-2 trial examined the effect of ticagrelor *vs* clopidogrel in non-STEMI patients with ACS and documented higher rate of platelet inhibition in the subgroup of the patients' cohort receiving ticagrelor[70]. Following these results, the PLATO (Platelet Inhibition and Patient Outcomes) multi-center, randomized, controlled trial compared the clinical outcomes of loading doses of ticagrelor *vs* clopidogrel in patients with ACS admitted to the hospital for prevention of cardiovascular death[71]. The results demonstrated significantly less incidence of the primary endpoint (time of occurrence of CV death, MI or stroke) in ticagrelor group than in clopidogrel group. Furthermore, the rates of major bleeding were not significantly different between the two groups. Nevertheless, after carefully analyzing bleeding events ticagrelor was associated with an increase in combined major and minor PLATO bleeding rates by 11% ($P = 0.008$)[72].

PFT-GUIDED INDIVIDUALIZED ANTIPLATELET THERAPY

Given the possibility to measure the response to clopidogrel and to use alternative antiplatelet agents in selected patients, investigators began to investigate PFT-guided antiplatelet protocols. The GRAVITAS study, a multicenter randomized double blind control trial, investigated the effect of high-dose *vs* standard-dose clopidogrel using the VerifyNow assay to identify HTPR in 2.214 patients undergoing PCI. Patients with HTPR were given high-dose platelet (600 mg loading dose and 150 mg daily doses) *vs* the standard-dose (300 mg loading dose and 75 mg daily doses). The study showed that although double-dose clopidogrel significantly reduced - but not completely abolished-HTPR, it failed to reduce MACEs at 6 mo follow-up. Specifically, HTPR was reduced by only 22% at one month[73]. This observation was further demonstrated by Alexopoulos *et al*[74] reporting that although double clopidogrel dose further inhibits platelet reactivity compared to standard dose, 35.8% of the patients under double dose remained non-responders, while for HTPR patients switching to prasugrel the percentage of non-responders was reduced to 7.0% ($P < 0.0001$).

However, two recent multi-center randomized controlled trials failed to demonstrate a clinical benefit PFT-guided antiplatelet therapy in CAD patients. The testing platelet reactivity in patients undergoing elective stent placement on clopidogrel to guide alternative therapy with prasugrel (TRIGGER-PCI) compared HTPR patients (PRU > 208) with stable CAD receiving prasugrel or clopidogrel following PCI using DES the study was prematurely terminated as the primary endpoint of death or MI at 6 mo occurred only in one patient of the clopidogrel group. The authors concluded that although prasugrel significantly reduced HTPR (mean PRU values from 245 to 80 at 3 mo) the small incidence of adverse events in elective DES procedures would not allow to prove the effectiveness of PFT-guided antiplatelet therapy[75].

The ARTIC trial compared conventional (1227 patients) *vs* PTF-guided (1213 patients) antiplatelet therapy after PCI for the composite endpoint of cardiovascular death, MI, stent thrombosis stroke and revascularization at one year follow-up. In total 37% of the patients suffered a non-ST-segment elevation acute coronary syndrome (NSTE-ACS), while the remaining had severe stable CAD. In the conventional therapy group 6% of the patients received prasugrel and only 12% in the PFT-guided group, while in the rest of non-responders double clopidogrel dose was used to treat HTPR. There was no significant difference in the primary outcome or bleeding events between the two study groups at one year follow-up[76] (Table 2). Nevertheless, the fact that the vast majority of the patients were offered double clopidogrel dose to overcome HTPR, a strategy previously reported as less effective compared

Table 2 Highlighted multicenter randomized control trials investigating platelet function tests-guided antiplatelet therapy

Study	Gravitas	Arctic	Trigger-PCI
Study population (*n*)	2214	2440	423
PFT assay	VerifyNow	VerifyNow	VerifyNow
High-dose clopidogrel	100%	80%	-
High-dose Aspirin	-	45%	-
Prasugrel	-	12%	100%
Results (primary endpoint)	2.3% *vs* 2.3%	31.1% *vs* 34.6%	0.0% *vs* 0.5%

PFT: Platelet function tests; PCI: Percutaneous coronary intervention.

to switching to novel antiplatelet agents (prasugrel or ticagrelor) in overcoming HTPR, as well as the small percentage of patients with ACS enrolled, probably negatively influenced outcomes in the PFT-guided group.

Finally, Aradi *et al*[77], conducted a meta-analysis to investigate the safety and efficacy of tailored antiplatelet therapy based on platelet reactivity testing in patients after PCI. The authors included 10 randomized controlled trials (5 multi-center, 2 double-center and 3 single-center) with a total of 4213 patients and concluded that PFT-guided intensified antiplatelet therapy was associated with decreased cardiovascular mortality and stent thrombosis. Nevertheless, the authors emphasized that the net benefit of personalized antiplatelet therapy depends on the risk of stent thrombosis and should be applied in patients at high risk. This extremely significant observation may also explain the early termination of the TRIGGER-PCI trial, where no stent thrombosis occurred in more than 400 HTPR patients, the results of the French VERIFRENCHY trial investigating patients of intermediate risk presenting with stable CAD and NSTE-ACS, as well as the negative results of the ARTIC trial where less than half of the patients suffered from ACS[78]. Of note, the ARTIC study was not included in this meta-analysis.

In the PAD arena, data about the clinical efficacy of novel antiplatelet agents and PFT-guided antiplatelet therapy modification are scarce. Torngren *et al*[78] in a study including PAD patients receiving ticagrelor because of previous ACS reported enhanced peripheral endothelial function compared to clopidogrel or prasugrel, while in a recently published post hoc analysis of the PLATO trial involving 1.144 patients with peripheral arterial disease, ticagrelor reduced the rate of cardiovascular death and MI to 16.7% compared to 21.5% in the clopidogrel group (P = 0.045)[79]. Spiliopoulos *et al*[80], recently published a study observing the clinical effect ticagrelor in 37 consecutive HTPR patients suffering from CLI undergoing angioplasty or stenting of complex lesions (long occlusions, advanced infrapopliteal disease). According to this initial experience switching therapy from clopidogrel to ticagrelor managed to overcome HTPR in all patients with documented increased platelet aggregation (PRU $\geqslant$ 234). Specifically, mean PRU during clopidogrel therapy (308.4 ± 41.8) was significantly reduced when switched to ticagrelor (67.0 ± 52.8; P < 0.0001). This was accompanied with very satisfactory clinical outcomes for the specific CLI cohort where major amputation can usually reach 25% at one year. Kaplan-Meier analysis estimated that the one-year primary composite endpoint of event-free survival was 92.0%, while revascularization-free survival rate was 67.3% at one year follow-up.

Currently, a global, multi-center, double blind, randomized, controlled, trial involving 900 sites in 25 countries (EUCLID trial; sponsored by AstraZeneca), enrolled approximately 13500 symptomatic PAD patients in order to investigate the safety and efficacy of ticagrelor *vs* clopidogrel. Primary outcome measures will be cardiovascular death, MI and ischemic stroke and results are expected within 2016. The authors speculate that individualized therapy using PTF as to identify CAD or PAD patients on increased ischemic or bleeding risk, will gradually earn its way in everyday clinical practice as long as future well-designed large-scale trials demonstrate its utility. Novel antiplatelet agents should be prescribed with consciousness as they have been related with increased bleeding events.

CONCLUSION

Following the CAPRIE trial in which clopidogrel achieved a further 24% relative risk reduction and 0.51% per year absolute risk reduction (P = 0.043) in major cardiovascular events compared to aspirin in symptomatic PAD patients, its use in every day clinical practice has been remarkably increased over the years[81]. It is generally a safe and effective drug commonly combined with aspirin, in selected patients undergoing coronary or peripheral revascularization procedures, to prevent cardiovascular ischemic events. However, a notable percentage of vascular patients present poor response to traditional antiplatelet therapy. High on-clopidogrel platelet reactivity, a clinical entity has recently emerged in the ambit of coronary and peripheral arterial disease seems to negatively affect clinical outcomes and certainly merits further investigation. The same phenomenon of low response to aspirin has been also described, however until today its clinical significance remains unproven. As modern clinical practice can support the routine use of platelet monitoring, given the fact that today platelet function tests are user-friendly, accurate and affordable in the immediate future personalized antiplatelet therapy could become a safe and efficient option in patients with low response to clopidogrel. Nonetheless, the potential risk of bleeding should always be under concern, especially in patients at high hemorrhagic risk. Consideration of the individual's genetic profile could also be an appropriate tool regarding tailored antiplatelet therapy. However, it is a fact that until today the benefit of PFT-guided personalized therapy in clinical outcomes remains to be determined and more data from meticulously designed trials are necessary.

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ORIGINAL ARTICLE

Prospective Study

Comparison of partners-heart failure algorithm *vs* care alert in remote heart failure management

Leonardo Calo', Annamaria Martino, Claudia Tota, Alessandro Fagagnini, Renzo Iulianella, Marco Rebecchi, Luigi Sciarra, Giuseppe Giunta, Maria Grazia Romano, Roberto Colaceci, Antonio Ciccaglioni, Fabrizio Ammirati, Ermenegildo de Ruvo

Leonardo Calo', Annamaria Martino, Claudia Tota, Alessandro Fagagnini, Renzo Iulianella, Marco Rebecchi, Luigi Sciarra, Ermenegildo de Ruvo, Division of Cardiology, Policlinico Casilino, 00169 Rome, Italy

Annamaria Martino, Giuseppe Giunta, Antonio Ciccaglioni, Cardiovascular, Respiratory, Nephrologic and Geriatrics Sciences Department, Umberto I Hospital, Sapienza University of Rome, 00185 Rome, Italy

Maria Grazia Romano, Roberto Colaceci, Fabrizio Ammirati, Department of Cardiology, G.B. Grassi Hospital, 00100 Rome, Italy

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Correspondence to: Leonardo Calo', MD, FESC, Division of Cardiology, Policlinico Casilino, ASL Rome B. Via M Buonarroti 16, 00169 Rome, Italy. leonardo.calo@tin.it
Telephone: +39-06-23188416
Fax: +39-06-23188410

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Abstract

AIM: To compare the utility of the partners-heart failure (HF) algorithm with the care alert strategy for remote monitoring, in guiding clinical actions oriented to treat impending HF.

METHODS: Consecutive cardiac resynchronization-defibrillator recipients were followed with biweekly automatic transmissions. After every transmission, patients received a phone contact in order to check their health status, eventually followed by clinical actions, classified as "no-action", "non-active" and "active". Active clinical actions were oriented to treat impending HF. The sensitivity, specificity, positive and negative predictive values and diagnostic accuracy of the partners-HF algorithm *vs* care alert in determining active clinical actions oriented to treat pre-HF status

and to prevent an acute decompensation, were also calculated.

RESULTS: The study population included 70 patients with moderate to advanced systolic HF and QRS duration longer than 120 ms. During a mean follow-up of 8 ± 2 mo, 665 transmissions were collected. No deaths or HF hospitalizations occurred. The sensitivity and specificity of the partners-HF algorithm for active clinical actions oriented to treat impending HF were 96.9% (95%CI: 0.96-0.98) and 92.5% (95%CI: 0.90-0.94) respectively. The positive and negative predictive values were 84.6% (95%CI: 0.82-0.87) and 98.6% (95%CI: 0.98-0.99) respectively. The partners-HF algorithm had an accuracy of 93.8% (95%CI: 0.92-0.96) in determining active clinical actions. With regard to active clinical actions, care alert had a sensitivity and specificity of 11.05% (95%CI: 0.09-0.13) and 93.6% respectively (95%CI: 0.92-0.95). The positive predictive value was 42.3% (95%CI: 0.38-0.46); the negative predictive value was 71.1% (95%CI: 0.68-0.74). Care alert had an accuracy of 68.9% (95%CI: 0.65-0.72) in determining active clinical actions.

CONCLUSION: The partners-HF algorithm proved higher accuracy and sensitivity than care alert in determining active clinical actions oriented to treat impending HF. Future studies in larger populations should evaluate partners-HF ability to improve HF-related clinical outcomes.

Key words: Heart failure; Cardiac resynchronization therapy; Defibrillators; Remote monitoring

Core tip: This is a multicenter observational registry that compared the utility of the partners-heart failure (HF) algorithm with the care alert strategy for remote monitoring, in guiding clinical actions oriented to treat impending HF in a population of 70 cardiac resynchronization therapy recipients followed over a mean follow-up period of 8 ± 2 mo. The partners-HF algorithm displayed high sensitivity (96.9%), specificity (92.5%), positive (84.6%) and negative (98.6%) predictive values for active clinical actions oriented to treat impending HF. The care alert exhibited lower sensitivity (11.1%), positive (42.3%) and negative (71.1%) predictive values.

Calo' L, Martino A, Tota C, Fagagnini A, Iulianella R, Rebecchi M, Sciarra L, Giunta G, Romano MG, Colaceci R, Ciccaglioni A, Ammirati F, de Ruvo E. Comparison of partners-heart failure algorithm *vs* care alert in remote heart failure management. *World J Cardiol* 2015; 7(12): 922-930 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i12/922.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i12.922

INTRODUCTION

Heart failure (HF) is a primary public health problem, with mortality and hospitalization rates of approximately 7.2% and 31.9% at one-year respectively[1]. Outpatient management, symptoms and daily weight often do not identify patients in time to prevent imminent HF. Modern implantable cardiac resynchronization therapy-defibrillators (CRT-D) with remote monitoring (RM) capabilities, continuously assess parameters, including heart rate, patient's activity (PA), intra-thoracic impedance, atrial fibrillation (AF), ventricular arrhythmias (VA), shock therapy delivered and system integrity[2-5].

Previous studies have demonstrated the ability of individual device diagnostic data, to predict HF events, to reduce the time from clinical event to treatment, length of hospitalization and quantity of in-office visits[6-10].

Earlier studies have shown that implantable device-measured parameters, such as intra-thoracic impedance, AF burden, mean heart rate, heart rate variability (HRV), patient activity (PA), frequency of premature ventricular contractions (PVCs), VA episodes, implantable cardioverter defibrillators (ICD) shocks and percentage of pacing of cardiac resynchronization therapy (%CRT), individuate subjects at risk of HF and facilitate early interventions[6-12]. Variations of intrathoracic impedance[3] as well as HRV and PA[2] occurs nearly two weeks before HF exacerbation. Low HRV indicate a sympathetic dominance in cardiac autonomic control and may be associated with exacerbation of atrial and VAs[13]. A prolonged AF duration, a rapid ventricular rate (VR) during AF and an increase in the burden of PVCs reduce %CRT[14] and are warning signs of HF, together with ICD shocks[15].

Each HF device diagnostic parameter, although validated in various studies, has several limitations. A previous study[16] showed that sensitivity values of individual parameters, ranged from 23.6% to 50.0%, whereas their combination displayed 65.4% sensitivity and 99.5% specificity for cardiovascular hospitalizations and deaths.

The partners-HF[11] is the largest cohort study to have evaluated the ability of combined HF device diagnostics, including Optivol™ Fluid index, AF duration, rapid VR during AF, low PA, high nocturnal heart rate (NHR), low HRV, low CRT pacing percentage, and ICD shocks, to identify patients at risk of acute HF in the subsequent 30 d. The retrospective analysis of the prospectively collected data of the partners-HF study, demonstrated that subjects with a positive partners-HF algorithm were at a greater risk (HR = 5.5; $P < 0.001$) of HF hospitalization during the next month.

The purpose of this multicenter, observational registry was to prospectively assess the utility of the partners-HF criteria, implemented within Discovery Link™, in guiding clinical actions oriented to treat pre-HF status and to prevent an acute decompensation in a population of HF individuals implanted with a Medtronic CRT-D device.

MATERIALS AND METHODS

Registry population and design

This study has been approved by our Institutional Review Board and is conform to the guiding principles of the Declaration of Helsinki.

Consecutive CRT-D candidates were enrolled by three Italian cardiology centers. The clinical status of the patients, including NYHA class, was initially assessed by the cardiologists involved in the project. All patients underwent implantation of a Medtronic CRT-D system (Model: Consulta™, Concerto™ Ⅱ, VIVA XT™, PROTECTA XT™; Medtronic Inc., Minnesota) equipped with the CareLink Medtronic®-RM system for RM.

Inclusion criteria were: Left ventricular ejection fraction ⩽ 35% + NYHA class Ⅱ, Ⅲ and ambulatory Ⅳ and broad QRS (> 120 ms if left bundle branch block was present, or otherwise > 150 ms + optimal pharmacological treatment for HF). Exclusion criteria were: acute coronary syndrome within 40 d, coronary artery revascularization within 3 mo, end-stage HF requiring inotropic support, ventricular assist devices or dialysis.

Each patient received a wireless CareLink Monitor which provided automatic transmission of clinical and technical parameters stored in the implanted device's memory to a Service Center where information was decrypted, uploaded to a secure website and periodically accessed by the nurses. Patients were followed up for at least 6 mo. Data were prospectively collected between January 2012 and October 2012 and classified on the basis of both the care alert and the partners-HF algorithms at the same time. Automatic "scheduled" transmissions were programmed every 15 d. "Care alert"-triggered transmissions and transmissions activated manually by the patients were also collected. Patients were instructed to manually activate transmissions in case of occurrence or exacerbation of HF-related symptoms (including shortness of breath, dyspnea, orthopnea, asthenia, pre-syncope or syncope) or signs (including weight increase, peripheral edema enlargement).

The project, including data collection, was approved by the Hospital Ethics Committees of each cardiology center involved in the registry using the Medtronic Clinical Service Project®, and every individual enrolled gave written informed consent to enrolment in the registry.

Partners-HF algorithm

The partners-HF application, based on the algorithm described by Whellan *et al*[11], was implemented within the Discovery Link™. The latter is a web environment enabling elaborated and aggregated information from the Medtronic CareLink Network® to be shown in interactive JavaScript charts. The partners-HF algorithm was adopted to process information in order to select the last transmission (including both manually and automatically triggered ones) from each device and to perform analysis. Statistics were calculated over the last 28 consecutive days[11]. Every two weeks, the first partners-HF profile of those patients who satisfied the partners-HF criteria was directly logged into the Discovery Link.

The partners-HF algorithm was considered positive in the following cases: Optivol™ Fluid index ⩾ 100 or any 2 of the following criteria met during a one-month period of evaluation: Long AF duration, rapid VR during AF, Optivol™ fluid index ⩾ 60, low PA, high nocturnal NHR, low HRV, low %CRT, and ICD shocks (Appendix).

Care alert

The Carelink system automatically triggered alerts in case of shocks delivered or if the following clinical and technical parameters exceed a programmable threshold: OptiVol™ Fluid Monitoring Index (> 60), AF duration (> 24 h), VR rate during AF (> 100 bpm), lead impedance, integrity and battery voltage alert (out of predefined range).

Adjudication of impending heart failure and classification of clinical actions

After every transmission, all patients received a phone contact and their health status was checked by nurses experienced in HF. At time of enrollment, patients were instructed to measure frequently their body weight and to check their pulse in order to identify HF-related signs (increase of heart rate, weight and/or peripheral edema) and symptoms (increase in shortness of breath, cough and/or asthenia, reduction of exercise tolerance, needing use of extra pillows during the night). Data on vital status, symptoms, quality of life, adherence to pharmacological treatment, hospitalizations and mortality were collected by nurses at every phone contact. Pre-specified boundaries for weight, blood pressure, pulse and symptoms were previously established for every patient. Adjudication of impending HF was based on the development of early HF-related signs and symptoms (see above) and on the exceeding from the prespecified boundaries, but still not requiring hospitalization[12]. RM transmissions suggestive of worsening HF or device malfunctioning were submitted to physicians.

