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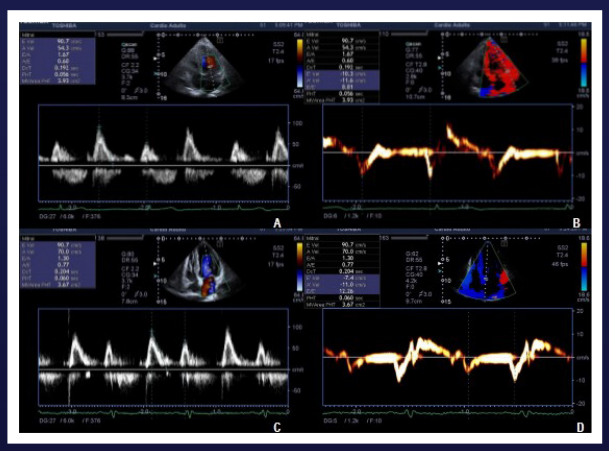

Figure 1 – *A and B: Pulsed Dopper images showing transmitral flow and septal tissue Doppler showing a normal diastolic pattern. C and D: Tissue Doppler images showing a reduced e' wave (7.4 cm/s), compatible with moderate diastolic dysfunction (pseudonormal pattern). Page 412*

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Cardiac Autonomic Modulation - The Search for an Ultimate Technique

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Abnormal parasympathetic activity on the heart has been implicated in several forms of symptomatic bradycardia, especially in young and otherwise healthy individuals. Neurally mediated syncope, sinus arrest and advanced atrioventricular (AV) block have high risk of injury and serious implications on quality of life. Nonetheless, until recently, their clinical management, mainly based on behavioral measures and pacing, have often proved to be ineffective or inadequate. An alternative approach to treat this specific population, avoiding device implants and continuous drug therapy seemed to be required.

Ganglionated plexus (GP) ablation was first described in 2005,[1] with the purpose of targeting the main parasympathetic ganglia and promoting a vagal attenuation. During the last decade, several authors[2-8] presented their clinical results, with significant remission of symptoms and electrocardiographic improvement, and autonomic modulation became an established therapeutic modality.

The cardiac autonomic nervous system, however, is not a simple target. Precisely the opposite: a complex interface between the central nervous system and the heart, comprising both extrinsic (Vagus nerve and sympathetic thoracic chain) and intrinsic components (epicardial GPs). It is widely recognized that most thoracic nerves and cardiac ganglia have both parasympathetic and sympathetic inputs (except the purely parasympathetic Vagus nerve and the purely sympathetic Stellate cardiac nerve), and present remarkable anatomic and functional heterogeneity. The variation and overlap of autonomic nervous supply to the myocardium makes interventional treatment difficult, although the predilection of certain structures for specific areas of the heart might be helpful for this purpose.

The intrinsic cardiac nervous system, a dense network of neuronal somata and connecting pre- and postganglionic fibers is the main target of the autonomic modulation procedures. Since almost all these epicardial ganglia have dual innervation, a titrated ablation with a net result of vagal attenuation must be reached.

Most authors considered as eligible for treatment those patients with no structural heart disease and recurrent functional bradycardia (cardioinhibitory syncope, advanced AV block or sinus arrest), after failure of medical treatment. Some authors[9] recommend that a pre-ablation atropine test must be performed (0.04mg/Kg) and a positive response required as eligible criteria. Denervation has also been proposed to treat extreme bradycardia (pauses longer than 10 seconds) in asymptomatic patients,[7] a very uncommon presentation with still unknown cardiovascular risks.

Several methods to identify the main GP implicated in functional bradycardia have been studied, alone and combined. Endocardial high-frequency stimulation has been widely used in previous articles,[2,5,6] despite limitations caused by immediate rhythm disturbances and the requirement of specific equipment.

Spectral analysis of endocardial electrograms is an alternative method. Areas with right-shifted spectra (> 120 Hz) correlate with vagal-evoked response sites,[10] and it has been used as a diagnostic parameter to identify GP sites.[1,3,7,9,11]

Due to technical limitations of above mentioned methods, the anatomic location alone, conducted based on previous anatomic studies[12,13] emerged as a simple and attractive mapping strategy in recent articles.[6-8,14] It is based on the concept that, although the structural organization of the intrinsic autonomic system varies from heart to heart, the most critical sites of innervation to the sinoatrial and AV nodes locate in predictable areas.

