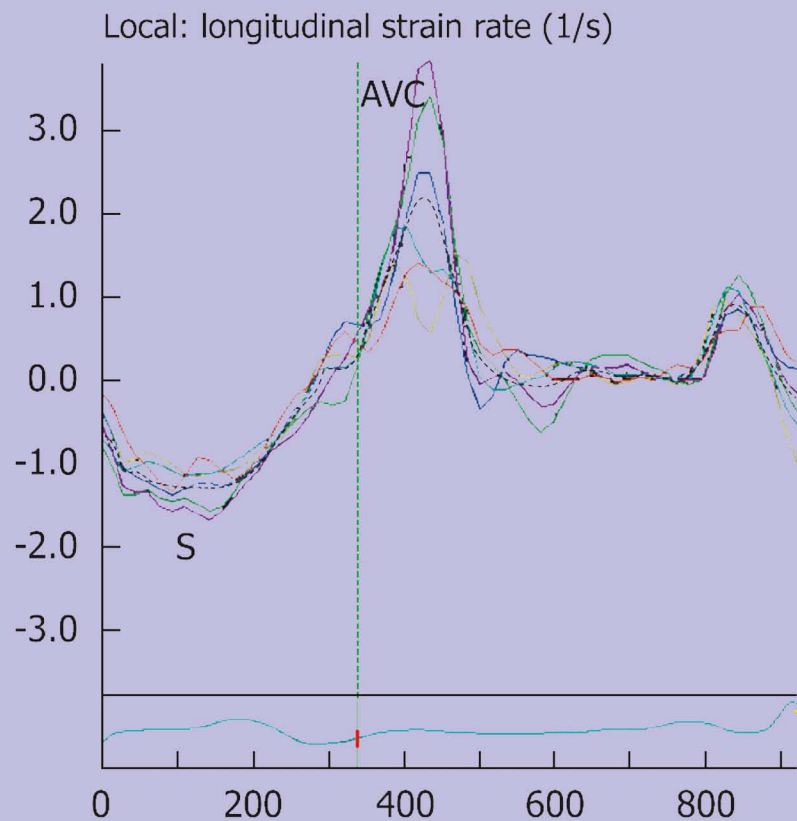
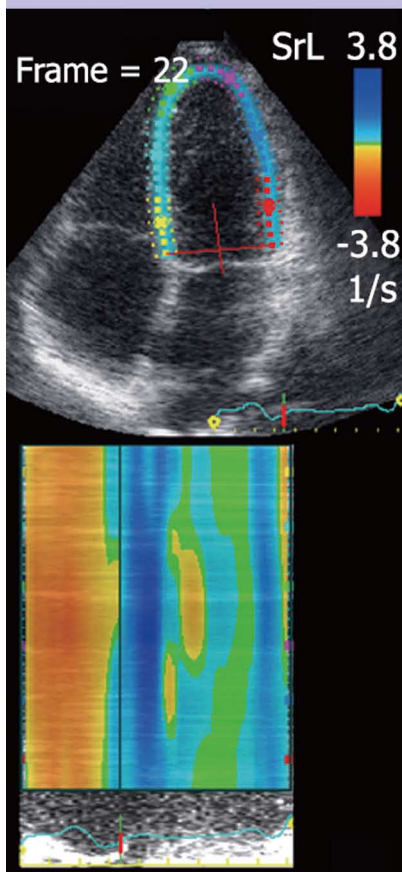


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Two-dimensional speckle tracking echocardiography for the assessment of atrial function

Tomás Francisco Cianciulli, María Cristina Saccheri, Jorge Alberto Lax, Alejandra Marina Bermann, Daniel Ernesto Ferreiro

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that it only measures regional strain and does not obtain information about the curved portion of the atrial roof. To overcome these limitations in the quantification of atrial function, the use of speckle tracking echocardiography (STE) strain has been proposed. This technique is not derived from Doppler but rather from 2D echocardiography; it is angle-independent and allows one to measure global as well as regional atrial strain. In this editorial, we describe the physical and pathophysiological concepts of STE and underline the clinical usefulness of this new technique.

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Key words: Atrial function; Speckle tracking echocardiography; Longitudinal atrial strain; Atrial reservoir strain; Passive conduit; Active pump

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Abstract

Echocardiography is the most common diagnostic method for assessing atrial function but the technique has some limitations. Traditionally, assessment of left atrial function has been performed by measuring volumes with 2D echocardiography. Additionally, it can be assessed with transmitral Doppler and pulmonary vein Doppler. Recently, an alternative method has been incorporated, namely, measurement of myocardial deformation with color tissue Doppler-derived strain. However, this method has several limitations, such as suboptimal reproducibility, angle-dependence, signal artifacts and the fact

INTRODUCTION

Echocardiography is a simple and widely available tool for the diagnosis of cardiovascular diseases, which provides information about cardiovascular structure, function and hemodynamics. Since its first clinical application, at the end of the 1970s, the technique has evolved and

expanded, improving its diagnostic capabilities, including the spectrum of: M-mode and 2D echocardiography; pulsed, continuous wave and color Doppler; transesophageal and stress echocardiography; contrast myocardial perfusion; second harmonic imaging; and 3D and 4D echocardiography.

In recent years, the magnitude and speed of such changes have continued to increase, with the advent of techniques that quantify myocardial velocity and deformation, thus allowing better assessment of ventricular function (pulsed tissue Doppler, color tissue Doppler, strain, strain rate (SR), tissue tracking, tissue synchronization imaging, speckle tracking, rotation and torsion). Additionally, digital storage techniques have allowed quantification regional myocardial function off-line.

During the past decade, numerous research studies have been published, which have described the usefulness of 2D strain obtained with speckle tracking, for the assessment of left ventricular function. However, there have been few studies describing the use of this new technique in the assessment of atrial function. The purpose of this editorial is to review the methodology and its usefulness in various clinical scenarios.

METHODOLOGY

The left atrial function contributes to left ventricular filling by means of its three components: a reservoir component, which receives blood from the pulmonary veins during ventricular systole; a passive conduit component during early diastole and diastasis; and a pump component, with active contraction during late diastole^[1].

Changes in atrial function during the different phases of the cardiac cycle can be assessed non-invasively with echocardiography, using conventional methods such as changes in atrial area and volume. Currently, the new 2D strain and SR techniques, derived from speckle tracking allow us to identify these three components of atrial function (Figure 1).

The reservoir phase begins with ventricular systole. During that phase, as it receives blood from the pulmonary veins, the atrial chamber distends in early diastole, atrial blood is suctioned by the ventricle, and the atrium acts as a passive conduit. In late diastole, the atrial muscles contract actively, and perform a pump function that completes ventricular filling.

In normal subjects, the atrial contribution as a reservoir, passive conduit and pump is approximately 40%, 35% and 25%, respectively. The reservoir function of the atrium is particularly relevant because 40% of the systolic volume is stored in the atrium during ventricular systole.

In cases of diastolic dysfunction, changes in ventricular filling occur, and the relative contributions of each of these components vary in order to maintain systolic ventricular volume. Prolonged ventricular relaxation, for example, leads to a decrease in conduit function, while the reservoir and pump functions increase. As

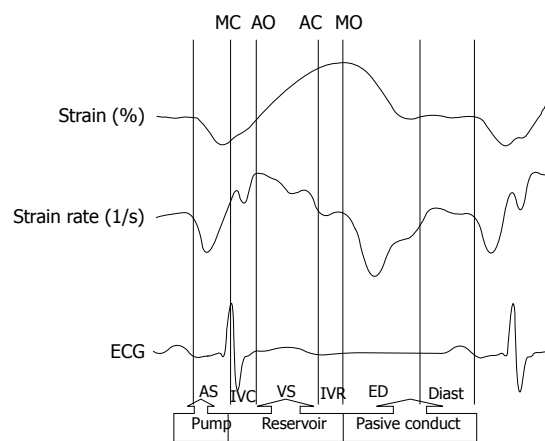


Figure 1 Atrial strain and strain rate during the phases of the cardiac cycle. MC: Mitral closure; AO: Aortic opening; AC: Aortic closure; MO: Mitral opening; AS: Atrial systole; IVC: Isovolumic contraction; VS: Ventricular systole; IVR: Isovolumic relaxation; ED: Early diastole; Diast: Diastasis.

diastolic dysfunction progresses, and the patient exhibits a pseudonormal or restrictive mitral flow, the passive conduit function increases, while the reservoir and active pump functions decrease significantly^[2].

Traditionally, assessment of left atrial function has been performed by measuring volume with 2D echocardiography. Additionally, it can be assessed with transmitral Doppler and pulmonary vein Doppler. Recently, an alternative method has been incorporated, namely, measurement of myocardial deformation with color tissue Doppler-derived strain^[3,4]. However, this method has several limitations, such as suboptimal reproducibility, angle-dependence, signal artifacts and the fact that it only measures regional strain and does not obtain information about the curved portion of the atrial roof.

To overcome these limitations in the quantification of atrial function, the use of speckle tracking echocardiography (STE) strain has been proposed. This technique is not derived from Doppler but rather from 2D echocardiography, it is angle-independent, and allows us to measure global as well as regional atrial strain^[5-7].

STE is a new technique of 2D echocardiography image analysis that allows the study of regional atrial myocardial deformation expressed by a dimensionless parameter, the strain (ϵ), which is defined as the percentage change from the original dimension. Deformation of atrial tissue occurs over time during the cardiac cycle, and the rate of this deformation, the SR, measures the velocity with which this myocardial deformation occurs.

STE allows accurate assessment of segmental strain deformation by grey-scale-based image analysis, frame by frame. The lack of angle-dependency is a great advantage because atrial strain can be tracked in 2D echocardiography imaging, along the direction of the wall and not along the ultrasound beam.

This new technique has a few limitations, namely, it is frame-dependent, cannot be used in patients whose 2D image quality is suboptimal (STE needs high quality

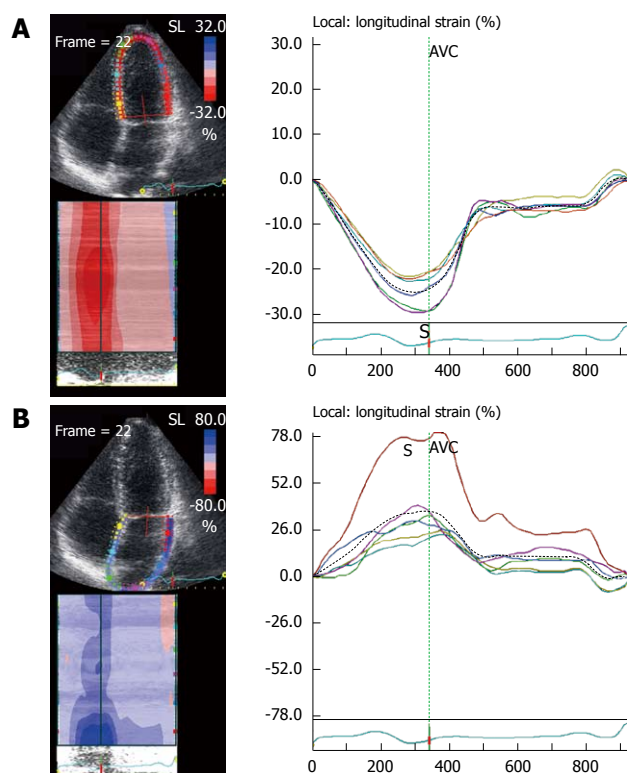


Figure 2 Ventricular strain (A) and atrial strain (B). Due to the fact that the atria relax while the ventricles contract, during ventricular systole, ventricular strain curves are negative, while atrial strain curves are positive. AVC: Aortic valve closure.

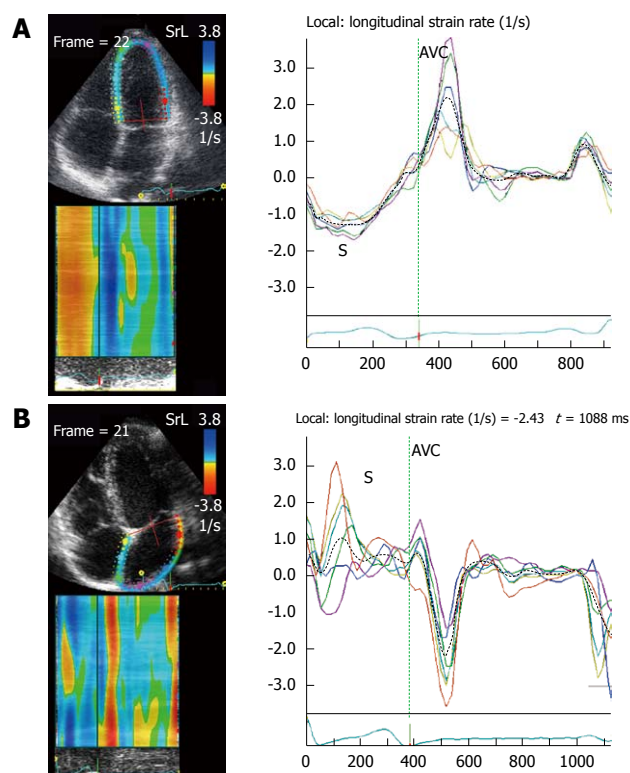


Figure 3 Ventricular strain rate (A) and atrial strain rate (B). Due to the fact that the atria relax while the ventricles contract, during ventricular systole, ventricular strain rate (SR) is negative, whereas atrial SR is positive. AVC: Aortic valve closure.

grey-scale images with an optimal frame rate between 50 and 70 frames/s), and requires a learning curve for the off-line analysis with the software used. Nonetheless, it is a very promising tool for the assessment of regional and global atrial function.

Global longitudinal left atrial strain and SR parameters determined by STE are feasible and reproducible indices for the evaluation of left atrial function.

The ventricular strain is different from atrial strain (Figure 2). The atria and ventricles move in opposite directions during the cardiac cycle, so the atrial myocardium lengthens during ventricular systole (positive strain), while the ventricular myocardium shortens during ventricular systole (negative strain). Ventricular SR curves are also opposite to atrial SR curves (Figure 3).

The apical four-chamber view permits 2D strain measurements of both atria. To calculate atrial strain, the atrial endocardium is first traced manually. The epicardial surface is calculated automatically, and after manually reducing the region of interest to the atrial thickness, the software automatically divides the atrial wall into six segments, two correspond to the interatrial septum, two to the lateral wall, and two to the roof of the left atrium (Figure 4).

Before acquiring the atrial strain from the apical four-chamber view, if speckle tracking is not adequate, the region of interest is manually adjusted to include only the atrial wall. If these steps are repeated from the apical two- and three-chamber views, strain values of the an-

terior, inferior and posterior walls of the left atrium are obtained, thus resulting in a bull's eye rendering of the 17 atrial segments (Figure 5). With this technique, atrial strain can be calculated in less than 3 min. In the apical three-chamber view, we only consider the posterior wall of the left atrium, because the opposite wall includes the ascending aorta. Of note, because there still is no software available to calculate atrial strain, we employ the same software that is used for the analysis of ventricular function. Once the longitudinal atrial strain curves have been obtained, two measurements are performed: peak atrial strain (during the reservoir phase), which is plotted as a positive curve (S) at the time of aortic valve closure; and strain during atrial systole (A), which is plotted as a negative curve with a peak after the P wave of the ECG. From the 2D atrial strain, SR curves are derived, which permits measurement of atrial SR during the three phases (Figure 6). Calculation of right atrial strain and right atrial SR is performed similarly to that of longitudinal 2D strain of the left atrium (Figure 7).

Radial strain of the atria is not obtained from the parasternal views because the atrial wall is thinner than the left ventricle wall, to allow speckle tracking analysis. The atrial reservoir strain is greater in the apical two-chamber view than in the four-chamber view. The cause of this discrepancy could be that there are two areas in which atrial strain is low: the interatrial septum and the area of the pulmonary veins where the heart is anchored to the mediastinum. During atrial contraction and re-

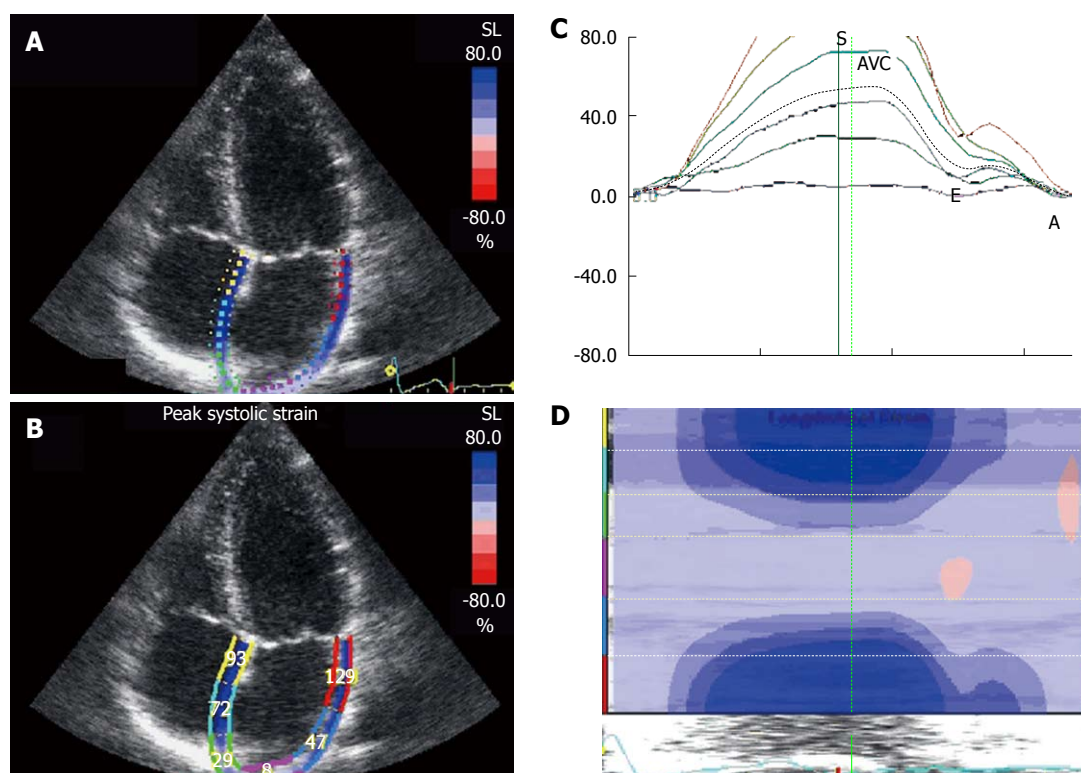


Figure 4 Measurement of 2D longitudinal atrial strain with speckle tracking. Quad view. A: Once the atrial endocardial border is traced, atrial thickness is traced automatically; B: Strain value of the reservoir in the six atrial walls; C: Longitudinal strain curves of the six atrial segments. The dotted line represents average atrial longitudinal strain. During the period in which the atrium acts as a reservoir (includes isovolumic contraction, ejection and isovolumic relaxation phases), atrial strain increases, reaching a peak (S) at the end of filling from pulmonary vein flow, just before mitral opening. During the conduit atrial phase, atrial strain decreases (E), with a plateau during diastasis and a negative peak at the end of atrial contraction (A); D: Curved anatomical M-mode: the light blue color indicates that atrial roof strain is lower than that of the rest of the walls, colored in blue. AVC: Aortic valve closure.

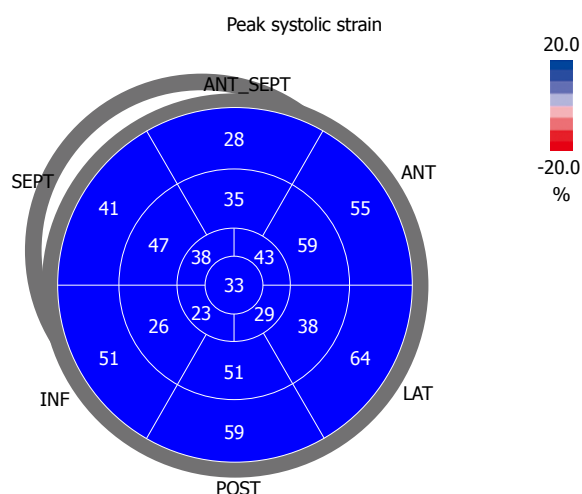


Figure 5 Bull's eye view of the 2D strain of the left atrial reservoir in a normal subject. Bull's eye with the longitudinal 2D strain values of the left atrium. It is colored blue because it represents the atrial strain of the reservoir phase, which increases during ventricular systole. Note that basal values are higher than medial values and further reduced at the center, which represents the atrial roof strain. Values of the antero-septal wall should be excluded, because they correspond to the ascending aorta.

laxation, a deformation gradient is observed from all views, with higher strain in the atrioventricular junction and lower strain in the atrial roof. Surprisingly, the strain of the atrial roof is not higher than in the atrial base, as

occurs with the left ventricle, but this could be because the atrial roof is fixed to the mediastinum by the pulmonary veins. In the assessment of left atrial function, the posterior wall exhibits the lowest strain. This could be because its motion is limited by attachment of the four pulmonary veins. By contrast, the inferior wall exhibits the highest strain and SR values^[8], which are attributable to its greater thickness, whereas the anterior wall, adjacent to Theile's transverse sinus, is thinner. In the assessment of right atrial function, the free wall exhibits the greatest strain and SR, compared to the rest of the atrial segments. This could be because its largest mass is conferred by the pectinate muscles, which could generate its greater mobility.

CLINICAL APPLICATIONS

Considering the limitations of the classic indices used to assess atrial function, 2D atrial strain measured with speckle tracking is a quick and simple technique that can clarify atrial function in several pathophysiological conditions associated with changes in atrial function, such as: mitral valve disease, supraventricular arrhythmias, hypertension, coronary heart disease, heart failure, atrial stunning and cardiomyopathy.

Assessment of atrial function is useful in distinguishing physiological from pathological hypertrophy. During

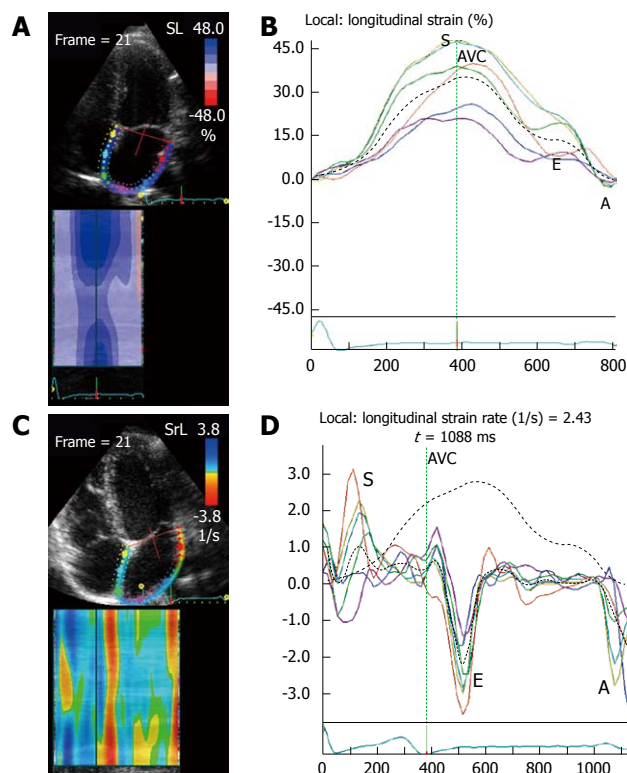


Figure 6 2D longitudinal strain and strain rate of the left atrium obtained from the apical four-chamber view in a normal subject. Quad view. A: Left atrial speckle tracking and curved anatomical M-mode of atrial strain during the whole cardiac cycle; B: Atrial strain curves depicted with different colors in the six segments (the dotted line indicates average strain). Positive curves indicate the atrial strain of the reservoir during ventricular systole (S), which coincides with aortic valve closure; Negative curves represent atrial strain during early diastole (E), and peak atrial contraction (A) occurs just after the P wave of the ECG; C and D: Similar to A and B: showing the left atrial longitudinal strain rate (SR) (the dotted line indicates the average SR). Positive curves (S), indicate atrial lengthening (relaxation) during the periods of: isovolumic contraction, ventricular ejection and isovolumic relaxation (reservoir SR). Negative curves indicate atrial SR during rapid ventricular filling (E) and atrial systolic SR (A) is seen after the P wave of the ECG. AVC: Aortic valve closure.

ventricular diastole (passive conduit and pump function phases), the left atrium is exposed to ventricular filling pressures. In healthy subjects, during exercise, the reservoir and pump strains increase to maintain ventricular filling at an optimal level during hemodynamic changes. In patients with hypertrophy secondary to hypertension, atrial pressure rises to maintain an adequate ventricular filling, and the rise in wall tension contributes to its dilatation. As a consequence, pump function increases while the reservoir function drops^[9], which leads to an increase in peak strain during atrial contraction and a decrease in the reservoir strain.