Clinical actions performed as a result of transmissions, according to each center's clinical practice, were registered on a Medtronic Clinical Service®-form and were classified as follows: "no-action", "non-active" and "active". No action: (1) consisted on telephonic contact; (2) non-active clinical action; (3) consisted on clinical examination without pharmacological treatment modification (PTM). Active clinical actions included PTM during telephonic contact; or (4) during clinical examination. In the event of manual or care alert transmissions, physicians could decide either to undertake clinical action immediately or to wait until the first partners-HF data from those specific transmissions

Table 1 Study population

Clinical characteristics of the study population	
Age (yr)	70.3 ± 8.3
Male (%)	78.3
EF (%)	27.5 ± 6.5
Etiology post-ischemic DC (%)	63.2
Idiopathic DC (%)	33.7
Valvular DC (%)	2
Congenital DC (%)	1.1
NYHA Ⅱ (%)	17.3
Ⅲ (%)	78.6
Ⅳ (%)	4.1
Optimized pharmacological treatment (%)	63.2[1]
Prevention: Primary (%)	73.7
Secondary (%)	18.3
SVT (%)	3.7
Syncope (%)	3.2
Cardiac arrest (%)	5.1
AF permanent (%)	16.3
Persistent (%)	7.4
Paroxysmal (%)	4.1
Devices: Consulta™ CRT-D	37.4
Concerto™ Ⅱ CRT-D (%)	26.3
Viva XT™ CRT-D (%)	22.1
Protecta XT™ CRT-D (%)	14.2

[1]The low percentage of optimized pharmacological treatment is related to reduce aldosterone antagonists administration in patients affected by chronic kidney disease. EF: Ejection fraction; DC: Dilated cardiomyopathy; NYHA: New York Heart Association; SVT: Sustained ventricular tachycardia; AF: Atrial fibrillation; CRT-D: Cardiac resynchronization therapy-defibrillator.

became available in the Discovery Link environment.

Study endpoints

The aim of this study was to determine the sensitivity, specificity, positive and negative predictive values and diagnostic accuracy of the partners-HF algorithm and of care alert in determining active clinical actions oriented to treat pre-HF status and to prevent an acute decompensation. Analyses of sensitivity, specificity, predictivity and accuracy were performed with respect to overall active clinical actions (3 + 2) *vs* the sum of clinical actions and no actions (1 + 0).

Statistical analysis

Continuous variables are summarized as mean ± SD and categorical variables as counts and percentages. Positive transmissions by the partners-HF algorithm and/or care alert were considered true positive when they were associated with acute HF and/or with pharmacological treatment modification due to impending HF. Positive transmissions by the partners-HF algorithm and/or care alert were considered as false positive in the remaining cases. Negative transmissions by the partners-HF algorithm and/or care alert were considered true negative when they were not associated to acute HF or PTM due to pre-HF (see above), and as false negative when they were not. The sensitivity of the partners-HF algorithm and of care alert was calculated as the ratio between the number of true positive transmissions and the sum of true positive and false negative transmissions. Specificity was calculated as the ratio between true negatives and the sum of true negatives and false positives. Positive predictive value was calculated as the ratio between true positives and the sum of true positives and false positives. Negative predictive value was calculated as the ratio between true negatives and the sum of true negatives and false negatives. Accuracy was calculated as the ratio between the sum of true positive and true negative transmissions and total transmissions. All the tests were performed by means of R 2.11.1 for Windows.

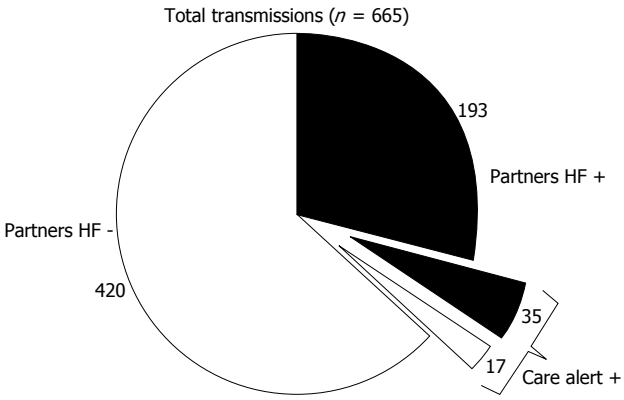

Figure 1 Distribution of total transmissions. Legend total transmissions are depicted in a pie-chart; positive partners-HF transmissions are shown in black; negative partners-HF transmissions are shown in white. Care alert transmissions are represented in separate slices. HF: Heart failure.

RESULTS

Study population

The characteristics of the study population are presented in Table 1. Patients were predominantly males and had mostly a moderate to advanced HF. All patients had QRS duration longer than 120 ms. The relatively low (63.2) percentage of optimized pharmacological treatment is due to reduced aldosterone antagonists administration in patients affected by chronic kidney disease.

Transmissions

During a mean follow-up of 8 ± 2 mo, 665 transmissions were received from 70 patients. Transmissions were classified as follows: 52 (7.8%) care alert, 149 (22.4%) manual and 464 (69.8%) scheduled.

Of all transmissions, 228 (34.3%) fulfilled the partners-HF criteria. Positive partners-HF transmissions were classified as: scheduled (136; 59.6%), manual (57; 25%), and care alert (35; 15.4%). Of the 437 negative partners-HF transmissions, 328 (75.1%) were scheduled, 92 (21%) were manual and 17 (3.9%) were triggered by a care alert. Figure 1 shows the distribution of partners-HF positive and negative transmissions, contemporarily triggered or not by care alert. Overall, the "care alert" transmissions met the partners-HF criteria in 67.3% of cases (Figure 1).

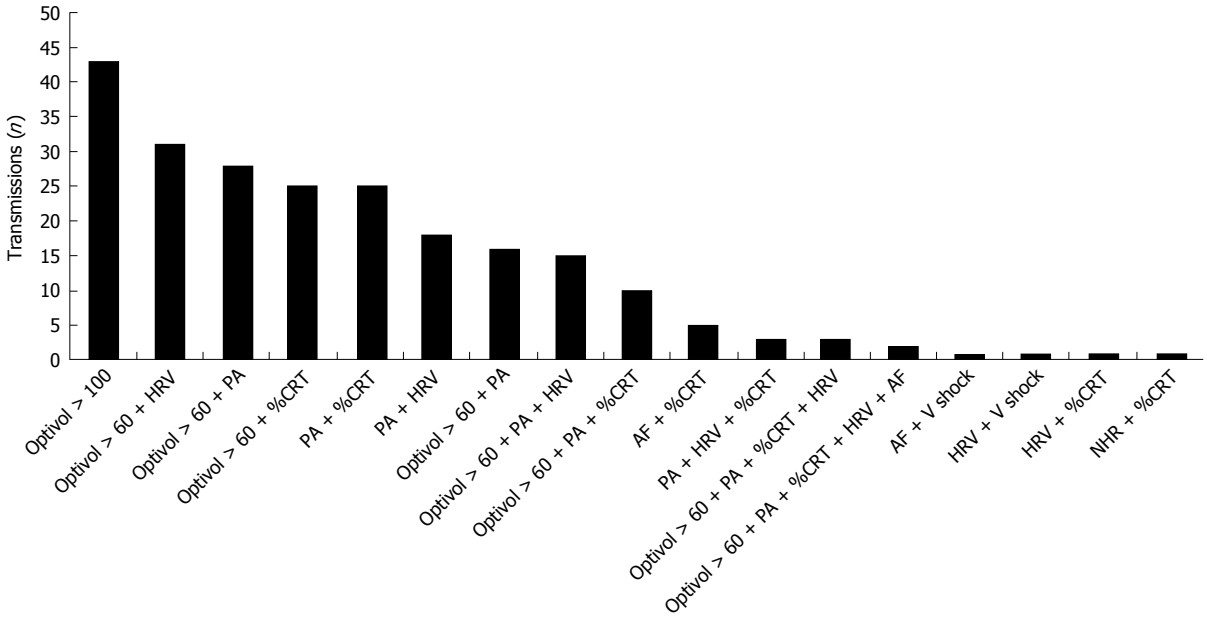

Figure 2 Positive partners-heart failure transmissions. HRV: Heart rate variability; PA: Patient activity; %CRT: Percentage of cardiac resynchronization therapy pacing; AF: Atrial fibrillation; V shock: Ventricular shock; NHR: Night heart rate.

Table 2 Pharmacological treatment modifications following positive or negative partners-heart failure transmissions and care alert

Active clinical actions	Partners-HF +	Care alert +
Pharmacological treatment modification during telephonic contact		
Diuretic dosage increase	120	25
BB dosage increase	57	22
AAD administration	2	2
ACE-I and ARA dosage increase	4	0
OAC administration	5	5
Clinical examination and pharmacological treatment modification		
Diuretic dosage increase	9	4
BB dosage increase	8	3
AAD administration	1	1
ACE-I and ARA dosage increase	1	1
Anti-platelet administration	0	0

+: Positive; BB: Beta-blockers; AAD: Anti-arrhythmic drugs; ACE-I: Angiotensin-converting-enzyme inhibitors; ARA: Angiotensin Ⅱ-receptor antagonists; OAC: Oral anti-coagulant; HF: Heart failure.

The most common reasons triggering a positive partners-HF transmission: Optivol fluid index ≥ 100 (18.8%) or optivol fluid index > 60 plus one of the following parameters: reduced HRV (13.6%), low PA (12.3%), or reduced %CRT (11%) (Figure 2). The 52 "care alert"-triggered transmissions (35 partners-HF positive and 17 partners-HF negative) were generated by OptiVol fluid index in 43 (82.7%) cases, AF duration and/or AF VR in 7 (13.5%) cases and shock for VAs in 2 (3.8%) cases.

Clinical actions following transmissions

During follow-up, no deaths or HF hospitalizations occurred. Of overall transmissions, 16 (2.4%) were followed by clinical examination and PTM, 183 (27.5%) by PTM during telephonic contact and 7 (1%) by clinical examination without PTM.

Of the 228 positive partners-HF transmissions, 11 (4.8%) were followed by clinical examination and PTM, 182 (79.8%) by PTM during telephonic contact and 6 (2.7%) by clinical examination without PTM (Figure 3A). PTM consisted of 19 drug dosage up-titrations and/or new treatment administrations during clinical examination and 188 during telephonic contact (Table 2). No pharmacological down titration was done.

Of the 437 negative partners-HF transmissions, 5 (1.1%) were followed by clinical examination and PTM, 1 (0.2%) by PTM during telephonic contact and 1 (0.2%) by clinical examination without PTM (Figure 3B). PTM consisted of 7 drug dosage up-titrations and/or new treatment administrations during clinical examination and 2 telephonic PTM, made as a consequence of a single care alert transmission. In these cases, diuretic and beta-blocker dosages were increased; no pharmacological down titration was reported.

Of the 52 care alert transmissions, 4 (7.7%) were followed by clinical examination and PTM and 18 (34.6%) by PTM during telephonic contact (Figure 3C). PTM consisted of 9 drug dosage up-titrations and/or new treatment administrations during clinical examination and 54 without in-office clinical examinations (Table 2).

Clinical actions following negative care alert transmissions consisted of 12 (1.9%) clinical examinations and PTM, 165 (27%) PTM during telephonic contact, 7 (1.1%) clinical examinations without PTM and 429 (70%) telephone contacts alone. PTM consisted of 17 drug dosage up-titrations and/or new treatment administrations during clinical examination and 136 without in-office clinical examinations.

Diagnostic accuracy of partners-HF and care alert

True positive, true negative, false positive and false

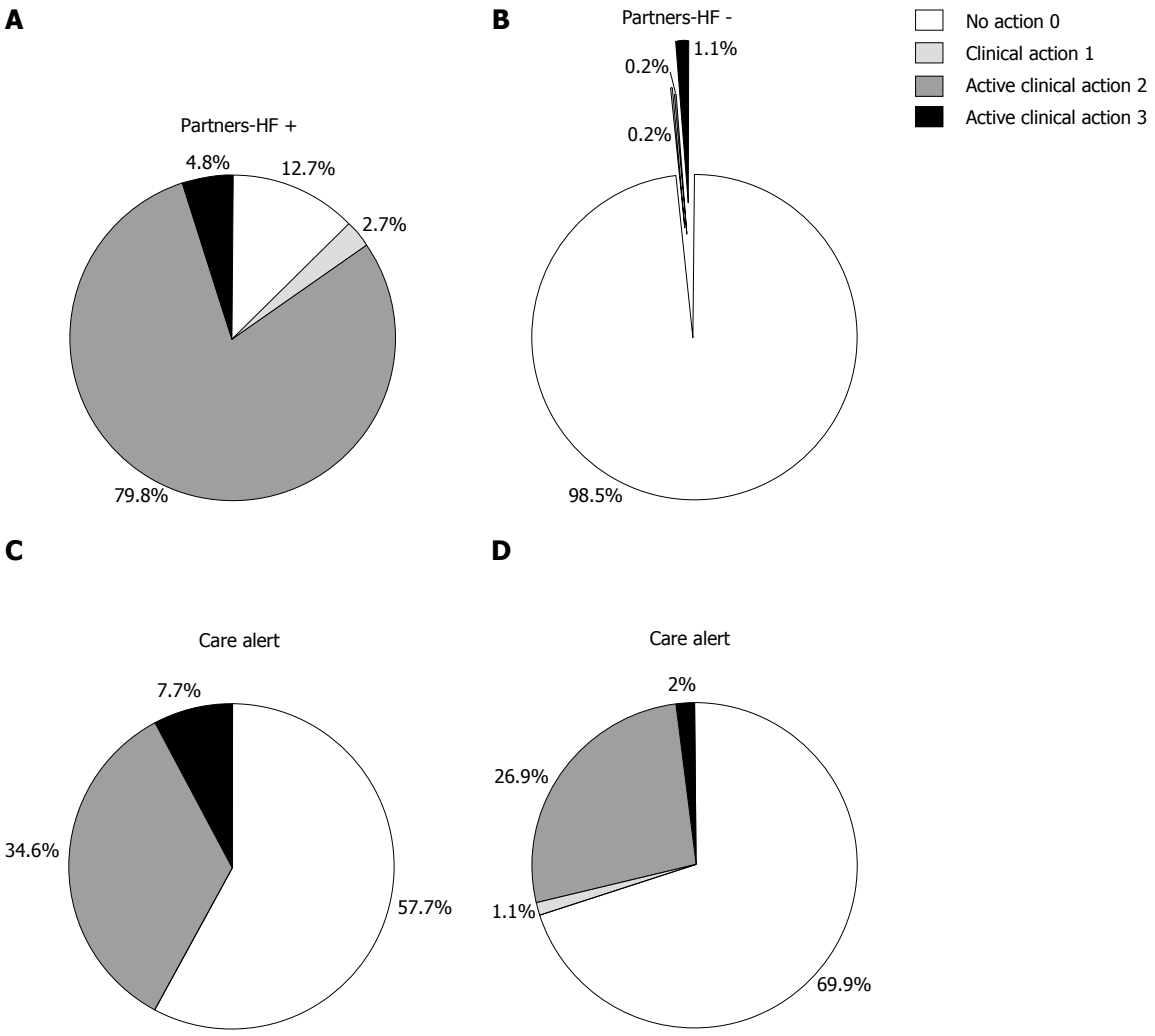

Figure 3 Clinical actions following positive partners-heart failure, negative partners-heart failure, positive care alert and negative care alert transmissions. No actions are depicted in white; clinical actions are depicted in light gray; active clinical actions are depicted in dark gray (pharmacological treatment modifications during telephonic contacts) and black (clinical examination and pharmacological treatment modifications). HF: Heart failure.

negative partners-HF transmissions with respect to active clinical actions are depicted in Figure 4A. The sensitivity and specificity of the partners-HF algorithm for active clinical actions (classes 2-3) were 96.9% (95%CI: 0.96-0.98) and 92.5% (95%CI: 0.90-0.94) respectively (Table 3). The positive and negative predictive values were 84.6% (95%CI: 0.82-0.87) and 98.6% (95%CI: 0.98-0.99) respectively. The partners-HF algorithm had an accuracy of 93.8% (95%CI: 0.92-0.96) in determining active clinical actions (Table 3).

Care alert true positive, true negative, false positive and false negative transmissions with respect to active clinical actions are depicted in Figure 4B. With regard to active clinical actions (classes 2-3), care alert had a sensitivity and specificity of 11.05% (95%CI: 0.09-0.13) and 93.6% respectively (95%CI: 0.92-0.95). The positive predictive value was 42.3% (95%CI: 0.38-0.46); the negative predictive value was 71.1% (95%CI: 0.68-0.74). Care alert had an accuracy of 68.9% (95%CI: 0.65-0.72) in determining active clinical actions (Table 3).

DISCUSSION

Main findings

In this registry we observed that: (1) The partners-HF algorithm has high sensitivity (96.9%), specificity (92.5%) and diagnostic accuracy (93.8%) in identifying patients with early HF-related symptoms and signs (pre-HF), at risk of acute HF, who benefit from active clinical actions; (2) The care alert displays good specificity (93.5%) but very low sensitivity (11.1%) in identifying patients with pre-HF who benefit from active clinical actions; (3) Of all the CRT-D remote transmissions, 34.3% fulfilled the partners-HF criteria and 7.8% were triggered by a care alert. Positive partners-HF transmissions also determined a care alert in 15.4% of cases, and care alert transmissions met partners criteria in 67.3% of cases; (4) The most common reasons triggering a Positive partners-HF transmission were: Optivol fluid index $\geqslant$ 100 (18.8%) or optivol fluid index

Table 3 Diagnostic accuracy of partners-heart failure and care alert in determining active clinical actions

	Active clinical actions (2-3)	Non-active/no clinical actions (0-1)	Total transmissions
Positive partners-HF transmissions	193	35	228
Negative partners-HF transmissions	6	431	437
Positive care alert transmissions	22	30	52
Negative care alert transmissions	177	436	613
Overall transmissions followed by an action	199	466	665
Appendix partners-HF algorithm			
Parameters	Criterion		
Fluid index	⩾ 60 d		
AT/AF duration	⩾ 6 h and not persistent AT/AF		
VR during AT/AF	AT/AF ⩾ 24 h and VR ⩾ 90 bpm		
Patient activity	< 1 h over 1 wk		
NHR	⩾ 85 bpm for 7 consecutive days		
HRV	< 60 ms for 7 consecutive days		
%CRT pacing	< 90% for 5 of 7 d		
Shock (s)	⩾ 1 shock		

AT: Atrial tachycardia; AF: Atrial fibrillation; VR: Ventricular rate; NHR: Night heart rate; HRV: Heart rate variability; CRT: Cardiac resynchronization therapy; HF: Heart failure.