The cardiac autonomic nervous fibers spread through out the entire atrial epicardial surface; therefore a comprehensive ablation would be necessary to promote a significant atria autonomic modulation. However, most of autonomic ganglia are concentrated in some specific areas of the atria, allowing that a tailored amount of radiofrequency promote sufficient autonomic modulation and avoiding extensive lesions and potential complications. Recent clinical observations have contributed to our understanding of the sinoatrial node and AV node innervation[2,3,6,7,8,14,15], and the interatrial septum emerged as a critical area, involved in most of the parasympathetic tone changes.[2,14] Ablation of both sides of the septum, near the anterior right GP and inferior right GP of the left atrium, and the superior and inferior GP of right atrium had the greatest impact on heart rate and atrial-His interval.[14] These data bring the perspective of performing ablation targeting the interatrial septum.

In specific cases, it was possible to observe that certain GP sites had differential effects on the sinoatrial and AV nodes, implying the presence of selective pathways in these structures.[8,14] These data raised the possibility of treating sinus node arrest with selective denervation of the sinus node, and managing advanced AV block with selective AV node therapy, although we still lack clinical evidence that these patterns are consistent in large populations.

Amongst all the technical aspects of the autonomic denervation procedure, finding the best endpoints to measure vagal modulation is one of the most controversial issues in the field.[14-20] Vagal-evoked response is a crude way to locate GP and, accordingly, evoked response abolition is a crude

Keywords

Bradycardia; Atrioventricular Block; Syncope, Vasovagal; Catheter Ablation; Ganglia, Autonomic.

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way to demonstrate clinical denervation. Spectral mapping with elimination of all right shifted signals can be useful but is a surrogate outcome and might result in large non-specific radiofrequency lesions.

Objective evaluation of the sinoatrial node and AV node function has been regarded as hard endpoints during denervation in recent articles.[3,8,14,21,22] Authors observed a significant shortening of heart rate, AH interval and Wenckebach cycle length that, combined with a negative or significantly blunted response to atropine (0,04 mg/Kg) were considered procedural primary endpoints.

A recent work by Pachon et al.[11] proposed a method of vagal stimulation by using an electrophysiological catheter placed in the internal jugular vein. This extracardiac technique has brought valuable data for denervation confirmation, although the presence of parasympathetic fibers also in most of the sympathetic thoracic nerves innervating the heart might limit its efficacy.[23]

Long-term follow-up results demonstrate that some technical aspects remain to be mastered, as symptom recurrence rates varying between 0 and 27%.[1,4,5,9,14] Clinical limitations of the cardiac denervation procedure lie in the complexity of the intrinsic autonomic system: GPs behave as integration centers with extensive signal modulation that makes a uniform and permanently successful outcome unlikely. The highly dense neuroanatomy of the atria raises the possibility that a significant portion of the innervation remain stunned but still functional after ablation. In that case, a redo procedure might be helpful.

Late vagal tonus recovery is another important cause of recurrences. Metaiodobenzylguanidine imaging studies revealed that denervation persists for at least 4 months,[24] but long-term evaluation are still lacking.

A large, multicenter clinical trial designed to determine the most proper method to perform denervation is of the essence. Yet, some available data seem consistent and valuable, as interventions on autonomic cardiac modulation became a worldwide standard procedure for management of functional bradycardia: 1- denervation is useful for refractory patients; 2- anatomic mapping emerge as a simple and effective approach; 3- the interatrial septum has a critical role, and 4- physiological evaluation (extracardiac stimulation, heart rate and AH interval shortening) combined with a negative atropine test seemed to be the most adequate endpoints.