In patients with hypertrophic cardiomyopathy (HCM), an atrial reservoir strain < 21% predicts the development of atrial fibrillation (AF) within less than 12 mo^[10]. In the rare cases of HCM with right atrial hypertrophy, the atrial reservoir strain is markedly decreased (Figure 8).

The pathophysiology of AF in patients with apparently normal hearts remains unclear. The increase in left atrial volume is one of the most important structural

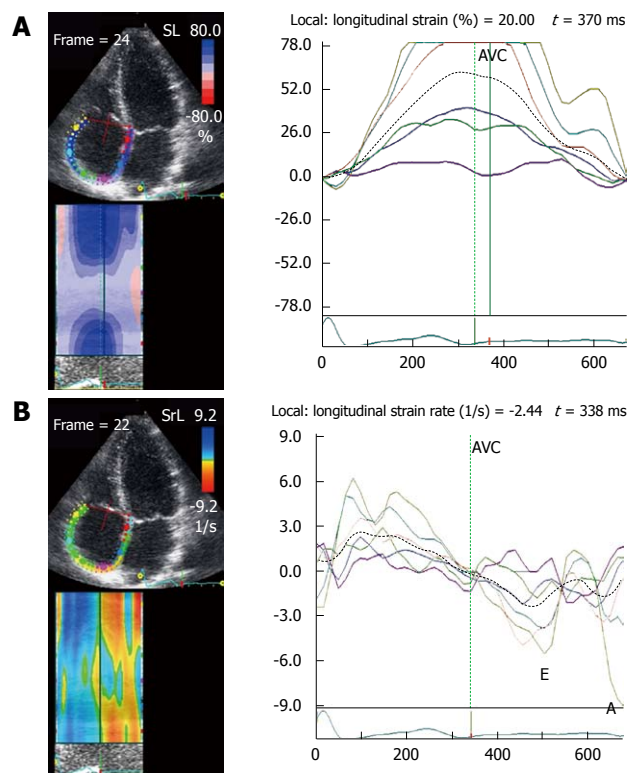


Figure 7 Right atrial strain and strain rate. Longitudinal 2D strain is obtained from the apical four-chamber view in a normal subject. A: Right atrial strain; B: Right atrial strain rate. Explanation as in Figure 6B. AVC: Aortic valve closure.

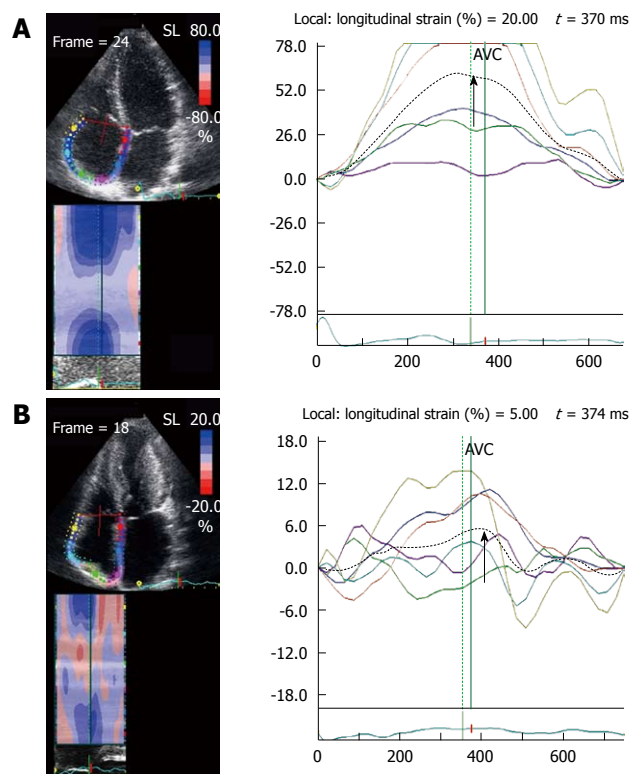


Figure 8 Average 2D longitudinal strain (dotted line) of the right atrium during the reservoir phase (arrow) in a normal subject (A), which is 60%, whereas in a patient with hypertrophic cardiomyopathy with right atrial hypertrophy (B), it is reduced to 6%. AVC: Aortic valve closure.

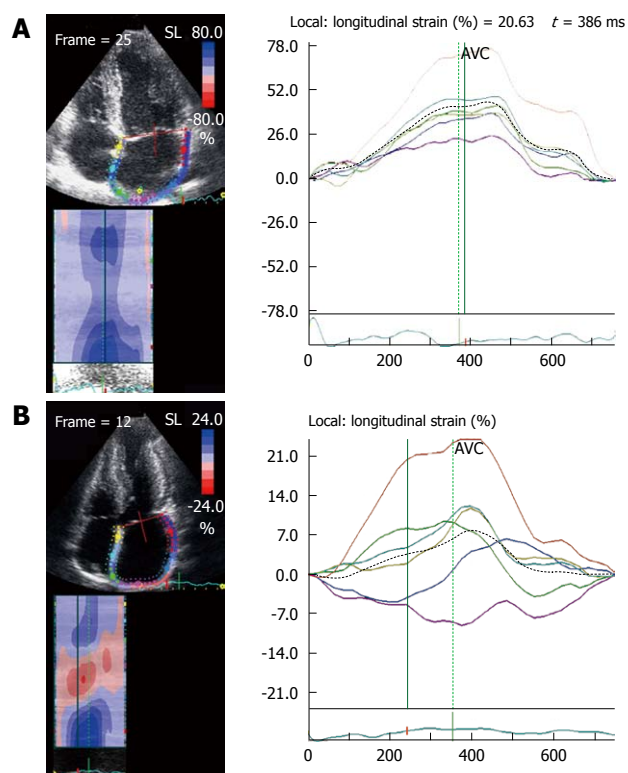


Figure 9 Left atrial 2D longitudinal strain during the reservoir phase in a normal subject (A) and in a patient with atrial fibrillation (B). Note that peak strain (dotted line) is 40% in the normal subject and 7% in the patient with atrial fibrillation. AVC: Aortic valve closure.

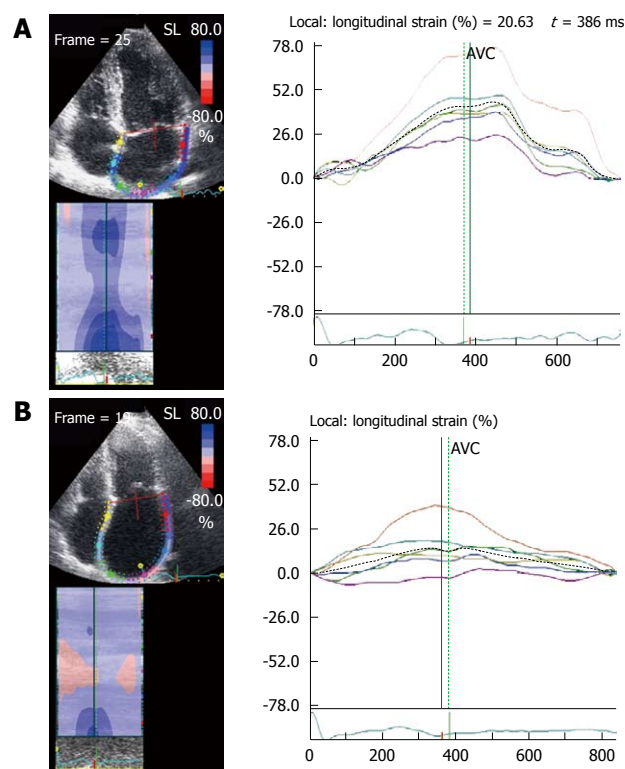


Figure 10 Left atrial 2D longitudinal strain during the reservoir phase in a normal subject (A) with an average reservoir strain (dotted line) of 40%, and in a patient with mitral stenosis (B) with a marked reduction in strain (13%). AVC: Aortic valve closure.

changes in AF. Stretching of the left atrial myocytes increases the intercellular matrix, collagen production and fibrosis. Atrial dilatation reflects not only a remodeling process, but also the effects of the rise in atrial pressure. It has been shown^[10] that the left atrium of patients with paroxysmal AF is larger and exhibits higher pressure. Atrial SR is the best predictor of paroxysmal AF, even better than atrial strain, probably because it allows better identification of the three phases of atrial function.

Another predictor of the new development of AF in patients with heart failure is the presence of intra-atrial asynchrony, which can be demonstrated with strain during the reservoir phase^[11]. Patients with heart failure exhibit a delay in contraction and in the reservoir relaxation phase of the left atrial free wall.

In patients with AF^[12], reservoir and conduit atrial strain and SR are decreased and strain and atrial strain are absent during late diastole, compared to patients with sinus rhythm (Figure 9). With restoration of sinus rhythm there is an increase in atrial reservoir and passive conduit strains. In contrast with the increase in peak velocity of the pulsed tissue Doppler A wave, peak atrial SR which reflects atrial pump function (A wave), is not normalized until 6 mo post restoration of sinus rhythm^[13].

Patients with AF who undergo electrical cardioversion have higher recurrence rates if they have multiple risk factors, including older age, and recurrence also depends on the cause and duration of the AF, functional

capacity, and degree of atrial dilatation. Severity of the impairment in atrial function, assessed by the decrease in 2D strain and SR during the reservoir phase and early diastole^[4], is an independent predictor of AF recurrence (Figure 9).

The extent of atrial fibrosis detected with late gadolinium enhancement by magnetic resonance correlates with this reduction in atrial strain and SR measured with speckle tracking^[14]. This finding explains why atrial reservoir strain and SR are useful predictors of the development of AF, and predict recurrence in patients with AF who undergo radiofrequency ablation^[12]. These findings suggest the usefulness of antiarrhythmic drugs in patients subjected to ablation who exhibit low atrial strain and SR values, which entail a high risk of recurrence.

Atrial stunning is characterized by a reduction in the mechanical function of the left atrium in AF after restoring sinus rhythm; it may last for several weeks and is associated with an increase in thromboembolic risk for the duration of the vulnerable period. Thomas *et al*^[15] have measured telediastolic atrial strain and have shown a gradual recovery of the left atrial pump function following cardioversion.

Patients with class III-IV heart failure, a wide QRS and intraventricular asynchrony benefit from cardiac resynchronization therapy (CRT). In addition to the clinical benefit (increase in functional capacity), there is an improvement in systolic function (ejection fraction increase

> 15%), reverse remodeling (end-systolic volume reduction < 15%), and an increase in right ventricular systolic function. These changes are accompanied by reverse atrial remodeling, which is identified by a reduction in left atrial area and volume. The contractile function of the left atrium, assessed by 2D speckle tracking strain and SR also increases at 3 mo after implantation of a biventricular pacemaker. Furthermore, there is an increase in strain values of the atrial reservoir and passive conduit, consistent with a rise in atrial compliance. The changes mentioned are only observed in patients with a good response to CRT^[16].

In mitral stenosis, there is a rise in left atrial pressure and volume, proportional to the severity of the stenosis. In spite of this increase in afterload, the left atrial pump function contributes less to ventricular filling, even in the presence of sinus rhythm, because atrial contraction cannot overcome the mechanical obstruction. Hence, atrial strain drops^[17] as the left atrium dilates (Figure 10). Patients with mitral stenosis with a greater impairment in the reservoir SR^[18] suffer more events (AF, pulmonary hypertension, or need for mitral valve repair or replacement). Future challenges will include defining an atrial strain value that can predict the development of AF and allow us to begin anticoagulation while the patient is still in sinus rhythm, before arrhythmia actually develops, and thus prevent embolic episodes.

In mitral regurgitation, the volume overload of the left atrium is proportional to the severity of regurgitation, but there is much less systolic dysfunction than with mitral stenosis; many patients even exhibit an increase in the reservoir SR, possibly due to a rise in atrial compliance. They also exhibit an increase in the passive conduit atrial SR, which is attributable to the rise in gradient during early diastole, and also shown by the increase in the peak E velocity of the transmitral flow. The left atrial pump function decreases due to the rise in left ventricular diastolic pressure, that is, the rise in atrial afterload. The increase in the reservoir and conduit atrial SR in mitral regurgitation might explain the different time sequence in the development of AF seen in mitral stenosis and mitral regurgitation^[17].

In atrial septal defect, atrial strain shows no change compared to healthy subjects, but when patients who underwent surgery were compared to those with percutaneous closure with an occluder, strain in both atria was only decreased in patients undergoing surgical closure^[19].

CONCLUSION

The assessment of atrial function with 2D speckle tracking strain and SR is feasible and reproducible and has several clinical applications. It permits evaluation of the three components of atrial function (pump, passive conduit and reservoir functions) in both atria. These new parameters of atrial function are more sensitive than traditional indices of atrial function and could be incorporated into the routine assessment of various pathophysiological scenarios.

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Clinical importance of aspirin and clopidogrel resistance

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Abstract

Aspirin and clopidogrel are important components of medical therapy for patients with acute coronary syndromes, for those who received coronary artery stents and in the secondary prevention of ischaemic stroke. Despite their use, a significant number of patients experience recurrent adverse ischaemic events. Interindividual variability of platelet aggregation in response to these antiplatelet agents may be an explanation for some of these recurrent events, and small trials have linked "aspirin and/or clopidogrel resistance", as measured by platelet function tests, to adverse events. We systematically reviewed all available evidence on the prevalence of aspirin/clopidogrel resistance, their possible risk factors and their association with clinical outcomes. We also identified articles showing possible treatments. After analyzing the data on different laboratory methods, we found that aspirin/clopidogrel resistance seems to be associated with poor clinical out-

comes and there is currently no standardized or widely accepted definition of clopidogrel resistance. Therefore, we conclude that specific treatment recommendations are not established for patients who exhibit high platelet reactivity during aspirin/clopidogrel therapy or who have poor platelet inhibition by clopidogrel.

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Key words: Aspirin; Clopidogrel; Antiplatelet agent; Aspirin resistance; Clopidogrel resistance; Cardiovascular outcome; Platelet aggregation

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INTRODUCTION

Platelets adhere to sites of vascular injury. Atherosclerotic lesions are associated with impaired endothelial function and hence are susceptible to platelet and leukocyte adhesion. Indeed, patients with atherosclerosis have enhanced baseline platelet activation, which is reflected by corresponding increases in urinary thromboxane (TX) metabolite excretion^[1-3]. It should be noted, however, that endothelial disruption is not a prerequisite for

platelet adhesion. Initially, platelets tether to the vessel wall via membrane integrins and selectins. Subsequent rolling and firm adhesion have been demonstrated by intravital microscopy in experimental models of microvascular injury. Shear stress augments adhesion receptor engagement and platelet activation (so-called “outside-in” signaling). This in turn triggers release or generation of soluble platelet activators such as TX, adenosine diphosphate (ADP), and thrombin. A layer of activated platelets forms and attracts other platelets and leukocytes. This is followed by either stable thrombus formation or rapid resolution.

Activated platelets release inflammatory and mitogenic proteins that promote leukocyte chemoattraction, vascular inflammation and further modify the endothelial phenotype^[2]. Indeed, there is growing evidence that platelet adhesion is involved in the earliest development of atherosclerotic lesions. On activation, the most densely expressed platelet, integrin II b β 3 [glycoprotein (GP) II b/IIIa], undergoes conformational change, binds soluble fibrinogen and von Willebrand factor and facilitates platelet aggregate formation. Notably, GP II b/IIIa gradually loses its binding capacity when platelets are stimulated by ADP alone. However, more potent agonists, such as thrombin, induce persistent fibrinogen binding. The cycle of initiation, propagation, and perpetuation of platelet activation creates the platelet mass that forms a nidus for coagulation. Fibrin generation and release of secondary platelet agonists propagate this process. Secondary agonists continuously activate integrins and importantly may be required to prevent disassembly of the early platelet aggregate. Six soluble ADP, TXA₂, soluble CD40 ligand, and the product of growth arrest specific gene 6 are prominent in these paracrine signaling pathways^[3].

Oral antiplatelet drugs are a cornerstone of modern pharmacotherapy in cardiovascular atherothrombotic diseases. The efficacy of acetylsalicylic acid (ASA, aspirin) and clopidogrel in decreasing the risk of adverse events in vascular disease patients has been well established in the past 20 years. Despite chronic oral antiplatelet therapy, a number of atherothrombotic events continue to occur. In recent years, a number of reports in the literature have shown possible relationships between residual platelet activity, as measured with a variety of laboratory tests, and clinical outcomes, raising the possibility that ‘resistance’ to oral antiplatelet drugs may underlie many such adverse events. The aim of our review was to collect articles showing the definition, detection, risk factors and clinical consequences of aspirin and clopidogrel resistance.

The effect of aspirin is mediated by the irreversible inactivation of cyclooxygenase (COX-1), leading to the prevention of thromboxane A₂ generation from arachidonic acid. Following oral administration, aspirin is effective as an antiplatelet agent within 60 min. COX-1 is rapidly resynthesized by nucleated cells, such as endothelial cells, and therefore the effect of aspirin on nucleated cells lasts only for a relatively short time^[4-8]. In contrast, the effect of aspirin on platelets (anucleate cells) lasts for the life of the platelets (7-10 d)^[9] (Figure 1).

Clopidogrel, an ADP-receptor antagonist, is a pro-drug requiring oxidation by the hepatic cytochrome P450 (CYP450) to generate an active metabolite^[10]. Only a small proportion of clopidogrel undergoes metabolism by CYP450; it is mostly hydrolyzed by esterases to an inactive carboxylic acid derivative that accounts for 85% of clopidogrel-related circulating compounds. CYP3A4 and CYP3A5 are the enzymes responsible for the oxidation of the thiophene ring of clopidogrel to 2-Oxo-clopidogrel, which is further oxidized, resulting in the opening of the thiophene ring and the formation of both a carboxyl and a thiol group^[10]. The latter forms a disulfide bridge with the two extracellular cysteine residues located on the ADP P2Y₁₂ receptor expressed on the platelet surface and causes an irreversible blockade of ADP binding for the platelet’s life span^[11]. Inhibition of platelet function is consistent with time-dependent, cumulative inhibition of platelet aggregation on repeated daily dosing and with slow recovery of platelet function on drug withdrawal (Figure 1).

LABORATORY ANTIPLATELET RESISTANCE

The definition of resistance

An exact definition of “resistance” to antiplatelet therapy on the basis of physiology does not exist. However, there is a significant prevalence of variable responses to dual antiplatelet regimens similar to different responses to anti-hypertensive therapy or statin therapy. Therefore, it is imperative to understand this variable response or hyporesponsiveness to aspirin and clopidogrel in some patients. A clear definition of this response should be established and, based on this, one may then be able to categorize patients as responders, hyporesponders, nonresponders, or resistant and thus manage their therapeutic regimen accordingly^[9-12].

Laboratory detection

Thromboxane A₂ production: Serum thromboxane B₂ (TxB₂) reflects the total capacity of platelets to synthesize TxA₂, which is the most specific test to measure the pharmacological effects of aspirin^[10,11].

The urinary levels of TxB₂ metabolite, 11-dehydro-TxB₂, represent a time-integrated index of TxA₂ biosynthesis *in vivo*. Because it is not formed in the kidney, detection of this TxA₂ metabolite in the urine reflects systemic TxA₂ formation, although about 30% of the urinary metabolite derives from extra-platelet sources. Therefore, the method is not highly specific for monitoring the effects of aspirin on platelet COX-1^[13,14].

Optical aggregometry: The historical “gold standard” is turbidometric platelet aggregometry, which measures platelet coaggregation in platelet-rich plasma^[4,10]. Samples are exposed to an agonist, such as collagen, epinephrine, ADP or arachidonic acid, and the increase in light transmittance resulting from platelet-platelet aggregation

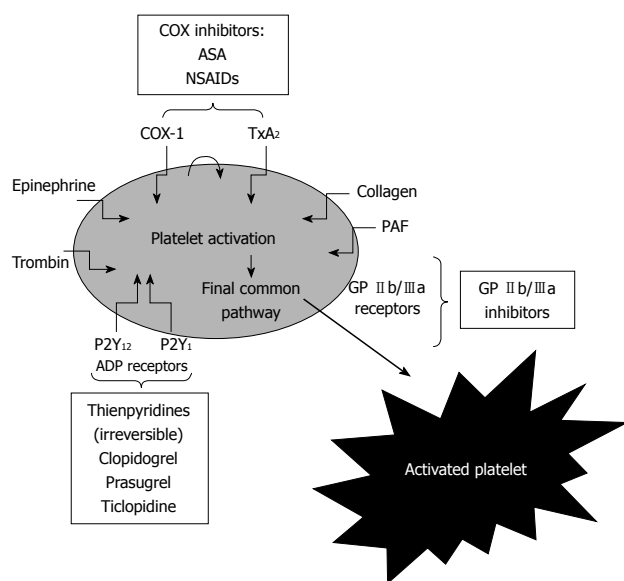


Figure 1 Pathways of platelet activation and mechanism of action of antiplatelet agents^[2]. COX: Cyclooxygenase; GP: Glycoprotein; TxA₂: Thromboxane A₂; ASA: Acetylsalicylic acid; NSAIDs: Nonsteroidal anti-inflammatory drugs; PAF: Platelet-activating factor.

is measured. Its disadvantages include the large sample volumes required, long processing times and complex sample preparation^[11,13,15]. Its advantages are that it can be used to monitor ASA, thienopyridines and platelet GP II b/III a inhibitor therapy^[15].

Platelet function analyzer-100 system: The platelet function analyzer (PFA-100) measures *in vitro* the cessation of high-shear blood flow by the platelet plug. It is a simple, rapid, point-of-care, whole blood method that requires low sample volumes and no sample preparation. Its disadvantages are that it is dependent on the Von Willebrand factor and hematocrit levels and that it requires pipetting^[10-12,15].

Kotzailias *et al*^[16] indicated that the current PFA-100 cartridges are not sufficiently sensitive to detect clopidogrel-induced platelet inhibition in stroke patients. A recent consensus paper concluded that this method is not recommended for monitoring of thienopyridines^[15]. On the other hand, Marcucci *et al*^[17] showed that this method (combined with optical aggregometry) can be useful in the detection of residual platelet activity, which was associated with worsening cardiovascular outcomes in 367 consecutive adult patients admitted to hospital, including 200 patients on dual antiplatelet agents (group A) and 167 on dual antiplatelet agents plus GP II b/III a inhibitors (group B), with a diagnosis of ST-segment elevation acute MI.

Impedance aggregometry: Impedance aggregometry measures the change in electrical impedance between two electrodes when platelets are aggregated by an agonist. The method is similar to light or optical aggregometry except that it can be done in whole blood, thus obviating the need for preparation of a platelet suspension. Impedance aggregometry can also be done in thrombocytopenic patients^[15].

Ultegra RPFA-ASA: The Ultegra RPFA-ASA (Accumetrics, San Diego, CA, USA) is a simple, rapid, point-of-care method that has several other advantages: required sample volumes are small, it uses whole blood and no pipetting is required^[15]. If aspirin/clopidogrel produces the expected antiplatelet effect, fibrinogen-coated beads will not agglutinate and light transmission will not increase.

Thromboelastogram platelet mapping system: The thromboelastogram platelet mapping system measures platelet contribution to clot strength. It is a point-of-care method that uses whole blood to assess platelet clot formation and clot-lysis data. It is able to monitor all 3 classes of antiplatelet therapies. However, it requires pipetting and has undergone only limited study^[10-12,15].

Vasodilator-stimulated phosphoprotein phosphorylation: Vasodilator-stimulated phosphoprotein (VASP) phosphorylation measures activation-dependent platelet signaling. Its advantages include small required sample volumes, the use of whole blood, stability (allowing samples to be shipped to a remote laboratory) and dependency on the P2Y₁₂ receptor, which is the site of action for clopidogrel. Its disadvantages are that it requires complex sample preparation, flow cytometry and experienced technicians^[12,15].