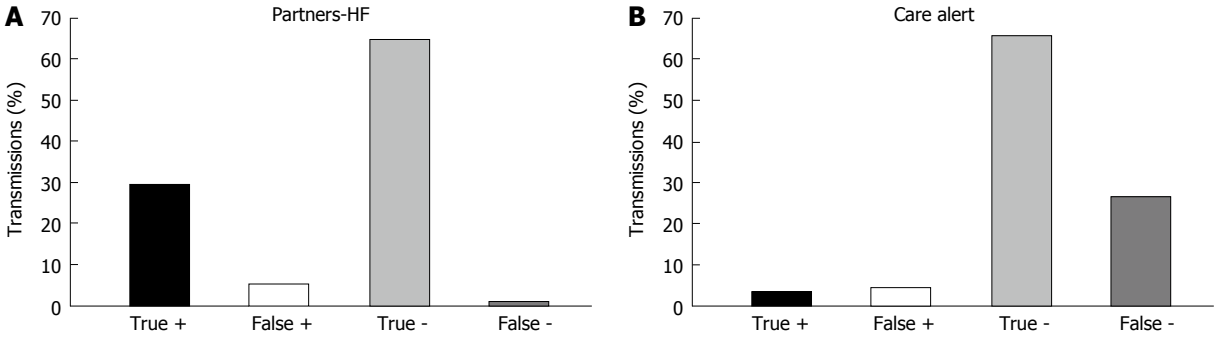

Figure 4 True and false positive and negative transmissions according to active clinical actions. A: True +: Positive partners-HF transmissions followed by active clinical actions (class 2 or 3); False +: Positive partners-HF transmissions followed by no actions or non-active clinical actions (class 0 or 1); True -: Negative partner-HF transmissions followed by no actions or non-active clinical actions (class 0 or 1); False -: Negative partners-HF transmissions followed by active clinical actions (class 2 or 3); B: True +: Positive care alert transmissions followed by active clinical actions (class 2 or 3); False +: Negative care alert transmissions followed by no actions or non-active clinical actions (class 0 or 1); True -: Positive care alert transmissions followed by no actions or non-active clinical actions (class 0 or 1); False -: Negative care alert transmissions followed by active clinical actions (class 2 or 3). All values are expressed in terms of percentage of total transmissions. HF: Heart failure.

> 60 plus one of the following parameters: reduced HRV (13.6%), low PA (12.3%), or reduced %CRT (11%); and (5) The most common active clinical action was HF-therapy titration, particularly of diuretics and beta-blockers, and the introduction of oral anticoagulation in patients with asymptomatic AF.

Despite advances in treatment of HF, it is still a major cause of cardiovascular mortality and hospitalization, especially in the early period after hospital discharge[1]. Prevention of HF relapses is important not only to reduce HF mortality and morbidity, but also health care costs[1]. Cardiac implantable electronic devices have nowadays remote monitoring capabilities that allow clinicians to have remote access to the complete device diagnostic information.

Previous studies

Earlier studies have shown that implantable device-measured variables, including intra-thoracic impedance, AF burden, mean heart rate, HRV, PA, frequency of PVCs, VA episodes, ICD shocks and %CRT, individuate subjects at risk of HF and facilitate early interventions[6-10]. Intrathoracic impedance[3], HRV and PA[2] reduction, occurs nearly two weeks before HF exacerbation. Low HRV indicate a sympathetic dominance in cardiac autonomic control and may be associated with exacerbation of atrial and VAs[13]. A prolonged AF duration, a rapid VR during AF and an increase in the burden of PVCs reduce %CRT[14] and are warning signs of HF, together with ICD shocks[15].

Although validated in various studies, the use of each device parameter in HF patients, is restricted by some limitations. In particular, variations of intrathoracic impedance may be related to lung inflammation; increased AF burden and prolonged AF duration are not useful in subjects with permanent AF; reduced mean heart rate, HRV or patient activity may reflect difficulty walking secondary to orthopedic diseases. Consequently, there is great interest in combining HF device diagnostic parameters for the management of CRT-D recipients.

The partners-HF[11] was a large cohort study explor-

ing the ability of the partners-HF criteria algorithm to dynamically stratify patients' risks of HF. A cohort of 694 CRT-D recipients with advanced HF (NYHA Ⅲ-Ⅳ) was prospectively evaluated in 100 centers. The retrospective evaluation of diagnostic CRT-D data demonstrated that subjects with a positive partners-HF algorithm had greater risk of hospitalization due to HF in the next month (adjusted HR = 5.5; $P < 0.001$). Moreover, the study demonstrated that increasing the frequency of reviewing the HF device diagnostics from quarterly (90 d) to monthly (30 d) but not to semimonthly (15 d), improved the ability to identify individuals at higher HF risk.

The prospective, multicenter observational Home Monitoring in CRT (Home-CARE) study[16] followed up for 1 year 377 CRT-D recipients who had been hospitalized for HF at least once within the 12 mo before enrollment. The following data were automatically retrieved every 24 h by the Home Monitoring (Biotronik, Berlin, Germany) algorithm: Mean heart rate, heart rate at rest, PA, frequency of PVCs, HRV, right ventricular pacing impedance, and painless shock impedance. The retrospective sensitivity values of individual parameters ranged from 23.6% to 50.0%, whereas their combination displayed 65.4% sensitivity and 99.5% specificity for cardiovascular hospitalizations and deaths.

Some studies have demonstrated favorable effects of RM in improving HF treatment, with potential benefits on clinical outcomes[6-10]. However, few and inconclusive data are available on the RM use in routine clinical practice and its impact on HF clinical outcomes. The Home Guide[17] registry proved RM highly effective in detecting clinical events, excluding deaths, with a sensitivity and a positive predictive values of 89% and 97%, respectively. RM sensitivity for atrial and VAs and device-related issues was > 90%, while it was < 35% for stroke, syncope and acute coronary syndromes and displayed an intermediate sensitivity (59%) for HF detection. Interestingly, 3 out of 4 events needing clinical intervention were asymptomatic and were effectively detected by RM, allowing a prompt reaction.

In our study the most common clinical reaction to partners-HF transmissions was drug therapy adjustment, while HF therapy titration and oral anticoagulation introduction in patients with asymptomatic AF were the most prevalent therapy interventions.

Clinical implications

This is the first multicenter observational registry prospectively assessing the clinical utility of partners-HF algorithm for risk stratification of HF patients in clinical practice. Remote monitoring of CRT-D recipients trough partners-HF algorithm, was not compared with usual care and this registry was not powered to explore the impact of the partners-HF algorithm on HF-hospitalizations and mortality. Our results prove that the partners-HF has significant diagnostic accuracy in determining active clinical actions oriented to treat pre-HF status and to prevent an acute decompensation. Given the high positive and very high negative predictive values, clinicians could contact only patients with positive partners-HF transmissions, thus avoiding a significant number of unnecessary telephone contacts.

Care alert displays very low sensitivity and a poor ability to identify patients needing active clinical action oriented to treat pre-HF status. Moreover, given its low positive predictive value, clinicians should be aware that an active clinical action oriented to preventing acute HF may be not necessary in case of care alert triggered transmissions.

Another important aspect is that this prospective analysis was conducted in patients with advanced HF (82.7%: NYHA classes Ⅲ/Ⅳ; mean EF: 27.5% ± 6.5%). This may explain the high percentage of manual and care alert transmissions collected and the high prevalence of positive partners-HF transmissions (35%). Considering that no HF hospitalization occurred in a population of advanced HF during a 6 mo follow-up, the partners-HF algorithm appears to be a powerful tool to identify and consequently treat pre-HF status in order to prevent acute decompensation.

According to the partners-HF study[11], positive partners transmissions were mostly triggered by the optivol fluid index, alone or in combination with low HRV, low PA or a low %CRT. The weight of each partners-HF criterion in the risk stratification of HF patients was not considered in this registry. A combined algorithm of HF diagnostic parameters could be utilized to stratify patients into high, medium and low risk of HF by using a specific risk stratification score, calculated by attributing a specific weight to each partners-HF criterion on the basis of its ability to detect pre-HF status. Finally, whether therapeutic interventions based on the partners-HF algorithm are effective in improving outcomes in HF, was not investigated.

The partners-HF algorithm proved to be a powerful predictor of a pre-HF status and was able to guide clinical actions oriented to avoiding acute HF. Future larger randomized prospective trials should be performed to confirm our results, to develop and validate a dynamic HF risk score based on the partners-HF algorithm and to ascertain whether the use of this algorithm for RM can improve the main clinical outcomes of HF patients.

COMMENTS

Background

Heart failure (HF) is a principal cause of death hospitalization and health care costs. The partners-HF algorithm retrospectively identified cardiac resynchronization-defibrillator (CRT-D) recipients at risk of HF relapses in the subsequent 30 d. However no studies have validated this algorithm prospectively and have compared it with the care alert strategy, that is commonly adopted for CRT-remote monitoring.

Research frontiers

Remote monitoring has emerged as a useful tool to prevent HF relapses, and to reduce cardiac hospitalization and mortality.

Innovations and breakthroughs

This is the first multicenter observational registry prospectively assessing the

clinical utility of partners-HF algorithm for risk stratification of HF patients in clinical practice.

Applications

The authors' prospective study showed that the partners-HF algorithm has significant diagnostic accuracy in determining active clinical actions oriented to prevent HF relapses. Moreover, it has a high positive and a high negative predictive value, allowing clinicians to contact only patients with positive partners-HF transmissions, thus avoiding a significant number of unnecessary telephone contacts.

Terminology

Remote monitoring: Wireless remote monitoring of cardiac electronic devices, including cardiac defibrillators and CRT.

Peer-review

This is a valuable research, because status of clinical actions is very important for patient's therapy and outcomes. Herein the traits of the partners-HF algorithm *vs* care alert in determining active clinical actions were explored and observed the effect of different methods on treatment or prevent heart failure.

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Salmonella Berta myocarditis: Case report and systematic review of non-typhoid *Salmonella* myocarditis

Pedro Villablanca, Divyanshu Mohananey, Garnet Meier, John E Yap, Sonam Chouksey, Ayokunle T Abegunde

Pedro Villablanca, Albert Einstein College of Medicine/ Montefiore Medical Center, Bronx, NY 10467, United States

Divyanshu Mohananey, Garnet Meier, John E Yap, Sonam Chouksey, Department of Internal Medicine, John H Stroger, Jr Hospital of Cook County, Chicago, IL 60612, United States

Ayokunle T Abegunde, Section of Digestive Diseases and Nutrition, Department of Medicine, Oklahoma University Health Sciences Center, Oklahoma City, OK 73104, United States

Author contributions: Villablanca P, Mohananey D, Meier G, Yap JE, Chouksey S and Abegunde AT contributed equally to the work; Villablanca P, Mohananey D and Abegunde AT conceptualized and designed the review; Villablanca P, Mohananey D, Meier G, Yap JE, Chouksey S and Abegunde AT contributed to search of literature and data extraction; Villablanca P, Mohananey D and Abegunde AT carried out the analysis and drafted the manuscript; all authors reviewed and approved the final manuscript as submitted.

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Correspondence to: Divyanshu Mohananey, MD, MSc, Department of Internal Medicine, John H Stroger, Jr Hospital of Cook County, 1900 W. Polk St. 9th Floor, Chicago, IL 60612, United States. divyanshu26@hotmail.com
Telephone: +1-786-3979971

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Abstract

AIM: To study trends in the epidemiology, clinical presentation, microbiology and prognosis of non-typhoid *Salmonella* (NTS) myocarditis.

METHODS: We performed a systematic literature search for all reported NTS cases. The search yielded 838 publications. A total of 21 papers were deemed eligible. No language restrictions were enforced. Articles that were not written in English were translated. Pre-specified data such as clinical presentation, electrocardiogram (ECG) changes, transthoracic echocardiographic findings, cardiac magnetic resonance findings, microbiology cultures, *Salmonella* species, inflammatory markers (erythrocyte sedimentation rate and C-reactive protein), cardiac biomarkers and severity of illness were collected using data extraction sheets. Cases were classified by age into 2 groups; pediatric cases (defined as < 18 years old) and adult cases (defined ⩾ 18 years old). The mean age of patients and standard deviations were calculated. The data was analyzed with IBM SPSS Statistics (Windows, Version 20.0. Armonk, NY: IBM Corp.) for demographic characteristics, presenting symptoms, microbiology, diagnostic methods, treatment modalities and outcome.

RESULTS: From the selected articles, we identified a total of 24 individual cases with verifiable data. There were 20 males with a male to female ratio of 5:1. The mean age at presentation was 30.8 years (range 1 mo-67 years), 16% of cases were children aged < 18 years. Most patients presented with chest pain, fever,

and abdominal pain. The most common ECG finding was ST elevation. Cardiac biomarkers were elevated in around 70% of cases. *Salmonella* Enteritidis was the most common NTS isolated. Definitive diagnosis was established by blood and stool cultures in most of the cases. The pediatric and adults cases had similar incidence of bacteremia (40% *vs* 36.8%) while the pediatric group had more stool cultures positive compared to the adult group (100% *vs* 63.1%). Eighty-three percent of patients received antibiotics and 58% were successfully treated through conservative management. The overall mortality was 24% and 42% of patients required intensive care.

CONCLUSION: This systematic review of published cases shows that NTS myocarditis occurs predominantly in young adults and carries a poor prognosis.

Key words: Diarrhea; Myocarditis; *Salmonella*; Non-typhoid

Core tip: Myocarditis is a rare extra-intestinal manifestation of non-typhoid *Salmonella* infection. In our review, the most common presenting symptoms were fever, abdominal pain, and chest pain and the most frequent electrocardiogram finding was ST segment elevation. Around 70% of patients had positive cardiac biomarkers (creatine kinase and/or troponin). *Salmonella Enteritidis* was the most common pathogen identified. Mortality appears to be high as is seen with all bacterial myocarditis, and intensive care unit admission is warranted in a large number of cases.

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INTRODUCTION

Salmonella species are gram-negative bacilli that are responsible for significant morbidity and mortality in both developing and developed nations. They are responsible for a wide spectrum of disease including enteric fever or typhoid fever [*Salmonella Typhi* (*S. Typhi*) and (*S. Paratyphi*)], as well as a range of clinical syndromes including diarrheal illness caused by a group of bacteria known as non-typhoid *Salmonella* (NTS)[1], *Salmonella* associated myocarditis is a rare entity described in case reports. Most case reports have described myocarditis associated with *S. Typhi* and Para-typhi infections. However, myocarditis associated with NTS, the species most commonly found in the western hemisphere, has been infrequently reported. Also, there is no existing structured analysis on this subject. We report an illustrative case and carefully analyze the available literature on NTS myocarditis in order to describe the epidemiological distribution, diagnostic trends and prognosis.

A 19-year-old male with no significant past medical history presented to the emergency department (ED) with a 2-d history of watery non-bloody diarrhea associated with diffuse abdominal cramping, fever, nighttime chills and sweats. He recalled eating jerk chicken from a local restaurant and a sausage-egg biscuit prior to onset of diarrhea. He ate alone and denied any sick contacts or recent travel. On admission his vitals were as follows: Temperature 38.8 ℃, heart rate (HR) 103/min and blood pressure (BP) 122/94 mmHg. Physical exam was unremarkable except for mild abdominal tenderness and dehydration. He received intravenous fluids and was discharged with a diagnosis of possible viral gastroenteritis. Forty-eight hours after discharge, he developed acute-onset chest pain (CP) and shortness of breath (SOB). The patient described the CP, as a retrosternal "squeezing" pain, 8/10 in severity, not radiating and associated with SOB at rest along with intermittent palpitations. He did not recognize any aggravating or relieving factors. When the symptoms persisted for 24 h, the patient came to the ED again and this time his vitals were as follows: BP 112/65 mmHg, HR 81/min, temperature 37.3 ℃ and respiratory rate 18 breaths/min with oxygen saturation of 97% on room air. Physical examination was unrevealing. Initial electrocardiogram (ECG) was significant for ST segment depression in leads V1 and V2 with ST segment elevations in leads V5 and V6 (Figure 1). Chest X-ray (CXR) did not show any cardiopulmonary process. Initial troponin I was 6.23 ng/mL (0.000-0.034 ng/mL) and a repeat assay 6 h later was 13.2 ng/mL. CBC showed hemoglobin of 12.5 g/dL, white blood cell count (WBC) of 6.9 × 10^9/L (4.4 × 10^9-10.6 × 10^9/L) with 26% bands. Kidney function and electrolytes were within normal limits. Liver enzymes showed alkaline phosphatase of 33 IU/L (50-120 U/L), aspartate aminotransferase of 42 IU/L (0-40 IU/L) and lactate dehydrogenase of 325 IU/L (85-210 IU/L). Table 1 summarizes the laboratory and imaging investigations for this patient. Within a few hours of admission, the patient became hypotensive with BP of 90/49 mmHg and the troponin went up to 18.9 ng/mL. He was subsequently transferred to the cardiac intensive care unit (ICU). Transthoracic echocardiogram (TTE) showed an ejection fraction (EF) of 40% with no regional wall motion abnormalities or pericardial fluid. Due to suspicion for myocarditis, a cardiac magnetic resonance imaging (CMRI) was done which showed multiple areas of abnormal sub-epicardial and mid-myocardial contrast hyper enhancement involving the posterior, inferior and anterior walls of the left ventricle, the anterior wall of right ventricle and the inter-ventricular septum reflecting multifocal biventricular myocarditis (Figure 2). Stool culture came back positive for *Salmonella*

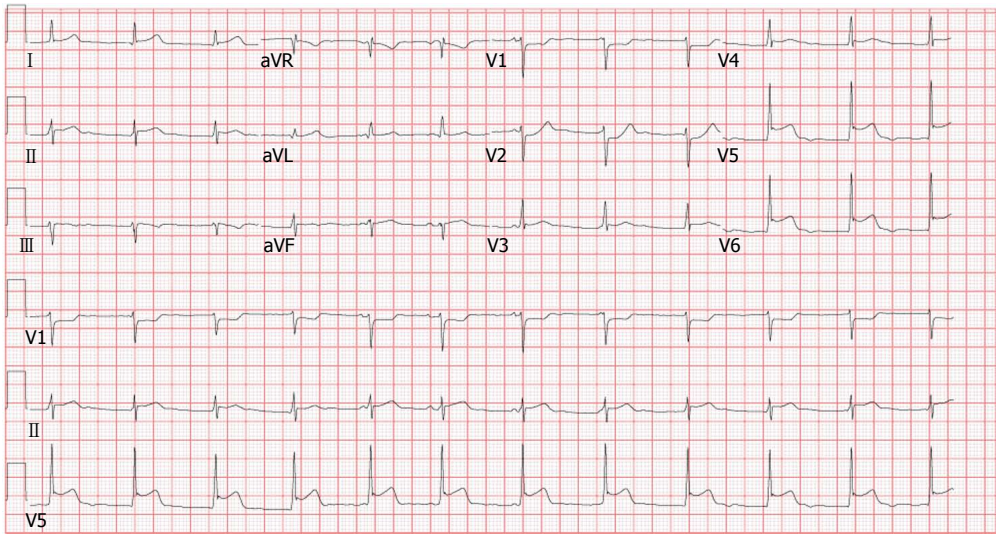

Figure 1 Electrocardiogram on admission with ST segment changes; ST segment depression in V1 and V2 with ST segment elevations in V5 and V6.