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The Freedom of Clinicians and the Art of the Impossible

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Systemic arterial hypertension – or simply hypertension, in casual language – is considered the major and most common risk factor for death and disability of non-communicable diseases.[1,2] Its prevalence in Europe ranges from 30% to 45%.[3] In the United States, two thirds of the adults older than 60 years are hypertensive.[4] In South Asia and Sub-Saharan Africa, hypertension has increased rapidly.[5] Recently, the world prevalence of hypertension was estimated at 31%.[6]

The last three decades have witnessed the development of several effective and safe drugs to treat hypertensives. However, although a blood pressure reduction by only 10 mm Hg in those patients is known to reduce the risk of cardiovascular death and stroke by 25-40% throughout life,[7] the threshold value or target value to be achieved in hypertensive adults in general, and in the elderly in particular, is controversial. In addition, several patients remain poorly controlled despite treatment, without reaching the target values of the ESC/ESH Recommendations[3] or those suggested as a result of the SPRINT study.[8]

Several guidelines/recommendations for the diagnosis and treatment of hypertension have been published by scientific societies or other international and national public agencies, without reaching absolute consensus. Regarding the systolic blood pressure levels originally proposed by the *5th Joint National Committee* (< 140 mmHg)[9] and those emerging from the SPRINT study (< 120 mmHg), there is an indecision/decision range, and although it is believed that "lower blood pressure is better" for patients in general, the clinicians should decide.

Recommendations in medicine, originally clinical practice guides suggesting an approach for the management of difficult clinical situations, let clinicians free to adjust therapy according to the patient's specificity. For example, in case of hypertension, clinicians could decide upon a more "aggressive" therapy for younger patients, even if asymptomatic, or upon a more conservative one (admitting higher systolic blood pressure levels) for the elderly, supposedly – what is still a matter of discussion – more susceptible to complications from treatment itself.

That initial therapeutic flexibility has diminished, although not explicitly. The recommendations, written and edited based on studies not rarely different from the real world, began to define what clinicians should do in each circumstance, under penalty of their performance being considered poor clinical practice. Briefly, the "recommendations" became "guidelines", and the semantic change in Portuguese says a lot.

It is worth noting that attending physicians should always, taking into account their patients' characteristics – cardiovascular risk, general well-being, weaknesses and options – and weighing the drawbacks from occasional adverse effects of treatment, make the best decisions.

In this scenario, the guidelines now published were created, intended for the Federation of the Portuguese Language Societies of Cardiology (*Federação das Sociedades de Cardiologia de Língua Portuguesa* - FSCLP - www.fsclp.org). The FSCLP was created in 2014 aimed mainly at *"promoting the development of Cardiology to serve the population of countries and territories whose official language is Portuguese"* – (statutes, 4th article). Prior to its foundation, Lusophone Cardiology Meetings were held in Cape Verde (2009) and Mozambique (2011). The first FSCLP Congress was held in Portugal in 2016, and the second one will be held in Brazil in November 2017.

In the already-mentioned statutes, the pathways to substantiate the major objective are succinctly enunciated, the most important being: to stimulate the study and investigation of the scientific issues related to cardiovascular disease; to analyze the social aspects of heart diseases and their prevention, as well as patient care; and to narrow the relationship between the physicians of Portuguese-speaking societies and communities dedicated to cardiology. Concisely, to develop Lusophone Cardiology.

To create more guidelines[10] for the FSCLP that would not repeat what is already written seemed like an impossible challenge. Nevertheless, these Guidelines for *"Arterial Hypertension Management in Primary Heath Care in Portuguese Language Countries"* emerge valuable. Firstly, they depict accurately the reality of the Lusophone space, with its similarities and differences. Secondly, avoiding excessive observations, they do not leave essential aspects out. Thirdly – and decisively – they emphasize the importance of hypertension prevention and treatment in primary health care, which is, after all, their objective. Finally, they take into account the medical, social and economic characteristics of the space they are destined for.

In addition, these Guidelines[10] published here have another very significant merit: they are the first scientific and pedagogical work by the FSCLP, which is something to be proud of. These Guidelines are aimed at meeting the goals of the FSCLP and at taking a big step towards the beginning of a *"continuous process, involving mainly educational actions, lifestyle changes and guaranteed access to medicines"* for hypertension, as stated in the document itself.

The authors of these Guidelines have outlined with Art what seemed Impossible.

Keywords

Hypertension; Risk Factors; Blood Pressure / control & prevention; Primary Health Care.