Activation-dependent changes on the platelet surface: Other methods assess activation-dependent changes on the platelet surface. These tests include measurement of levels of platelet surface P-selectin, activated GP II b/III a and leukocyte-platelet aggregation. Their advantages include the small sample volumes required and the use of whole blood; disadvantages include complex sample preparation, the requirement for flow cytometry and experienced operators and lack of commercial availability^[12,15].

Comparism of methods

In a study by Lordkipanidzé *et al*^[18], 201 patients with stable coronary artery disease receiving daily aspirin therapy (≥ 80 mg) were recruited. They found that platelet function tests (light transmission aggregometry, whole blood aggregometry, PFA-100 system, VerifyNowAspirin and urinary 11-dehydro-TxB₂ concentrations) were not equally effective in measuring aspirin's antiplatelet effect and correlated poorly amongst themselves. Their results have been confirmed by other studies^[19-21].

On the other hand, a recent study based on healthy volunteers found high concordance ($> 90\%$) between the examined assays (light transmission aggregometry, PFA-100, VerifyNow, and urinary 11-dehydro-TxB₂)^[22].

The assessment of platelet function inhibition by clopidogrel is also highly test-specific. Lordkipanidzé *et al*^[23] examined 116 patients with stable coronary artery disease requiring diagnostic angiography. Agreement between assays (light transmission aggregometry (ADP 5 and 20 mmol/L as the agonist), whole-blood aggregometry (ADP 5 and 20 mmol/L), PFA-100 (Collagen-ADP cartridge) and VerifyNow P2Y₁₂) to identify patients with insufficient inhibition of platelet aggregation by clopidogrel

was also low. Their result was in concordance with other studies^[24,25].

The broad use of statins, angiotensin receptor blockers and selective serotonin reuptake inhibitors may be, in part, responsible for the lack of agreement^[26]. Our previous results showed the effect of different cardiovascular drugs on the laboratory efficacy of aspirin and clopidogrel^[27,28].

How to define antiplatelet resistance?

Based on the recent position paper of the Working Group on antiplatelet drugs resistance appointed by the Section of Cardiovascular Interventions of the Polish Cardiac Society, endorsed by the Working Group on Thrombosis of the European Society of Cardiology, the term 'laboratory resistance' to oral antiplatelet agents should be reserved for situations when the expected effect from an oral antiplatelet drug cannot be obtained due to changes in the target enzyme or receptor (pharmacodynamic 'resistance'). Such situations can be ascertained with a good approximation *in vitro*.

For the assessment of ASA-specific effects, the proposed test is the use of aggregation induced by arachidonic acid and of TXB₂ concentrations in serum (or in the supernatant after aggregation). For further evaluation, the *in vitro* addition of ASA can be performed before aggregation or the preparation of serum to exclude pharmacokinetic 'resistance'.

For the assessment of a clopidogrel-specific effect, the proposed test is aggregation induced with ADP or VASP phosphorylation. For further evaluations, the *in vitro* addition of the active metabolite of the P2Y₁₂ receptor antagonist can be performed before such tests to exclude pharmacokinetic 'resistance'.

In the case of abnormal results from non-specific tests, one should only use the term 'elevated platelet reactivity despite treatment'. To detect the reason for this, more specific tests for a given drug should be used^[29]. On the other hand, no specific method or agonist dose was mentioned in the detection of this phenomenon^[12].

CLINICAL IMPORTANCE OF ANTIPLATELET RESISTANCE

Aspirin resistance

Despite lacking a definition of resistance and the association of platelet function tests, aspirin resistance seems to be associated with worsening clinical outcome. Based on a recent meta-analysis, the prevalence of laboratory aspirin resistance ranged from 5% to 65%. In the 12 studies eligible for pooling, comprising 1813 patients, the mean prevalence of laboratory aspirin resistance was 27%. The pooled odds ratio of all cardiovascular outcomes was 3.8 (95% CI: 2.3-6.1) for laboratory aspirin resistance. This systematic review and meta-analysis showed that patients biochemically identified as having laboratory aspirin resistance were more likely to also have "clinical resistance" to aspirin because they exhibited significantly higher risks of recurrent cardiovascular events compared with patients who were identified as (laboratory) aspirin sensitive^[30]

(Figure 2). This result was confirmed by another meta-analysis considering 20 studies totalling 2930 patients with cardiovascular disease. Overall, 810 patients (28%) were classified as aspirin resistant. A cardiovascular related event occurred in 41% of patients (OR 3.85, 95% CI: 3.08-4.80), death in 5.7% (OR 5.99, 95% CI: 2.28-15.72) and an acute coronary syndrome in 39.4% (OR 4.06, 95% CI: 2.96-5.56). Therefore, patients who were resistant to aspirin were at a greater risk of clinically important cardiovascular morbidity long term compared to patients who were sensitive to aspirin. This result was confirmed by other studies^[31-33]. Interestingly, aspirin resistant patients did not benefit from other antiplatelet treatment^[31].

Clopidogrel resistance

We found only one meta-analysis focusing on clopidogrel resistance^[12,32]. The authors identified 25 eligible studies that included a total of 3688 patients. Mean prevalence of clopidogrel nonresponsiveness was 21% (95% CI: 17%-25%) and was inversely correlated with time between clopidogrel loading and determination of nonresponsiveness and loading dose. The pooled odds ratio of cardiovascular outcomes was 8.0 (95% CI: 3.4-19.0). Therefore, laboratory clopidogrel nonresponsiveness could be found in approximately 1 in 5 patients undergoing PCI. Patients who were *ex vivo* labeled nonresponsive were likely to be also "clinically nonresponsive", as they exhibited increased risks of worsened cardiovascular outcomes (Figure 3). Their results indicated that use of a 600-mg clopidogrel loading dose would reduce these risks, which needed to be confirmed in large prospective studies^[34].

Very recently a comparison of platelet function tests in predicting clinical outcomes in patients undergoing coronary stent implantation was published to evaluate the capability of multiple platelet function tests to predict clinical outcomes. It was a prospective, observational, single-center cohort study of 1069 consecutive patients taking clopidogrel undergoing elective coronary stent implantation between December 2005 and December 2007. On-treatment platelet reactivity was measured in parallel by light transmittance aggregometry, VerifyNow P2Y₁₂ and Plateletworks assays and the IMPACT-R and PFA-100 system (with the Dade PFA collagen/ADP cartridge and Innovance PFA P2Y). Cut-off values for high on-treatment platelet reactivity were established by receiver operating characteristic curve analysis. Of the platelet function tests assessed, only light transmittance aggregometry, VerifyNow, and Plateletworks were significantly associated with the primary end point. However, the predictive accuracy of these tests was only modest. None of the tests provided accurate prognostic information to identify low-risk patients at higher risk of bleeding following stent implantation^[35].

RISK FACTORS OF ANTIPLATELET RESISTANCE

Aspirin resistance

Based on a number of large trials and meta-analyses,

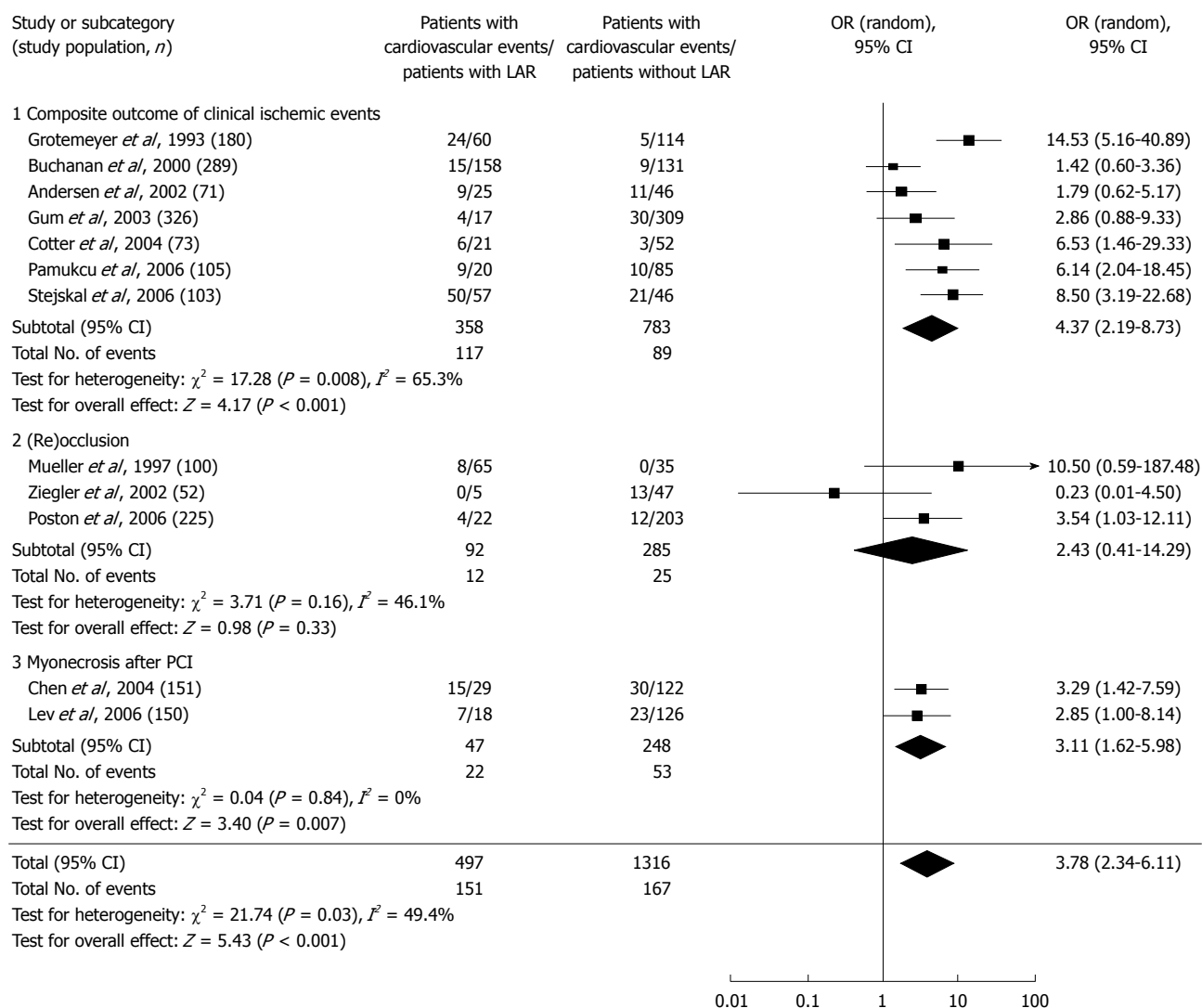


Figure 2 The clinical importance of aspirin resistance^[30].

low doses of aspirin (75 to 150 mg/d) are comparatively safe and sufficient to inhibit platelet COX-1 and are as effective in preventing vascular events as higher aspirin doses (500 to 1500 mg/d)^[4]. In some patients, the failure to suppress platelet COX-1 may be due to an inadequate dosage and reduced bioavailability of aspirin. In some cases, this may well relate to poor patient adherence (compliance), concurrent administration of nonsteroidal anti-inflammatory drugs (e.g. ibuprofen and indomethacin) and COX-2 inhibitors (which may compete with aspirin for platelet COX-1) or even a reduced absorption (or increased metabolism) of aspirin^[36-38]. Such concerns have been highlighted in a recent meta-analysis of 6 studies focusing either on nonadherence or premature discontinuation of aspirin in over 50 000 patients at high risk of coronary artery disease, where a 3-fold increased risk of cardiac events (OR 3.14, 95% CI: 1.75-5.61, $P = 0.0001$) was related to nonadherence or the unjustified withdrawal of aspirin^[39].

Age, weight and intake of proton pump inhibitors may also reduce the bioavailability of low-dose aspirin,

mainly due to increased inactivation of ASA by gastrointestinal mucosal esterases and reduced absorption of active ASA^[38]. Although low-dose aspirin may potentially be a cause of apparent aspirin resistance through reduced absorption, the use of higher doses of aspirin seems unjustifiable and is outweighed by an increased risk of gastrointestinal bleeding^[40]. However, in conditions accompanied by increased platelet turnover (e.g. acute coronary syndromes, coronary artery bypass grafting and other surgical procedures, acute or chronic infection and inflammation), a temporary increase of aspirin dose seems reasonable, albeit unproven^[38]. Circumstantial evidence for this claim is available as aspirin resistance (as defined by PFA-100) and is twice as common in acute coronary syndromes complicated by pneumonia compared with those cases without infectious complications (90% *vs* 46%)^[41]. In addition, there appears to be an independent association between CRP and aspirin resistance in these patients. Thus, in conditions that are associated with both infection and inflammation, nonplatelet sources of TxA₂ production (e.g. monocytes, macrophages and endothelial cells)

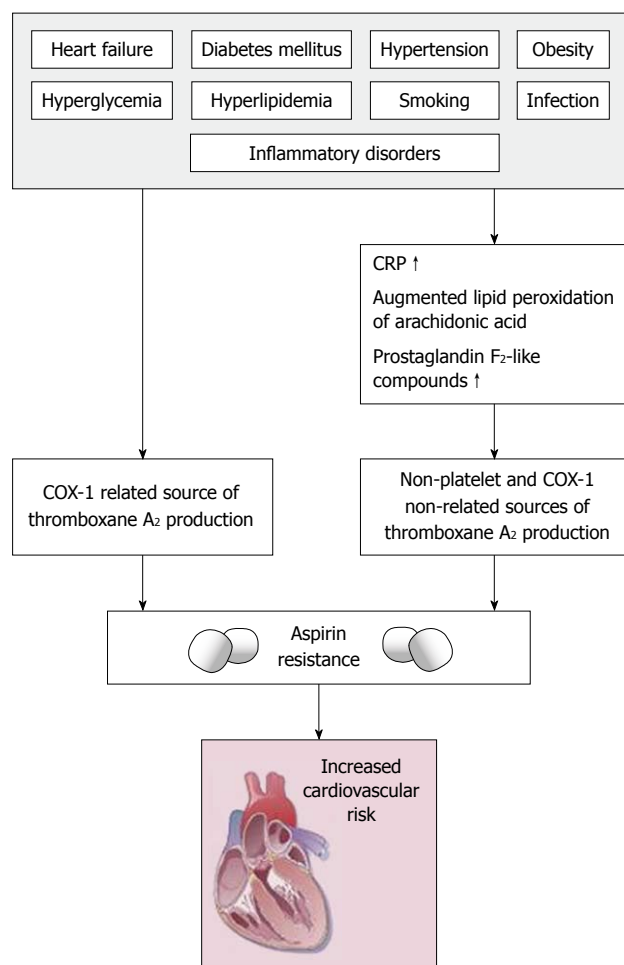


Figure 3 The possible background of aspirin resistance^[38]. COX: Cyclooxygenase.

and up-regulation of the COX-2 enzyme coupled with increased levels of F₂-isoprostanes may lead to uncontrolled thromboxane synthesis. Such COX-1-independent mechanisms are especially relevant to patients with diabetes mellitus, hyperlipidemia, smoking and heart failure; all of which are associated with augmented lipid peroxidation of arachidonic acid and consequent overproduction of isoprostanes^[38,42-49] (Figure 3).

In our recent work, 599 patients with chronic cardiovascular and cerebrovascular diseases (355 men, mean age 64 ± 11 years; 244 women, mean age 63 ± 10 years) who were taking aspirin 100-325 mg/d were examined^[28]. Compared with aspirin-resistant patients, patients who demonstrated effective aspirin inhibition had a significantly lower plasma fibrinogen level (3.3 g/L *vs* 3.8 g/L , $P < 0.05$) and significantly lower RBC aggregation values (24.3 *vs* 28.2 , $P < 0.01$). In addition, significantly more patients with effective aspirin inhibition were hypertensive (80% *vs* 62% , $P < 0.05$). Patients who had effective platelet aggregation were significantly more likely to be taking beta-adrenoceptor antagonists (75% *vs* 55% , $P < 0.05$) and ACE inhibitors (70% *vs* 50% , $P < 0.05$), whereas patients with ineffective platelet aggregation were significantly more likely to be taking HMG-CoA reductase inhibitors (statins) (52% *vs*

38% , $P < 0.05$). Use of statins remained an independent predictor of aspirin resistance even after adjustment for risk factors and medication use (OR 5.92, 95% CI: 1.83-16.9, $P < 0.001$). The importance of impaired hemorheological parameters in the development of aspirin resistance was confirmed by another study conducted by our workgroup and it was also confirmed by independent studies^[50,51]. One potential explanation is when plasma fibrinogen levels increase red blood cells adhere and release ADP, which is a potential agonist of platelet aggregation. On the other hand, the aggregated red blood cells migrate in the center of blood flow, displacing other cells (platelets) in small vessels, so they can easily contact the endothelium. Furthermore, platelets from aspirin-resistant patients appeared to be more sensitive and activable by ADP. This hypersensitivity could provide a possible explanation for the so-called aspirin resistance, and this could justify therapeutic improvement with alternative antiplatelet agents^[52].

Individual differences in the rate of platelet activation and reactivity markedly influence normal hemostasis and the pathological outcome of thrombosis. Such individual variability is largely determined by environmental and genetic factors. These are known to either hamper platelets' responses to agonists, and thereby mimic the pharmacological modulation of platelet function, or mask the therapy effect and sensitize platelets. We recently reviewed the possible role of different polymorphisms in the development of aspirin resistance, which may affect the efficacy of antiplatelet therapy. Variation in the way patients respond to aspirin may, in part, reflect heterogeneity in COX-1, COX-2, GP I b α , GP I a/II a, GP II b/III a, UGT1A6*2, P2Y(1), and P2Y(12) genotypes. On the other hand, very recently within 31 studies, 50 polymorphisms in 11 genes were investigated in 2834 subjects. The PLA1/A2 polymorphism in the GP IIIa platelet receptor was the most frequently investigated, with 19 studies in 1389 subjects. The PLA1/A2 variant was significantly associated with aspirin resistance when measured in healthy subjects (OR 2.36, 95% CI: 1.24-4.49, $P = 0.009$). Combining genetic data from all studies (comprising both healthy subjects and those with cardiovascular disease) reduced the observed effect size (OR 1.14, 95% CI: 0.84-1.54, $P = 0.40$). Moreover, the observed effect of a PLA1/A2 genotype varied depending on the methodology used for determining aspirin sensitivity/resistance. No significant association was found with aspirin resistance in four other investigated polymorphisms in the COX-1, GP I a, P2Y1 or P2Y12 genes^[53]. The lack of association among aspirin resistance and different gene haplotypes were confirmed by recently published studies^[54,55].

Clopidogrel resistance

Clopidogrel is a prodrug that is metabolized by CYP450 into an active metabolite, which irreversibly inhibits binding of ADP to the P2Y₁₂ receptor on the platelet^[56,57]. Increased body mass index, hemoglobin A1c, C-peptide levels, and von Willebrand factor were significant factors of clopidogrel resistance^[58]. Matetzky *et al*^[59] reported that smokers were more likely to be responders. Gurbel *et al*^[60]

Table 1 The role of clopidogrel and statin interaction based on recent clinical trials

Study	Sample size	Comparison	Primary end point	Comment
CREDO substudy ^[66]	1159	Post hoc analysis categorizing baseline statin use to those predominantly metabolized by CYP3A4 or not	1 yr composite endpoint of death, myocardial infarction and stroke	No detrimental effect
GRACE ^[67]	15 693	Four groups: group I received aspirin alone, group II aspirin and clopidogrel, group III aspirin and statin and group IV aspirin, clopidogrel and statin	6 mo mortality adjusted for baseline characteristics, in-hospital medications and procedures, re-hosp and revascularization	No detrimental effect
MITRA plus ^[68]	2086	Two groups: group I received atorvastatin and clopidogrel, group II other statins (both lipophilic and non-lipophilic) and clopidogrel	Long-term mortality	No detrimental effect
Mukherjee <i>et al</i> ^[69]	1651	Two groups: group I received CYP3A4 statin plus clopidogrel, group II received non-CYP3A4 statin plus clopidogrel	In-hospital and 6 mo mortality	No detrimental effect
Brophy <i>et al</i> ^[70]	2927	Two groups: group I received clopidogrel and atorvastatin, group II clopidogrel alone	30-d rates of adverse cardiovascular events (composite of death, myocardial infarction, unstable angina, stroke or transient ischaemic attack and repeat revascularization procedures)	Worse outcome associated with statins
CHARISMA substudy ^[71]	10 078	Post hoc analysis categorizing baseline statin use to those predominantly metabolized by CYP3A4 or not	Composite of myocardial infarction, stroke or cardiovascular death at median follow-up of 28 mo	No detrimental effect

Taken from Bhindi R, Ormerod O, Newton J, Banning AP, Testa L. Interaction between statins and clopidogrel: is there anything clinically relevant? *QJM* 2008; 101: 915-925^[72].

reported that patients with longer stents were more likely to be resistant to clopidogrel; however, Angiolillo *et al*^[61] did not find a correlation between stent length and non-responsiveness. Lev *et al*^[62] found that 50% of their aspirin-resistant study participants were also resistant to clopidogrel. In their study, patients with dual drug resistance were more likely women (67.7% *vs* 26.9%, $P = 0.02$) with an elevated body mass index ($33.8 \pm 7.9 \text{ kg/m}^2$ *vs* $29.7 \pm 5 \text{ kg/m}^2$, $P = 0.03$) than those with dual drug sensitivity. In our previous study, 157 patients with chronic cardio- and cerebrovascular diseases (83 males, mean age 61 ± 11 years, 74 females, 63 ± 13 years) taking 75 mg clopidogrel daily (not combined with aspirin) were included. Compared with clopidogrel-resistant patients [35 patients (22%)], patients who demonstrated effective clopidogrel inhibition had a significantly lower body mass index (26.1 kg/m^2 *vs* 28.8 kg/m^2 , $P < 0.05$). Patients with ineffective platelet aggregation were significantly more likely to be taking benzodiazepines (25% *vs* 10%) and selective serotonin reuptake inhibitors (28% *vs* 12%, $P < 0.05$). After an adjustment to the risk factors and medications BMI (OR 2.62, 95% CI: 1.71-3.6, $P < 0.01$), benzodiazepines (OR 5.83, 95% CI: 2.53-7.1, $P < 0.05$) and SSRIs (OR 5.22, 95% CI: 2.46-6.83, $P < 0.05$) remained independently associated with clopidogrel resistance^[27].

Concurrent medication use may interfere with the ability of clopidogrel to decrease platelet reactivity. Gurbel *et al*^[60] reported that high doses of calcium-channel blockers and angiotensin-converting enzyme inhibitors possibly contribute to a decreased response to clopidogrel. Studies that have evaluated clopidogrel resistance and statins have not been uniformly reproducible either. Atorvastatin is the most frequently studied statin in clopidogrel trials. Lau *et al*^[63] showed that atorvastatin promoted clopidogrel resistance at 10 mg, 20 mg and 40 mg ($P = 0.027$, $P = 0.002$ and $P = 0.001$, respectively). On

the other hand, Mitsios *et al*^[64] reported that daily doses of 10 mg of atorvastatin did not result in a decreased clopidogrel response over a 5-wk period. In the same study, clopidogrel significantly attenuated platelet aggregation in 3 different concentrations of ADP in the presence of no statin, atorvastatin, or pravastatin ($P < 0.01$, $P < 0.01$, and $P < 0.02$ at 2 μmol , 5 μmol , and 10 μmol of ADP, respectively). Also, Müller *et al*^[65] reported that antiplatelet activity was not reduced in patients who were given a 600 mg loading dose of clopidogrel and 1 of these statins: atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin or cerivastatin. There is some evidence supporting a possible pharmacokinetic interaction between statins and the anti-platelet drug clopidogrel. In particular, it has been suggested that this interaction is more likely with lipophilic statins, which share the same CYP450 metabolizing isoenzyme (Table 1^[66-72]). However, discordance between *ex vivo* data, which points in favour of an interaction, and the majority of clinical studies, which failed to detect a clinically relevant effect, has to be acknowledged^[72].

Lau *et al*^[73] reiterated the contribution of CYP3A4 activity to the phenomenon of clopidogrel resistance. A significant inverse correlation was observed between platelet aggregation and CYP3A4 activity as measured by the erythromycin breath test in healthy volunteers. The investigators also demonstrated that by enhancing CYP3A4 activity with rifampin in 10 healthy volunteers, 3 initial non-responders (platelet inhibition $< 10\%$) and one low responder (platelet inhibition between 10% to 29%) to clopidogrel exhibited enhanced platelet inhibition that met the definition of a clopidogrel responder (platelet inhibition $> 30\%$). This was in concordance with our results and later articles^[27,74].