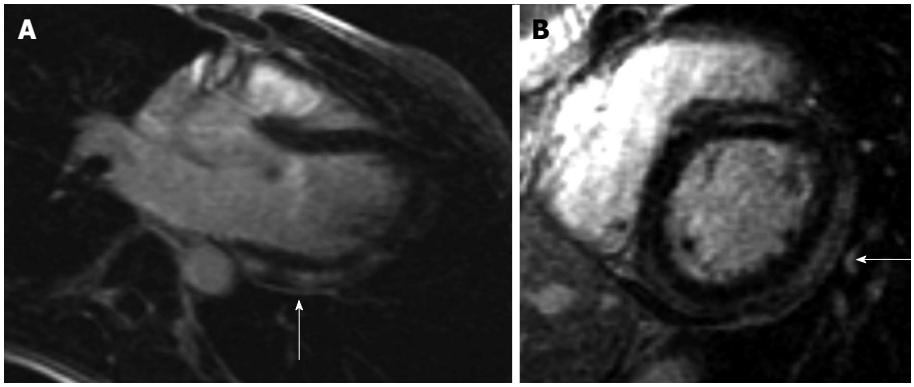

Figure 2 Cardiac magnetic resonance imaging findings. Cardiac magnetic resonance demonstrates pathological delayed gadolinium enhancement (as indicated by arrows). A: Long axis view: Delayed enhancement of the myocardium demonstrates subepicardial and mid myocardial enhancement indicative of myocarditis; B: Short axis view: Delayed enhancement of the myocardium demonstrates subepicardial and mid myocardial enhancement indicative of myocarditis.

Berta and therapy was initiated with sulfamethoxazole/trimethoprim. The patient gradually improved over the next 4 d and his CP resolved. He received 14 d of therapy during which symptoms resolved completely and EF normalized.

MATERIALS AND METHODS

This systematic review was conducted according to the PRISMA guidelines[2]. A computer-assisted literature search of PubMed, EMBASE CENTRAL and Google search engine was conducted. We also performed manual searches of the reference lists of studies, reviews, editorials, and letters, as well as related conference proceedings. Search terms keywords included "*Salmonella* myocarditis", "bacterial myocarditis", "non-typhoidal *Salmonella*" as well as combinations of these terms. No language restrictions were enforced. Articles that were not written in English were translated.

Inclusion criteria for publications in this systematic review: (1) Articles reporting original data; (2) Articles including patients with at least 1 blood and/or stool culture or tissue finding confirming diagnosis of *Salmonella*; (3) Articles including patients with clinical, electrocardiographic, or imaging evidence suggesting myocardial involvement; and (4) Articles providing data on at least one of the following: Clinical presentation, ECG description or original ECG, serum cardiac markers, any radiologic images. Exclusion criteria are listed as follows: (1) Articles including patients with myocarditis and infection with *Salmonella* typhi or para-typhi; (2) Articles reporting conditions that might present with clinical and imaging abnormalities similar to *Salmonella* myocarditis; (3) Articles on *Salmonella* infection affecting other organs; and (4) Review articles.

The following data was extracted: age, gender, presenting complaints (CP SOB, diarrhea) fever > 37.5 ℃, white blood cell count, serum cardiac markers [creatine kinase (CK) and Troponin], ECG characteristics, microbiology cultures, inflammatory markers [erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)], TTE findings, CMRI findings, CXR

Table 1 Diagnostic testing for reported case

Diagnostic test	Result
Hemoglobin	12.5 g/dL
WBC	6.9×10^9/L
Kidney function and electrolytes	Within normal limits
Alkaline phosphatase	33 IU/L
Aspartate aminotransferase	42 IU/L
Lactate dehydrogenase	325 IU/L
Troponin I (at presentation)	6.23 ng/mL
Troponin I (6 h later)	13.2 ng/mL
ECG	ST depression in V1 and V2 with ST elevations in V5 and V6
TTE	EF of 40% with no regional wall motion abnormalities or pericardial fluid
CMR	Multiple areas of abnormal sub-epicardial and mid-myocardial contrast hyper enhancement involving the posterior, inferior and anterior walls of the left ventricle, the anterior wall of right ventricle and the inter-ventricular septum reflecting multifocal biventricular myocarditis

WBC: White blood cell count; ECG: Electrocardiogram; TTE: Transthoracic echocardiogram; CMR: Cardiac magnetic resonance imaging; EF: Ejection fraction.

findings, hemodynamics (BP and HR), *Salmonella* species and possible source of infection, need for ICU admission and outcomes (dead or alive). There was no age restriction for inclusion of cases in the study. Cases were classified by age into 2 groups; pediatric cases (defined as < 18 years old) and adult cases (defined ⩾ 18 years old). The mean age of patients and standard deviations were calculated. All available ECG descriptions for each case were obtained and original data was analyzed. The ECG data was grouped into the following categories: ST segment elevation, ST segment depression, affected walls, T wave inversion, and others findings that included specific abnormalities such as prolonged QT, atrioventricular blocks (AVB), premature ventricular complex (PVC) were also recorded. Serum cardiac markers (CK and/or troponin) inflammatory markers (CRP and/or ESR) were classified as normal or elevated. Fever was defined as temperature > 37.5 ℃, leukocytosis as WBC count > 10000/mm^3, reduced EF as < 50%, hypotension as systolic BP < 90 mmHg and diastolic BP < 60 mmHg, tachycardia as HR > 100/min. If data was reported without quantification but the description matched the criteria it was considered a positive finding. CXR and CMRI findings were obtained from the report or direct data analysis if there was an available image. The prevalence of the different measured variables was calculated from the extracted data. The "not available" data cases were not considered in the calculation. Data was analyzed with IBM SPSS Statistics (Windows, Version 20.0. Armonk, NY: IBM Corp) for demographic characteristics, presenting symptoms, microbiology, diagnostic methods, treatment modalities and outcome. The analysis was reviewed by

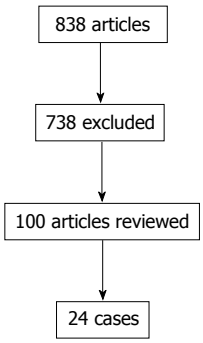

Figure 3 Flow chart of literature review.

Villablanca P (MD MS-Clinical Research).

RESULTS

The literature search yielded a total of 838 publications. Following the exclusion criteria, 816 citations were excluded after examining titles and abstracts, leaving twenty-one articles with 24 patients for detailed evaluation (Figure 3)[3-23].

Demographic characteristics

There were 19 adult and 5 pediatric cases, 20 males with a male-to-female ratio of 5:1. Female prevalence was higher in pediatric population (60%) as compared to adults (5.2%). The age of the patients ranged from 1 mo to 67 years. The mean age at presentation was 30.8 years. The mean age for adults and pediatrics cases was 36.6 years (range: 18-67 years) 9.6 years (range: 0.1-16 years) respectively.

Diagnostic evaluation

NTS species were identified either by blood cultures or stool cultures; only one patient had both cultures positive. One pediatric case was confirmed with myocardial biopsy after the patient died (Table 2). The pediatric and adults cases had similar incidence of bacteremia (40% *vs* 36.8%) while the pediatric group had more stool cultures positive compared to the adult group (100% *vs* 63.1%). *S. Enteritidis* was the most common pathogen found among all the reported cases, with a total of 10 cases (41.6%). *Salmonella typhymurium* was the most frequently reported pathogen in the adult group (36.8% of cases) and *S. Enteritidis* was the most frequently reported pathogen in the pediatric group (80% of cases) (Figure 4).

Presenting signs and symptoms

Fever, abdominal pain, and CP were the most common reported symptoms. Fever was present in 66.6% of the cases. SOB, chills and sweating were less prevalent. Of note, less than 25% of the cases had associated diarrhea. More than half of the cases of NTS presented with tachycardia and around 20% with hypotension. There was history of recent travel in 8 cases with a wide distribution around the world including Pakistan,

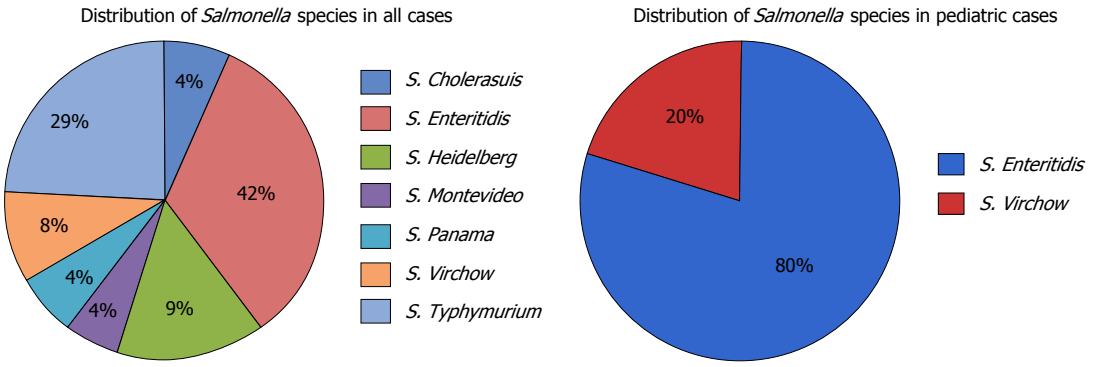

Figure 4 Distribution of *Salmonella* species in all cases, including pediatric cases.

Table 2 Clinical, electrocardiographic, laboratory, and imaging findings of non-typhoid *Salmonella* myocarditis

	n (%)	*n*[1]
Culture		
Blood	7 (29.1)	24
Stool	16 (66.6)	24
Myocardial biopsy	1 (4.1)	24
Presenting sign or symptom		
Dyspnea	6 (25)	24
Chest pain	15 (62.5)	24
Fever	16 (66.6)	24
Diarrhea	5 (22.7)	22
Abdominal pain	15 (71.4)	21
Hypotension	5 (21.7)	23
Tachycardia	13 (56.5)	23
ECG abnormalities		
ST elevation	12 (52.1)	23
ST depression	4 (17.3)	23
T-wave inversion	6 (26)	23
Infero-lateral	12 (52.1)	23
Antero-lateral	6 (26)	23
Inflammatory markers		
WBC > 10000/mm^3	8 (40)	20
Elevated troponin	9 (69.2)	13
Elevated CK	10 (66.6)	15
Elevated ESR or CRP	9 (100)	9
TTE		
Reduced ejection fraction	4 (36.4)	11
Regional wall motion abnormality	5 (45.5)	11
Chest X-ray		
Cardiomegaly	3 (27.7)	11
Pulmonary edema	3 (27.7)	11
CMR	2 (100)	2

[1]Based on reported data. ECG: Electrocardiogram; WBC: White blood cell count; CK: Creatine kinase; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; TTE: Transthoracic echocardiogram; CMR: Cardiac magnetic resonance imaging.

Bali, Dominican Republic, Spain and Eastern Europe. Average prodrome was 4.6 d. Duration of prodromal symptoms was slightly longer in the pediatric cases (8.1 d). Four cases recalled a possible source of infection, which included eating chicken, rice-eggs, sausages with spoiled meat and dumplings. Table 2 illustrates the frequency of presenting signs and symptoms.

Electrocardiographic findings

The most common finding on ECG was the presence of ST segment elevation (52.1%). ST segment depression was seen in a small number of the cases with only one pediatric case. Adults had a higher prevalence of more serious ECG findings including 3rd degree AVB (10.5%, *n* = 2) and ventricular fibrillation (5.2%, *n* = 1). More benign findings like 1st degree AVB, prolonged QT, PVC, right bundle branch block and low voltage were present in both groups. Table 2 shows the frequency of different ECG abnormalities along with area of involvement.

Markers of inflammation and cardiac injury

Variability in the choice of biomarkers was seen in the cases reviewed. Older cases used CK since troponin was not available. The prevalence of elevated CK and/or troponin in patients presenting with *Salmonella* myocarditis was near 70%. Prevalence of reported elevated troponin was high, in both, adults (71.4%, *n* = 10) and pediatric cases (40%, *n* = 2). However, troponin assay was not available at that time when most of the pediatric cases were reported. Most of the cases measured troponin I (55%, *n* = 5), followed by troponin T (33%, *n* = 3), and one case did not specify the kind of troponin used. Among the cases reviewed, the prevalence of elevated white count > 10000/mm^3 was 40% of which 25% had a left shift. Where it was measured, elevation in ESR or CRP was noted in 100% of cases of *Salmonella* myocarditis. Frequency of abnormalities in inflammatory and cardiac biomarkers is illustrated in Table 2.

Imaging

Almost 30% patients had evidence of pulmonary edema on CXR and less than 1/3 had cardiomegaly. On TTE, 36.4% reported reduced EF (less than 50%). More than 50% of the adults had regional wall motion abnormalities. Pericardial effusion was seen exclusively in children (3 cases). Out of the 24 cases, only 2 received CMRI scans, both of which revealed delayed gadolinium enhancements. Frequency of abnormal imaging studies is shown in Table 2.

DISCUSSION

Myocarditis is an inflammatory condition of the myocardium with both infectious and non-infectious etiologies.

It most commonly presents as non-ischemic cardiomyopathy but manifestations can range from sudden death, new onset atrial or ventricular arrhythmias, complete heart block or an acute myocardial infarction[24]. Viral infections are the most common cause of myocarditis with an epidemiological change from Coxsackie B virus and adenovirus being identified in the past to parvovirus B19 being the most common etiological agent currently[24]. Bacterial myocarditis is uncommon with a prevalence ranging from 0.2% to 1.5%[25]. However, it should always be considered in patients with sepsis and ventricular dysfunction[26].

Salmonella species are gram-negative bacilli that are responsible for significant morbidity and mortality in both developing and developed nations. Both typhoidal and NTS can have extra-intestinal manifestations however myocarditis is an uncommon extra-intestinal manifestation[27]. NTS are food-borne pathogens that are responsible for diarrheal illness. There is about a 5% incidence of invasion beyond the gastrointestinal tract present most commonly in immunocompromised hosts[28]. In our review, the major presenting symptoms were fever and CP with only around 20% having diarrhea. A large study of over 7000 human *Salmonella* infections noted cardiac involvement in the form of endocarditis in 20 patients all of whom had a rapidly fatal outcome[29]. It can be postulated that myocardial damage occurs secondary to involvement of endocardium or due to direct bacterial invasion from bacteremia. In addition to this, sepsis induced myocardial depression and subsequent remodeling may also play a part as it does in other bacterial myocarditis[26].

ECG analysis in myocarditis usually shows sinus tachycardia with non-specific ST segment changes and T wave abnormalities[24]. In our review, the most common ECG abnormality was ST elevation. Troponins have been shown to have high specificity (89%) and low sensitivity (34%) for diagnosis of myocarditis[30]. In our review troponins were elevated in majority of the patients with NTS myocarditis. Even though the gold standard for diagnosis remains endomyocardial biopsy, CMRI is slowly replacing the need for more invasive procedures[31]. In a recent study of 82 patients with troponin elevation without significant coronary artery disease, late gadolinium enhancement CMRI established a diagnosis of myocarditis in 80% of the patients in comparison to 88% diagnosed with endomyocardial biopsy[31]. In our review, only 2 patients received CMRI with both cases showing evidence of myocarditis. TTE showed a reduced EF in around 36% of the cases with around 50% of adult cases showing regional wall motion abnormalities. Interestingly, all of the pediatric cases showed pericardial fluid suggestive of increased incidence of pericardial disease in pediatric population affected by *Salmonella*.

The most common etiological agent overall was *S. Enteritidis* (40%). This is in concordance with a Malaysian retrospective analysis of 55 patients with NTS bacteremia, which showed that *S. Enteritidis* had the maximum blood invasiveness[28]. The overall mortality in our patients was around 20% with 40% of patients requiring ICU stay. Antibiotic therapy is not recommended for NTS gastrointestinal infections. However, it should be considered if patients are at risk for invasive disease (age > 50 or neonates, immunosuppressed, sickle cell patients and those with vascular abnormalities)[27]. Treatment of myocarditis caused by NTS has not been detailed in any study, but in essence it can be treated as NTS bacteremia or as a life threatening infection. In those cases affected by life threatening infections, treatment should be started with both, a third-generation cephalosporin and a fluoroquinolone until the susceptibility is known[32]. Ninety percent of the patients included in this review received antibiotic treatment.

Results of this systematic review of published cases shows that NTS myocarditis occurs predominantly in young adults and carries a poor prognosis. The initial diagnostic approach is similar to myocarditis due to other etiologies and includes ECG, TTE and CXR. Upon diagnosis, patients should receive supportive therapy for myocarditis in addition to antibiotics. Mortality appears to be high as with all bacterial myocarditis and ICU admission is warranted in a large number of cases. *Salmonella* infections are a rare cause of myocarditis but should always be considered in cases presenting with features of myocarditis and evidence of *Salmonella* infection in the absence of viral etiology.

COMMENTS

Background

Salmonella species are bacteria that are responsible for significant morbidity and mortality in both developing and developed nations. They are responsible for a wide spectrum of disease including enteric fever or typhoid fever (*S. Typhi* and *S. Paratyphi*) and a range of clinical syndromes including diarrheal illness caused by a group of bacteria known as non-typhoid *Salmonella* (NTS). In rare circumstances *Salmonella* can cause inflammation of the myocardium (myocarditis).

Research frontiers

To the best of our knowledge, no systematic review of NTS myocarditis has previously been published. The authors carefully analyze the available literature on NTS myocarditis in order to describe the epidemiological distribution, diagnostic trends and prognosis of this condition.

Innovations and breakthroughs

Salmonella Enteritidis was the most common pathogen identified in these cases. Around 30% of patients had bacteremia and 100% of pediatric patients had either stool or blood culture positive for *Salmonella*. Fever, abdominal pain and chest pain were the most common presenting symptoms and ST segment elevation was the most frequent electrocardiogram finding. Around 70% of patients had positive cardiac biomarkers (creatine kinase and/or Troponin). Mortality appears to be high as with all bacterial myocarditis and intensive care unit admission is warranted in a large number of cases.

Applications

Salmonella infections are a rare cause of myocarditis but should always be considered in cases presenting with features of myocarditis and evidence of *Salmonella* infection in the absence of viral etiology.

Terminology

Salmonella species are gram-negative bacteria that cause a wide range of

diseases. *S. Berta*, *S. Typhi*, *S. Paratyphi*, *S. Enteritidis, etc*., are all serotypes of the Genus *Salmonella*. However, based on clinical syndromes, *i.e.*, causation of enteric fever, salmonella can be divided into typhoid salmonella and NTS. Myocarditis is an inflammatory condition of the muscular wall of the heart.

Peer-review

NTS infection involving myocarditis is rare, and the current case presentation and systemic review of the disease is therefore unique.

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SYSTEMATIC REVIEWS

Adherence to cardiovascular medications in the South Asian population: A systematic review of current evidence and future directions

Julia M Akeroyd, Winston J Chan, Ayeesha K Kamal, Latha Palaniappan, Salim S Virani

Julia M Akeroyd, Winston J Chan, Salim S Virani, the Health Policy, Quality and Informatics Program, Michael E DeBakey Veterans Affairs Medical Center, Health Services Research and Development Center for Innovations, Houston, TX 77030, United States

Ayeesha K Kamal, Section of Neurology, Department of Medicine, Aga Khan University Hospital, Karachi 74800, Pakistan

Latha Palaniappan, General Medicine Disciplines, Stanford School of Medicine, Stanford, CA 94304, United States

Salim S Virani, Health Services Research and Development, Michael E DeBakey Veterans Affairs Medical Center, Houston, TX 77030, United States

Salim S Virani, Section of Cardiovascular Research, Department of Medicine, Baylor College of Medicine, Houston, TX 77030, United States

Salim S Virani, Center for Cardiovascular Disease Prevention, Methodist DeBakey Heart and Vascular Center, Houston, TX 77030, United States

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Correspondence to: Salim S Virani, MD, PhD, Health Services Research and Development, Michael E DeBakey Veterans Affairs Medical Center, 2002 Holcombe Blvd., Houston, TX 77030, United States. virani@bcm.edu
Telephone: +1-713-4404410
Fax: +1-713-7487359

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Abstract

AIM: To review methods of assessing adherence and strategies to improve adherence to cardiovascular disease (CVD) medications, among South Asian CVD patients.