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2017 Guidelines for Arterial Hypertension Management in Primary Health Care in Portuguese Language Countries

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Introduction

The World Health Organization (WHO) goal to reduce mortality due to chronic non-communicable diseases (CNCD) by 2% per year requires a huge effort from countries.[1-4] This challenge for health professionals asks for a global political action on control of social measures, with cost-effective population interventions to reduce CNCD and their risk factors (RF). Health professionals should demand from their government the implementation of acceptable cost measures, such as tobacco cessation counseling, guidance on healthy feeding practices and need for regular physical exercise, systemic arterial hypertension (SAH) control, and promotion of teaching and updating activities in programs directed to those issues. Those measures would contribute with around 70% of the goal of 2% per year reduction in CNCD.[2,5] Dyslipidemia, SAH and obesity are highly prevalent multifactorial diseases in Portuguese language countries (PLC).[5,6] Systemic arterial hypertension is the major RF for complications, such as stroke, acute myocardial infarction and chronic kidney disease, corresponding in importance to dyslipidemia and obesity for the development of atherosclerotic diseases.[5,6] In addition to their significant epidemiological impact, the non-pharmacological treatment of those cardiovascular RF plays a relevant economic role in the expenditures of the Ministries of Health, Social Security and Economy, because those affections are major causes directly or indirectly involved with absenteeism in the workplace. There is evidence that preventive actions are more promising in the primary health care setting.

The number of adults with SAH increased from 594 million in 1975 to 1.13 billion in 2015, being 597 million men and 529 million women. That increase might be due to both population aging and increase in number.[6] When analyzing the trends in blood pressure (BP) levels of 19.1 million adults from several population studies in the past four decades (1975-2015), the elevated levels shifted from high-socioeconomic-level countries to low-intermediate-socioeconomic-level countries of South Asia and Sub-Saharan Africa. However, BP levels remain high in Eastern and Central Europe and Latin America.[6]

Several trends were identified when analyzing the proportional mortality and percentage change in the mortality rates due to hypertensive diseases and their outcomes, ischemic heart diseases (IHD) and stroke, in the PLC from 1990 to 2015 (Table1). The highest proportional mortality rates due to hypertensive diseases were observed in Brazil, Mozambique and Angola. Portugal had the highest human development index (HDI) in 2015 and the highest mortality due to stroke.[7-9] The reduced access, around 50-65%, to essential pharmacological treatment in low-and low-intermediate-socioeconomic-level countries might have contributed to those results. In addition, in 40% of those countries there is less than 1 physician per 1000 in habitants, and a small number of hospital beds for the care of the uncontrolled-SAH-related outcomes.[7] Thus, joint actions to implement primary prevention measures can reduce the outcomes related to hypertensive disease, especially IHD and stroke. It is mandatory to ensure the implementation of guidelines for the management of SAH via a continuous process, involving educational actions, lifestyle changes and guaranteed access to pharmacological treatment.

Diagnosis and classification

The risk resulting from high BP levels increases with age, and every 2-mmHg elevation is associated with a 7% and a 10% increase in the risk of death due to IHD and stroke, respectively.[2] At the medical office, BP can be assessed by use of either the automated or auscultatory method, being elevated when systolic BP (SBP) ≥ 140 mm Hg and/or diastolic BP (DBP) ≥ 90 mm Hg, at least on two occasions.

The diagnosis of SAH is based on the measurement at the doctor's office of two or more high BP values on at least two occasions. The classification of BP according to measurements taken at the medical office, for individuals older than 18 years, is shown in Table 2. Ambulatory BP monitoring for 24 hours (ABPM)

Keywords

Hypertension / complications; Chronic Disease / mortality; Dyslipidemias; Obesity; Community of Portuguese-Speaking Countries.

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Table 1 – Proportional mortality and annual percentage of change in mortality rates in both sexes, all ages, from 1990 to 2015, due to hypertensive disease, ischemic heart disease and stroke, in addition to human development index (HDI) and population in 2015