Proton pump inhibitors are among the competitive inhibitors of CYP450 2C19, the other major isoenzyme

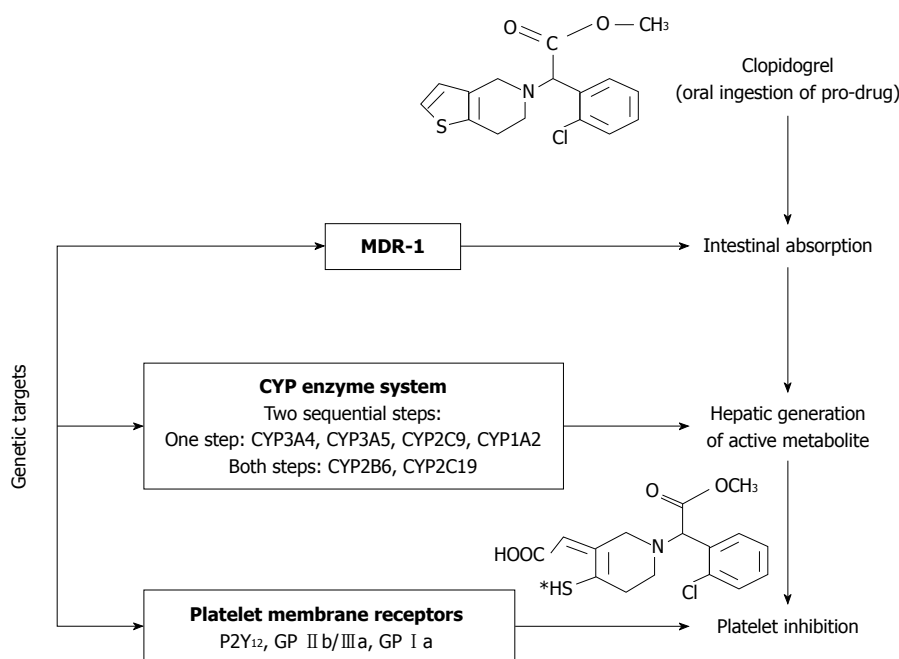


Figure 4 Possible genetical background of clopidogrel resistance^[82]. GP: Glycoprotein.

involved in the activation of clopidogrel. In a prospective, randomized, double-blind placebo-controlled study involving patients undergoing elective coronary artery stenting who received clopidogrel, co-administration of the proton pump inhibitor omeprazole was associated with decreased CYP450 2C19-dependent inhibition of platelet aggregation (i.e. a decreased platelet inhibitory effect of clopidogrel)^[75]. Juurlink *et al*^[76], using a population-based nested case-control study design, reported on their investigation of the potential association of a CYP450 2C19-dependent drug-drug interaction between clopidogrel and proton pump inhibitors and the risk of readmission to hospital because of myocardial infarction among patients 66 years or older who received clopidogrel therapy following hospital discharge after acute myocardial infarction. Patients who experienced reinfarction within 90 d after discharge were more likely than event-free patients in the control group to have received concomitant therapy with clopidogrel and proton pump inhibitors. The authors estimated that, compared with no treatment, CYP450 2C19-inhibiting proton pump inhibitors were collectively associated with a 40% relative increase in the risk of recurrent myocardial infarction. An exception was the proton pump inhibitor pantoprazole, which did not show the above associations^[76,77]. On the other hand, recent trials and meta-analysis could not confirm their findings^[77-79]. At this point, concomitant therapy with a CYP450 2C19-inhibiting proton pump inhibitor and clopidogrel should be administered when there is a sound clinical indication. For example, patients taking clopidogrel and warfarin therapy who require a proton pump inhibitor may need to avoid pantoprazole, since warfarin is metabolized primarily by CYP450 2C9. Alternatively, treatment strategies may be considered that use drugs not dependent on the CYP450

2C19 isoenzyme, such as pantoprazole and H2-receptor antagonists^[80].

Variation in the way patients respond to clopidogrel may in part reflect heterogeneity in GP IIb/IIIa, P2Y1, P2Y12, CYP2C9, CYP3A4 and CYP3A5 genotypes^[11,81,82] (Figure 4). The very recently conducted FAST-MI study (French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction study) and the TRITON-TIMI 38 study (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction 38) demonstrated a greater than 3-fold increase in the risk of adverse cardiovascular events among patients undergoing percutaneous coronary intervention who were homozygous or heterozygous for any of the *CYP2C19* alleles known to result in a nonfunctional protein (*CYP2C19**2, *3, *4 and *5), as compared with patients who had the wild-type *CYP2C19**1 allele^[83,84].

TREATMENT OF ANTIPLATELET RESISTANCE

Possible treatment of aspirin resistance

There are only few studies examining the possible treatment of aspirin resistance^[10,11]. Epidemiological studies suggest that Mediterranean diets are associated with a reduced risk of cardiovascular disease. It has been proposed that resveratrol is one of the most important dietary constituents involved in vasculoprotection. Stef *et al*^[85] in an *in vitro* study including 50 high-risk cardiac patients showed that resveratrol effectively inhibited collagen- and epinephrine-induced aggregation of platelets from aspirin resistant patients, which may contribute to its cardioprotective effects in this population.

In the last decade, numerous studies have revealed a central role for NAD(P)H oxidases in cardiovascular pathophysiology^[86]. Importantly, there is increasing evidence that NAD(P)H oxidase(s) play an important role in platelet aggregation^[85]. In another *in vitro* study Stef *et al*^[86] also showed that inhibition of NAD(P)H oxidase effectively suppressed collagen and epinephrine-induced aggregation of platelets from aspirin-resistant patients, which may represent a novel pharmacological target for cardioprotection in high-risk cardiac patients.

Aprotinin, a drug effective in limiting blood loss in patients undergoing surgery, was first approved in the United States in 1993 for use in high-risk patients needing coronary artery surgery. Aspirin is the only drug proven to reduce saphenous vein graft failure, but aspirin resistance (ASA-R) frequently occurs after off-pump coronary artery bypass grafting (OPCAB). Poston *et al*^[87] proposed that thrombin production during OPCAB stimulates this acquired ASA-R. They found that ASA-R is a common post-OPCAB event whose frequency may be reduced by intraoperative use of aprotinin, possibly *via* TF and thrombin suppression. Improved perioperative PLT function after OPCAB may also inadvertently enhance the clinical relevance of these potential antithrombotic effects.

A previous *in vitro* study showed the association between increased platelet response to ADP and aspirin resistance^[88]. Eikelboom *et al*^[89] raised the possibility that the clinical benefits of adding clopidogrel to aspirin may be greatest in patients whose platelets are least inhibited by aspirin. In another study, the addition of clopidogrel to aspirin provided greater inhibition of platelets and could overcome aspirin resistance^[90]. Pamukcu *et al*^[91,92] found an association between aspirin resistance and poor clinical outcome in AICS patients and also showed that the prevalence of major acute cardiac events in patients who were on clopidogrel treatment for 12 mo. Poor clinical outcomes were significantly lower compared to those who were on a clopidogrel treatment for the first 6 mo. In another study, aspirin resistance was also associated with worsening clinical outcomes, but the poor outcomes increased just after cessation of clopidogrel therapy. On the other hand, in their meta-analysis, Krasopoulos *et al*^[31] showed that concomitant therapy with clopidogrel or tirofiban (an inhibitor of platelet GP IIb/IIIa), or both, provided no benefit to those patients identified as aspirin resistant. Further studies are needed to clarify their findings.

Very recently, Tirnaksiz *et al*^[93] suggested a possible effect of atorvastatin therapy on aspirin resistance and it was confirmed by another study^[94].

Biondi-Zoccai *et al*^[39] undertook a systematic review to appraise the hazards inherent to aspirin withdrawal or non-compliance in subjects at risk for or with CAD. They concluded that non-compliance or withdrawal of aspirin treatment has ominous prognostic implication in subjects with or at moderate-to-high risk for CAD.

Possible treatment of clopidogrel resistance

The American College of Cardiology/American Heart

Association/Society for Cardiovascular Angiography and Interventions guidelines state that “in patients in whom stent thrombosis may be catastrophic or lethal”, platelet aggregation studies may be considered and the dose of clopidogrel increased to 150 mg/d if less than 50% inhibition of platelet aggregation is demonstrated.” This is a Class II b, level C recommendation, indicating that there is disagreement over whether the intervention is considered beneficial, and that the recommendation reflects only consensus opinion, not data from randomized clinical trials. Finally, the method to assess platelet inhibition is not described^[15,95]. We collected articles examining the possible association between laboratory and clinical clopidogrel resistance based on different platelet function assays (Tables 2-4)^[59,62,96-108].

Bonello *et al*^[109] concluded from a prospective, randomized, multicenter study that clopidogrel resistance was defined as a VASP index of more than 50% after a 600-mg loading dose. Patients with clopidogrel resistance undergoing coronary stenting were randomized to a control group or to the VASP-guided group, in which patients received additional bolus clopidogrel to decrease the VASP index below 50%. A total of 162 patients were included. The control ($n = 84$) and VASP-guided groups ($n = 78$) had similar demographic, clinical and biological characteristics. In the VASP-guided group, dose adjustment was efficient in 67 patients (86%) and VASP index was significantly decreased (from 69.3 ± 10 to 37.6 ± 13.8 , $P < 0.001$). Eight major adverse cardiac events (5%) were recorded during the 1-mo follow-up, with a significantly lower rate in the VASP-guided group compared with the control group (0% *vs* 10%, $P = 0.007$). There was no difference in the rate of major and minor bleeding (5% *vs* 4%, $P = 1$). This was the first study to suggest that adjusting the clopidogrel loading dose according to platelet monitoring using the VASP index is safe and may significantly improve the clinical outcome after PCI in patients with clopidogrel resistance despite a first 600 mg loading dose.

A total of 119 patients undergoing PCI were blindly randomized in a 2:1 fashion to receive clopidogrel loading 600 mg on the table immediately before PCI and 75 mg 2 times per day for 1 mo (high-dose group) *vs* standard dosing (300 mg loading and 75 mg/d; low-dose group)^[110]. Platelet aggregation was measured using light transmission aggregometry at baseline, 4 h and 30 d. The composite of cardiovascular death, myocardial infarction and target vessel revascularization was studied at 30 d in addition to major and minor bleeding. Baseline characteristics and baseline platelet aggregation were similar in the 2 groups. Percent inhibitions of platelet activity were 41% and 27% in the high-dose group *vs* 19% and 10% in the low-dose group at 4 h and 30 d ($P = 0.046$ and 0.047 , respectively). Composite clinical end points were 10.3% in the high-dose group and 23.8% in the low-dose group ($P = 0.04$). No difference was noted in major or minor bleeding. In conclusion, a higher loading and maintenance dose of clopidogrel in patients undergoing PCI resulted in superi-

Table 2 Clinical studies based on optical aggregometry

Study	Method	Patient population	Dosage	Adjunct antiplatelet therapy	No. of patients (clopidogrel sensitive/clopidogrel resistant)	Outcome measures	Result
Geisler <i>et al</i> ^[96]	Optical aggregometry	PCI	600 mg	No	363 (341/22)	Cardiovascular event within a 3-mo follow-up	Low responder had a significantly higher risk of major cardiovascular events (22.7 <i>vs</i> 5.6%, OR, 4.9, 95% CI: 1.66–14.96, <i>P</i> = 0.004)
Buonamici <i>et al</i> ^[97]	Optical aggregometry	PCI	Loading dose of clopidogrel followed by 75 mg daily	GP II b/ III a inhibitor, 325 mg aspirin	804 (699/105)	Stent thrombosis during a 6-mo follow-up	The predictors of stent thrombosis was: nonresponsiveness to clopidogrel (HR 3.08, 95% CI: 1.32–7.16, <i>P</i> = 0.009)
Müller <i>et al</i> ^[98]	Optical aggregometry	PCI	600 mg loading dose followed by 75 mg daily	100 mg aspirin	105 (90/15)		Their data showed that 5 patients who developed a stent thrombosis were non-responders
Wenaweser <i>et al</i> ^[99]	Optical aggregometry	PCI	300 mg loading dose followed by 75 mg daily	100 mg aspirin	82 (60/21)	Presence of stent thrombosis	Combined ASA and clopidogrel resistance was more prevalent in patients with stent thrombosis (52%) compared with controls (38%, <i>P</i> = NS) and volunteers (11%, <i>P</i> < 0.05)
Soffer <i>et al</i> ^[100]	Optical aggregometry	PCI	450 mg clopidogrel before the procedure	325 mg aspirin	72 (divided into two groups based on angina classification)	Angina class	In multivariate analysis, higher angina class was independently associated with lower inhibition of platelet aggregation (<i>P</i> = 0.018)
Buonamici <i>et al</i> ^[97]	Optical aggregometry	PCI	600 mg loading dose followed by 75 mg daily	GP II b/ III a inhibitor, 325 mg aspirin	804 (699/105)	Stent thrombosis	The incidence of stent thrombosis was 8.6% in nonresponders and 2.3% in responders (<i>P</i> < 0.001)

ASA: Acetylsalicylic acid; GP: Glycoprotein; NS: Not significant.

Table 3 Clinical studies based on optical aggregometry combined with another method

Study	Method	Patient population	Dosage	Adjunct antiplatelet therapy	No. of patients (clopidogrel sensitive/clopidogrel resistant)	Outcome measures	Result
Lev <i>et al</i> ^[62]	Optical aggregometry, RPFA	Elective PCI	300 mg clopidogrel followed by 75 mg daily	No	150 (114/36)	Markers of myonecrosis	Myonecrosis occurred more frequently in clopidogrel-resistant <i>vs</i> clopidogrel-sensitive patients (32.4% <i>vs</i> 17.3%, <i>P</i> = 0.06)
Bliden <i>et al</i> ^[101]	Optical aggregometry, TEG	PCI	Previously 75 mg daily, 300–600 mg loading dose followed by 75 mg daily	325 mg aspirin	100	Cardiovascular event/ revascularisation	Patients receiving chronic clopidogrel therapy who exhibit high on-treatment ADP-induced platelet aggregation are at increased risk for postprocedural ischemic events
Gurbel <i>et al</i> ^[102]	Optical aggregometry, TEG	PCI	300–600 mg loading dose followed by 75 mg daily	325 mg aspirin	192 (154 patients without and 38 patients with ischaemic events)	Cardiovascular outcome/ revascularisation	Posttreatment ADP-induced aggregation by LTA (63% ± 12% <i>vs</i> 56% ± 15%, <i>P</i> = 0.02) was significantly higher in patients with events (<i>n</i> = 38)
Matetzky <i>et al</i> ^[59]	Optical aggregometry, cone and platelet analyzer	PCI	300 mg clopidogrel followed by 75 mg daily	300 mg of aspirin followed by 200 mg/d	60 (patients were stratified into 4 quartiles)	Cardiovascular event	Whereas 40% of patients in the first quartile sustained a recurrent cardiovascular event, only 1 patient (6.7%) in the second quartile and none in the third and fourth quartiles suffered a cardiovascular event (<i>P</i> = 0.007)

ADP: Adenosine diphosphate.

or platelet inhibition and decreased cardiovascular events without increasing bleeding complications.

On the other hand, the use of a 150 mg maintenance dose of clopidogrel in patients with type 2 diabetes with

< 50% platelet inhibition was associated with enhanced antiplatelet effects, however, the antiplatelet effects achieved were nonuniform, and a considerable number of patients persisted with inadequate platelet inhibition^[111].

Table 4 Clinical studies based on optical aggregometry combined with activation-dependent changes on the platelet surface or with vasodilator-stimulated phosphoprotein phosphorylation

Study	Method	Patient population	Dosage	Adjunct antiplatelet therapy	No. of patients (clopidogrel sensitive/clopidogrel resistant)	Outcome measures	Result
Bonello <i>et al</i> ^[103]	VASP phosphorylation	PCI	300 mg loading dose followed by 75 mg daily	100 mg aspirin	144 patients were divided into quintiles according to PRI	Cardiovascular events	Patients in quintile 1 of VASP analysis had a significantly lower risk of MACE as compared with those among the four higher quintiles (0 <i>vs</i> 21, <i>P</i> < 0.01)
Barragan <i>et al</i> ^[104]	VASP phosphorylation	PCI	Ticlopidin or clopidogrel	250 mg aspirin	36 (20 healthy volunteers and 16 stented patients)	Presence of stent thrombosis	VASP phosphorylation analysis may be useful for the detection of coronary SAT
Serebruany <i>et al</i> ^[105]	Optical aggregometry, and whole blood flow cytometry	AICS or ischaemic stroke	75 mg	81-325 mg aspirin	359 (359/0)		Lack of nonresponse
Gurbel <i>et al</i> ^[106]	Optical aggregometry, GP II b/III a receptor, VASP phosphorylation	PCI	300-600 mg loading dose followed by 75 mg daily	No information	120 (20 patients with stent thrombosis and 120 patients without stent thrombosis)	Stent thrombosis	The SAT patients had significantly higher mean platelet reactivity than those without SAT by all measurements
Cuisset <i>et al</i> ^[107]	Optical aggregometry, P-selectin	NSTEMI followed by PCI	300-600 mg loading dose followed by 75 mg daily	160 mg aspirin	106 (94 patients without and 12 with cardiovascular event)	Cardiovascular event	Low responders to dual antiplatelet therapy had increased risk of recurrent CV events
Cuisset <i>et al</i> ^[108]	Optical aggregometry, P-selectin	NSTEMI followed by PCI	300-600 mg loading dose followed by 75 mg daily	160 mg aspirin	392 (146 patients with 300 mg loading dose clopidogrel and 300 patients with 600 mg loading dose of clopidogrel)	Cardiovascular event	The ADP-induced platelet aggregation and expression of P-selectin were significantly lower in patients receiving 600 mg than in those receiving 300 mg. During the 1-mo follow-up, 18 CV events (12%) occurred in the 300-mg group <i>vs</i> 7 (5%) in the 600-mg group (<i>P</i> = 0.02); this difference was not affected by adjustment for conventional CV risk factors (<i>P</i> = 0.035)

VASP: Vasodilator-stimulated phosphoprotein; GP: Glycoprotein; ADP: Adenosine diphosphate.

Ticlopidine could be an alternative agent in the treatment of clopidogrel resistance as previous studies have suggested^[112,113]. A recent case report presented three patients with acute stent thrombosis showing biological non-responsiveness to clopidogrel, despite overdosing to 150 mg/d and a sufficient duration of the treatment. Platelet P2Y₁₂ inhibition was finally obtained with a standard regimen of ticlopidine. The effects of possible poor compliance would appear limited because each patient was his/her own control and was under surveillance in hospital^[114]. This replacement should of course be subject to hematological monitoring in order to avoid any serious neutropenia.

Wolak *et al*^[115] studied 1519 consecutive patients who underwent 2020 stent implantations and were discharged on dual antiplatelet regimens of either aspirin and ticlopidine or aspirin and clopidogrel given for up to 4 wk. Thrombotic stent occlusion (TSO) was defined as ST elevation myocardial infarction in the stented artery territory associated with angiographic demonstration of complete stent occlusion. Mortality follow up was obtained for all patients by linkage to the Population Register. Follow up duration was 12 mo. TSO occurred in 37 stents at a me-

dian of 29 d post procedure. Of these cases, six occurred in the ticlopidine group (0.7%) and 31 in the clopidogrel group (2.8%, *P* < 0.01). The median time to TSO was 34 d and 28 d in ticlopidine and clopidogrel treated patients, respectively (*P* < 0.01). After controlling for multiple demographic, clinical and angiographic variables clopidogrel (*vs* ticlopidine) treatment remained the sole predictor of TSO (OR 5.4, 95% CI: 1.2-24.1, *P* = 0.028). Of even more concern, clopidogrel treatment was associated with an increased risk of 1 year mortality (OR 1.8, 95% CI: 1.2-2.8).

Newer drugs may overcome the limitations of current antiplatelet drugs. Prasugrel is a third-generation thienopyridine that is not as dependent as clopidogrel on biotransformation to an active metabolite. In preclinical studies, it was shown to have greater potency and achieve more rapid platelet inhibition than clopidogrel when given orally^[116]. The JUMBO-TIMI trial found prasugrel to have a comparable safety profile to clopidogrel^[117]. However, the recent TRITON TIMI-38 trial found that prasugrel reduced ischemic events in an ACS population undergoing PCI, at the cost of increased major bleeding. Those assigned to clopidogrel received a 300 mg loading dose immediately before or

during PCI, whereas 600 mg is now more commonly used clinically as it may be more effective. Although this raised the question of dose equivalence, platelet function analysis in PRINCIPLE-TIMI 44 has shown that the dose of prasugrel used in TRITON leads to greater platelet inhibition than clopidogrel at the higher loading and maintenance doses^[118]. Subgroup analysis of TRITON suggested prasugrel may have the greatest benefit over clopidogrel in the highest-risk patients, such as those with diabetes.

Based on very recent trials, among persons treated with clopidogrel, carriers of a reduced-function CYP2C19 allele had significantly lower levels of the active metabolite of clopidogrel, diminished platelet inhibition and a higher rate of major adverse cardiovascular events, including stent thrombosis, than did noncarriers^[83,84]. On the other hand, common functional CYP genetic variants do not affect active drug metabolite levels, inhibition of platelet aggregation, or clinical cardiovascular event rates in persons treated with prasugrel. These pharmacogenetic findings are in contrast to observations with clopidogrel, which may explain, in part, the different pharmacological and clinical responses to the two medications^[119].

Ticagrelor is an oral, reversible, direct-acting inhibitor of the ADP receptor P2Y₁₂ that has a more rapid onset and more pronounced platelet inhibition than clopidogrel^[120]. In patients who have an acute coronary syndrome with or without ST-segment elevation, treatment with ticagrelor as compared with clopidogrel significantly reduced the rate of death from vascular causes, myocardial infarction or stroke without an increase in the rate of overall major bleeding but with an increase in the rate of non-procedure-related bleeding^[121].

In a very recent trial, ticagrelor therapy overcame non-responsiveness to clopidogrel, and its antiplatelet effect is the same in responders and nonresponders. Nearly all clopidogrel nonresponders and responders treated with ticagrelor had platelet reactivity below the cut off points associated with ischemic risk^[122].

CONCLUSION

We previously reviewed the possible clinical importance of aspirin and clopidogrel resistance in some aspects^[10,11]. The current review is an updated article of the topic (containing the possible risk factors of this phenomenon) including the clinical consequences of clopidogrel resistance.

In its broadest sense, resistance refers to the continued occurrence of ischaemic events despite adequate antiplatelet therapy and compliance. The lack of a standard definition of resistance, as well as the lack of a standard diagnostic modality, has hampered the field in identifying and treating this clinical entity. Attempts have been made to develop a more meaningful definition with the goal of correlating laboratory tests with clinical outcomes, but there is no current definition that unifies the biochemical and clinical expression of failed treatment.

On the other hand, despite the presence of statistical heterogeneity among studies, likely reflecting methodolog-

ical differences, almost all included studies have suggested a positive association between the risk of cardiovascular events and laboratory antiplatelet nonresponsiveness.

The optimal treatment of resistance is also unclear. These results suggest that a new era of individualized antiplatelet therapy may arise with routine measurements of platelet activity in the same way that cholesterol, blood pressure and blood sugar are followed, thus improving care for millions of people.

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RAAS and adrenergic genes in heart failure: Function, predisposition and survival implications

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Abstract

It is well appreciated that several neurohormones and signaling cascades are activated that promote long-term deterioration of cardiac function and structure. Activation of the renin-angiotensin-aldosterone system (RAAS) and the adrenergic system is closely related to heart failure. Common gene variants that encode neurohormonal, adrenergic and intracellular proteins have been demonstrated to modulate the course and consequences of heart failure. However, the literature is replete with conflicting results and it remains uncertain as to whether particular gene variants predispose heart failure. Therefore, the main purpose of this review was to discuss the effects of single nucleotide polymorphisms (SNPs) that are located in genes encoding elements of the RAAS and the adrenergic system on the predisposition to and survival from heart failure. Most studies indicate that common SNPs encoding elements of the RAAS and the adrenergic system do not predispose individuals to heart failure. Conversely, it has been demonstrated that

ARB1 Arg389Gly, GRK5 Gln41Leu, ACE I/D, CYP11B2 C-344T and AGTR1 A+1166C modulate pharmacological responses and have a considerable impact on cardiac-related survival. It should not be expected, however, that a single polymorphism determines survival, given that multiple gene products and environmental factors contribute to the pathogenesis of heart failure. Therefore, future studies should consider the interaction effects of multiple genes in populations that are as homogeneous as possible with respect to environmental characteristics.