METHODS: We conducted a systematic review of English language studies that examined CVD medication adherence in South Asian populations from 1966 to April 1, 2015 in SCOPUS and PubMed. Working in duplicate, we identified 61 studies. After exclusions, 26 studies were selected for full text review. Of these, 17 studies were included in the final review. We abstracted data on

several factors including study design, study population, method of assessing adherence and adherence rate.

RESULTS: These studies were conducted in India (*n* = 11), Pakistan (*n* = 3), Bangladesh (*n* = 1), Nepal (*n* = 1) and Sri Lanka (*n* = 1). Adherence rates ranged from 32%-95% across studies. Of the 17 total publications included, 10 focused on assessing adherence to CVD medications and 7 focused on assessing the impact of interventions on medication adherence. The validated Morisky Medication Adherence Scale (MMAS) was used as the primary method of assessing adherence in five studies. Three studies used validated questionnaires similar to the MMAS, and one study utilized Medication Event Monitoring System caps, with the remainder of the studies utilizing pill count and self-report measures. As expected, studies using non-validated self-report measures described higher rates of adherence than studies using validated scale measurements and pill count. The included intervention studies examined the use of polypill therapy, provider education and patient counseling to improve medication adherence.

CONCLUSION: The overall medication adherence rates were low in the region, which suggest a growing need for future interventions to improve adherence.

Key words: Assessing medication adherence; South Asia; Cardiovascular disease medication

Core tip: The overall adherence rate in South Asia is quite low. Only 7 of 17 publications conducted interventions geared toward improving adherence. Even fewer (*n* = 3) utilized community health care workers, which provide a unique resource in these resource constrained environments. Just over half of the studies found in our review utilized validated or gold standard methods (*n* = 9) with the rest using non-validated self-reported measures. Additionally, there was a lack of usage of technology despite the majority of these countries benefitting from a high cell phone density.

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide, with an estimated 17.5 million people dying from CVD in 2012[1]. Approximately one-fifth of the global population resides in South Asia (India, Pakistan, Bangladesh, Nepal, and Sri Lanka), where patients suffer from a disproportionately high rate of CVD-related morbidity and mortality[2-6]. In a large international, case-control study of first myocardial infarction (MI), results indicated that the mean age for first MI was significantly lower in South Asian participants (53.0 years) than in participants from other countries (58.8 years)[5]. In approximately 10% of these cases, first MI in South Asian participants occurred in those aged 40 or below. These data indicate a growing epidemic of premature CVD in South Asian populations.

Use of beta-blockers, angiotensin-converting-enzyme (ACE) inhibitors, statins and antiplatelet drugs are successful means of secondary prevention of CVD. However, the use of these drugs varies widely by population. Results of a large-scale epidemiological study examining use of secondary prevention drugs for CVD in high-income, middle-income, and low-income countries showed that use was highest in high-income countries [antiplatelet drugs 62.0%, β blockers 40.0%, ACE inhibitors or angiotensin Ⅱ receptor blockers (ARBs) 49.8%, and statins 66.5%] and lowest in low-income countries (including India, Bangladesh and Pakistan) (8.8%, 9.7%, 5.2%, and 3.3% for antiplatelets, β blockers, ACE inhibitors or ARBs, and statins, respectively)[7].

An important factor in the use of appropriate medications to manage CVD risk is medication adherence. Adherence is critical to the effectiveness of all drug therapies, but is particularly important for medications prescribed for chronic conditions[8]. In a study of 37154 patients with established atherothrombotic disease, non-adherence to medications at baseline and one year were both significantly associated with increased risk of cardiovascular death, myocardial infarction, or stroke at 4 years[9]. This is of particular importance to South Asian countries for various reasons. First, low availability of electronic medical records in most health care settings precludes accurate assessment of medication adherence by health care providers in these countries and therefore, poses specific challenges in the assessment of medication adherence. Second, the overall health literacy and the opportunities to improve provider and patient awareness of the importance of medication adherence may be limited. Lastly, low availability of pharmacy records and medication refill data also limit the use of traditional measures used to assess medication adherence (*i.e.,* medication possession ratio or proportion of days covered).

Therefore, the overall aim of this review is to examine the current methods of assessing adherence to CVD medications as well as explore current interventional strategies to improve medication adherence, among CVD patients in South Asia.

MATERIALS AND METHODS

Search strategy and study selection

To identify eligible studies, we conducted a systematic search of the literature using the electronic databases PubMed (1966 to April 1, 2015) and SCOPUS (1966 to

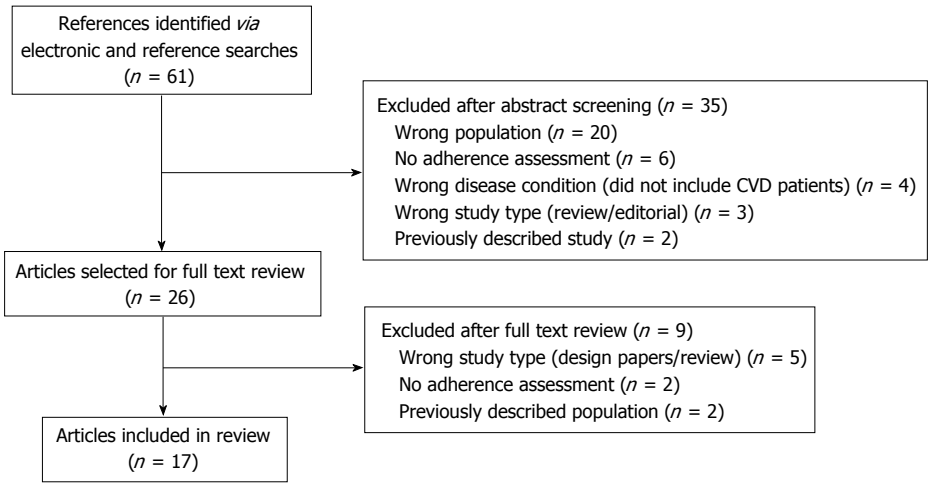

Figure 1 Results of systematic search. CVD: Cardiovascular disease.

April 1, 2015) and reviewed reference lists for relevant articles. Major search terms included "medication adherence" OR "adherence medication" OR "enhancing medication adherence" OR "measuring medication adherence" OR "medication adherence scale" OR "Morisky medication adherence scale" OR "interventions for enhancing medication" AND "South Asia" OR "South Asian" OR "India" OR "Pakistan" OR "Sri Lanka" OR "Nepal" OR "Bangladesh" AND "cardiovascular disease" OR "cardiovascular disease medication" OR "cardiovascular disease medicine".

Working in duplicate, reviewers screened all abstracts and full-text publications for eligibility. The following details were abstracted from included publications: Patient population, sample size, country, study design, adherence measure, definition of adherence and adherence rate.

Eligibility criteria

Eligible studies were defined as fully published (English language) studies that examined CVD medication adherence as the primary or secondary outcome in South Asian populations. English is widely spoken in post-colonial South Asia, and there are very few scientific studies published in native languages in this region. We excluded studies conducted outside of the target population (India, Pakistan, Sri Lanka, Nepal or Bangladesh), studies not reporting the method of adherence assessment, studies not focused on CVD medications, studies with previously described populations, published reviews and editorials, and studies reporting no results (rationale/design papers) (Figure 1).

RESULTS

Results of our systematic search of the literature are shown in the Figure 1. A total of 61 studies were identified through electronic and reference searches, and 35 were excluded after abstract review. The majority of abstracts were excluded because the studies were conducted outside of South Asia (*n* = 20), there was no assessment of adherence reported (*n* = 6), the therapeutic area was not CVD (*n* = 4), the paper was a review or editorial (*n* = 3), or the study population was already included in our review through a different publication (*n* = 2). Of the 26 articles included in the full-text review, we further excluded 9 articles. The reasons for exclusion of full text articles were that the papers were design/rationale or review papers (*n* = 5), no adherence assessment was reported (*n* = 2) and the study population was previously described in an included paper (*n* = 2). Therefore, the final review included 17 articles. A summary of the included studies is shown in the Table 1.

The majority of the studies included in this review were conducted in India (*n* = 11). The remainder of the studies were conducted in Pakistan (*n* = 3), Bangladesh (*n* = 1), Nepal (*n* = 1) and Sri Lanka (*n* = 1). Below, we provide a synthesis of these results in terms of strategies used to measure adherence followed by interventions to improve adherence to CVD medications in South Asian populations.

Adherence assessments

Adherence is defined as "the extent to which a person's behaviour - taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider"[10]. Of the 17 publications included in this review, 10 focused on assessing adherence to CVD medications. Adherence was defined and measured using a variety of methods across studies. These studies largely used self-report to determine adherence rate, including the use of the Morisky Medication Adherence Scale (MMAS)[11] and other validated questionnaires, interview questions and pill counts.

Five studies used the validated MMAS[11] as their primary method of adherence assessment. These studies utilized the 4-item MMAS, which scores 1 point for each "no" response and 0 points for each "yes" response, with total scores ranging from 0 (non-adherent) to 4 (fully adherent). Hashmi *et al*[12] used the 4-item MMAS

Table 1 Summary of studies included in the systematic review

Ref.	Patient population	Sample size	Country	Design	Adherence measure	Definition of adherence	Adherence rate
Joshi *et al*[25]	Hypertensive patients seeking treatment	139	India	Cross-sectional	Pill count	⩾ 80% pills taken	79% in controlled hypertensives 39% in uncontrolled hypertensives
Ponnusankar *et al*[32]	Patients with chronic conditions (hypertension, diabetes mellitus, cardiovascular conditions, bronchial asthma)	90	India	Cross-sectional	Pill count Self-assessment	Prescribed doses taken Rating system	92.29% ± 4.5% for counseled group 84.71% ± 11.8% for usual care
Hashmi *et al*[12]	Patients prescribed anti-hypertensive medication for at least 1-mo prior	438	Pakistan	Cross-sectional	Pill count 4-item MMAS	⩾ 80% pills taken ⩾ 0 scores	Pill count : 77% Morisky: Mean overall score = 2.5 ± 1.3
Qureshi *et al*[29]	Patients on anti-hypertensive medications	178	Pakistan	Randomized controlled trial	MEMS bottles	Prescribed doses taken	Intervention arm: 48.1%, 95%CI: 35.8%-60.4% Control arm: 32.4%, 95%CI: 22.6%-42.3%
Kar *et al*[30]	Adults with SBP ⩾ 140	1010	India	Cross-sectional	Self-report	All prescribed doses taken daily in the past 15 d	Baseline: 37.9% Follow-up: 58.3%
Bahl *et al*[26]	Adults with hypertension	1175	India	Prospective	Self-report	If all doses were taken since last visit	100% at each follow-up point
Palanisamy *et al*[31]	Post discharge patients prescribed at least 1 anti-hypertensive medication	43	India	Cross-sectional	4-item MMAS	At least 1 yes response was classified as non-adherent	95.4%
Dennis *et al*[19]	Hypertensive adults with at least 6 mo treatment history	608	India	Cross-sectional	BMQ	⩾ 1 indicates non-adherence	50.33%
Saleem *et al*[21,22]	Hypertension adults using anti-hypertensive drugs for last 6 mo	385	Pakistan	Cross-sectional	DAI-10	⩾ 0 survey scores	Overall mean score: -1.74 ± 2.154
Soliman *et al*[27]	Adults with an estimated 10-yr total CVD risk score greater than 20%	203	Sri Lanka	Randomized clinical trial	Pill count	Not reported	Intervention arm: Over 80% with > 80% pill compliance Usual care arm: Results not provided
Simkhada[16]	Hypertensive patients taking anti-hypertensive medication for at least 6 mo	147	Nepal	Cross-sectional, prospective study	6-item questionnaire	⩽ 1 scores	17.34%
Fathima *et al*[13]	Patients at a CVD clinic	162	India	Cross-sectional	4-item MMAS	⩾ 0 scores	Mean score = 3.2
Thom *et al*[28]	Adults with established CVD, or estimated 5-yr CVD risk ⩾ 15%	2004	Europe and India	Randomized clinical trial	Self-report	Taking medication at least 4 d during the week preceding visit	Intervention arm: 86.3% Usual care arm: 64.7%
Khanam *et al*[23]	Randomly selected hypertensive adults from 3 rural surveillance sites	29960	Bangladesh	Cross-sectional	Self-report	Continued medication use at time of interview	73.8%
Kumar *et al*[15]	Adults on anti-hypertensive medication for more than 6 mo	120	India	Cross-sectional	8-item MMAS Self-reporting	⩽ 2 scores Not-defined in paper	54.2% 96.7%
Rao *et al*[24]	Adults with hypertension and/or diabetes	426	India	Cross-sectional	Self-report	⩾ 80% pills taken	Hypertensive 82.2% Diabetic 83.6%
Venkatachalam *et al*[14]	Adults with hypertension for ⩾ 1 yr	473	India	Cross-sectional	4-item MMAS	⩾ 0 scores	24.1%

MMAS: Morisky medication adherence scale; MEMS: Medication event monitoring system; SBP: Systolic blood pressure; CVD: Cardiovascular disease; BMQ: Brief medication questionnaire; DAI: Drug attitude inventory.

to evaluate adherence to anti-hypertensive therapy in 460 patients from two tertiary care hospitals in Pakistan. Additionally, patients were asked to report the number of pills they were prescribed each week and the number of pills they took and missed over the previous 3, 5, and 7 d. Adherence rate was calculated as pills taken divided by pills prescribed for each time point, with patients taking 80% or more of their prescribed medication classified as adherent, and those taking less than 80% classified as non-adherent. According to the 80% cutoff level, 77% of patients were adherent (mean = 98% ± 5%) and 23% were non-adherent (mean = 39% ± 29%). The mean overall MMAS score was 2.5. Results indicated that adherence by pill count was significantly associated with MMAS score (β = 0.016 for the linear relationship between pill count and

MMAS; $P < 0.001$). Fathima *et al*[13] used the 4-item MMAS to evaluate adherence to prescribed medications among 162 patients with hypertension, diabetes or ischemic heart disease in Bangalore, India. Total scores ranged from 0 (non-adherent) to 4 (fully adherent), with scores of 1, 2, and 3 classified as moderately adherent. The mean MMAS score was 3.2, with 40.1% classified as fully adherent, 58.6% classified as partially adherent, and 1.3% classified as non-adherent. Results showed a significant association between age and adherence, with a significantly higher proportion of patients 60 years of age and older fully adherent (48.1%) compared to those under 60 (32.5%), $P < 0.05$. Similarly, a significantly higher proportion of patients who perceived that their medication was not expensive were fully adherent (51.2%) compared to those who perceived their medication as expensive (27.6%), $P < 0.05$. Venkatachalam *et al*[14] examined determinants of adherence to hypertension medication in 473 individuals residing in South India. Adherence was assessed using the 4-item MMAS, where patients were classified as non-adherent if they failed to meet any one of the four MMAS criteria. Overall adherence was 24.1%, with 51.6% of patients forgetting to take medication regularly, 59.8% having difficulty remembering to take their medication, 53.6% stopping medication upon feeling better, and 55.2% stopping medication upon feeling worse. Kumar *et al*[15] looked at factors associated with medication adherence in 120 hypertensive patients at a tertiary care hospital in India. Adherence was assessed using the 8-item MMAS and through self-report (not well defined in the publication). The 8-item MMAS was scored 1 point for every "yes" response and 0 points for every "no" response, with a score of 2 or greater classified as low adherence, 1-2 as medium adherence, and 0 as high adherence. Despite a self-reported adherence rate of 96.7%, MMAS scores indicated that 45.8% of the study sample had low adherence, 54.2% had medium adherence, and 0% had high adherence.

Simkhada[16] conducted a cross-sectional prospective study of blood pressure control and predictors among 147 hypertensive patients in Nepal. Adherence was assessed using a 6-item questionnaire adapted from Choo *et al*[17] and Morisky *et al*[11] as used by Rose *et al*[18] in a previous publication. The 6 questions assessed difficulty taking medications, forgetting to take medications, number of days medications were missed in the past week, number of days an extra pill was taken in the past week, medications taken less because the patient felt better, and medications taken more because the patient felt worse. Patients who responded "yes" to two or more questions were classified as non-adherent. Results showed that only 29.9% of patients were adherent to blood pressure medications.

Two studies included in this review used validated questionnaires other than the MMAS to assess adherence. Dennis *et al*[19] examined barriers to medication adherence in 608 hypertensive patients in India. Adherence was measured using the Brief Medication Questionnaire (BMQ), and through detailed patient interviews. The BMQ is a self-reported survey that measures barriers to adherence through four screens. The regimen screen (previously validated against Medication Event Monitoring System caps)[20] consists of 5 questions assessing patient behavior towards taking medication. Each question is worth 1 point, where scores greater than 1 indicate non-adherence. Overall, 50.3% of patients were adherent to anti-hypertensive therapy. Many patients believed that either their medications were not working (37.8%), or that medications would bother them (5.9%). Access to medication was also an issue, with 78.6% of patients reporting difficulty paying for medication and 54.9% reporting difficulty getting refills on time.

Saleem *et al*[21] examined the association between knowledge and medication adherence among 385 hypertensive patients in Quetta City, Pakistan. Adherence was measured using the 10-item drug attitude inventory (DAI-10). Scores ranged from -10 to 10, with negative scores indicating non-adherence, 0-5 indicating moderate adherence, and 6-10 indicating high adherence. The DAI-10 was pilot-tested with 40 hypertensive patients for reliability and validity (Cronbach's $\alpha = 0.7$) and translated from English to Urdu. The hypertension fact questionnaire was used to assess patient's knowledge of hypertension, its causes, treatment and management. The overall mean score of the DAI-10 was -1.74 ± 2.15. In this study, 64.7% of patients were classified as non-adherent, 35.3% as moderately adherent, and none as highly adherent. There was a significant, inverse relationship between knowledge and adherence, which investigators noted was a conflicting outcome. Saleem *et al*[22] published another study on the same population, using the same assessment of adherence, looking at the association between adherence and quality of life. These results showed no relationship between quality of life and medication adherence.