Countries	Hypertensive disease	Ischemic heart disease	Stroke	HDI 2015	Population 2015*
	Proportional mortality (annual % change in mortality rates)				
Brazil	1.77 (+1.79)	14.44 (+0.44)	10.61 (+0.12)	0.754*	205,002,000
Mozambique	1.46 (+0.27)	3.84 (+1.25)	5.37 (+0.52)	0.418*	25,727,911
Angola	1.28 (-0.97)	4.65 (-0.96)	5.35 (-1.09)	0.533*	25,789,024
Portugal	1.08 (+1.20)	12.71 (-1.32)	14.96 (-2.32)	0.843*	10,374,822
Guinea-Bissau	0.53 (-0.43)	4.87 (+0.25)	5.07 (+0.22)	0.424*	1,844,000
East Timor	1.33 (+0.38)	11.84 (+1.16)	10.02 (+0.57)	0.605*	1,212,107
Macao	NA	NA	NA	0.566 #	642,900
Cape Verde	0.75 (-0.62)	11.74 (+1.34)	13.74 (-0.18)	0.648*	524,833
Saint Thomas and Prince	0.44 (-0.55)	8.18 (-0.41)	10.22 (-0.18)	0.574*	190,000

** last year available - 2015, # last year available - 2014, NA: not available. Source:[7-9]*

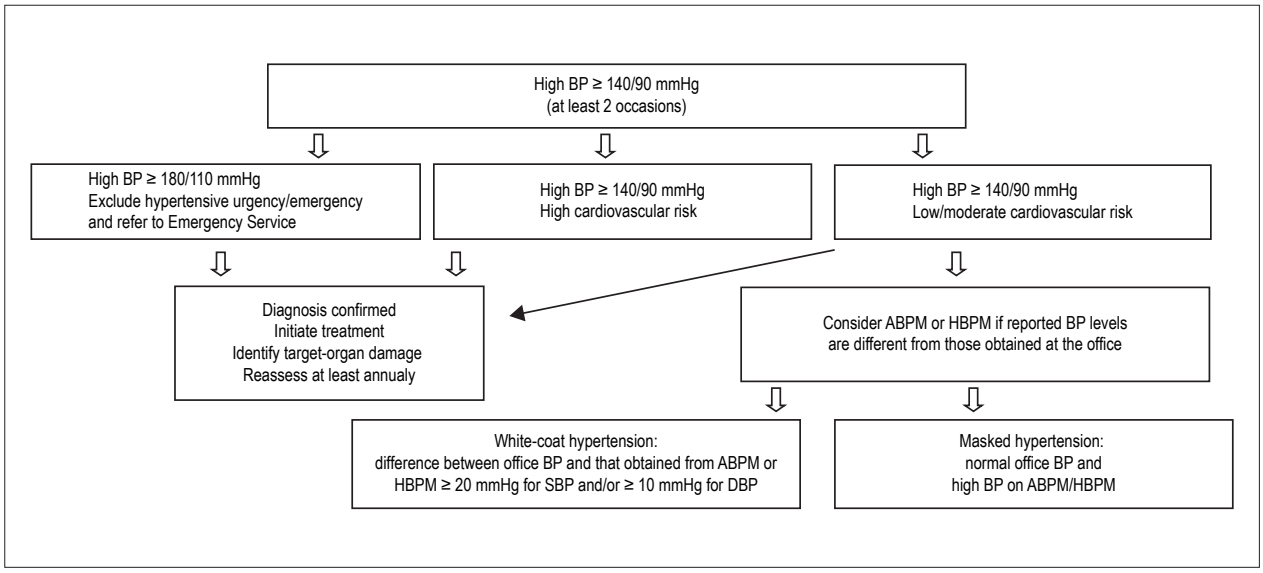

Figure 1 – *Flowchart for the diagnosis of arterial hypertension. BP: blood pressure; ABPM: ambulatory BP monitoring; HBPM: home BP monitoring; SBP: systolic BP; DBP: diastolic BP.*

or home BP monitoring (HBPM) can help in the diagnosis of white-coat hypertension (WCH) and masked hypertension (MH). The WCH relates to the difference between BP measured at the office (high) and that measured with ABPM or HBPM (normal). In MH, the situation is the opposite (Figure 1). In view of the suspicion of WCH and MH, ABPM is mandatory, and may be replaced by HBPM in communities where ABPM is not available. Figure 1 shows the flowchart for the diagnosis of SAH.

The ABPM enables the identification of circadian BP changes, especially those related to sleep. In ABPM, BP is considered increased when BP in 24 hours ≥ 130/80 mmHg, ranging from wakefulness ≥ 135/85 mm Hg to sleep ≥ 120/70 mmHg. For HBPM, BP is considered elevated when ≥ 135/85 mmHg.[1]

Recommended technique for measuring blood pressure

Initially the patients should be informed about the procedure, and the steps on Table 3 should be followed.[3,10,11] Blood pressure should be measured by all health professionals on every clinical assessment and at least once a year.