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Key words: Cardiac failure; Polymorphisms; Susceptibility; Mortality

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INTRODUCTION

In recent years, the incidence of heart failure has continued to increase along with sustained elevated rates of morbidity and mortality^[1]. It is now well appreciated that several neurohormonal and signaling cascades are activated that promote long-term deterioration of cardiac function and structure^[2] (Figure 1). These changes, collectively referred to as cardiac remodeling, are modulated by genetic factors^[3].

The renin-angiotensin-aldosterone system (RAAS) plays a pivotal role in the processes of heart failure. In response to sustained activation of the RAAS, the angio-

tensin- II receptors are deregulated in the human failing heart^[4]. Several downstream intracellular signaling effectors are overexpressed and activated in tandem with cardiac hypertrophy^[5]. The adrenergic system is also closely related to heart failure. Adrenergic receptors are deregulated in human heart failure in concert with impaired ventricular contraction and relaxation^[6].

It seems that the molecular portrayal of heart failure is incomplete as molecular and genetic studies continue to recognize genes and signaling cascades that participate in the development and progression of heart failure^[7]. In addition to the discovery of disease-causing (rare) mutations, common variants in genes that encode neurohormonal, adrenergic, intracellular and interstitial proteins have been demonstrated to modulate the course and consequences of heart failure. The literature, however, is replete with conflicting results and intense debate exists as to whether gene polymorphisms determine susceptibility to developing heart failure. Considering the prominent role of the RAAS and adrenergic receptors in the pathophysiology of heart failure, this review focuses on functional single nucleotide polymorphisms (SNPs) that appear to be related to the predisposition to and the survival from heart failure.

FUNCTIONAL PROPERTIES OF GENE POLYMORPHISMS

β -adrenergic receptors

The gene encoding the β_1 -adrenergic receptor is located on chromosome 10q24-26 and contains no introns. Several SNPs have been described in this gene, with two having been demonstrated to be functional and relevant for heart failure^[8,9]. One of these polymorphisms is derived from a transition between the amino acids arginine and glycine in residue 389 (*Arg389Gly*)^[10]. Given that this variant is located in the carboxyl terminal, it follows that variant alleles may have a distinct binding to G proteins and thus may have distinct signaling characteristics. The other variant is located at residue 49, wherein amino-acid serine is replaced by glycine (*Ser49Gly*)^[11]. The functional properties of these SNPs have been demonstrated in transgenic animals as well as studies *in vitro*, *ex vivo* and *in vivo*. For example, numerous *in vitro* studies have demonstrated that cells expressing the human variant *Arg389* have increased adenylyl cyclase activity in response to agonists compared to those expressing *Gly389*^[10,12]. Upon agonist activation, the former variant appears more prone to desensitization and may predispose to heart failure under certain conditions; e.g. increased catecholamine stimulation^[13]. These findings have been substantiated in numerous *ex vivo* and *in vivo* human and animal studies^[10,14-16] in which mice expressing *Arg389* have increased contractile responses to agonists and exhibited faster and greater desensitization upon activation^[17]. Nonetheless, most human studies have shown identical hemodynamic responses to exercise in both variants^[18,19]. While not in all studies, it has been demonstrated that

Gly49 has similar characteristics to *Arg389*, showing increased contractile responses and desensitization upon stimulation with agonists^[11,20]. Together, these data indicate that individuals who harbor *Arg389* and *Gly49* may have increased cardiac remodeling under adverse conditions.

The gene encoding the β_2 -adrenergic receptor is located on chromosome 5q31-32. Among numerous gene variants, two non-synonymous polymorphisms, namely *Arg16Gly* and *Gln27Glu*, have to some extent been related to cardiac functional changes^[8,9]. Although consistent evidence has shown that these SNPs have no influence on agonist-mediated contractile responses, *in vitro* studies have reported that *Gly16* and *Gln27* are more prone to desensitization than *Arg16* and *Gln27*^[21]. Thus, these variants might be relevant to heart failure. However, some *ex vivo* and *in vivo* human studies showed that *Gly16* is more resistant to agonist-mediated desensitization than *Arg16*^[22]. The contrasting results are not easily explained, but may stem from the predominant desensitization of *Gly16* from endogenous catecholamines^[9,23]. In contrast, another SNP (*Thr164Ile*) has been shown to modulate cardiac contractile responses *in vitro* and *in vivo*. Consistent evidence indicates that *Ile164* presents reduced basal and agonist-mediated intracellular effector activation, contractile response and heart rate as compared to *Thr164*^[24-26]. However, most humans possess two copies of threonine, casting doubts as to whether this variant is relevant to heart failure.

α -adrenergic receptors

The α_1 -adrenergic receptors encompass three subtypes, the genes for which are located in different chromosomes. Nonetheless, only α_{1A} and α_{1B} receptors seem to be translated and are functional in the human heart^[27]. Several gene variants have been reported, though many are uncommon or non-functional (for a review, see reference^[28]). A common variant of α_{1A} receptors, resulting from the substitution of arginine for cysteine, is located in the residue 347 (*Arg347Cys*) and has been expressed *in vitro*^[29]. There were no differences with respect to antagonist and agonist binding affinities, intracellular calcium concentrations or receptor desensitization upon stimulation with noradrenaline. In contrast, another gene polymorphism located in the intracellular loop seems functional; the *Gly247Arg* variant enhances G-protein binding, inositol phosphate production and cellular growth^[30]. Moreover, non-synonymous polymorphisms located in transmembrane domains have been reported to decrease ligand binding and receptor activation, including the *Arg166Lys* and *Val311Ile*^[30]. To our knowledge, however, these three polymorphisms have never been studied in patients with heart failure.

The α_2 adrenergic receptors also comprise three subtypes. The α_{2A} and α_{2C} are encoded by single genes and are both important in controlling noradrenaline release in pre-synaptic nerve terminals^[31]. Among the α_{2A} receptors, a particular gene polymorphism appears to be functional

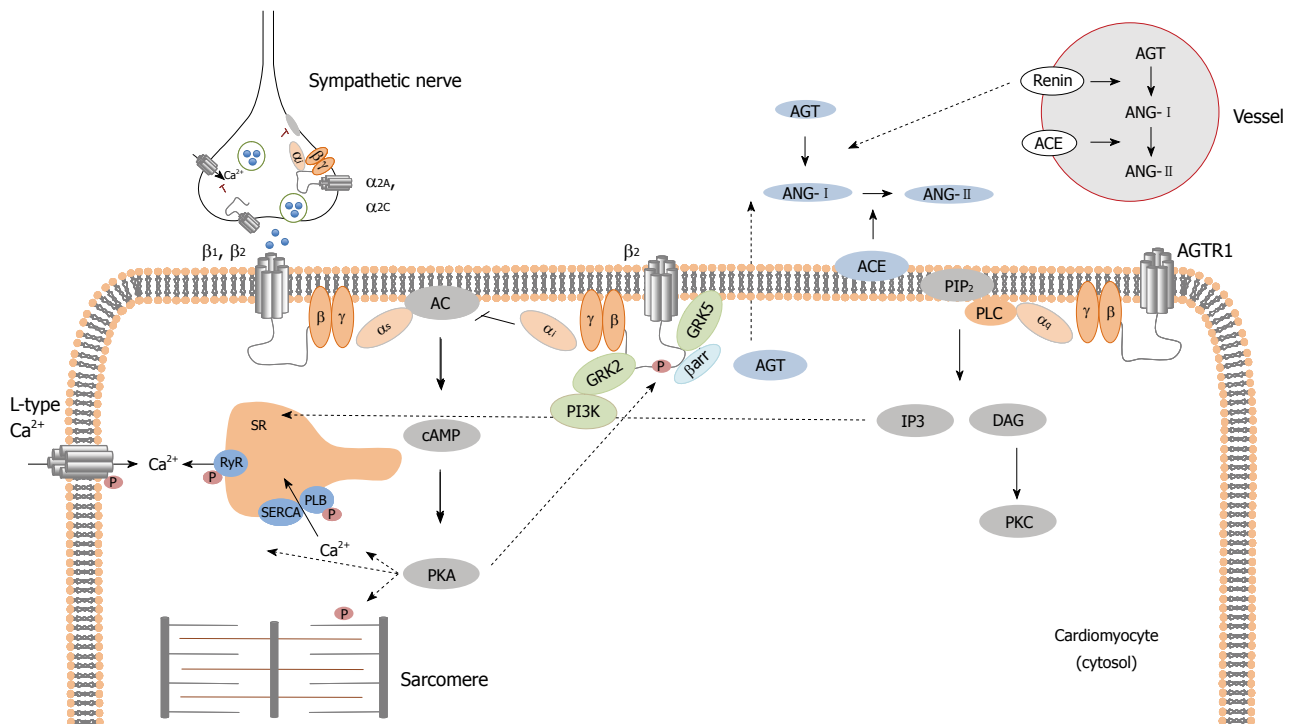


Figure 1 Adrenergic and renin-angiotensin-aldosterone system signaling pathways. AC: Adenylyl cyclase; ACE: Angiotensin-converting enzyme; AGT: Angiotensinogen; ANG: Angiotensin; cAMP: Cyclic Adenosine Monophosphate; β arr: β -arrestin; DAG: Diacylglycerol; GRK2: G-protein coupled receptor kinase 2; GRK5: G-protein coupled receptor kinase 5; IP3: Inositol trisphosphate; PI3K: Phosphoinositide 3-kinase; PIP2: Phosphatidylinositol 4,5-bisphosphate; PKA: Protein kinase A; PKC: Protein kinase C; PLC: Phospholipase C; SR: Sarcoplasmic reticulum.

and has been described in the context of heart failure. This polymorphism is located in a cytoplasmic domain and occurs in amino acid 251, where an asparagine or lysine is present (*Asn251Lys*)^[32]. Transfected cells with *Lys251* have shown enhanced coupling to Gi-proteins, effective inhibition of adenylyl cyclase and activation of mitogen-activated protein kinases^[32]. Thus, this variant could reduce noradrenaline release and confer protection under conditions of increased catecholamine stimulation, such as heart failure. In α_{2C} receptors, a polymorphism has been demonstrated to be functional and related to heart failure. The Del322-325 derives from a 12 nucleic acid deletion and generates a receptor that lacks four amino acids (glycine, alanine, glycine, proline) in the third intracellular loop^[33]. As a result, the generated receptors have reduced ligand-binding affinity, adrenaline-promoted coupling to Gi-proteins, inositol phosphate production and stimulation of mitogen-activated protein kinases^[33].

G-protein receptor kinases

Besides second-messenger protein kinases, the particular class of G-protein coupled receptors kinases (GRK) modulates agonist-promoted desensitization and internalization of G-protein coupled receptors^[34]. Among seven isoforms, GRK2 and GRK5 predominate in the myocardium and have been demonstrated to be important during heart failure^[34]. While GRK2 showed no non-synonymous polymorphisms, four non-synonymous variants have been found in GRK5^[35]. Of these, the substitution of leucine by glutamine in amino acid 41 (*Gln41Leu*) enhanced

isoproterenol-promoted desensitization and decreased signaling of β_1 -adrenergic receptors. Consistent with these observations, the *Leu41* allele has shown a protective effect against experimental cardiomyopathy induced by catecholamines. Nonetheless, this gene polymorphism is extremely uncommon in Caucasians^[35].

RAAS POLYMORPHISMS AND HEART FAILURE

Activation of the RAAS is one of the earlier and critical steps in heart failure. Although important to maintaining circulatory homeostasis, unremitting activation imposes a significant load on the heart and activates an immense and intricate signaling pathway^[36]. As a consequence, several elements of this system are deregulated in heart failure, including angiotensinogen, angiotensin-converting enzyme (ACE), angiotensin- II receptors and aldosterone^[2,4].

Angiotensinogen

The gene encoding angiotensinogen is located on chromosome 1q42-q43 and contains various gene polymorphisms^[37]. Two of these polymorphisms have been investigated particularly in heart failure^[38]. A polymorphism on exon 2 causes the substitution of methionine to threonine in amino acid 235 (*M235T*). This polymorphism is in close linkage disequilibrium with another polymorphism in the promoter region, the -6 G/A variant, and is associated with plasma angiotensinogen concentrations^[39].

Another polymorphism results from a threonine to methionine substitution in position 174. This polymorphism has been related to changes in cardiac function in patients with heart failure, although its functional implications remain unknown^[38].

ACE

The ACE converts angiotensin- I into angiotensin- II, which activates angiotensin- II receptors for modulating various cardiovascular responses, including vasoconstriction and cardiac growth^[36]. The gene that encodes ACE is located on chromosome 17q23. Despite the fact that more than 100 polymorphisms have been found in the ACE gene, a variant based on the presence (insertion) or absence (deletion) of 287 base pairs in intron 16 have been described and tested more than any other polymorphism^[37]. An initial study demonstrated that the *ACE I/D* polymorphism accounts for more than 40% of the total variance in serum ACE, with subjects harboring *D* alleles having increased concentrations^[40]. Nonetheless, its functional role *in vivo* is still under strong debate, as numerous studies have reported no association between *ACE I/D* genotypes and hypertension^[41].

Aldosterone

Another important element is aldosterone, whose synthesis is stimulated by angiotensin- II^[42]. Aldosterone is synthesized in the adrenal gland by aldosterone synthase, whose gene (*CYP11B2*) is located on chromosome 8q22^[43]. A common polymorphism at position -344 within the promoter region (*C-344T*) has been described to be functional *in vitro* and determines concentrations of aldosterone^[44,45]. In particular, the -344*C* allele has four times more binding affinity to the steroidogenic transcription factor 1 than the *T* allele and has been associated with increased production of aldosterone^[45,46].

Angiotensin-II receptors

Two distinct subtypes of angiotensin- II receptors mediate the predominant actions of the renin-angiotensin system^[47]. The gene encoding the type I receptor (*AGT1R*) is located on chromosomes 3q21-3q25. A particular gene polymorphism (*-4+1166C*) in the 3' untranslated region has been demonstrated to determine receptor expression and has been associated with hypertension. In particular, the presence of the +1166*C* allele seems to eliminate a particular microRNA (*mir-155*) binding site, preventing the receptors downregulation that occurs in the +1166*A* allele. The net result is increased receptor expression in +1166*C*^[48]. Aligned with these observations, the +1166*C* allele has been associated with hypertension^[49].

THE ROLE OF GENE POLYMORPHISMS ON THE PREDISPOSITION TO HEART FAILURE

To determine whether gene polymorphisms increase the chances of developing heart failure, several studies have

investigated whether the proportion of functional alleles differs among affected and unaffected populations. Although contrasting results are abundant, most studies have found no differences in allele frequencies among patients and the general population (Tables 1 and 2).

Adrenergic receptors and G-protein receptor kinases

Given their role in β_1 -adrenoceptors signaling and receptor desensitization, *Arg389* and *Gly49* alleles have been hypothesized to predispose heart failure. However, most studies to date have found no differences in allele frequencies between patients and healthy individuals^[50-58]. Likewise, numerous investigations have demonstrated a similar genotype within β_2 adrenergic receptors in heart failure patients and the general population^[51,59]. On the other hand, two studies reported that *Gly16* and *Glu27* were more frequent in end-stage heart failure patients^[52,60]. Given that these variants are more resistant to desensitization, patients could be more exposed to intracellular signaling and maladaptive cardiac hypertrophy. However, this association remains to be established. In addition, a foremost study advocated that genetic variation in α_{2C} adrenoreceptors might predispose people to heart failure^[54]. African Americans who are homozygous for the *De322-325* allele presented five times the odds for developing heart failure. The odds were augmented markedly among persons who were homozygous for both *De322-325* and *Arg389*. It makes reasonable sense that these variants predispose for heart failure by combining increased noradrenaline release with increased adrenergic signaling. However, more recent studies have not replicated these observations^[53,56]. Furthermore, the *GRK5* functional polymorphism also seems unrelated to heart failure predisposition. In a leading study, the proportion of *Glu41* alleles was similar among African- and European-American patients and controls^[35]. Together, these data show that most, if not all, polymorphisms play no role in the predisposition to heart failure. These observations have been substantiated in a recent large-scale genome-wide scan association study^[74]. This study included more than 23 000 individuals, among whom almost 3000 developed heart failure during a 13-year follow-up, and assessed almost 2.5 million markers. Only two markers exceeded the genome-wide threshold for significance. One SNP was found in individuals with European ancestry and was located near the ubiquitin-specific protease gene 3. The other one was detected in individuals of African ancestry and was located close to the leucine-rich repeats and immunoglobulin-like domains 3 gene. Fourteen additional loci were identified, one of which is located in the *GNA15* gene, which codes for a Gq-protein. The Gq-proteins are important mediators of α -adrenergic, endothelin and angiotensin-receptors signal transduction.

RAAS

The association between the *ACE I/D* polymorphism and heart failure was suggested in a leading study^[62]. In that study, the proportion of *ACE DD* genotype was

Table 1 Effect of adrenergic gene polymorphisms on the predisposition to patients with heart failure

Gene/SNP	Ref.	Cases/controls	Ethnic group	Association	Risk allele frequency
β adrenergic receptor type 1					
Arg389Gly	[50]	201/141	Mixed	No	0.74 vs 0.76
	[51]	256/230	Italian	No	0.69 vs 0.73
	[52]	189/378	Italian	Yes	0.74 vs 0.67
	[53]	91/119	Japanese	No	0.80 vs 0.81
	[54]	78/84	African-American	No	0.52 vs 0.56
	[54]	81/105	Caucasians	No	0.74 vs 0.76
	[55]	426/395	French	No	0.77 vs 0.75
	[56]	403/429	South Africans	No	0.70 vs 0.69
	[57]	260/230	Italian	No	0.69 vs 0.73
	[50]	201/141	Mixed	No	0.15 vs 0.15
Ser49Gly	[58]	184/77	Swedish	No	0.18 vs 0.13
	[52]	189/378	Italian	Yes	0.14 vs 0.08
	[53]	91/119	Japanese	No	0.16 vs 0.16
β adrenergic receptor type 2					
Gly16Arg	[51]	256/230	Italian	No	0.61 vs 0.60
	[52]	189/378	Italian	Yes	0.67 vs 0.59
	[59]	259/212	Mixed	No	0.60 vs 0.63
	[60]	520/328	Mixed	Yes	0.62 vs 0.59
Gln27Glu	[51]	256/230	Italian	No	0.32 vs 0.31
	[52]	189/378	Italian	No	0.38 vs 0.33
	[59]	259/212	Mixed	No	0.44 vs 0.42
	[60]	520/328	Mixed	No	0.42 vs 0.40
Thr164Ile	[61]	58/111	Canadians	No	0.41 vs 0.47
	[52]	189/378	Italian	No	0.02 vs 0.01
	[59]	259/212	Mixed	No	0.02 vs 0.01
	[60]	520/328	Mixed	No	0.01 vs 0.01
5'LC-Arg19Cys	[52]	189/378	Italian	No	0.36 vs 0.31
α adrenergic receptor type 2					
Del 322-325	[53]	91/119	Japanese	Yes	0.04 vs 0.11
	[54]	78/84	African-Americans	Yes	0.61 vs 0.41
	[54]	81/105	Caucasians	No	0.10 vs 0.04
	[56]	403/429	South Africans	No	1.00 vs 1.00
G-protein receptor kinase 5					
Gln41Leu	[35]	242/107	African-Americans	No	0.76 vs 0.77
	[35]	568/406	European-Americans	No	0.98 vs 0.99

SNP: Single nucleotide polymorphism.

more than 50% higher in end-stage heart failure patients than in healthy controls. However, these observations have been called into question by numerous subsequent studies^[63-69,75]. Likewise, the angiotensinogen *M235* variant has not been associated with a predisposition to heart failure in most studies^[61,66,70-72]. Similar results were reported for *T174* and *-6G/A* variants^[70-72]. One single study reported an association for these variants, although its small sample size precludes solid conclusions^[61]. The aldosterone synthase polymorphism (*-344C*) is not more frequent in patients than controls^[66,61,70], and neither are the *AGT1R* (*+1116C*) and *AGT2R* (*G1675*) polymorphisms^[61,70,73]. Collectively, these data indicate that gene polymorphisms encoding elements of the RAAS also do not indicate predisposition for heart failure, at least in Caucasians.

THE ROLE OF GENE POLYMORPHISMS IN SURVIVAL FROM HEART FAILURE

Although most studies have shown that gene polymorphisms do not increase the risk for heart failure, functional gene polymorphisms might determine survival

once heart failure develops. Stimulation of neurohormonal and interstitial proteins increases once the disease onsets. Hence, instead of predisposing to the disease, genetic variants might compromise heart function and survival by increasing function and expression of adverse proteins and/or by suppressing the favorable ones. However, even here the results are somewhat diverse and conflicting (Tables 3 and 4).

Adrenergic receptors and G-protein receptor kinases

Several lines of evidence have led to the belief that genes encoding β-receptors have no effect on clinical endpoints, including survival, hospitalization or heart transplantation^[76-78]. Nonetheless, many clinical trials have not accounted for mortality for cardiac reasons and/or have not controlled for potential pharmacological confounding effects. For example, Liggett *et al.*^[15] demonstrated that patients who are homozygous for *Arg389* and treated with bucindolol survive longer than patients treated with a placebo. In contrast, patients with *Gly389* have not benefited from treatment with bucindolol. Biolo *et al.*^[50] also demonstrated that treatment dosage modulates survival in

Table 2 Effect of renin-angiotensin-aldosterone system gene polymorphisms on the predisposition to patients with heart failure

Gene/SNP	Ref.	Cases/controls	Ethnic group	Association	Risk allele frequency
Angiotensin-converting enzyme I/D	[62]	214/79	Caucasian	Yes	0.58 vs 0.56
	[63]	193/77	Swedish	No	0.57 vs 0.56
	[64]	229/230	Italian	No	0.58 vs 0.60
	[65]	99/364	Caucasian	No	0.57 vs 0.54
	[66]	157/225	South-Africans	No	0.64 vs 0.69
	[67]	90/287	Czech	No	0.54 vs 0.57
	[68]	79/102	Chinese Han	No	0.43 vs 0.40
	[69]	104/183	Chinese	No	0.36 vs 0.37
	[70]	433/401	French	No	0.54 vs 0.57
Angiotensinogen M235T	[71]	88/122	Japanese	No	0.39 vs 0.36
	[61]	58/111	Canadians	No	0.64 vs 0.62
	[66]	157/225	South-Africans	No	0.83 vs 0.87
	[72]	158/200	Czech	No	0.51 vs 0.43
	[72]	40/63 (women)	Czech	Yes	0.56 vs 0.39
	[70]	433/401	French	No	0.40 vs 0.43
	[71]	88/122	Japanese	No	0.80 vs 0.80
	[61]	58/111	Canadians	Yes	0.48 vs 0.31
	[70]	433/401	French	No	0.12 vs 0.14
T174M	[71]	88/122	Japanese	No	0.05 vs 0.10
	[61]	58/111	Canadians	Yes	0.18 vs 0.10
	[72]	158/200	Czech	No	0.59 vs 0.58
G(-6)A	[70]	433/401	French	No	0.28 vs 0.28
	[61]	58/111	Canadians	No	0.31 vs 0.33
	[73]	193/77	Swedish	No	0.29 vs 0.30
Angiotensin-II type 1 receptor A1166C	[70]	433/401	French	No	0.16 vs 0.19
	[70]	433/401	French	No	0.16 vs 0.19
A-153G	[70]	433/401	French	No	0.16 vs 0.19
	[70]	433/401	French	No	0.16 vs 0.19
	[70]	433/401	French	No	0.16 vs 0.19
Aldosterone T-344C	[66]	157/225	South-Africans	No	0.21 vs 0.18
	[70]	433/401	French	No	0.43 vs 0.46
	[61]	58/111	Canadians	No	0.39 vs 0.45

SNP: Single nucleotide polymorphism; T174M: Threonine to methionine substitution in position 174.

patients harboring *Arg389*. Once again, all patients carrying *Gly389* survived irrespective of treatment conditions. These data indicate that medication attenuates the negative impact that variant *Arg389* has on survival. In contrast, mounting evidence shows that *Gly49* has no impact on survival or heart transplantation endpoints, irrespective of medication^[77,79]. Moreover, most studies to date have reported that *Gly16* and *Gln27* are not associated with survival related to heart failure^[59,77,79]. Nonetheless, it may be that, rather than single polymorphisms, the haplotype may determine the chances of survival. Indeed, one investigation reported that patients carrying two copies of *Gly16* and *Gln27* have an increased risk of adverse events^[78].