Two of the included studies used interview questions to assess adherence. Khanam *et al*[23] measured adherence to anti-hypertensive medication in 29960 patients residing in rural Bangladesh. Adherence was measured through self-reported interview questions using a two-part questionnaire on chronic disease lifestyle risk factors and management. The questionnaire was translated from Bangla to English and back in order to check for consistency of the meaning. Additionally, the questionnaire was piloted and pre-tested. Non-adherence was defined as discontinuation of medication at the time of the interview and was classified as a dichotomous various variable (yes or no) based on this definition. The overall rate of non-adherence in this population was 26.2%. Non-adherence was higher among men (29.2%) than women (24.3%), ($P < 0.001$), decreased with age ($P < 0.001$) and was less common among wealthy people. Non-adherence was greater when hypertension was diagnosed by unqualified providers (community health workers or informal health providers) (OR = 1.67, 95%CI: 1.42-1.97). Those who reported cardiovascular

comorbidities (angina, heart attack or stroke) were more likely to be adherent to medication (OR = 0.78, 95%CI: 0.64-0.97 for non-adherence). Rao *et al*[24] examined medication adherence among 426 patients with either hypertension or type-2 diabetes mellitus in southern India. Adherence to either hypertension or diabetes medication was assessed through self-reported interview questions. Patients were asked to report how they had been taking their medication in the week preceding the interview. Patients who reported taking less than 80% of their prescribed medications in the week preceding the interview were classified as non-adherent. The interview questions were pilot tested and the validity was appraised by experts. Among hypertensive patients, 82.2% reported adherence to treatment, while 83.6% of diabetics reported adherence to treatment. Adherence was higher among females (87.4%) than males (72.2%) ($P < 0.05$). High cost of treatment and asymptomatic disease were the most commonly cited reasons for non-adherence among those with hypertension (39% and 35% respectively) and diabetes (30% and 48%, respectively).

Joshi *et al*[25] examined the relationship between medication adherence and blood pressure control in 156 hypertensive patients in urban India. Adherence was assessed at the end of three month follow-up by pill count. Subjects who took less than 80% of their medication were classified as non-adherent. Adherence was 79% among patients with controlled hypertension, and 39% among patients with uncontrolled hypertension. Additionally, non-adherence was a significant predictor of uncontrolled hypertension in this population (OR = 6.23, 95%CI: 2.36-16.48, $P > 0.0001$).

Strategies for improving adherence

Of the 17 publications included in this review, 7 publications examined interventions aimed at improving adherence to CVD medications in South Asian populations as a primary or secondary outcome. Three of these interventions examined the use of combination or polypill therapy to improve adherence. Bahl *et al*[26] conducted an observational, open-label study to examine the use of a fixed combination therapy of perindopril and amlodipine on the management of hypertension among 1250 patients in India. Adherence was measured *via* self-report through interview questions asked on days 15, 30 and 60 of follow-up. Patients were asked if they had missed any doses and what time they took their medication the day prior to follow-up. All patients who completed the study (94%) adhered to their treatment as specified in the study protocol. It is important to note that there was no usual care group in this study. It is also important to point out that this is a clinical trial, in which participants are generally more adherent than free-living populations. Soliman *et al*[27] examined the effects of a polypill (75 mg aspirin, 20 mg simvastatin, 10 mg Lisinopril and 12.5 mg hydrochlorothiazide) *vs* usual care (not specified) on the prevention of CVD in 216 patients without established CVD. Adherence was assessed *via* monthly self-reports using pill counts (no further details provided). Results showed that the polypill intervention arm had 80% of patients with greater than 80% adherence, 6% with 60%-79% adherence, 3% with 40%-59% adherence, and 10% with < 40% adherence. Results for the usual care group were not reported. Thom *et al*[28] studied the effects of a fixed-dose combination (FDC) therapy (either aspirin, 75 mg; simvastatin, 40 mg; lisinopril, 10 mg; and atenolol, 50 mg or aspirin, 75 mg; simvastatin, 40 mg; lisinopril, 10 mg; and hydrochlorothiazide, 12.5 mg) *vs* usual care (treated at the discretion of their usual physician) on medication adherence and risk factors in patients at high risk of CVD. They utilized data from the Use of a Multidrug Pill in Reducing Cardiovascular Events randomized clinical trial of 1004 patients from Europe, and 1000 from India. Adherence was measured through self-report during a follow-up visit and defined as taking medication at least 4 d during the week before the visit. For patients in India, adherence in the FDC group *vs* the usual care group was 89.1% and 63.6%, respectively (RR = 1.40; 95%CI: 1.30-1.51). For patients in Europe, adherence in the FDC group *vs* the usual care group was 83.6% and 65.8%, respectively (RR = 1.27; 95%CI: 1.18-1.37). The effects of the FDC strategy on adherence did not differ significantly between patients from India *vs* patients from Europe ($P = 0.07$ for interaction).

Qureshi *et al*[29] used MEMS bottles to determine the impact of an education package aimed at general practitioners to increase adherence to anti-hypertensive drugs in 200 patients in Karachi, Pakistan. Components of the education package included non-pharmacological (diet, exercise, weight loss, smoking cessation) and pharmacological interventions (prescribing low cost and appropriate generic drugs; preferential use of single dose drug regimens; scheduled follow-up visits; stepped care approach for titration of drugs to achieve target blood pressure levels; and satisfactory consultation sessions for patients, with explanations of treatment and use of appropriate communication strategies). Adherence, defined as percentage of prescribed doses taken, was measured using MEMS bottles, which electronically monitored and recorded the time each cap was opened. Adherence was recorded by community health workers at the end of weeks 1, 3 and 6 of the study. Adherence was significantly better in the intervention group (unadjusted mean percentage days with correct dose) *vs* the standard of care group (48.1% *vs* 32.4%, $P = 0.048$) over the 6 wk period. Greater adherence was significantly associated with higher levels of education ($P < 0.001$), patients being encouraged by family members to take medications ($P < 0.001$), patient's belief in drug effects ($P < 0.001$) and having the purpose of the drug explained to the patient ($P < 0.001$).

Three small scale interventions used counseling to improve adherence. Kar *et al*[30] conducted a community intervention to implement the World Health Organization (WHO) CVD risk management package in India. The

intervention was implemented among 1010 adults from a randomly chosen cluster of households, and 79 hypertensive patients were followed at 1, 3, and 5 mo to reinforce risk prevention and adherence to medications. Adherence was assessed by self-report and defined as daily intake of all prescribed anti-hypertensive medication in the past 15 d. In the intervention households, regular intake of medication increased from 37.9% to 58.3% ($P < 0.05$), while in the control households, it decreased from 43.5% to 34.8%. Palanisamy *et al*[31] conducted a non-randomized intervention to improve adherence among 43 post discharge hypertensive patients in India, and analyzed differences in adherence pre and post intervention. After discharge, patients were given counseling on drugs and lifestyle modification, were provided with a medication schedule reminder and received frequent telephone reminders from study pharmacists. Adherence was assessed using a version of the Morisky scale, which included a 5-point response option (never/rarely/sometimes/often/always) and a set of open-ended questions regarding reasons for non-adherence. Scores ranged from 0-4 for the response option questions and 0-16 for the open-ended questions, with higher scores indicating poorer adherence. All patients who answered "yes" to at least one question were considered non-adherent. At baseline, 100% of the patients were considered non-adherent, whereas at the second interview, 51.2% were non-adherent and at the third interview, only 4.6% were non-adherent. Ponnusankar *et al*[32] conducted a randomized study of 90 patients with chronic conditions to assess the impact of patient medication counseling on adherence. Patients in the intervention group received a 15-20 min medication counseling session from a pharmacist. In order to determine adherence, patients were asked to bring back all remaining medications and empty foils along with medication receipts, as well as to complete an adherence self-assessment form. As measured by pill count at follow-up, adherence was 92.2% ± 4.5% for counseled group and 84.7% ± 11.8% for the usual care group. As measured by the self-assessment form, 75% of patients in the intervention group and 67% of patients in the usual care group rated themselves as adherent.

Two additional interventions were identified, however, only the design/rationale papers have been published at this point. Fathima *et al*[33] reported the design of the Primary pREvention strategies at community level to Promote Adherence of treatment to pREvent cardiovascular diseases (PrePare) study, a multi-center, household-level, cluster-randomized trial to improve systolic blood pressure (BP) and medication adherence in at-risk households in India. The intervention consists of household visits by community health workers (CHWs) every two months. The CHWs will assess adherence by administering a questionnaire and inspecting the empty blister packets or purchase receipts, measure BP and ascertain if BP values meet the preset targets, ensure that individuals adhere to the prescribed treatment by using educational messages to target tobacco use, adherence to medication, and promote lifestyle changes and set goals for BP and weight and reduction in tobacco use and weight reduction goals for the next visit. Kamath *et al*[34] reported the design of the Secondary Prevention of Acute Coronary Syndromes study, a hospital-based, open-label, randomized trial of community health worker interventions *vs* usual care in India. The intervention includes a patient diary with information on ischemic heart disease risk factors, treatments and importance of treatment compliance, as well as calendar checklist, on which patients mark every time they take a dose of their medications. Adherence will be calculated based on the percentage of doses taken of those prescribed. The 4-item MMAS will also be used to assess adherence at follow-up visits. If patients prematurely stopped taking their medications, the reasons for doing so will be elicited.

DISCUSSION

In this systematic review, we provide a summary of the methodologies used to assess and interventions to improve adherence to CVD medications in South Asians. The most common method for assessing adherence was patient self-report ($n = 16$). While self-reporting does open the door to recall bias, it provides one of the most economically feasible methods for data collection. All of these studies were conducted in developing countries or in resource limited settings, which could be a contributing factor to the use of self-report rather than "gold standard" methods such as MEMS caps or pharmacy refill data. Usage of MEMS caps in resource-limited settings is most likely constrained due to their high costs (approximately $100 USD per cap)[35-37]. Only 1 study in our review included the use of MEMS caps, in a region where the gross national income per capita is $1483 USD, which is the lowest in the world[38]. As expected, pharmacy refill data, which relies on complete pharmacy records, was not used by any of the included studies. Without reliable and interoperable electronic pharmacy records, tracking where patients get their medication from can prove to be largely difficult and resource intensive.

In addition to primarily relying on self-report, several of the studies did not use validated instruments. Of the 16 studies using self-reported measures, 7 used validated questionnaires, while 9 studies used non-validated interview or survey questions. Furthermore, there was a general lack of information on these interview or survey questions. Details on how adherence rates were calculated or how data was collected were missing in several of these publications. There was also a general lack of detail on what class and type of medications was being measured for adherence. From the studies that did report medication type, the majority were anti-hypertensive medications. There was little data on other CVD medications such as anti-platelets and cholesterol lowering medications.

It is important to note that the adherence rates from non-validated, self-reported interview or survey

questions were typically higher than those of the validated scale measurements. The most glaring of disparities appears in Kumar *et al*[16] where 96.7% of patients were classified as adherent according to a self-report assessment form and only 54.2% of patients were classified as medium adherent and 0% classified as highly adherent according to the 8-item MMAS. Therefore, researchers in this region should consider using validated measures such as the MMAS as opposed to non-validated measures when assessing adherence. Additionally, several of these papers did not assess whether a higher medication adherence to hypertensive medications was associated with an improvement in an intermediate outcome measure such as blood pressure control. Generally the adherence rates were low which shows a growing need for future interventions geared towards improving adherence.

Overall, very few studies (n = 7) evaluated the impact of interventions to improve adherence. One intervention targeted provider education. While important, provider based interventions are typically considered quality improvement and are generally considered weak interventions[39]. Three interventions focused on the use of allied health care providers, such as community health workers or pharmacists, to educate or counsel patients on the importance of adhering to medication. Two of these interventions provided drug counseling from pharmacists to patients in an attempt to improve medication adherence, and both showed promising results. The third followed the WHO CVD risk management package utilizing community health care workers to assess risk and provide counseling on lifestyle changes to manage that risk. Community health care workers are a unique resource available to most if not all of these countries and could form the backbone for interventions directed at improving medication adherence in these low resource environments. The final 3 interventions were geared towards reducing pill burden through the use of polypills. No interventions targeted patient-provider communication, which may be an important determinant of a patient's medication adherence[40].

An important distinction to note is that non-adherence can be classified as either (1) intentional; or (2) unintentional. Intentional non-adherence is related to patient's beliefs and knowledge and health seeking behaviors, while unintentional non-adherence is related to demographics and comorbid illness[41]. Unintentional and intentional non-adherence to medications are related to different patient characteristics. A review of medication adherence in native and immigrant South Asians noted that the primary factors related to non-adherence in the included studies were forgetfulness, side-effects and choosing not to take the medication[42]. This suggests that both unintentional and intentional nonadherence contribute to overall non-adherence in South Asian populations and successful interventions aimed at improving adherence in this population should address both mechanisms. The interventions included in this review did not make such a distinction, and thus the generalizability of the results is limited.

Several studies cited behavioral barriers to medication adherence. Interventions geared towards patient education could improve patient self-efficacy and help break down these barriers. Additionally, there was a general lack of system-wide interventions such as audit and feedback and decision support systems. Although the lack of electronic medical record data in this region would lead to a low overall yield for decision support systems. However, audit and feedback interventions could be utilized more in resource constrained settings. Furthermore, we found a lack of interventions utilizing technology to assist patient adherence. Several of these regions benefit from a high cell phone density[43]. However, none of the interventions included in this review used cell phone reminders through text messages or other means to improve medication adherence. Although most interventions used in these studies showed promising results, further research is needed to determine which interventions or combination of interventions is the most effective strategy for improving medication adherence in this resource constrained environment. This research along with cost-effectiveness of each of the above mentioned strategies would be informative as policy makers decide which of these interventions could be scaled up to the population level.

In conclusion, we found that self-report measures were the most commonly used method to assess adherence. Although several interventions were directed towards providers, allied health care professionals and community health care workers, there is a need to employ strategies directed towards provider-patient communication. Additionally, there is a need to better incorporate technology such as cell phones, which are readily available for most people living in these countries. Examples of this type of intervention include the use of tailored and specific Short Text Message (SMS) reminders to improve medication adherence[44] and the use of text and a voice SMS in local language to improve health literacy and medication adherence[45].

Future research should focus on using more validated self-reported measurements. These scales should be validated in South Asian populations specifically, since health literacy may vary from previously studied populations. Additionally, there is a need to tie these adherence measurements to intermediate outcome measures such as blood pressure or cholesterol control as well as determine their effects on CVD outcomes.

COMMENTS

Background

Cardiovascular disease (CVD) is the leading cause of death worldwide, with an estimated 17.5 million people dying from CVD in 2012. Approximately one-fifth of the global population resides in South Asia, where patients suffer from a disproportionately high rate of CVD-related morbidity and mortality. Adherence to medication is critical to the effectiveness of CVD risk management. Therefore, the aim of this review is to examine current methods of assessing adherence and strategies to improve adherence to CVD medications, among

South Asian CVD patients.

Research frontiers

Current research indicates a growing epidemic of premature CVD in South Asian populations. Establishing suitable strategies to assess adherence to CVD medications is of particular importance to South Asian countries for various reasons. First, low availability of electronic medical records in most health care settings precludes accurate assessment of medication adherence using electronic medication refill data by health care providers. Second, the overall health literacy and the opportunities to improve provider and patient awareness of the importance of medication adherence may be limited. Lastly, low availability of pharmacy records and medication refill data also limit the use of traditional measures used to assess medication adherence.

Innovations and breakthroughs

Understanding adherence to CVD medications in South Asian populations is an important research question. This study focuses on the specific challenges and complexities related to CVD medication adherence in native South Asians.

Applications

This review demonstrates the need to identify a gold standard for assessment of adherence related to CVD medications in South Asians. Additionally, there is a need to employ intervention strategies directed towards provider-patient communication and to tie these interventions to intermediate outcome measures in order to determine their effects on CVD outcomes.

Terminology

Adherence in this review is defined as "the extent to which a person's behaviour - taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider". The Morisky Medication Adherence Scale is a 4 or 8-item self-report questionnaire that results in a score ranging from 0 (non-adherent) to 4 or 8 (fully adherent).

Peer-review

In this manuscript, Virani *et al* reviewed mainly adherence to antihypertensives in South Asian populations and the methodology of studies conducted in this field. It is an interesting topic and the presentation of data is impressive.

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SYSTEMATIC REVIEWS

Global myocardial strain assessment by different imaging modalities to predict outcomes after ST-elevation myocardial infarction: A systematic review

Abhishek Shetye, Sheraz A Nazir, Iain B Squire, Gerald P McCann

Abhishek Shetye, Sheraz A Nazir, Iain B Squire, Gerald P McCann, Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester LE3 9QP, United Kingdom

Author contributions: McCann GP designed the concept of the review; Shetye A performed the literature review and drafted the manuscript; Nazir SA, Squire IB and McCann GP reviewed the manuscript and provided critical revisions; all authors were involved in the interpretation of the results and approved the final manuscript.

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Correspondence to: Dr. Gerald P McCann, National Institute of Health Research Career Development Fellow/Honorary Consultant Cardiologist, Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Groby Road, Leicester LE3 9QP,
United Kingdom. gerry.mccann@uhl-tr.nhs.uk
Telephone: +44-116-2583977

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Abstract

AIM: To conduct a systematic review relating myocardial strain assessed by different imaging modalities for prognostication following ST-elevation myocardial infarction (STEMI).

METHODS: An online literature search was performed in PubMed and OVID® electronic databases to identify any studies that assessed global myocardial strain parameters using speckle-tracking echocardiography (STE) and/or cardiac magnetic resonance imaging (CMR) techniques [either myocardial tagging or feature tracking (FT) software] in an acute STEMI cohort (days 0-14 post-event) to predict prognosis [either development of major adverse cardiac events (MACE)] or adverse left ventricular (LV) remodelling at follow-up ($\geqslant$ 6 mo for MACE, $\geqslant$ 3 mo for remodelling). Search was restricted to studies within the last 20 years. All studies that matched the pre-defined search criteria were reviewed and their results interpreted. Due to considerable heterogeneity between studies, meta-analysis was not performed.

RESULTS: A total of seven studies (n = 7) were identified that matched the search criteria. All studies used STE to evaluate strain parameters - five (n = 5) assessed global longitudinal strain (GLS) (n = 5), one assessed GLS rate (GLS-R) (n = 1) and one assessed both (n = 1). Three studies showed that GLS independently predicted the development of adverse LV remodelling by multivariate analysis - odds ratio between 1.19 (CI: 1.04-1.37, P < 0.05) and 10 (CI: 6.7-14, P < 0.001) depending on the study. Four studies showed that GLS predicted the development of MACE - hazard ratio (HR) between 1.1 (CI: 1-1.1, P = 0.006) and 2.34 (1.10-4.97, P < 0.05). One paper found that GLS-R could significantly predict MACE -

HR 18 (10-35, $P < 0.001$) - whilst another showed it did not. GLS < -10.85% had sensitivity/specificity of 89.7%/91% respectively for predicting the development of remodelling whilst GLS < -13% could predict the development of MACE with sensitivity/specificity of 100%/89% respectively. No suitable studies were identified that assessed global strain by CMR tagging or FT techniques.

CONCLUSION: GLS measured acutely post-STEMI by STE is a predictor of poor prognosis. Further research is needed to show that this is true for CMR-based techniques.

Key words: Strain; Speckle tracking; Tagging; Feature tracking; Myocardial infarction; Major adverse cardiac events; Remodelling

Core tip: Global myocardial strain is an objective measure of cardiac function. It can be assessed using post-processing analysis on different imaging modalities such as speckle-tracking echocardiography (STE) and cardiac magnetic resonance imaging (CMR) - tagging and feature tracking. We performed a systematic review that showed global longitudinal strain (GLS) measured acutely by STE following ST-elevation myocardial infarction (STEMI) predicted clinical outcomes and adverse left ventricular remodelling, a surrogate marker of poor prognosis. No relevant studies were found for CMR techniques. GLS may refine risk stratification in the STEMI population but further work is needed to support this.