Clinical assessment and risk stratification

Complementary assessment is aimed at detecting target-organ damage (TOD), aiding cardiovascular risk stratification and identifying signs of secondary SAH. Table 4 shows the recommended complementary tests (routine and for specific populations).

- Target-organ damage should be investigated with the complementary tests shown in Table 4, in addition to the following exams:
- Left ventricular hypertrophy, assessed on electrocardiogram: Sokolow-Lyon index [S in V1 + R in V5 or V6 (whichever is larger)] > 35 mm; RaVL > 1.1 mV; Cornell index [S in V3 + R in aVL > 28 mm (men), and S in V3 + R in aVL > 20 mm (women)]; or on echocardiogram: left ventricular mass index ≥ 116 g/m² (men), and ≥ 96 g/m² (women);

Atherosclerotic disease in other sites and chronic kidney disease ≥ stage 3 [estimated glomerular filtration rate (eGFR) > 60 mL/min/1.73 m²] (Table 5).

Table 2 – Blood pressure classification according to measurements taken at the office for individuals older than 18 years

Classification	SBP (mm Hg)	DBP (mm Hg)
Normal	≤ 120	≤ 80
Prehypertension	121 – 139	81 – 89
Stage 1 hypertension	140 – 159	90 – 99
Stage 2 hypertension	160 – 179	100 – 109
Stage 3 hypertension	≥ 180	≥ 110

When SBP and DBP are in different categories, the highest should be used to classify BP.
Systolic hypertension is considered isolated if SBP ≥ 140 mm Hg and DBP < 90 mm Hg, and it should be classified into stages 1, 2 and 3. SBP: systolic blood pressure; DBP: diastolic blood pressure. Source: 7th Brazilian guideline for arterial hypertension management, 2016.[1]

Table 3 – Recommended technique for measuring office blood pressure by using the auscultatory method

• BP should be measured with a validated, calibrated and accurate sphygmomanometer, with cuff size adequate to arm circumference (according to the manufacturer's recommendation): usually cuff width close to 40% and cuff length covering 80-100% of arm circumference.
• The cuff should be placed snugly, 2-3 cm above the cubital fossa, with its compressive part centralized on the brachial artery, and the arm supported at heart level.
• The patient should rest at a calm environment for 5 minutes, sitting in a chair with back supported, legs uncrossed and feet on the floor. The patient should be relaxed, having neither exercised in the previous 30 minutes, nor consumed tobacco, alcohol or energetic foods (including coffee) in the previous 1 hour.
• In addition, BP will be measured after 2 minutes in the supine position with the arm supported, especially for diabetics and the elderly, and when orthostatic hypotension is suspected. It is worth noting that measuring BP in the sitting position will be useful for therapeutic decision-making, while that in the orthostatic position, for treatment changes in case of orthostatic hypotension.
• The cuff should be inflated rapidly up to 30 mm Hg above the level the radial pulse can no longer be palpated, and then deflated at approximately 2 mm Hg/beat. SBP will be determined by auscultation of the first sound (Korotkoff phase I), and DBP, by disappearance of the sounds (Korotkoff phase V). If the heart beats persist until level zero, determine DBP on the muffling of sounds (Korotkoff phase IV).
• The first reading should be discarded, and two sequential readings in both members should be taken, the highest one being recorded. If arrhythmia is present, more measurements should be taken to determine mean BP.
• Record the BP reading obtained for the patient. Reassess BP levels at least monthly until control is achieved, and then every 3 months.

BP: blood pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Risk stratification should consider the classical RF, relating them to BP levels as shown in Table 5.

The following risk factors are considered:

- male sex and age (men > 55 years and women > 65 years);
- smoking habit, dyslipidemia (triglycerides > 150 mg/dL; LDL-C > 100 mg/dL; HDL-C < 40 mg/dL), obesity (body mass index ≥ 30 kg/m^2), abdominal obesity (abdominal circumference > 102 cm for men, and > 88 cm for women), diabetes mellitus, abnormal oral glucose tolerance test or fasting glycemia of 102-125 mg/dL, and family history of premature cardiovascular disease (men < 55 years, and women < 65 years).