A number of studies have reported that individuals carrying the *Thr164Ile* variant have a worse prognosis than those carrying the *Thr164* homozygous variant^[59,80]. Even though *Thr164Thr* patients have improved survival rates upon treatment with β -blockers, individuals who carry the *Thr164Ile* variant seem to present the opposite response^[81].

Similarly, *GRK5* gene variants are associated with different responses to medication and impact on survival. Liggett *et al.*^[35] showed that survival times were longer in *Gln41* patients under treatment with β -blockers than medication naïve patients. Nonetheless, survival

times were similar to *Leu41* carriers who were not under treatment. These findings prompted the authors to conclude that the *Leu41* variant provides similar effects as β -blockers (genetic β -blockade). A large prospective study supported these outcomes^[88]. Among untreated African Americans, *Leu41* carriers had longer survival times than those who were *Gln41* homozygous. On the contrary, there were no differences in survival times among African Americans treated with β -blockers, indicating that medication attenuated the negative impact of the *Gln41* variant. Even though *GRK5* variants seem to be associated with pharmacological responses and survival rates in African-Americans, they are uncommon in Caucasians. However, since recent evidence points out that Caucasians who are homozygous for *Gln41* and *Gly389* have shown prolonged survival with treatment, as is the case with African Americans, future studies should consider gene-gene interaction effects.

Contrasting results have been reported for α_2c adrenoceptor polymorphisms. The variant *Del322-325* was surprisingly associated with a decreased event rate and reduced death rate in patients with dilated cardiomyopathy^[82]. However, more than 90% of the patients were Caucasians, and similar to previous studies, had no individuals who were homozygous for the deletion variant.

Table 3 Effect of adrenergic gene polymorphisms on survival of patients with heart failure

Ref.	Sample, design ¹	Endpoints	Follow-up (mo)	Mortality rate (%)	SNP	Main findings
β adrenergic receptor type 1						
[15]	1040 ²	AD, HZ	48	19	Arg389Gly	Increased survival in Arg389 homozygous treated with bucindolol
[58]	184 ³	AD, HT	24-60	38	Ser49Gly	Decreased survival in Ser49Ser patients
[76]	600 ²	AD, HZ	7-17	26	Arg389Gly	No association with endpoints
[50]	201 ³	AD, CD	18-62	28	Arg389Gly, Ser49Gly	Increased survival in Arg389 allele carriers on high dose beta-blockers
[77]	444 ³	CD, HT	41 (median)	25	Arg389Gly, Ser49Gly	No association with endpoints
[78]	227 ³	AD, HT	48	18	Arg389Gly, Ser49Gly	No association with endpoints
[79]	637 ³	AD, HT	35 (mean)	23	Arg389Gly, Ser49Gly	No association with endpoints
β adrenergic receptor type 2						
[59]	259 ³	AD, HT	22 (mean)		Gly16Arg, Gln27Glu, Thr164Ile	Increased risk of death in Thr164Ile patients
[77]	444 ³	CD, HT	41 (median)	25	Gly16Arg, Thr164Ile	No association with endpoints
[78]	227 ³	AD, HT	48	18	Gly16Arg, Gln27Glu	Increased risk of death (haplotype)
[80]	31 ³	AD, HT	24	3	Thr164Ile	Worsening HF in Thr164Ile patients
[81]	443 ³	AD	36 (median)		Thr164Ile	Improved survival in Thr164Thr patients treated with beta-blockers
α adrenergic receptor type 2						
[78]	227 ³	AD, HT	48	18	Del322-325	No association with endpoints
[79]	637 ³	AD, HT	35 (median)	23	Del322-325	No association with endpoints
[82]	345 ³	AD, HT	60 (mean)	18	Del322-325	Reduced risk of death and end-points
G-protein kinase receptor 5						
[35]	375 ²	AD, HT	30 (mean)		Gln41Leu	Increased survival in Leu41 African-American patients treated with beta-blockers. No impact on Caucasians

¹Study design; ²Placebo controlled randomized trial; ³Non-randomized, single group assignment. SNP: Single nucleotide polymorphism; AD: All cause mortality; CD: Cardiac mortality; HT: Heart transplantation; HZ: Hospitalizations.

Table 4 Effect of renin-angiotensin-aldosterone system gene polymorphisms on survival of patients with heart failure

Ref.	Sample, design ¹	Endpoints	Follow-up (mo)	Mortality rate (%)	SNP	Main findings
Angiotensinogen						
[75]	82 ²	AD, HZ	12	24	M235T	No association with endpoints
[78]	227 ³	AD, HT	48	18	M235T	No association with endpoints
[83]	451 ³	AD	48	49.7	M235T, T174M	Increased mortality in 174M patients
Angiotensin-converting enzyme						
[75]	82 ²	AD, HZ	12	24	I/D	No association with endpoints
[73]	194 ³	AD, HT	60	42	I/D	Increased risk of death in DD patients
[78]	227 ³	AD, HT	48	18	I/D	No association with endpoints
[84]	328 ³	AD, HT	3-38	23	I/D	Decreased survival in D allele patients untreated with beta-blockers. No differences in treated patients
[85]	479 ³	AD, HT	3-62	28.6	I/D	Decreased survival in D allele patients untreated with β-blockers. No differences in treated patients and decreased impact with high dose ACE inhibitors
[86]	323 ³	AD, HZ	10 (median)	9.6	I/D	Associated with severity of disease (NYHA class)
Angiotensin- II receptor type 1						
[73]	194 ³	AD, HT	60	42	A1166C	Not associated with end-points. Increased risk of mortality as haplotype (ACE DD)
[75]	82 ²	AD, HZ	12	24	A1166C	No correlation with mortality rate
[78]	227 ³	AD, HT	48	18	A1166C	No association with endpoints
Aldosterone						
[87]	354 ²	AD, HZ	12	3.4	-344 T/C	Decreased survival in C allele patients. Isosorbide dinitrate and hydralazine improved composite score in TT genotype but had no impact on C allele

¹Study design; ²Placebo controlled randomized trial; ³Non-randomized, single group assignment. SNP: Single nucleotide polymorphism; AD: All cause mortality; CD: Cardiac mortality; HT: Heart transplantation; HZ: Hospitalizations; ACE: Angiotensin-converting enzyme.

More recent studies disputed these observations, among which there was no association between this variant and

survival related to heart failure^[75,78,79]. It is not reasonable to expect that a single polymorphism will exert a marked

influence on survival, given that multiple gene products and environmental factors contribute to the pathogenesis of the disease. Therefore, future studies should consider the combined effect of several genes involved in the progression of heart failure in populations that are as homogeneous as possible in regard to their environmental characteristics.

RAAS

Even though the RAAS genes are not associated with a predisposition for heart disease, numerous genes encoding elements of the RAAS have been associated with varied responses to pharmacological treatment and survival. One such example concerns the *ACE I/D* polymorphism. An initial investigation reported that survival was lower in patients harboring the *DD* genotype^[63] and numerous subsequent studies have supported these observations. McNamara *et al.*^[84,85] demonstrated that survival was lower in untreated *DD* patients, *albeit* similar among those who received β -blockers and ACE inhibitors. Hence, in tandem with other polymorphisms, the adverse effects induced by the *ACE DD* genotype seem to be attenuated by standard heart failure medication. However, other studies have not replicated these findings^[73,78,86]. The same group showed that the aldosterone -344C polymorphism has a scaled impact on survival, being considerably poorer in *CC* genotype patients^[87]. Unlike other polymorphisms, *TT* patients, rather than *CC*, were the ones who benefited the most with isosorbide dinitrate and hydralazine. Therefore, individuals with the *CC* genotype might be at particular risk of death once they develop heart failure. Once more, allele frequencies were different between African-Americans and Caucasians.

The angiotensin- II receptor polymorphisms also are expected to have a negative impact upon survival following heart failure, because their sustained activation has serious cellular and cardiovascular consequences. Nonetheless, the results are contradictory. Evidence exists that 1166C is associated with a more severe disease condition and increased risk of death when combined with *ACE DD*^[73]. However, in a recent study, survival and heart transplantation endpoints were not associated with either 1166C or 1166A^[78]. In addition, studies are unanimous in demonstrating that the angiotensinogen M235T polymorphism does not influence survival from heart failure^[75,78,83]. On the other hand, the M174 allele was associated with increased mortality^[83]. Overall, angiotensinogen polymorphisms seem to have a slight effect on survival, however, thus far, studies have included somewhat small samples and are of insufficient number for drawing definitive conclusions. In addition, some of these studies have not taken into consideration the potential attenuating effects that medication has on certain gene variants.

CONCLUSION

Although gene products related to the RAAS and the adrenergic system are strongly implicated in the pathogenesis

of heart failure, functional genetic variations that enhance or suppress their function and/or expression do not seem to predispose the development of heart failure. These observations are not entirely unexpected as the production of these end products markedly increases once heart failure onsets. In contrast, when the disease develops, genetic variants that adversely modify the function/expression of proteins are expected to lead to a worse outcome and possibly to poorer prognosis. Indeed, numerous studies have demonstrated that some SNPs not only modify responses to medication but also have implications for survival related to heart failure. This has been the case for ARB1 *Arg389Gly*, GRK5 *Gln41Leu*, ACE *I/D*, CYP11B2 *C-344T* and AGTR1 *A+1166C*.

However, conflicting results abound in the literature, wherein positive associations reported in initial studies often were not supported by subsequent investigations. Several reasons may explain these discrepant results, including limitations in design, technical procedures (mistyping) and analysis (unconditional). Numerous studies have included small sample sizes, which may compromise the power to detect common small effects in genetic association studies of multifactorial traits, such as heart failure^[38]. In addition, numerous studies have not matched or grouped sample cohorts according to the severity of heart failure. With respect to survival, many studies were retrospective and have not controlled for potential confounding pharmacological effects. As outlined previously, standard heart failure medications (e.g. β -blockers and ACE inhibitors) appear to offset the adverse impact that some gene variants exert on survival. Furthermore, several studies involved various SNPs and multiple testing may generate false-positive results^[8]. On the other hand, the impact of one SNP on survival is expected to be small and can be counteracted by the presence of other SNPs, at least in theory. Given that multiple gene products and environmental factors contribute to heart failure, future studies should consider studying haplotypes and the interacting effects of multiple genes implicated in the pathogenesis of heart failure. Future studies should be carried out in populations that are as homogeneous as possible regarding etiology, gender, race and environmental characteristics.

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Myocardial perfusion imaging and infarct characterization using multidetector cardiac computed tomography

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INTRODUCTION

During the past few years, computed tomography coronary angiography (CTCA) has rapidly emerged as an alternative to invasive coronary angiography, particularly in patients at intermediate risk of coronary artery disease (CAD). Multicenter studies have confirmed the high predictive accuracy of CTCA, and further demonstrated comparable results to conventional angiography regarding the prediction of revascularization^[1,2]. The high predictive accuracy of CTCA, and particularly its negative predictive value, has lead to the ongoing incorporation of the technique in the diagnostic work-up of patients with suspected CAD.

However, recent data suggesting that revascularization does not improve the prognosis of patients with intermediate coronary artery stenosis if the lesion does not impair flow during stress, renders mere anatomical assessment of coronary stenosis without myocardial perfusion imaging (MPI) a very useful, albeit insufficient approach for clinical decision making^[3,4]. In this regard, MPI has shown to be a useful and accurate tool in the diagnosis and prognosis of patients with CAD^[5]. Until recently, CTCA was restricted to the anatomical assessment of coronary stenosis, whereas the functional significance of coronary lesions remained outside of its scope. Nevertheless, the kinetics of iodinated contrast is similar to gadolinium-diethylenetriamine pentaacetic acid used in contrast-enhanced magnetic resonance

Abstract

Until recently, computed tomography coronary angiography was restricted to the anatomical assessment of coronary stenosis, whereas the functional significance of coronary lesions remained outside of its scope. Nevertheless, the kinetics of iodinated contrast is similar to gadolinium-diethylenetriamine pentaacetic acid used in contrast-enhanced magnetic resonance imaging, allowing assessment of myocardial perfusion and viability by cardiac computed tomography.

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Key words: Computed tomography coronary angiography; Coronary artery disease; Ischemia; Non-invasive imaging; Myocardial viability

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imaging (MRI), allowing assessment of myocardial perfusion and viability by cardiac CT^[6-14]. Accordingly, a number of studies confronted this limitation by demonstrating that myocardial perfusion during first-pass contrast-enhanced adenosine stress CTCA is feasible and related to microsphere-derived myocardial blood flow^[15,16].

In parallel, owing to a volumetric acquisition and to ECG-triggering, CTCA allows submillimetric reconstructions from every possible angle at different time points of the cardiac cycle, consequently creating an optimal scenario for morphological and functional assessment, and enabling a wide array of non-coronary applications.

ASSESSMENT OF MYOCARDIAL PERFUSION WITH CT

It has been a long time since MPI was established as a reference standard for risk stratification and decision making in patients with CAD.

During the past 30 years, the field of MPI has been lead by single photon emission computed tomography (SPECT) and positron emission tomography (PET), supported by robust evidence establishing that prognosis is highly related to the presence of inducible ischemia^[5]. Nevertheless, these methods portray a number of inherent limitations, namely attenuation artifacts, expenses, ionizing radiation, and restricted availability of PET.

More recently, myocardial perfusion assessment using MRI has been established as an alternative to evaluate myocardial perfusion by means of an ionizing radiation free technique with superior spatial resolution, which allows discrimination between subendocardial and transmural infarction^[17,18].

The rapidly evolving field of CTCA has lead to the exploration of non-coronary applications of CTCA^[19]. The anatomical nature of CT has been one of the most debated limitations of CTCA for the purpose of evaluating patients with a high probability of CAD, thus significant efforts have been undertaken to attempt a simultaneous assessment of myocardial perfusion. Myocardial perfusion at rest has already been investigated in the past using electron beam CT^[20]. The physiopathological substrate of this concept is similar to MRI with gadolinium, since both contrast media have similar kinetics. Accordingly, a conceptual congruence exists between MRI and CTCA regarding the assessment of myocardial perfusion and viability. In brief, during the first pass of iodinated contrast through the left ventricle, areas with diminished perfusion have a reduced delivery of contrast to the myocardium resulting in a characteristic hypoattenuation (Figure 1)^[15]. The evaluation of myocardial perfusion defects at rest over the simultaneous assessment of coronary artery stenosis appears to be an interesting strategy for the assessment of patients with acute chest pain. It is noteworthy that myocardial perfusion at rest can be evaluated during conventional CTCA acquisitions, with no additional contrast administration or radiation dose required. Myocardial

perfusion defects in first pass contrast-enhanced MDCT correlate closely with the presence of ischemic myocardium and myocardial necrosis, as determined by increases in troponin levels^[15,22].

Since the assessment of myocardial perfusion by CTCA is based on the myocardial signal density, it is pivotal to determine the normal values of myocardial signal density and to identify potential mechanisms of misinterpretation of perfusion defects. A recent study that included consecutive asymptomatic patients without history of CAD and low pre-test likelihood, reported a mean myocardial signal density at the basal, mid and apical myocardium of 97.4 ± 17.3 Hounsfield units (HU), with significant differences between inferobasal and all American Heart Association segments. Indeed, the inferobasal segments commonly showed a considerable myocardial signal density drop mimicking perfusion defects, which appears to be attributed to beam hardening effect artifacts from the spine^[23].

ASSESSMENT OF MYOCARDIAL VIABILITY BY CARDIAC CT

The ability to discriminate between dysfunctional but viable myocardium and necrotic myocardium has important clinical implications, regarding both clinical outcome and election of optimal therapeutical strategy. Indeed, a large meta-analysis demonstrated an 80% reduction in mortality in patients with CAD and left ventricular dysfunction with viable myocardium treated with revascularization, and no benefit for revascularization in patients without viability^[24].

In the setting of acute myocardial infarction, the presence of microvascular obstruction and reduced capillary density lead to a delayed arrival of contrast to the infarct core during the first pass of iodinated contrast through the left ventricle. Likewise, those necrotic regions display a delayed enhancement of contrast, attributed mainly to an increment in the volume of distribution and to a delayed contrast wash-out (Figure 2)^[6-14]. Both patterns are highly reproducible and have been extensively validated in animal and clinical studies, with good concordance with SPECT and MRI, although the contrast-to-noise ratio is significantly higher with MRI^[6-14].

An emergent clinical application of delayed enhancement using cardiac CT is for early assessment of myocardial viability in the setting of ST-segment elevation acute myocardial infarction (STEMI). A recent study that explored this concept in STEMI patients undergoing non-contrast cardiac CT immediately after primary percutaneous coronary intervention, demonstrated that although door-to-balloon target times were accomplished and optimal epicardial results (TIMI 3) were obtained, myocardial delayed enhancement (Figure 2) was detected in half of the patients. Similarly, even though 6-mo clinical outcome did not differ significantly, the presence of myocardial delayed enhancement was related to poor microvascular flow, greater enzyme elevation, worse left

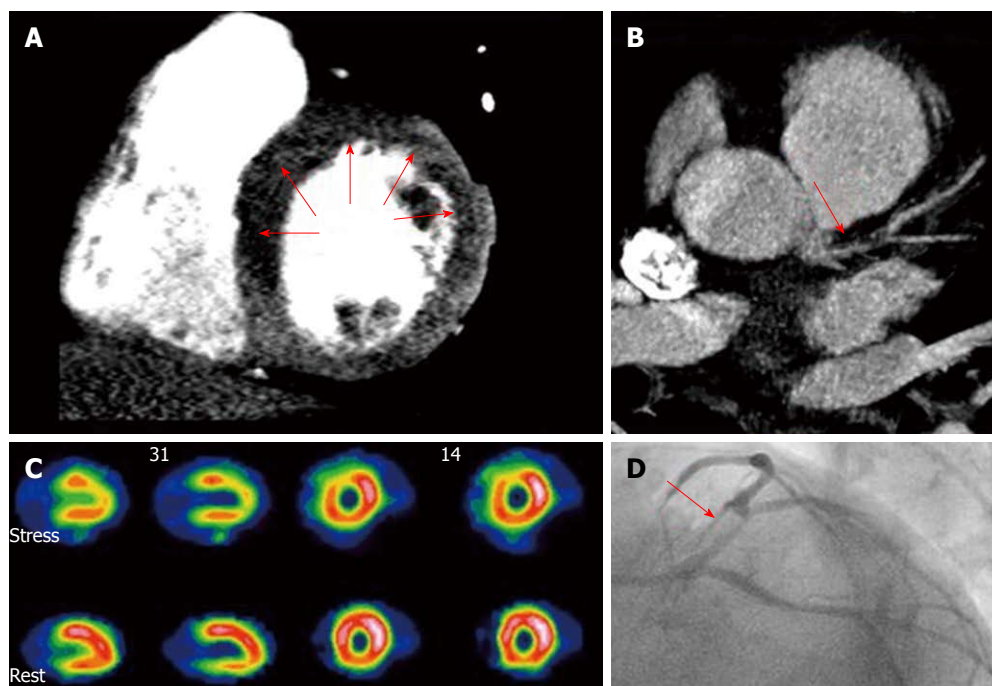


Figure 1 Combined assessment of myocardial perfusion at pharmacological (adenosine) stress and coronary angiography within a single-session cardiac computed tomography. Cardiac computed tomography (CT) (panels A and B) showed diminished perfusion of the left ventricle anterior and septal wall (A, arrows) and a significant lesion at the proximal left anterior descending artery (B, arrow), findings confirmed by single photon emission computed tomography (SPECT) (C) and invasive coronary angiography (D, arrow). A: CT perfusion (stress); B: Coronary CT angiography; C: SPECT myocardial perfusion imaging; D: Invasive angiography. With permission of Blankstein *et al*^[21], *J Am Coll Cardiol* 2009; 54: 1072-1084.

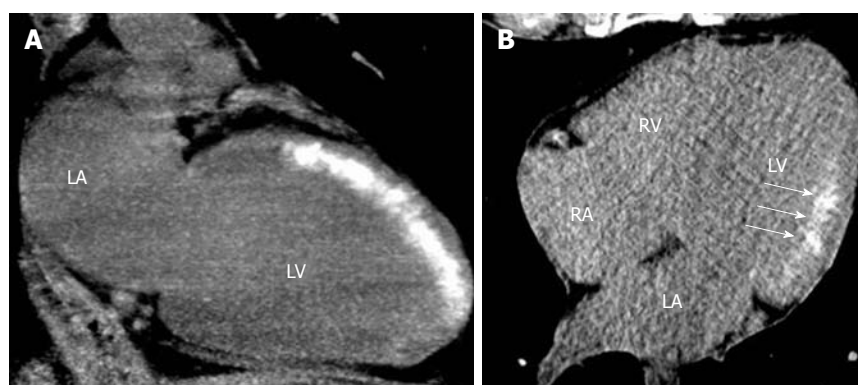


Figure 2 Early assessment of myocardial viability immediately after primary percutaneous coronary intervention in patients with anterior (A) and inferolateral (B) ST-segment elevation acute myocardial infarction. Delayed enhancement of iodinated contrast administered during percutaneous coronary intervention is observed using non-contrast enhanced cardiac computed tomography, without heart rate control and using a low dose-saving protocol. Discrimination between transmural (A) and subendocardial (B, arrows) extent of the irreversible myocardial damage (delayed enhancement) can be achieved using this technique. LA: Left atrium; LV: Left ventricle; RA: Right atrium; RV: Right ventricle.

ventricular function, and a higher incidence of complications during hospitalization^[14]. It should be noted that this application of the technique uses the contrast administered during the invasive procedure, thereby precluding the need for contrast administration during the CT scan. In addition, since coronary assessment is not required and contrast enhancement is usually readily evident if present, β -blockers are not required and radiation dose can be significantly reduced up to 5.5 mSv using retrospective gating acquisitions. Indeed, Chang *et al*^[25] recently demonstrated good agreement between retrospective and

prospective ECG-gated delayed enhancement CT regarding infarct size estimation, enabling a further significant decrease in radiation dose (930.1 ± 62.2 mGyXcm *vs* 42.4 ± 2.3 mGyXcm, $P < 0.001$).

Finally, it should be stressed that delayed enhancement studies also allow evaluation of the presence of microvascular obstruction, an independent predictor of events after acute myocardial infarction, as hypoattenuated regions within an area of delayed enhancement^[13,26].

The transmural extension of the delayed enhancement is significantly related to the likelihood of im-

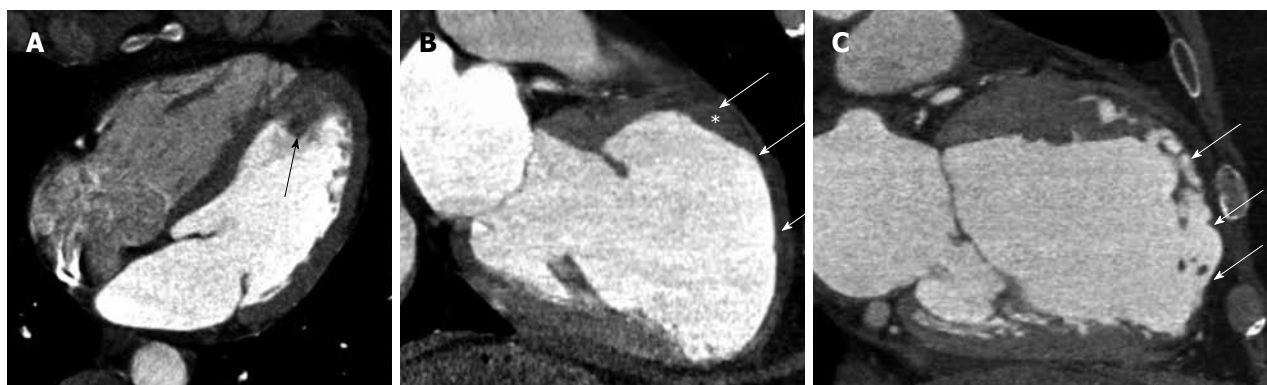


Figure 3 Acute and chronic sequelae of myocardial infarction. A: A four-chamber view of a patient with acute chest pain and an occluded mid left descending coronary artery. A fresh, mobile, pedunculated thrombus is observed at the left ventricular apex (arrow); B: A two chamber view of a patient with recent onset heart failure and history of previous myocardial infarction. A left ventricle aneurysm is detected at the anterior wall, with significant wall thinning, pericardial effusion (arrows) and a fixed thrombus (*); C: An anterior wall chronic myocardial infarction, with significant wall thinning and lipomatous metaplasia (arrows).

provement in regional function after revascularization^[27]. In parallel to cardiac MRI, the spatial resolution of CT allows the ability to discriminate between transmural and subendocardial infarcts using delayed enhancement CT (Figure 2)^[14].