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INTRODUCTION

Ischaemic heart disease (IHD) presents a significant burden to healthcare services and is one of the leading causes of death worldwide[1]. Acute myocardial infarction (MI) results from spontaneous coronary artery occlusion due to thrombus formation as a result of plaque rupture and subsequent platelet aggregation - most commonly seen with the background of IHD[2]. ST-elevation myocardial infarction (STEMI) is an acute emergency that requires prompt reperfusion by either primary percutaneous coronary intervention (PPCI) or thrombolysis, ideally within two hours of symptom onset[3].

Timely reperfusion has led to a reduction in mortality from acute MI[4]. However, despite receiving current best therapy, a significant number of patients still develop complications post-MI that includes new-onset heart failure (HF)[5] - 20.4% of patients develop HF on admission and 8.6% subsequently[6]. The incidence of HF has increased over the past few decades[7] and it is especially prevalent amongst the elderly[8]. Long-term mortality from HF still remains high, even with the best contemporary pharmacological and non-pharmacological interventions[9]. The increase in HF incidence may partly be a result of improved survival post-MI, albeit with greater morbidity in some survivors.

Outcomes after STEMI

Major adverse cardiac events: Major adverse cardiac events (MACE) are often used in cardiovascular studies as a measure of clinical outcomes after STEMI. It is an umbrella term that includes a variety of measures - including all-cause mortality, hospital readmission due to HF, recurrence of MI, need for revascularisation, and occurrence of stroke. Demographic features associated with poor outcomes post-STEMI include age[10], diabetes[11], hypertension[12], infarct location (*i.e.*, anterior MI)[13], large infarct size (IS)[14] and presence of microvascular obstruction[15].

"Hard events" such as mortality are the best markers of outcome. However, these are relatively rare occurrences and so require a considerable sample size to demonstrate statistically significant association with a biomarker, or effects of intervention[16] and some authors believe that studies reporting these need to have a sample size of $n > 1000$ to be statistically robust[17]. Such large, multi-centre trials are challenging to conduct and need to be carried out over a considerable period of time in order to accrue the required sample sizes and numbers of events. Consequently, surrogate markers of poor outcome such as adverse left ventricular (LV) remodelling can be used in lieu of hard outcomes with much smaller sample sizes to achieve statistically significant results.

Adverse LV remodelling: Adverse LV remodelling post-MI is thought to be the main process underpinning the development of HF and is defined as: "A change in size, shape and function of the heart resulting from cardiac load or injury"[18]. It is a complex process that progresses over a period of weeks to months post-infarct (Figure 1). Adverse LV remodelling post STEMI can be defined as either an increase in end-diastolic volume (EDV) of > 20% or end systolic volume (ESV) of > 15%, at follow-up compared to baseline. However, there is no consensus on which definition is better. Several cellular, extracellular, inflammatory, and neuro-hormonal pathways have been implicated to play a role in development of LV remodelling; these include neutrophils[19], macrophages[19], collagen fibres[20], various metallo-proteinases[20] and activation of the sympathetic nervous system along with the renin-angiotensin-aldosterone system (RAAS)[7,18] amongst others. The exact role of

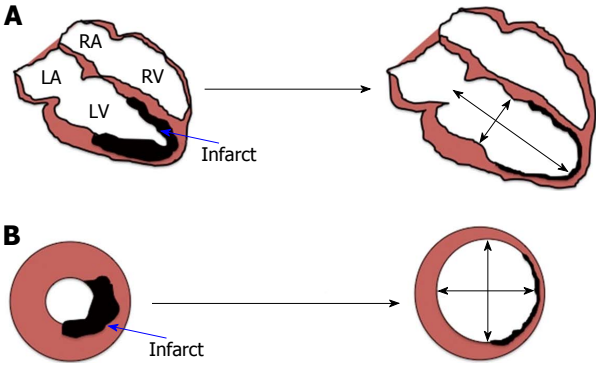

Figure 1 Development of adverse left ventricular remodelling post-myocardial infarction in (A) long axis view and (B) short axis view. LA: Left atrium; LV: Left ventricle; RA: Right atrium; RV: Right ventricle.

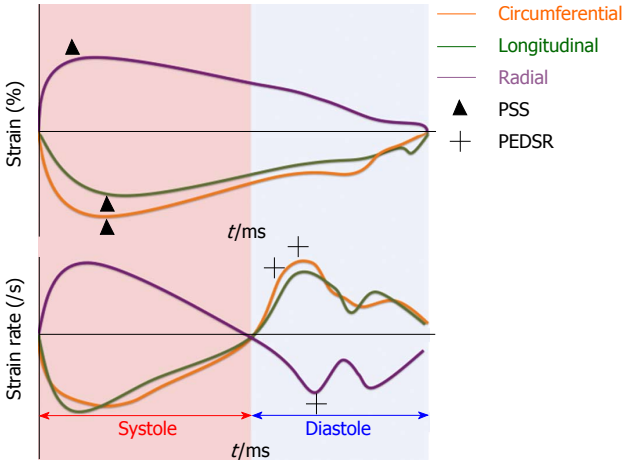

Figure 3 Strain and strain rate values as a function of time - peak systolic strain and peak early diastolic strain rate are annotated. PSS: Peak systolic strain; PEDSR: Peak early diastolic strain rate.

these components has not yet been elucidated and there is still some controversy over the initial trigger of remodelling[21]. There is good evidence to suggest that angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, and aldosterone antagonists attenuate the process of adverse remodelling by inhibiting RAAS[18].

Early identification of high-risk patients who are likely to undergo adverse LV remodelling may allow targeted therapeutic intervention in these patients to counteract remodelling processes. Parameters that reflect myocardial dysfunction can potentially be utilised to help identify such patients as cardiac function is often affected post-MI, which usually precedes development of overt HF.

LV dysfunction post-infarction

Traditionally, the systolic phase of the cardiac cycle is often used as a measure of LV function in a clinical setting. A region of myocardium affected by an infarct may have impaired contractility due to death of myocytes in that zone. Ejection fraction (EF) is the most commonly used method to assess systolic function and a

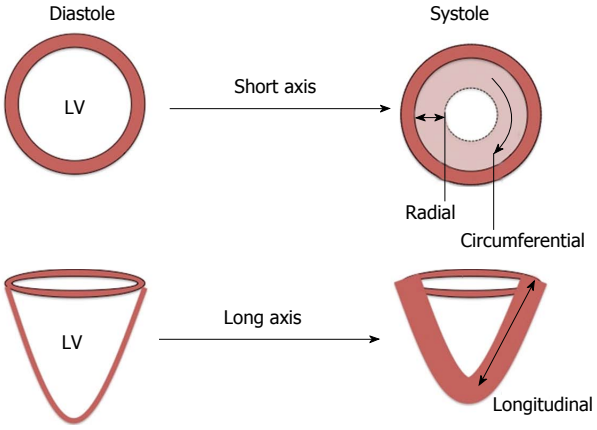

Figure 2 Myocardial contraction in three vectors - circumferential, longitudinal and radial. LV: Left ventricle.

reduced EF, commonly measured by echocardiography, is known to be associated with a poor outcome[22]. However, EF is relatively insensitive to regional differences in myocardial function and has been shown to be a poor predictor of late myocardial dysfunction when measured acutely after reperfusion therapy[23]. Wall Motion Score Index (WMSI) has also been used in addition to EF but it has the inherent shortcoming of being a subjective measure based on the experience of the assessor. WMSI is based on either the 16-segment[24] or the 17-segment model[25] of the LV.

An infarct is also thought to affect LV compliance by increasing wall stiffness and hence reducing active relaxation of the myocardium - this can cause diastolic dysfunction[26]. Recent evidence suggests that diastolic dysfunction post-MI measured by echocardiography confers a poor outcome[27,28].

The optimal marker of LV dysfunction would: (1) Be objective and "angle independent"; (2) Be sensitive to myocardial dysfunction early after an MI; (3) Offer an evaluation of both regional and global LV contractility; (4) Provides an assessment of both systolic and diastolic heart function; and (5) Be reproducible and easy to measure.

Myocardial strain

Strain is defined as the change in length of an object relative to its original length[29]. In the heart, myocardial strain is a sensitive measure of contractility. Strain can be calculated at both the segmental and global level and in the three axes of myocardial contraction - circumferential, longitudinal and radial (Figure 2). Strain rate (SR) measures the change in strain for a given vector as a function of time and can also be assessed. Systolic and diastolic strain rates vary throughout the cardiac cycle (Figure 3).

Anatomically, myocardial fibres are orientated longitudinally in the sub-endocardium and circumferentially in the mid-myocardium[30]. This suggests that longitudinal strain (LS) can provide a reflection of sub-endocardial function whilst circumferential strain can inform mid-myocardial function. Radial strain, whilst

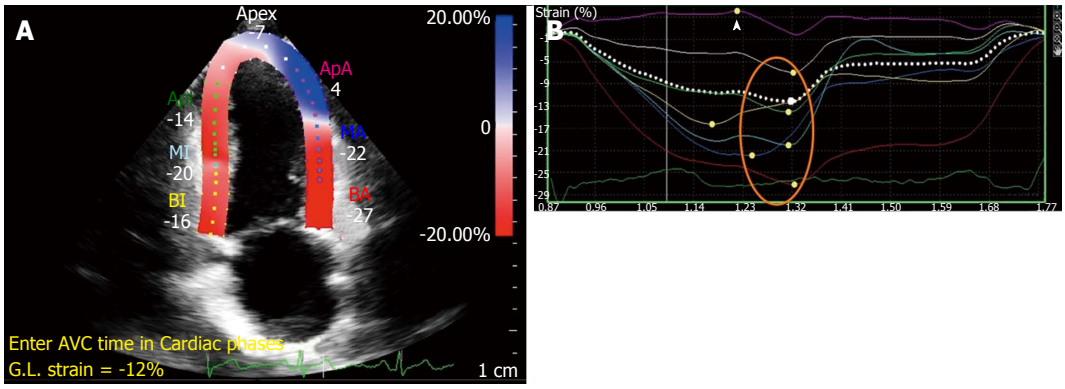

Figure 4 Peak systolic strain calculated by speckle-tracking echocardiography. A: Segmental strain after definition of endocardial and epicardial contours; B: Graphical illustration of segmental peak systolic strain - normal values annotated by orange circle, impaired strain by arrowhead.

being potentially informative of myocardial contraction in the short axis, has been shown to have high intra- and inter-observer variability[31] making it unsuitable for routine clinical practice. Peak systolic strain (PSS) is commonly used to assess myocardial contraction whilst peak early diastolic strain rate (PEDSR) is a marker of diastolic function[32]. Consequently, strain/diastolic strain rate assessment provides a comprehensive evaluation of myocardial contractility and compliance.

Myocardial Strain/strain rates can be assessed by a number of different imaging modalities - most frequently by echocardiography, but also by cardiac magnetic resonance imaging (CMR).

Echocardiography

Tissue Doppler imaging can assess myocardial strain but this technique is extremely angle dependent and has been superseded by speckle tracking echocardiography (STE)[33,34]. The ultrasonic images obtained by echocardiography consist of a large number of "speckles" which have individual properties[35]. These "acoustic markers"[34] can be identified and tracked as they move from one frame to the other throughout the cardiac cycle. Endocardial and epicardial borders are pre-defined by the operator and each speckle within this region of interest (ROI) is tracked. The tracking of such movement can be used to derive measures of strain[36] and strain rate[33]. STE is entirely a post-processing analysis. The only minor requirements are a short duration of breath holding by the patient so that respiratory motion does not affect the tracking of cardiac motion and a high frame rate to optimize temporal resolution.

Common echocardiographic imaging protocols include the acquisition of two-, four-chambered and three chamber views from which global LS (GLS) is derived (Figure 4). Short axis views allow circumferential and radial strain to be derived but it is difficult to accurately obtain global measures due to uncertainty of the imaging plane location.

STE-derived global strain parameters in the setting of an acute STEMI have shown good reproducibility - intra- and inter-observer variability of 0.92 and 0.85 by Intra-class Correlation Coefficient (ICC) respectively[37]. Repeatability is a measure of the "variation in repeat measurements made on the same subject under identical conditions made within a short period of time over which the underlying value can be considered to be constant"[38]. It is another method of establishing reliability. However, no studies to date have reported the repeatability of global strain measured by STE in acute STEMI.

CMR

CMR is another non-invasive imaging modality and is an alternative method of imaging to echocardiography. CMR can be used in the diagnosis, risk-stratification, and prognosis of a number of cardiac disorders[39,40], including acute MI[41-43]. Typically, strain is assessed on CMR using specialised myocardial tissue tagging sequences that involves the superimposition of horizontal and vertical lines on a cine image that appear in the form of a "grid"[44]. These grids or "tags" are formed onto the tissue by changing the local magnetisation through the use of selective radiofrequency saturation pulses perpendicular to the plane of image acquisition[45]. Tags deform along with the myocardium through the cardiac cycle and this deformation can be used to assess strain. Tagged images are commonly acquired using spatial modulation of magnetisation (SPAMM)[46] and complementary SPAMM sequences[47]. Post-processing analysis of tagged data can be performed using Harmonic phase analysis[48] and local sine wave modelling[49] and they have been shown to have good agreement[50]. Tagging has been validated against other invasive methods of strain assessment such as sonomicrometry[51] and has been used in a variety of animal models[52-54]. Tagging-derived strain parameters have a good intra- and inter-observer variability - ICC of 0.8 for both - along with acceptable test-retest repeatability - ICC of 0.74[55].

Tagging sequences however involve relatively long breath holds that may be difficult in the context of a recent STEMI. In addition, analysis is also labour-intensive and time-consuming[56]. Tagging, particularly with SPAMM sequences, cannot reliably calculate diastolic strain as the tags fade after systole especially at the 1.5 T field strength[45,57]. This can be overcome

Table 1 Advantages and disadvantages of speckle-tracking echocardiography *vs* cardiac magnetic resonance imaging

Advantages	Disadvantages
Cheaper than CMR scan	Cannot acquire SAX views easily - needed to calculate circumferential strain
Can be performed at the bedside	Cannot routinely obtain stress imaging as part of acquisition protocol
Short duration: 10-20 min for STE *vs* 45-60 min for CMR	Not possible to ascertain infarct size, oedema, microvascular obstruction
Significant contraindications for CMR - for example, pacemaker/ICD, brain aneurysmal clip, claustrophobia, eGFR < 30 mL/min per 1.73 m^2 - *vs* almost none for STE	CMR has much higher spatial resolution than STE. Consequently, a greater percentage of images are analysable by CMR than STE

CMR: Cardiac magnetic resonance imaging; eGFR: Estimated glomerular filtration rate; ICD: Insertable cardioverter defibrillator; SAX: Short axis; STE: Speckle-tracking echocardiography.

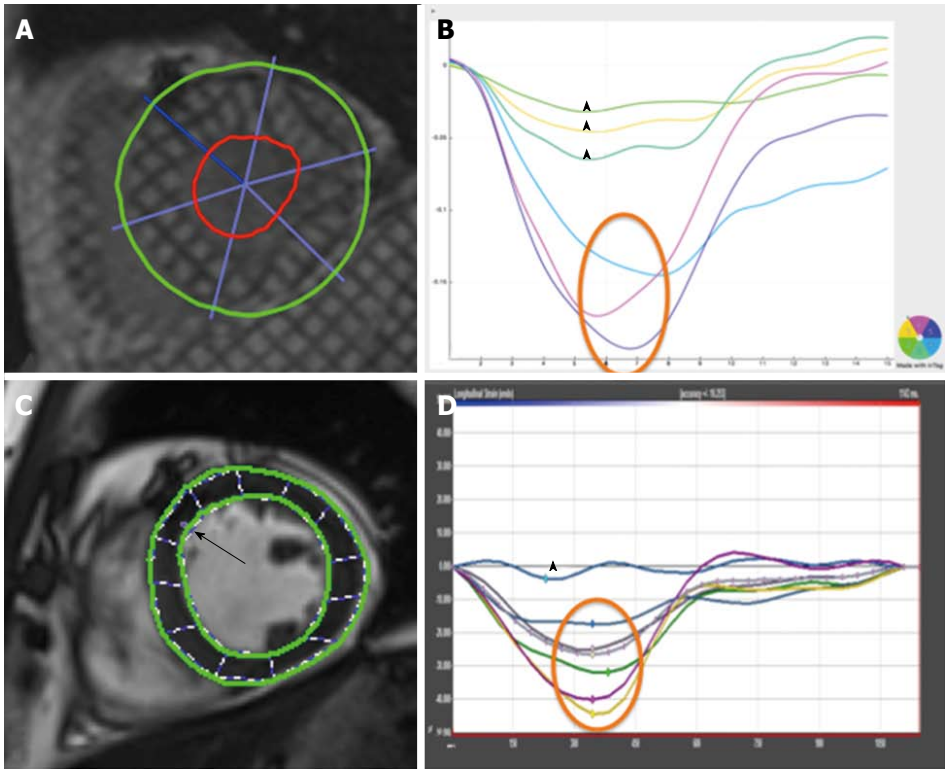

Figure 5 Comparison of tagging (A and B) and feature tracking (C and D) for evaluation of global circumferential strain - normal peak systolic strain annotated by orange circle, impaired peak systolic strain by arrowhead.

by using a stronger magnetic field strength (3.0 T) and Steady State Free Precession (SSFP) sequences[45]. However, true reproducibility is poor at 3.0 T CMR[58]. This may in part be due to the fact that by 3.0 T CMR images are also more susceptible to artefacts due to increase in inhomogeneity within the magnetic field[59].

To overcome the issues of tagging, myocardial motion tracking through the cardiac cycle on routinely acquired cine SSFP sequences can be performed by means of the novel feature tracking (FT) software[60]. FT is analogous to STE - endocardial and epicardial borders are defined and then subsequently propagated through the cardiac cycle. The software tracks the motion of the defined ROI from one frame to the next - PSS and PEDSR can be derived from this motion[60]. FT has shown excellent reproducibility - intra- and inter-observer variability of 0.988 and 0.971 in terms of ICC[61] - and acceptable test-retest repeatability - ICC of 0.77[56] - for PSS. Additionally, PSS by FT can predict global recovery of LV function in terms of EF[62].

Figure 5 illustrates a comparison of global circumferential strain (GCS) evaluation by tagging and FT.

STE vs CMR to assess strain

STE has several advantages over CMR in the assessment of strain (Table 1). There is good agreement between STE-derived and CMR derived global values of strain - this is true both for tagging[63,64] and FT[65]. This suggests that these methods could be used interchangeably in the assessment of global strain. A detailed comparison of different imaging modalities to be used in the setting of an acute MI can be found elsewhere[36].