Treatment

Blood pressure reduction is followed by a significant cardiovascular risk reduction, which is higher in individuals at high cardiovascular risk, with a relative residual risk reduction in the other individuals.[2,11] Non-pharmacological therapy with changes in lifestyle (CLS) should be initially implemented for all stages of SAH and for individuals with BP of 135-139/85-89 mmHg (Table 6). For stage 1 hypertensives at low or intermediate cardiovascular risk, management can start with CLS, and 3 to 6 months can be waited before deciding to start pharmacological treatment. For the other stages, antihypertensive agents should be initiated as soon as the diagnosis is established.

A BP target lower than 130/80 mm Hg is recommended for patients at high cardiovascular risk, including those with diabetes mellitus, and lower than 140/90 mm Hg for stage 3 hypertensives. For patients with coronary artery disease, BP should not be lower than 120/70 mm Hg because of the risk of coronary hypoperfusion, myocardial damage and cardiovascular events. For elderly hypertensives ≥ 80 years, BP levels should be lower than 145/85 mm Hg. Special attention should be paid to patients with dark skin phenotype who will benefit more from the use of calcium-channel blockers.[12-14] Figure 2 shows the pharmacological approach to SAH.

When angiotensin-converting enzyme inhibitors (ACEI) are not tolerated, they should be replaced with low-cost angiotensin-receptor blockers (ARB). Beta-blockers should be considered for young individuals intolerant to ACEI and ARB, lactating women, individuals with increased adrenergic tone, and those with IHD or heart failure (HF). In case of intolerance to calcium-channel blockers (CCB) because of edema, or HF or suspected HF, diuretics can be used:

Table 4 – Recommended complementary tests (routine and for specific populations)

Routine tests for all hypertensive patients	
Urinalysis	Fasting glycemia and HbA1c
eGFR	Total cholesterol, HDL-C and serum triglycerides
Conventional ECG	Serum levels of creatinine, potassium and uric acid
Recommended tests to search for TOD in specific populations	
Chest X ray	Clinical suspicion of cardiac and/or pulmonary impairment. Aortic dilatation or aneurysm (if echocardiogram is not available). Suspicion of aorta coarctation.
Echocardiogram	Evidence of LVH on ECG or patients with clinically suspected HF. LVH = LV mass corrected for BS ≥ 116 g/m^2 (men) or 96 g/m^2 (women)
Albuminuria	Diabetic hypertensive patients, with metabolic syndrome or at least two RF. Normal values < 30 mg/24h.
Carotid US	Carotid murmur, CbVD signs, atherosclerotic disease in other sites. IMT values > 0.9 mm and/or atherosclerotic plaques.
Renal US or Doppler	Patients with abdominal masses or abdominal murmurs.
Exercise test	Suspicion or family history of CAD, DM.
Brain MRI	Patients with cognitive disorders and dementia. Detection of silent infarctions and micro hemorrhages.

HbA1c: glycated hemoglobin; eGFR: estimated glomerular filtration rate; TOD: target-organ damage; ECG: electrocardiogram; LVH: left ventricular hypertrophy; HF: heart failure; LV: left ventricular; BS: body surface; RF: risk factors; US: ultrasonography; CbVD: cerebrovascular disease; IMT: intima-media thickness; CAD: coronary artery disease; DM: diabetes mellitus; MRI: magnetic resonance imaging.