ASSESSMENT OF CHRONIC MYOCARDIAL INFARCTION SEQUELAE BY CARDIAC CT

Cardiac CT also allows an accurate evaluation of characteristics of chronic myocardial infarction and its sequelae (Figure 3). It is worth mentioning there is a higher prevalence of apical thrombus detected with CT compared to echocardiography^[28], with the difference possibly attributed to a higher spatial resolution and to the ability to evaluate the entire cardiac volume without “window” restrictions. Indeed, Carlsson *et al*^[29] demonstrated a similar diagnostic accuracy of CT and MR in detecting heterogeneous microinfarcts in a swine model.

On the other hand, chronic myocardial infarction characterization using CTCA has revisited the concept of lipomatous metaplasia. Despite the fact that adipose tissue can be easily detected using histopathology, the presence of myocardial fat replacing scar tissue remained unreported until 1997^[30]. Recently, Su *et al*^[31] demonstrated the presence of adipose tissue in 84% of evaluated chronic myocardial infarctions. Furthermore, using specific sequences to detect fat, a magnetic resonance study identified adipose tissue in 78% of infarcts older than 6 mo^[32].

CT has the ability to discriminate between air, water, fat and bone, with fat presenting a density of approximately -120 HU. Consequently, a number of studies have recently been carried out to explore the characterization of chronic myocardial infarction using CTCA. These studies have confirmed the notion that infarcted tissue is gradually replaced by adipose tissue, although attenuation levels (HU) are usually slightly higher than those of pericardial fat, indicating possibly a compound of adipose tissue, fibrosis, and myocardial fibers within the infarct

core^[28,33]. Overall, these results counterminimize the long-standing concept of myocardial scar, whose Latin origin is derived from the production and contraction of fibrous tissue.

The high prevalence of adipose tissue in myocardial infarction represents a possibility to attempt identification of chronic MI by means of CT without the addition of contrast media. This has been recently explored using conventional coronary calcium scoring acquisitions, showing a sensitivity and specificity of 66% and 100%, respectively, for the detection of chronic myocardial infarction (Figure 3). The presence of lipomatous metaplasia seems to be highly related to the infarct age. We have recently shown that patients with myocardial hypoenhancement on contrast CT had older infarcts than did patients without hypoenhancement (24 mo, interquartile range, 12-48 mo *vs* 6 mo, interquartile range, 3-33 mo, $P = 0.04$). Similar results were found with non-contrast CT (36 mo, interquartile range, 13-60 mo *vs* 11 mo, interquartile range, 4-24 mo, $P < 0.001$)^[28].

COMBINED ASSESSMENT OF CORONARY ANATOMY AND STRESS MYOCARDIAL PERFUSION: ONE-STOP SHOP?

Recently, two invasive studies that evaluated the coronary reserve flow have stressed the importance of functional assessment by showing that revascularization of patients with intermediate lesions does not confer a significant clinical benefit if stenoses are not flow limiting during stress^[3,4]. In the same line, Meijboom *et al*^[34] demonstrated that both conventional angiography and CTCA show a poor correlation with coronary flow reserve ($r = -0.30$ and $r = -0.32$, respectively), rendering a diagnostic accuracy of 67% and 71%, respectively. These findings represent a challenge for non-invasive imaging techniques and warrant the search for a “one-stop shop” tool that would allow a combined assessment of the coronary anatomy

and of the functional significance of coronary lesions. Even with the advent of dual source CT scanners, diffuse calcification is commonly related to a significant drop in positive predictive values, reinforcing the need for functional assessment by MPI^[35]. As aforementioned, CTCA allows the evaluation of myocardial perfusion at rest. In addition, recent advances in multidetector-row CT have allowed a simultaneous assessment of coronary imaging and MPI during pharmacological stress with adenosine. A seminal study using a canine model of left anterior descending artery stenosis demonstrated a myocardial blood flow in stenosed *vs* remote territories of 2.54 ± 0.93 mL/g per min and 8.94 ± 5.74 mL/g per min, respectively ($P < 0.05$) during adenosine infusion, with a myocardial signal density (HU) of 92.3 ± 39.5 HU in stenosed *vs* 180.4 ± 41.9 HU in remote territories ($P < 0.001$)^[15].

More recently, Blankstein *et al*^[21] have shown the feasibility of the combined (sequentially during the same procedure) morphological-functional approach: (1) adenosine-stress myocardial perfusion; (2) rest myocardial perfusion; (3) coronary angiography (simultaneous with rest perfusion); and (4) delayed enhancement 7 min post-contrast. This combined approach has been carried out with an effective radiation dose of 12.7 mSv, similar to that observed with SPECT in the same study. Adenosine-stress myocardial perfusion yielded a 79% and 80% sensitivity and specificity, respectively, to detect $> 50\%$ diameter stenoses by conventional angiography; whereas SPECT showed a sensitivity and specificity of 67% and 83%, respectively. In turn, using $> 70\%$ diameter stenoses as a reference standard, adenosine-stress myocardial perfusion yielded an 86% and 68% sensitivity and specificity, respectively, whereas SPECT showed a sensitivity and specificity of 73% and 73%, respectively. It should be stressed that this study included a high risk population, with a high prevalence of previous MI (35%), previous revascularization (38%), diabetes (32%), hypertension (88%), dyslipidemia (85%) and obesity (41%)^[21].

With the advent of new generation dual source CT scanners, cardiac acquisitions can be accomplished within a single heart beat, allowing a substantial reduction in radiation exposure. Rocha-Filho *et al*^[36] recently demonstrated that adding stress CT perfusion to coronary CT angiography during a single session has a significant incremental value for the detection of hemodynamically significant CAD, with the area under the receiver operating characteristic curve increasing from 0.77 to 0.90 ($P < 0.005$). Interestingly, this was achieved at an acceptable mean effective radiation exposure of 11.8 mSv.

It should be stressed that such a combined approach might gain relevance particularly in patients with a high probability of CAD with diffusely calcified vessels.

CLINICAL PERSPECTIVE

During the past decade, CTCA has evolved as the non-invasive diagnostic tool with the highest diagnostic performance to detect coronary artery stenosis, particularly

driven by its ability to exclude CAD. A number of investigators have subsequently established its ability to predict revascularization, its significant prognostic value over clinical risk factors, and the excellent prognosis of a negative study. In spite of these accomplishments, two main limitations have limited the incorporation of CTCA as a clinically established tool in diagnostic algorithms: radiation dose and lack of functional assessment hampering accurate assessment in patients with diffusely calcified vessels.

As mentioned previously, several investigators have convincingly demonstrated the ability of cardiac CT to evaluate myocardial perfusion, myocardial viability and the sequelae of chronic myocardial infarction. Indeed, cardiac CT might appear in the near future as a potential one-stop shop diagnostic tool.

Concerns regarding radiation exposure and potential association of the procedure with the risk of cancer represent other limitations of the technique, although such assumptions are based on Monte-Carlo simulations without demonstration of cause-effect^[37]. In addition, the lifetime-risk of cancer attributable to CTCA is similar to the risk related to a stress-rest SPECT or a chest or abdomen CT^[38]. It is noteworthy, though, that the currently widely established application of prospective ECG gating acquisitions allows a significant reduction in radiation doses to as low as 1-3 mSv^[39]. Furthermore, the recent incorporation of high pitch spiral scanners achieves effective radiation doses up to 1-2 mSv^[40].

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Impaired coronary microvascular endothelial function in men with metabolic syndrome

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Abstract

AIM: To assess coronary endothelial function of conduit and resistance vessels in patients with metabolic syndrome (MS).

METHODS: Seventy-eight men (mean age, 57 years) with chest pain and angiographically normal coronary arteries were included in the study. Patients with coronary spastic angina were excluded. Changes in coronary artery diameter and coronary blood flow (CBF) in response to acetylcholine (ACh) were determined using quantitative coronary angiography and Doppler velocity measure-

ments. Coronary flow reserve was calculated as the ratio of coronary blood velocity after adenosine triphosphate infusion relative to baseline values. Patients were divided into two groups based on the presence or absence of MS.

RESULTS: There were 24 patients in the MS group (31%). The increase in CBF in response to ACh infusion was impaired in the MS group ($P < 0.0001$) compared to the non-MS group, whereas changes in coronary artery diameter in response to ACh infusion did not differ between the two groups. Multivariate regression analysis revealed that MS was a significant factor associated with the lesser change in CBF induced by ACh infusion at 30 $\mu\text{g}/\text{min}$ ($P < 0.0001$, $r^2 = 0.46$).

CONCLUSION: Coronary endothelial dysfunction was present at the level of resistance vessels but not conduit vessels in the MS patients included in our study.

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Key words: Endothelial dysfunction; Metabolic syndrome; Doppler flow; Conduit vessels; Resistance vessels

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INTRODUCTION

The metabolic syndrome (MS) is characterized by abdominal obesity, elevated blood pressure, hypertriglyceridemia,

low high-density lipoprotein (HDL) cholesterolemia, and hyperglycemia^[1]. Several studies have revealed that MS is a factor that may be responsible for future cardiovascular events, independent of racial differences^[2-6].

Studies have also indicated the presence of endothelial dysfunction assessed using several modalities, such as positron emission tomography, flow-mediated dilation in the brachial artery, and venous occlusion plethysmography, in MS patients^[7-13]. However, no studies have investigated coronary endothelial function using quantitative coronary angiography and Doppler velocity measurements, which can provide insight into the dynamic biology of the endothelium of coronary arteries at the level of conduit and resistance vessels and can also provide prognostic information for risk stratification in the later clinical phase^[14,15]. Therefore, we investigated the relationship between coronary endothelial function at the level of conduit and resistance vessels and the presence of MS in patients with chest pain and angiographically normal coronary arteries.

MATERIALS AND METHODS

Patient population

Seventy-eight Japanese men who underwent coronary angiography to evaluate chest pain were included in this study. All patients had angiographically normal epicardial coronary arteries, normal left ventricular function (contrast ventriculographic ejection fraction $\geq 60\%$), and normal coronary flow reserve (CFR > 2.0). We excluded patients with coronary spastic angina, previous myocardial infarction, left ventricular hypertrophy (LVH), moderate-severe valvular disease detected by echocardiography (UCG), heart failure or other serious diseases. Written informed consent was obtained from all patients prior to entry into the study. The protocol was approved by the Ethics Committee of our institution.

Study protocol

All anti-anginal agents were discontinued at least 48 h prior to catheterization, except for the unrestricted use of sublingual nitroglycerin, which was withheld for 1 h prior to catheterization. Diagnostic left heart catheterization and coronary angiography were performed using a standard percutaneous brachial approach. A 6F guide catheter was introduced into the left main coronary artery. A 0.0014-inch Doppler flow guidewire (Volcano FloWire; Volcano Therapeutics Inc., Rancho Cordova, CA) was advanced through the guide catheter into the proximal segment of the left anterior descending coronary artery. The wire tip was positioned in a straight segment of the vessel to obtain a reliable flow-velocity signal.

After baseline control conditions were established, incremental doses of acetylcholine (ACh) were infused into the left coronary artery (3 $\mu\text{g}/\text{min}$ and 30 $\mu\text{g}/\text{min}$) for 2 min with 5-min intervals between consecutive doses. After re-establishment of control conditions, nitroglycerin was infused intracoronarily at the rate of 200 $\mu\text{g}/\text{min}$ for 1 min. Finally, adenosine triphosphate (20 μg) was infused. ACh and nitroglycerin were infused directly into the left coronary ostium using an infusion pump (TE-311; Terumo, Tokyo, Japan) at the rate of 1 mL/min.

Coronary angiography was performed under controlled conditions and at the end of each drug infusion. Coronary blood flow (CBF) velocity was monitored continuously using a 12-MHz pulsed Doppler velocimeter (FloMap; Volcano Therapeutics Inc. Rancho Cordova, CA). Arterial pressure, heart rate and ECG were monitored continuously and recorded using a multichannel recorder (Polygraph 1600; Nihon Electric Corporation, Tokyo, Japan).

Quantitative coronary angiography

The method for measuring coronary diameter has been previously described in detail^[16,17]. The coronary segment 2 mm distal to the Doppler wire tip was selected for quantitative analysis. In each patient, luminal diameters of selected segments of the left anterior descending coronary artery were measured by a single investigator blinded to angiographic and clinical data in order to determine the effects of different drugs on epicardial coronary diameter. Luminal diameters were measured on an end-diastolic frame using a computer-assisted coronary angiographic analysis system (CAAS II/QUANTCOR; Siemens, Berlin and Munich, Germany). Means of triplicate measurements of luminal diameter were used for analysis. Changes in coronary diameter in response to ACh and nitroglycerin infusions are expressed as percent change from the baseline measurement on the angiogram obtained prior to infusion. Intra- and inter-observer variability have previously been reported to be excellent^[18].

Estimation of coronary blood flow and coronary flow reserve

CBF was calculated as the product of CBF velocity and vessel diameter using the following formula: $\pi \times \text{average peak velocity} \times 0.125 \times \text{diameter}^2$. For CBF calculations, the internal diameter of the vessel at the location of the flow measurements (2 mm distal to the wire tip) was measured using the method described above. CFR was calculated as the ratio of CBF velocity after an adenosine triphosphate infusion to the baseline velocity.

The definition of metabolic syndrome

The presence of MS was determined according to the final report of the National Cholesterol Education Program's Adult Treatment Panel III criteria^[1]. The above-mentioned criteria may not be suitable for determining abdominal obesity in Japanese patients; therefore, we adopted the Japanese criteria for abdominal obesity (waist circumference ≥ 85 cm in men) in the present study. Consequently, we defined MS as the presence of at least three of the following factors: (1) waist circumference ≥ 85 cm; (2) fasting triglycerides > 150 mg/dL; (3) HDL cholesterol < 40 mg/dL; (4) hypertension (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg, or use of antihypertensive drug therapy); and (5) fasting glucose ≥ 110 mg/dL. The patients were divided into the following two groups based on the presence or absence of MS: the MS group comprising patients with MS and the non-MS group comprising patients without MS. In addition, the MS score was defined as the sum of the MS factors (0-5) that were present.

Table 1 Patients' characteristics regarding metabolic parameters (mean \pm SE)

Variables	MS group	Non-MS group	P value
n (%)	24 (31)	56 (69)	
Age (yr)	57 \pm 2	57 \pm 2	NS
Body mass index (kg/m ²)	26.4 \pm 0.6	24.3 \pm 0.3	0.0089
Waist circumference (cm)	90 \pm 1	86 \pm 1	0.0114
MS risk factors, n (%)			
Abdominal obesity	22 (92)	32 (59)	0.0042
Elevated triglyceride	18 (75)	22 (41)	0.0012
Low HDL cholesterol	11 (46)	5 (9)	0.0002
Hypertension	17 (71)	15 (28)	0.0004
Hyperglycemia	11 (46)	8 (15)	0.0032
Average MS score	3.3 \pm 0.1	1.5 \pm 0.1	< 0.0001

MS: Metabolic syndrome; NS: Not significant; HDL: High-density lipoprotein.

Other parameters

In each patient, total cholesterol, triglycerides, HDL-cholesterol, low-density lipoprotein cholesterol, glucose, insulin, hemoglobin A1C, and high-sensitive C-reactive protein (CRP) were measured. The homeostasis model assessment-insulin resistance (HOMA-IR) index was calculated as the fasting glucose (mg/dL) \times fasting insulin level (μ U/mL)/405.

Statistical analysis

All data were expressed as mean \pm SE. Baseline characteristics of the two groups were compared with Student's unpaired *t*-test or by χ^2 analysis, as appropriate. Serial changes in hemodynamic variables and changes in coronary vasoreactivity in response to drug infusion were compared using a one-way analysis of variance. If the analysis of variance showed a significant difference between means, the level of significance was determined by contrast analysis. Serial percent changes in the coronary vascular response to ACh infusion were compared between groups using a two-way analysis of variance. We used Spearman's rank correlation to investigate the relationship between the MS score and change in CBF induced by ACh infusion. We performed uni- and multivariate regression analyses to identify factors associated with percent changes in CBF induced by ACh. A *P* value < 0.05 indicated statistical significance.

RESULTS

Patients' characteristics and biochemical parameters

The patients' characteristics are indicated in Table 1. There were 24 patients with MS (31%). Body mass index, waist circumference and the frequency of having each MS factor were higher in the MS group than in the non-MS group. The average MS score was significantly higher in the MS group than in the non-MS group.

Data for other conventional risk factors, including biochemical parameters and medications taken, are also indicated in Table 2. The triglyceride, fasting blood sugar, hemoglobin A1C, and HOMA-IR levels were higher in the MS group and the level of HDL cholesterol was lower in the MS group compared with the non-MS group. The

Table 2 Patients' characteristics regarding other risk factors and medications (mean \pm SE) n (%)

Variables	MS group	Non-MS group	P value
Other risk factors			
Smoking	13 (54)	16 (35)	NS
Hypercholesterolemia ¹	6 (25)	10 (18)	NS
Total cholesterol (mg/dL)	206 \pm 7	194 \pm 5	NS
Triglyceride (mg/dL)	205 \pm 17	157 \pm 11	0.0218
HDL cholesterol (mg/dL)	44 \pm 3	52 \pm 2	0.0204
LDL cholesterol (mg/dL)	121 \pm 6	111 \pm 4	NS
Diabetes mellitus	5 (21)	5 (9)	0.0189
Fasting blood sugar (mg/dL)	107 \pm 3	98 \pm 2	0.0189
Hemoglobin A1C	5.7 \pm 0.1	5.4 \pm 0.1	0.0445
HOMA-IR	2.5 \pm 0.2	1.7 \pm 0.1	0.0046
C-reactive protein (mg/L)	1.7 \pm 0.3	1.3 \pm 0.2	NS
Medications			
Statins	1 (4)	6 (11)	NS
ACE or ARB	2 (8)	1 (2)	NS

¹On therapy and/or low-density lipoprotein (LDL)-cholesterol \geq 140 mg/dL. MS: Metabolic syndrome; NS: Not significant; HDL: High-density lipoprotein; HOMA-IR: Homeostasis of assessment-insulin resistance; ACE: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin receptor blocker.

frequency of medication intake was similar in the two groups.

Results of coronary vasoreactivity

The results of hemodynamics and coronary vasoreactivity are indicated in Table 3. The mean blood pressure was higher in the MS group. The baseline coronary artery diameter and CBF were similar in the two groups. Changes in coronary artery diameter in response to ACh infusion and NTG-induced coronary dilation also did not differ between the two groups (Figure 1 and Table 3). However, the increase in CBF in response to ACh infusion was impaired in the MS group compared to the non-MS group (*P* < 0.0001, Table 3 and Figure 2). However, CFR did not differ between the two groups (Table 3). Statistical significance between the MS and non-MS groups was more prominent in percent change in CBF induced by ACh infusion at a dose of 30 μ g/min, and subsequent analyses were performed using this value. The total MS scores were negatively associated with the increase in CBF in response to infusion of ACh at 30 μ g/min (*r* = -0.51, *P* < 0.0001, Figure 3).

Factors responsible for coronary microvascular endothelial dysfunction

Analysis of individual MS factors indicated that elevated triglycerides (*P* = 0.0246), low HDL cholesterol (0.0409), elevated blood pressure (0.0032), and hyperglycemia (*P* = 0.0309) were associated with a lower change in CBF in response ACh infusion at 30 μ g/min. Univariate analysis revealed that the presence of MS (*P* < 0.0001), reduced CFR (*P* = 0.0003) and an elevated CRP level (*P* = 0.0027) were associated with a lower CBF response induced by ACh infusion at 30 μ g/min; a high heart rate at baseline also tended to be associated with the reduced response. Multivariate regression analysis using these parameters demonstrated that the presence of MS (*P* < 0.0001) as well as reduced CFR (*P* = 0.0005) and elevated CRP (*P* = 0.0234) were signifi-

Table 3 Hemodynamics and angiographic results (mean \pm SE)

Variables	MS group		Non-MS group	
	Value	% change	Value	% change
Baseline mean blood pressure (mmHg)	111 \pm 2 ^a		104 \pm 2	
Baseline heart rate (/min)	67 \pm 2		64 \pm 1	
Coronary diameter (mm)				
Baseline	3.26 \pm 0.11	0	3.11 \pm 0.07	0
ACh at 3 μ g/min	3.30 \pm 0.12	1.6 \pm 1.4	3.18 \pm 0.08	2.6 \pm 0.7
ACh at 30 μ g/min	3.23 \pm 0.13	-0.5 \pm 1.9	3.08 \pm 0.09	-0.5 \pm 1.3
Nitroglycerin	3.67 \pm 0.12	13.9 \pm 2.3	3.54 \pm 0.08	14.7 \pm 1.6
Coronary blood flow (mL/min)				
Baseline	77 \pm 6	0	65 \pm 4	0
ACh at 3 μ g/min	110 \pm 14	38 \pm 9 ^b	114 \pm 9	73 \pm 6
ACh at 30 μ g/min	137 \pm 20 ^a	82 \pm 18 ^d	190 \pm 13	198 \pm 12
Coronary flow reserve	3.3 \pm 0.2		3.6 \pm 0.1	

MS: Metabolic syndrome; ACh: Acetylcholine. ^a $P < 0.05$ vs values in non-MS group; ^b $P < 0.01$, ^d $P < 0.001$ vs % change in non-MS group.

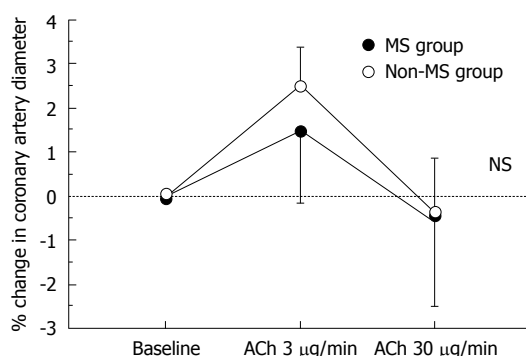


Figure 1 Percentage changes in epicardial coronary artery diameter in response to acetylcholine infusion. The changes in coronary artery diameter in response to acetylcholine infusion were similar between patients with metabolic syndrome (MS; solid circles) and patients without MS (open circles). Vertical bars represent SE. NS: Not significant.

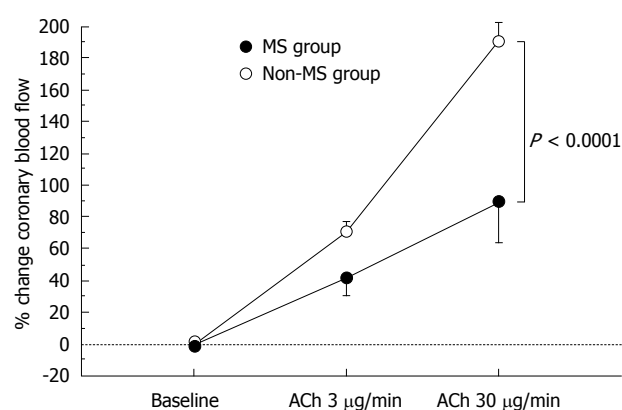


Figure 2 Percentage changes in coronary blood flow in response to acetylcholine infusion. The increase in coronary blood flow in response to acetylcholine infusion was attenuated in metabolic syndrome (MS) patients (solid circles) relative to non-MS patients (open circles). Vertical bars represent SE.

cant factors associated with the reduced CBF response induced by ACh infusion at 30 μ g/min ($r^2 = 0.46$, Table 4).