Aims of systematic review

Global myocardial strain can objectively evaluate LV dysfunction post-STEMI and can be measured by

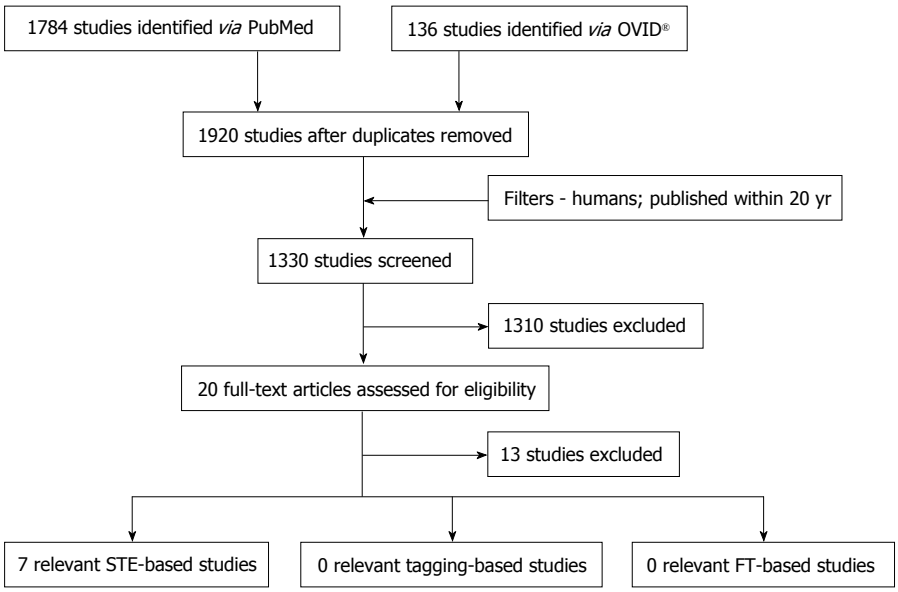

Figure 6 Flowchart illustrating the search for relevant studies. FT: Feature tracking; STE: Speckle-tracking echocardiography.

Table 2 Eligibility criteria for systematic review

Type of characteristic	
Population type	Acute STEMI
Measured parameters	Global longitudinal and/or circumferential strain and/or strain rate - PSS or strain rate (PSS-R) or PEDSR
Imaging modalities	STE or cardiac MRI tagging or cardiac MRI FT
Timeframe for baseline scan	Days 0-14 post-STEMI
Outcomes reported	MACE or adverse LV remodelling
Timeframe for follow-up	MACE - ⩾ 6 mo Adverse LV remodelling - s ⩾ 3 mo
Year published	Within the last 20 yr

STEMI: ST-elevation myocardial infarction; PSS: Peak systolic strain; PEDSR: Peak early diastolic strain rate; STE: Speckle-tracking echocardiography; FT: Feature tracking; MACE: Major adverse cardiac events; LV: Left ventricular; MRI: Magnetic resonance imaging.

STE and CMR techniques with good reproducibility and repeatability. We looked to review the literature for studies that evaluated the ability of global strain measured acutely post-STEMI by either STE or CMR to predict either MACE or development of adverse LV remodelling.

MATERIALS AND METHODS

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocol[66].

Eligibility criteria

Table 2 highlights the eligibility criteria for the review. Studies were limited to acute STEMI patients to represent the setting of an acute MI - NSTEMI patients were excluded since the diagnosis is more complex, heterogeneous presentations and that their subsequent management is based on risk-stratification[67]. There was no limitation placed on the management of the STEMI - both in terms of method of revascularisation (PPCI or thrombolysis) and success/failure. Strain parameters were restricted to peak systolic GCS and GLS and PEDSR in the same two vectors. Both segmental strain values and radial strain parameters were excluded since they both have been shown to have poor intra- and inter-observer variability[31,58]. We limited the timeframe for the baseline scan to be 0-14 d post- to limit the effects of subsequent remodelling. The timeframe for outcome measures were ⩾ 3 mo for adverse LV remodelling (since it is a dynamic process that takes months to fully develop[68]). Minimum follow-up time for development of MACE was six months. We included studies that quoted either changes in EDV or ESV.

Search protocol

The literature search was performed in PubMed and OVID® electronic databases. The final date on which the online search was performed was January 27th, 2015 (Table 3) for list of keywords used.

Study selection

Figure 6 highlights the process of study selection. Initial electronic search yielded 1920 studies; 1330 remained after addition of relevant filters. The titles and abstracts of these studies were then screened to assess for eligibility for inclusion in the systematic review (Table 2). A majority of the studies were deemed inappropriate for inclusion based on the aforementioned criteria (n = 1310). The remaining 20 papers were further scrutinised by searching for and evaluating the full-text article. A further 13 studies were excluded - some did not actually assess strain at all (n = 4), some assessed torsion (n = 3), three had included NSTEMI patients and the rest did not have full-text articles available as they were presented as posters (n = 3). Consequently,

Table 3 Keywords used for search of electronic databases

"Cardiac MRI" OR "CMR" OR "magnetic resonance imaging [MeSH Term]" OR "cardiac magnetic resonance" OR "feature tracking" OR "tissue tracking" OR "tagging" OR "tag" OR "tagged" OR "SPAMM" OR "CPSAMM" OR "HARP" OR "SinMOD" OR "Echocardiography [MeSH Term]" OR "Speckle tracking", "2D speckle" OR "3D speckle" OR "two dimensional speckle" OR "three dimensional speckle". MIs were searched using "myocardial infarction [MeSH Term]" OR "acute MI" OR "STEMI" OR "ST elevation". Strain was searched using "strain" OR "myocardial strain" OR "strain rate" OR "deformation" OR "myocardial deformation" OR "systolic" OR "diastolic" OR "PSS" OR "PEDSR" OR "longitudinal" OR "circumferential". Outcomes were searched using "Predict" OR "Outcome" OR "Risk" OR "Prognosis" OR "Logistic Models [MeSH Term]" OR "risk" OR "multivariable" OR "multivariate" OR "odds" OR "MACE" OR "mortality [MeSH Term]" OR "remodelling" OR "remodelling" OR "adverse" OR "cardiac" OR "left ventricular"
Note: MeSH terms were only available on PubMed

MRI: Magnetic resonance imaging; CMR: Cardiac magnetic resonance imaging; STEMI: ST-elevation myocardial infarction; PSS: Peak systolic strain; PEDSR: Peak early diastolic strain rate.

there were seven studies that matched our inclusion criteria for the review.

RESULTS

Strain measured by STE

Seven STE-based studies that matched our inclusion criteria were found (Table 4) highlights studies that assessed global strain to predict adverse LV remodelling and Table 5 highlights studies that used global strain to predict MACE. Six studies reported peak systolic longitudinal strain parameters to predict outcomes - only one study used diastolic strain. All the patients were treated with PPCI.

Multivariate analyses in all the studies have shown that peak systolic GLS can independently predict both adverse LV remodelling and MACE. Such analyses have shown that this is independent of factors such as age, diabetes, location of infarct, EF and WMSI. One study showed that global longitudinal SR (GLS-R) also had significant impact on prognosis[69] - patients with impaired GLS-R, and GLS, were 18-times more likely to suffer from composite endpoint of mortality, readmission due to HF, revascularisation, or re-infarction. One study showed that a cut-off GLS > -12.5% (*i.e.*, LV unable to contract more than 12.5% of its original length in the longitudinal vector) could predict development of remodelling - OR 1.19 (1.04-1.37), $P < 0.05$, sensitivity/specificity of 69%/79%[70]. Another showed a cut-off of GLS = 10.85% - OR 0.39 (0.26-0.57), $P < 0.01$, sensitivity/specificity of 89.7%/91.7%[71]. A cut-off for prediction of MACE ranged from GLS > -13% [HR = 2.34 (1.10-4.97), $P < 0.05$, sensitivity/specificity of 100%/89%][72] to GLS > -9.55% [OR = 0.56 (0.34-0.91), $P = 0.02$, sensitivity/specificity of 83.3/83.5%][71].

PEDSR was only measured in one study[73]. There was no significant difference in PEDSR in between patients that reached clinical endpoints and those that did not.

Strain measured by CMR

There were no studies that used CMR-based strain measurement techniques - either tagging or FT - to predict outcomes post-STEMI that matched our eligibility criteria.

DISCUSSION

This systematic review has shown that certain strain parameters measured by STE - namely, GLS[70-72,74,75] and GLS-R[69] - are independent predictors of adverse outcomes post-STEMI. Impaired GLS can predict both clinical endpoints and adverse LV remodelling, a surrogate marker of poor prognosis. When combined with routine clinical functional parameters such as EF and WMSI, strain provides incremental value in the prognostication of STEMI patients.

However, studies that monitored "hard" events such as mortality could not match the large sample size of $n > 1000$ that some authors believe is important for the evidence to be considered statistically robust[17]. Only one of the studies we assessed had such a large sample size - but the authors monitored remodelling and not "hard" events[74].

Some of the studies that monitored MACE had only a small number of patients that had reached their defined endpoints. Despite this, they constructed models for multivariate analysis that included a large number of independent variables (in addition to GLS). It is believed that one variable should be added for every 10 events to ensure that the regression estimates have reasonable precision[76]. Therefore, all of these studies may have included an inappropriately high number of variables to assess independent predictors of clinical endpoints and the models are likely to suffer from over-fitting.

PEDSR does not seem to provide any benefit at predicting these outcomes although has only been assessed in one study. Consequently, further studies are surely needed to determine if diastolic dysfunction has any role to play in prognostication after a STEMI[27].

Data in this review is limited to GLS measured by STE. We cannot comment on whether GCS is of any added value or has similar predictive properties as GLS since no studies assessed these two parameters together.

Evidence suggests that GLS measured by STE is related to IS[37,77]. The question remains as to whether GLS provides additional information to IS in post-

Table 4 All studies that have used speckle-tracking echocardiography-based strain to predict adverse left ventricular remodelling

Ref.	Age (yr)	Sample size (male)	Baseline ejection fraction (%)	Timeframe baseline scan	Timeframe follow-up scan(s)	Definition of adverse remodelling	Other parameters in multivariate model	Results	Limitations
Bochenek *et al*[70]	59.6 ± 10.3	66 (53)	49.7 ± 9.2	4-6 d post-infarct	3 mo	EDV > 20%	Diabetes Anterior MI Leuk. Count Time to reperfusion WMSI Max. Trop ST-elevation max pre-PCI	22 patients remodelled; GLS can predict LV remodelling - OR = 1.19 (1.04-1.37), $P < 0.05$ - shown by multivariate analysis GLS > -12.5% can predict remodelling - AUC = 0.77 for ROC, sensitivity/specificity of 69%/79% respectively	Only longitudinal strain measured. Too many variables in multivariate analysis
Joyce *et al*[74]	60 ± 12	1041 (792)	47.0 ± 9.0	2 d post-PPCI	3 and 6 mo	EDV ⩾ 20%	Male sex LAD infarct Max. Trop Discharge heart rate LA volume index WMSI	GLS > -15% can predict remodelling at 3 and 6 mo *vs* GLS < -15% (both $P < 0.001$): OR = 6.7 (2.8-11) for 3 mo; OR = 10 (6.7-14) for 6 mo	Only longitudinal strain measured; Prognostic data divided categorically - *i.e.*, GLS > -15% or < -15%; Excluded patients with re-infarction before follow-up and cardiogenic shock - could potentially have been used as another endpoint
Cong *et al*[71]	59.9 ± 11.6	127 (103)	51.8 ± 5.1	1 d post-PPCI	6-9 mo	ESV ⩾ 15%	Anterior MI Time to reperfusion ΣST before PPCI ΣST post-PPCI Raised CK-MB/Trops Baseline ESV/EF WMSI	41 patients developed remodelling; GLS predicted remodelling - OR = 0.39 (0.26-0.57), $P < 0.01$; GLS = -10.85% had sensitivity/specificity of 89.7%/91.7% respectively by ROC to predict remodelling	Only longitudinal strain measured; Too many variables in the multivariate analysis

AUC: Area under curve; CK: Creatine kinase; EDV: End diastolic volume; ESV: End systolic volume; GLS: Global longitudinal strain; LA: Left atrium; LAD: Left anterior descending; LV: Left ventricle; OR: Odds ratio; PPCI: Primary percutaneous coronary intervention; ROC: Receiver operator characteristic; WMSI: Wall motion score index; ΣST: Sum of ST-elevation.

STEMI prognostication and it can only be adequately answered using CMR. However, no studies were found that showed global strain measured by CMR could predict development of remodelling or MACE.

Limitations

We could rule out publication bias - unpublished data were not included as part of our review and could possibly affect our results, especially if it contradicted the seven studies that were assessed. We did not include three search results that were presented as posters since we could not access either the poster itself or the full-text articles associated with it. Regardless, we do not feel this exclusion would significant affect the results of the review since the titles of all three posters stated that GLS could predict post-STEMI outcomes. There is outcome data available in strain measured by TDI but we decided to exclude it from our review since its major limitation of "angle dependence" has been superseded by STE.

Conclusion

Global longitudinal strain when measured by STE is an independent predictor of both adverse LV remodelling and MACE after STEMI and provides incremental prognostic value when combined with traditional LV functional parameters such as EF and WMSI. No such data exist for CMR, but this modality could inform us as to whether strain provides prognostic data in addition to IS.

Table 5 All studies that have used speckle-tracking echocardiography-based strain to predict major adverse cardiac events

Ref.	Age (yr)	Sample size (male)	Baseline ejection fraction (%)	Timeframe baseline scan	Follow-up period	Outcome measures	Other parameters in multivariate model	Results	Limitations
Antoni *et al*[69]	60 ± 12	759 (517)	46.0 ± 8.0	2 d post-PPCI	21 ± 13 mo	GLS and/or GL-strain rate to predict: A: Mortality; B: Composite of revascularisation/ readmission for HF/re-infarction	Age (A) HTN (A) Multi-vessel disease (A/B) Peak Trop (A) QRS duration (A/B) EF (A/B) Severe MR (A) Smoking (B) Diabetes (B)	179 patients reached one or more endpoints; GLS independent predictor of all-cause mortality - HR = 1.2 (1.1-1.3), $P = 0.002$; GLS-R independent predictor of B endpoints - HR = 22 (11-48), $P < 0.001$; Both GLS and GLS-R independent predictors of combined A and B endpoints - HR = 1.1 (1 -1.1, $P = 0.006$) and 18 (10-35, $P < 0.001$) respectively	Sample size $n < 1000$ - potentially not large enough to predict "hard" events like mortality; Only longitudinal strain measured; SR analysis feasible in only 89% of segments
Shanks *et al*[73]	59.7 ± 11.6	371 (288)	45.2 ± 8.0	2 d post-PPCI	17.3 ± 12.2 mo	GL-PEDSR to predict: Mortality; Readmission for HF; Re-infarction; Revascularisation	EF TIMI 0-1 ESV-index Iso-volumetric relaxation SR	Combined clinical endpoints occurred in 84 patients; GL-PEDSR does not predict clinical outcomes	Sample size potentially too small to assess "hard" endpoint such as mortality; No measure of GLS; Only longitudinal parameters obtained
Woo *et al*[72]	64.4	98 (65)	52.6 ± 12.0	Pre-PPCI and 3 d post-PPCI	13.1 ± 3.8 mo	GLS to predict: Mortality; Readmission for HF	Initial Trop Initial NT-pro BNP EF (baseline) WMSI (follow-up) E/e'sr EF (follow-up) WSMI (follow-up)	7 patients developed endpoints; Pre-PPCI GLS predictor of outcomes - HR = 1.41 (1.01-1.98), $P < 0.05$; Post-PPCI GLS more likely to predict outcomes - HR = 2.34 (1.10-4.97), $P < 0.05$; Pre-PPCI GLS < 14% had sensitivity/specificity of 85%/75% respectively - post-PPCI GLS < 13% of 100%/89%	Very small sample size; Only longitudinal strain measured; Too many variables in multivariate analysis
Munk *et al*[78]	63.1	576 (446)	50.0 ± 10.0 (without composite endpoint), 47.0 ± 12.0 (with composite endpoint)	1 d post-PPCI	24 (IQ range 13-61) mo	GLS to predict: Mortality/re-infarction/ stroke/hospitalisation for HF; Crude mortality	EF WMSI ESV-index (Separately and in combination with each other)	162 patients experienced composite endpoints; GLS alone predicted outcomes within 1 yr post-MI - HR = 1.2 (1.12-1.29), $P < 0.01$; GLS alone could not predict outcomes later than 1yr post-MI	GLS could only be obtained in 74% of 576 patients - 26% excluded due to poor image quality (no difference in event rates, however); Only longitudinal strain measured
Cong *et al*[71]	59.9 ± 11.6	127 (103)	51.8 ± 5.1	1 d post-PPCI	16.9 ± 1.6 mo	GLS to predict: Mortality; Development of HF	Anterior MI Time to reperfusion ΣST before PPCI ΣST post-PPCI Raised CK-MB/Trops Baseline ESV/EF WMSI	GLS predicted outcomes - OR = 0.56 (0.34-0.91), $P = 0.02$; GLS > -9.55% had sensitivity/specificity of 83.3%/83.5% respectively	Sample size could potentially be too small to significantly predict "hard" events such as mortality

CK: Creatine kinase; ESV: End systolic volume; GLS: Global longitudinal strain; HF: Heart failure; OR: Odds ratio; HR: Hazard ratio; HTN: Hypertension; IQ: Inter-quartile range; MR: Mitral regurgitation; PEDSR: Peak early diastolic strain rate; PPCI: Primary percutaneous coronary intervention; TIMI: Thrombolysis in myocardial infarction; WMSI: Wall motion score index; ΣST: Sum of ST-elevation; MI: Myocardial infarction.

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COMMENTS

Background

Left ventricular (LV) dysfunction is an important determinant of prognosis following ST-elevation myocardial infarction (STEMI). Routinely used measures of LV dysfunction such as ejection fraction (EF) may not be able to detect subtle changes in cardiac function. Myocardial strain describes the relative change in length of myocardium through the cardiac cycle and is an objective measure of LV function. It can be measured during both systole and diastole and hence provides a reflection of both systolic and diastolic LV contractility. Acutely measured strain post-STEMI may help in predicting markers of poor prognosis [such as development of adverse LV remodelling or major adverse cardiac events (MACE)] at follow-up.

Research frontiers

Strain can be assessed using post-processing speckle-tracking echocardiography (STE) or cardiac magnetic resonance imaging (CMR)-based techniques [such as tagging or novel feature tracking (FT) software]. Such techniques can quantify strain at a segmental and global level and may provide additional information to LV volumes and EF.

Innovations and breakthroughs

This is the first paper to review the literature and present all the studies that have assessed acutely measured global strain parameters to predict markers of outcome post-STEMI. Three studies have shown that global longitudinal strain (GLS) measured by STE is a predictor of adverse remodelling following STEMI whilst four studies have shown that it can predict MACE at follow-up. Therefore, GLS may be a useful clinical measure of identifying patients at a "high risk" of developing poor outcomes. There were also no CMR-based studies assessing strain and its relation to prognosis following STEMI.

Applications

GLS may help improve risk stratification following STEMI but further studies are required to show that this improves outcome.

Terminology

Myocardial strain describes the relative change in length of myocardium through the cardiac cycle -GLS is a measure of LV contractility in the longitudinal vector; STE is an echocardiography-based post-processing software that analyses myocardial deformation parameters (such as global strain) by tracking the motion of "speckles" from one frame to another through the cardiac cycle; Tagging is a post-processing CMR-based software that evaluates strain on tagged sequences - examples of such sequences include spatial modulation of magnetisation (SPAMM) and complementary SPAMM; FT is a post-processing software that assesses strain on cine steady-state free precession images, a type of sequence that is routinely acquired during a clinical CMR scan.

Peer-review

The article is interesting, well-written and supported by updated references.

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