DISCUSSION

The present study revealed that coronary endothelial function at the level of resistance vessels is impaired in MS patients while that at the level of conduit vessels is similar among both MS and non-MS patients. Multivariate regression analysis demonstrated that the presence of MS was a significant factor associated with impaired coronary endothelial function at the level of resistance vessels.

Many reported studies have used several modalities to investigate the relationship between MS and coronary microvascular circulation. PET analysis has revealed that the increase in myocardial blood flow in response to a cold pressor test is impaired in MS patients^[7], indicating the presence of coronary microvascular endothelial dysfunction; this is in accordance with the results obtained in the present study. On the other hand, Pirat *et al*^[19], using UCG, have reported an impaired CFR in the LAD of coronary arteries in patients with MS. Furthermore, Turhan *et al*^[20] reported an impaired CBF using the Thrombolysis in My-

ocardial Infarction frame count method in MS patients with angiographically normal coronary arteries. The purpose of the present study was to assess ACh-induced coronary vasomotion and circulation, and thus our study protocol excluded patients with several conditions, such as severely reduced CFR (< 2.0) or LVH, which are frequently observed in MS patients. Therefore, differences in patient selection and other patient characteristics may contribute to the discrepancy in the results. In all the above-mentioned studies, the presence of coronary endothelial dysfunction at the level of the resistance vessels was shown in MS patients; however, no studies have investigated coronary endothelial function at the level of the conduit vessels in MS patients. The present study revealed that coronary endothelial function at the level of the conduit vessels was not reduced in response to ACh. This finding suggests that coronary endothelial function at the level of the resistance vessels is impaired earlier and is more prominent than that at the level of conduit vessels. The patients in our study exhibited chest symptoms, even in the non-MS group, and it was not clarified whether coronary endothelial function, especially at the level of the conduit vessels, was preserved in such patients. Nonetheless, the finding that coronary

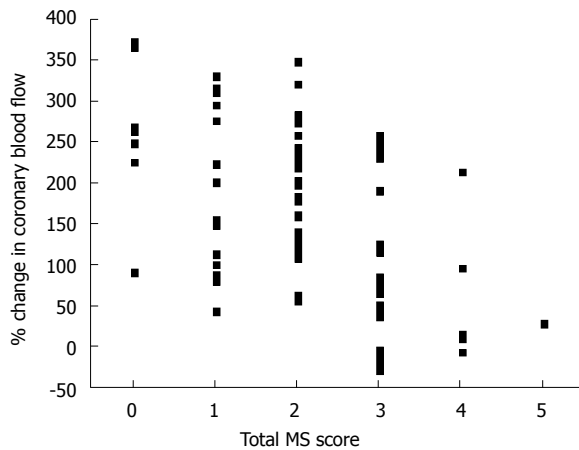


Figure 3 Relationship between the change in coronary blood flow induced by acetylcholine at 30 µg/min and the total metabolic syndrome score. The total metabolic syndrome score was inversely associated with the change in coronary blood flow induced by acetylcholine infusion at 30 µg/min ($r = -0.51$, $P < 0.0001$). MS: Metabolic syndrome.

microvascular endothelial dysfunction was severely impaired in the MS group is certain and clarifies the impact of MS on pathogenesis of the coronary artery vasculature. If the degree of MS is severe and its duration is longer, coronary endothelial dysfunction at the level of conduit vessels may be evident after the establishment of coronary microvascular endothelial dysfunction.

There are several possible mechanisms responsible for MS-induced coronary endothelial dysfunction. Several studies have revealed that insulin resistance may induce endothelial dysfunction mediated by oxidative stress^[21,22] and decreases in insulin-dependent activation of endothelial nitric oxide synthase (eNOS)^[23]. Furthermore, it has been reported that adiponectin may play a role in the phosphorylation of eNOS^[24] and reduced adiponectin, which is often recognized in MS patients, may lead to endothelial dysfunction. In addition, it has been reported that pericardial fat tissue, which is increased in MS patients, discharges several cytokines systemically and locally^[25,26], indicating the possibility that impairment of coronary endothelial function may be caused by pericardial fat tissue. These mechanisms may solely and/or multifactorially contribute to coronary endothelial dysfunction in MS patients.

The present study revealed that coronary endothelial dysfunction at the level of resistance vessels was more prominent than that at the level of conduit vessels in MS patients. Until now, there has been no data available indicating which is the stronger factor; i.e. coronary endothelial dysfunction at the level of conduit vessels or at that of resistance vessels, affecting future cardiovascular events. However, Halcox *et al.*^[15] have reported that if coronary endothelial dysfunction is present either at the level of conduit or resistance vessels, future cardiovascular events occur more frequently. Thus, the finding that coronary microvascular endothelial dysfunction is more prominent in MS patients may provide important information clarifying the pathogenesis of MS-induced cardiovascular events. The present study also demonstrated that coronary microvascular endothelial function declined in association

Table 4 Multivariate analysis of variables influencing % change in coronary blood flow induced by acetylcholine at 30 µg/min

Variables	% change in coronary blood flow induced by ACh 30 µg/min	
	<i>t</i> value	<i>P</i> value
Presence of MS	-5.07	< 0.0001
Coronary flow reserve	3.62	0.0005
C-reactive protein	-2.76	0.0073
Baseline heart rate	-1.13	0.262

ACh: Acetylcholine; MS: Metabolic syndrome.

with increased total MS score, even in non-MS patients with a moderate MS score such as 2; careful follow-up may be needed for such subjects.

There are several limitations to the present study. First, all the patients in our study had chest symptoms and had undergone coronary angiography; thus, they may represent a specific group. In addition, MS patients met the minimal criteria for MS and several non-MS patients also had a moderate MS score. Therefore, the results of the present study may not always represent endothelial function in all MS patients. Second, our data showed that CFR was not different in the two groups. However, we excluded patients with a CFR < 2.0 and/or LVH in order to accurately measure ACh-induced coronary circulation. However, in general, many such patients may be regarded as MS patients. If we had added them in the MS group in the present study, CFR in the MS group might have been lower than that in the non-MS group. There have been many studies showing impaired CFR in patients with MS^[19,27] and we do not mean to imply that CFR is preserved in MS patients. Finally, we did not measure biochemical parameters associated with MS, such as adiponectin, interleukin-6 and tumor necrotizing factor- α . Therefore, we cannot report on the precise mechanisms of MS-induced coronary microvascular endothelial dysfunction in the present study.

In conclusion, these findings suggest that coronary microvascular endothelial dysfunction is present in MS patients who have chest pain but angiographically normal coronary arteries. Such coronary microvascular endothelial dysfunction may be involved in the pathogenesis of MS-induced cardiovascular events.

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COMMENTS

Background

Metabolic syndrome (MS) is a major cause of future cardiovascular events. Endothelial dysfunction is thought to be involved in the pathogenesis of MS-induced cardiovascular events. However, coronary endothelial function in patients with MS remains to be elucidated.

Research frontiers

The purpose of the present study was to assess the coronary endothelial function of the conduit and resistance vessels in patients with MS and angiographically normal coronary arteries.

Innovations and breakthroughs

Several studies investigating coronary endothelial function in patients with MS have been reported and their results have identified coronary endothelial dysfunction at the level of the resistance vessels. However, no study has investigated coronary endothelial function at the level of the conduit vessels. Quantitative coronary angiography and Doppler velocity measurements, which we adopted in the present study, can assess coronary endothelial function at the levels of the conduit and resistance vessels simultaneously. Our study demonstrates that the increase in coronary blood flow in response to acetylcholine (ACh) infusion was impaired in MS patients compared with non-MS patients, whereas changes in coronary artery diameter in response to ACh infusion did not differ between the two groups. These findings suggest that coronary endothelial function at the level of the resistance vessels is impaired earlier and is more prominent than that at the level of the conduit vessels.

Applications

These findings suggest that coronary microvascular endothelial dysfunction is present in MS patients who have chest pain but angiographically normal coronary arteries. Such coronary microvascular endothelial dysfunction may provide a vital evidence for the pathogenesis of MS-induced cardiovascular events.

Peer review

This is an interesting study confirming previous findings on how MS affects coronary function.

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Stenting for left main coronary artery occlusion in adolescent: A case report

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Li JJ, Xu B, Chen JL. Stenting for left main coronary artery occlusion in adolescent: A case report. *World J Cardiol* 2010; 2(7): 211-214 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v2/i7/211.htm> DOI: <http://dx.doi.org/10.4330/wjc.v2.i7.211>

Abstract

Acute total or subtotal occlusion of left main coronary artery (LMCA) is a catastrophic and mostly fatal event. Patients may present with cardiogenic shock and die whenever this event occurs. Survival is strongly dependent on the presence of collateral blood flow to the left coronary artery or a dominant right coronary artery, and emergency intervention for preserving the left ventricular function. Here, we present a case of a 14-year-old boy with subtotal occlusion of the LMCA accompanying acute myocardial infarction probably caused by congenital syphilis according to his positive serum syphilis antibody. His survival was closely associated with a dominant right coronary artery and timely thrombolytic therapy. Finally, he was treated with angioplasty and paclitaxel-eluting stent implantation. He was followed up after stenting and was doing quite well at the time when we wrote this paper.

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Key words: Left main coronary artery; Paclitaxel-eluting stent; Adolescent; Acute myocardial infarction

INTRODUCTION

Acute total or subtotal occlusion of left main coronary artery (LMCA) is an emergency condition and a catastrophic event. Almost all such patients would die before their admission to a hospital. It was reported that the hospital mortality of patients with acute occlusion of LMCA is around 50%^[1]. However, the recanalization time is a critical point affecting the mortality of patients. It has been shown that the time from onset of the disease to the recanalization is quite short in patients with a higher survival rate^[2,3]. Additionally, several factors are apparently involved in survivors suffering from acute total or subtotal occlusion of LMCA, including the presence of collateral blood flow to the left coronary artery or a dominant right coronary artery, or rapid revascularization^[4,5]. In this condition, the left ventricular function can be preserved once the event occurs^[4,5].

Cardiovascular abnormalities are well-known manifestations of tertiary syphilis infection, including syphilitic coronary artery ostial stenosis^[6]. We here present a rare case of a 14-year-old boy with acute subtotal occlusion of LMCA accompanying acute myocardial infarction probably caused by congenital syphilis. He was successfully treated with thrombolytic therapy, angioplasty, and drug-eluting stent implantation.

CASE REPORT

On June 5, 2005, a 14-year-old boy was transferred to our hospital due to acute myocardial infarction. At admission, he complained of only uncomfortable chest. In the afternoon of May 29, 2005, he played football and then developed severe back and substernal chest pain accompanying syncope after his game. He was admitted to the emergency department of a local hospital where he had an episode of ventricular fibrillation. Electrocardiogram demonstrated an elevated ST segment in leads V₁-V₆. Although the patient had transient hypotension and pulmonary edema after attack of the disease, he survived after intravenously thrombolytic therapy with urokinase. The patient had no history of known heart disease, hypertension, hyperlipidemia, diabetes or cigarette smoking, or a known family history of coronary artery disease. Physical examination showed no pathological signs. Laboratory tests were normal except for positive serum syphilis antibody.

Selective coronary angiography was performed for him on June 7, 2005, showing a dominant right coronary artery and a subtotal occlusion (approximately 99%) of the distal LMCA, but no other obvious angiographic evidence for coronary atherosclerosis (Figure 1A and B). No right-to-left coronary artery collateral was observed. Therefore, a 3.5 mm × 12 mm paclitaxel-eluting stent (Boston Scientific Corporation, Natick, Massachusetts, USA) was implanted cross the proximal of the left anterior descending artery to the ostial of LMCA for the patient (Figure 2A and B). The patient was followed up for 12 and 24 mo after stent implantation, and he was doing quite well at the time when we wrote this paper.

DISCUSSION

Total or subtotal occlusion of LMCA is an uncommon event at cardiac catheterization due to its lethal nature. Since the left coronary system supplies blood for most of the left ventricular myocardium, acute total occlusion of left coronary system in the absence of collateral circulation leads to cardiogenic shock, which is usually fatal^[1]. The prevalence of total occlusion of LMCA in patients undergoing coronary angiography is very low (0.04%-0.06%) and extremely rare in patients with acute ST-elevation myocardial infarction undergoing percutaneous coronary intervention (PCI)^[3].

Survival, although uncommon, after total or subtotal occlusion of LMCA, is linked to a large and dominant right coronary artery, even if no collateral has been identified at angiography. It has been reported that survival after occlusion of LMCA is dependent on the severity of occlusion and development of right-to-left collateral blood flow^[1]. The mortality is higher in patients with occlusion of LMCA even in hospital regardless of age. In our case, subtotal occlusion of LMCA was diagnosed by angiography and history of acute myocardial infarction. Apparently, his survival was associated with a dominant right coronary artery and subsequent thrombolytic therapy.

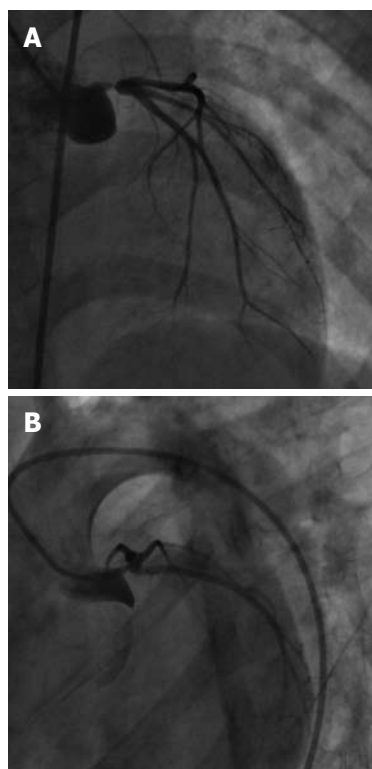


Figure 1 Selective angiography showing subtotal occlusion (approximately 99% stenosis) of the distal left main coronary artery (A) and the left coronary artery (B).

Total or subtotal occlusion of LMCA in elderly patients is usually due to atherosclerotic plaque rupture and not uncommon^[7]. However, LMCA occlusion in a young patient may be associated with embolization, hyper-coagulability, arteritis, spasm, spontaneous coronary artery dissection. Yamasa *et al*^[8] have reported a case of myocardial infarction secondary to thrombus in LMCA. Their patient had a past medical history of splenectomy for idiopathic thrombocytopenic purpura. Khan *et al*^[9] have also reported a healthy young male case of huge thrombus in LMCA, who survived after heart transplantation. Bush *et al*^[10] have recently described ST-elevation acute myocardial infarction involving the LMCA in a middle-aged man who was treated with primary angioplasty in combination with implantation of sirolimus-eluting stents. Cases of acute myocardial infarction due to blunt chest trauma are also available^[11]. Our case of acute myocardial infarction was treated with thrombolytic therapy, and subsequently coronary stenting for LMCA.

Cardiovascular involvement is a rare complication of congenital syphilis, and syphilis diagnosed at any stage should be immediately treated with appropriate antibiotics, preferably penicillin^[6]. The occlusion of LMCA in our patient might be due to congenital syphilis according to his positive serum syphilis antibody, and subsequently thrombotic formation triggered by strenuous exercise. The mechanism underlying thrombotic formation in our patient is unclear, but may be associated with hypercoagulability or spasm due to arteritis. Kennedy *et al*^[6] have

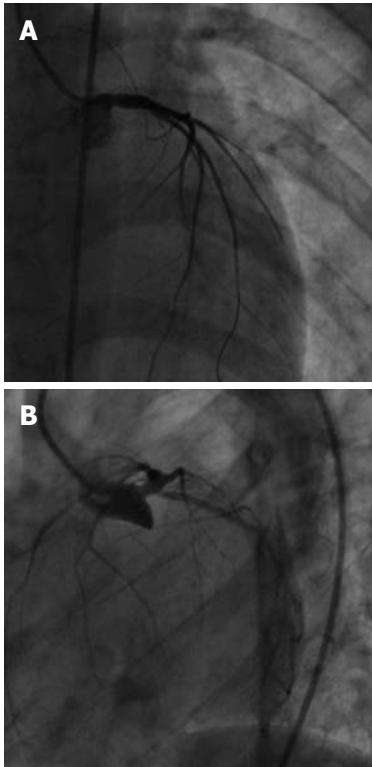


Figure 2 Selective angiography showing no residual stenosis in the left main coronary artery (A) and in the left coronary artery (B) after implantation of a 3.5 mm × 18 mm paclitaxel-eluting stent.

reported a case of a 32-year-old female who died of myocardial infarction due to coronary artery ostial stenosis secondary to syphilitic aortitis. In our patient, no evidence of either dissection flap or severe chest blow was found on coronary angiography. Laboratory tests were negative except for positive serum syphilis antibody, indicating that “congenital syphilis” may be a reasonable cause of left main coronary orifice stenosis. However, atherosclerotic origin could not be absolutely excluded.

It has been shown that fatty streaks appearing in aorta of most children over 3 years of age are increased in adolescence with coronary arteries involved approximately a decade later^[12,13]. Evidence of coronary atherosclerosis (ranging from insignificant disease to total or subtotal occlusion) in young individuals has also been demonstrated in autopsy studies^[12]. The prevalence of coronary atherosclerosis was 77% in the Korean War (mean age 22.1 and 20.5 years) and 45% in the Vietnam War (mean age 22.1 years)^[14,15]. During the Vietnam War, severe coronary atherosclerosis occurred in about 5% of all cases^[15]. It has been recently reported that coronary atherosclerosis can also occur in young trauma patients (age < 35 years, mean age 25.6 years)^[15]. The prevalence of coronary atherosclerosis in this study is consistent with that in the Korean War^[14]. Signs of coronary atherosclerosis have been found in 78.3% of patients with over 50% narrowing observed in 20.7% of them. Lesions producing over 75% narrowing have been observed in 9% of the patients. LMCA or significantly diseased 2- or 3-vessels

can be found in 20% of the patients^[16]. The proximal segment of the left anterior descending and circumflex arteries appears to be involved more often than that of the distal vessels. Major risk factors are smoking and family history of coronary heart disease. Although coronary atherosclerosis in young individuals has been well documented, coronary atherosclerosis was not detectable in this patient.

The therapeutic strategies for total or subtotal occlusion of LMCA include thrombolytic therapy, PCI, and emergency coronary artery bypass grafting. Drug-eluting stent has been widely used for stenosis or total/subtotal occlusion of LMCA. Bush *et al.*^[10] have reported a case of a middle-age man with ST-elevation acute myocardial infarction involving LMCA, who was successfully treated with primary angioplasty and sirolimus-eluting stenting, suggesting that PCI is a promising procedure for acute total or subtotal occlusion of LMCA.

ACKNOWLEDGMENTS

The authors certify that they comply with the ethical principles^[17].

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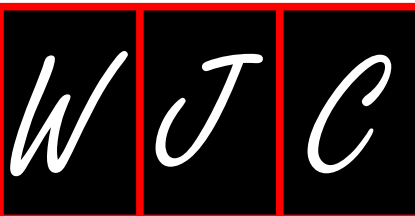
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Meetings

Events Calendar 2010

January 12-13
Riyadh, Saudi Arabia
1st International Cardiovascular
Pharmacotherapy Conference

January 17-21
Hollywood, United States
22nd Annual International
Symposium on Endovascular Therapy

January 20-23
Sao Paulo, Brazil
World Cardiology, Metabolism and
Thrombosis Congress

January 21-24
Phoenix, United States
13th Society for Cardiovascular
Magnetic Resonance Annual
Scientific Sessions

January 28-30
Brussels, Belgium
29th Belgian Society of Cardiology
Annual Scientific Meeting

January 28-31
Nashville, United States
31st Annual Meeting of
The American Academy of
Cardiovascular Perfusion

February 3-6
Snowbird, United States
35th Annual Cardiovascular
Conference at Snowbird

February 4-5
Leuven, Belgium
Leuven Symposium on Myocardial
Velocity and Deformation Imaging

February 6-9
St. Petersburg, United States
10th Annual International
Symposium on Congenital Heart
Disease

February 8-10
Tel Aviv, Israel
10th International Dead Sea
Symposium on Cardiac Arrhythmias
and Device Therapy

February 11-12
London, United Kingdom
2nd National Chronic Heart Failure
and Hypertension

February 18-21
Istanbul, Turkey
The 2nd World Congress on
Controversies in Cardiovascular
Disease (C-Care)

February 22-25
Maui, United States
Arrhythmias & the Heart
Symposium

February 22-26
Cancun, Mexico
15th Annual Cardiology at Cancun-
Advances in Clinical Cardiology and
Multi-Modality Imaging

February 25-28
Valencia, Spain
First International Meeting on
Cardiac Problems in Pregnancy

February 26-28
Hong Kong, China
International Congress of
Cardiology

February 28-March 4
Scottsdale, United States
International Congress XXIII on
Endovascular Interventions

February 28-March 5
Keystone, United States
Keystone Symposia: Cardiovascular
Development and Repair (X2)

March 3-5
Kish Island, Iran
Islamic Republic of 4th Middle East
Cardiovascular Congress

March 4-7
Newport Beach, United States
30th Annual CREF: Cardiothoracic
Surgery Symposium

March 7-12
Snowmass Village, United States
Interventional Cardiology 2010: 25th
Annual International Symposium

March 14-16
Atlanta, United States
American College of Cardiology
59th Annual Scientific Session

March 18-20
Rome, Italy
VIII Congress of the Italian Society
of Cardiovascular Prevention

March 18-20
Prague, Czech Republic
XI International Forum for the
Evaluation of Cardiovascular Care

March 24-25
Jeddah, Saudi Arabia
12th KFAFH Cardiovascular
Conference: A balanced approach to
treatment of cardiovascular diseases

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The 12th South China International
Congress of Cardiology

April 14-15
Tel Aviv, Israel
The 57th Annual Congress of the
Israel Heart Society in Association
with The Israel Society of
Cardiothoracic Surgery

April 15-18
Izmir, Turkey
59th European Society for
Cardiovascular Surgery
International Congress

May 5-7
Prague, Czech Republic
EuroPrevent 2010-Cardiovascular
Prevention: a Lifelong Challenge

May 8-9
St. Paul, United States
Controversies in Cardiovascular
Disease: Practical Approaches to
Complex Problems: Medical and
Surgical

May 12-16
Marrakesh, Morocco
7th Metabolic Syndrome, type
II Diabetes and Atherosclerosis
Congress

May 17-20
Whistler, Canada
6th IAS-Sponsored HDL Workshop
on High Density Lipoproteins

May 21-22
Sydney, Australia
3rd Cardiovascular CT, Concord
Conference 2010

May 29-June 1
Berlin, Germany
Heart Failure Congress 2010

June 1-4
Seoul, Korea, Republic of
9th Asian-Pacific Congress of
Cardiovascular & Interventional
Radiology (APCCVIR 2010)

June 16-19
Beijing, China
World Congress of Cardiology
Scientific Sessions

June 17-19
Port El Kantaoui, Tunisia
The 7th Tunisian and Europeans
Days of Cardiology Practice

July 1-3
Singapore, Singapore
6th Asian Interventional
Cardiovascular Therapeutics
Congress

July 16-19
Berlin, Germany
Frontiers in CardioVascular Biology
2010-1st Meeting of the CBCS of the
ESC

July 24-27
Vancouver, Canada
15th World Congress on Heart
Disease, Annual Scientific Sessions
2010

August 13-15
Krabi, Thailand
East Meets West Cardiology 2010

September 16-18
Athens, Greece
5th International Meeting of the
Onassis Cardiac Surgery Center

September 25-29
Belo Horizonte, Brazil
65th Brazilian Congress of
Cardiology

September 30-October 2
Berlin, Germany
5th International Symposium
on Integrated Biomarkers in
Cardiovascular Diseases

October 10-13
Rochester, United States
26th Annual Echocardiography
in Pediatric and Adult Congenital
Heart Disease Symposium

October 16-19
Copenhagen, Denmark
Acute Cardiac Care 2010

October 20-23
Boston, United States
2010 Cardiometabolic Health
Congress

November 25-26
London, United Kingdom
13th British Society for Heart Failure
Annual Meeting

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^a $P < 0.05$, ^b $P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, ^c $P < 0.05$ and ^d $P < 0.01$ are used. A third series of *P* values can be expressed as ^e $P < 0.05$ and ^f $P < 0.01$. Other notes in tables or under illustrations should be expressed as ¹F, ²F, ³F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

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- 2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaobua Zazhi* 1999; **7**: 285-287

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

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Patent (list all authors)

- 16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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

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