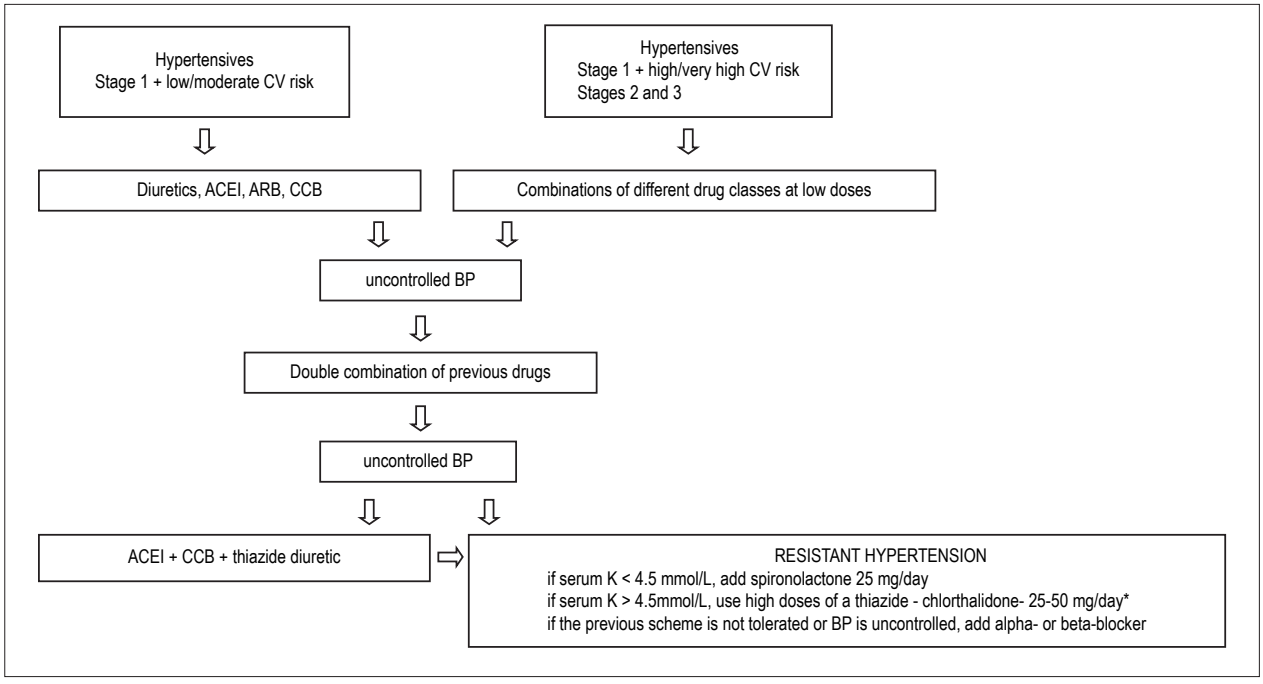

Figure 2 – *Flowchart for the treatment of arterial hypertension. (adapted from Malachias et al[1])*
*CV: cardiovascular; BP: blood pressure; ACEI: angiotensin-converting-enzyme inhibitor; ARB: angiotensin-receptor blocker; CCB: calcium-channel blocker. * the high doses of chlorthalidone should be used to replace another thiazide, as long as not used before.*

thiazide diuretics (chlorthalidone - 12.5-25 mg 1X day; indapamide - 1.5-2.5 mg 1X day). Individuals with dark skin phenotype should have ARBs rather than ACEIs for pharmacological combinations.[2,11-14]

Approximately two thirds of the patients will need combinations of at least two drugs to control BP. The advantage of the association is the synergism of different mechanisms of action, with dose reduction and consequent decrease in adverse effects, in addition to higher therapeutic adherence.

There is no preference for a therapeutic class of drug to treat a hypertensive patient with a previous stroke, but a BP lower than 130/80 mm Hg should be targeted.

Table 5 – Stratification based on risk factors, target-organ damage and cardiovascular or kidney disease

	SBP 130-139 or DBP 85-89	Stage 1 SAH SBP 140-159 or DBP 90-99	Stage 2 SAH SBP 160-179 or DBP 100-109	Stage 3 SAH SBP ≥ 180 or DBP ≥ 110
No risk factor	No additional risk	Low risk	Intermediate risk	High risk
1-2 risk factors	Low risk	Intermediate risk	High risk	High risk
≥ 3 risk factors	Intermediate risk	High risk	High risk	High risk
Presence of TOD, CVD, CKD or DM	High risk	High risk	High risk	High risk

BP: blood pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure; SAH: systemic arterial hypertension; TOD: target-organ damage; CVD: cardiovascular disease; CKD: chronic kidney disease; DM: diabetes mellitus. Source: 7th Brazilian guideline for arterial hypertension management, 2016.[1]

Table 6 – Recommendations for the non-pharmacological treatment of arterial hypertension

Measure	Recommendations	
Body weight control	Maintain BMI < 25 kg/m^2 up to 65 years of age;	
	Maintain BMI < 27 kg/m^2 after 65 years of age;	
	Maintain AC < 88 cm for women and < 102 cm for men.	
Dietary pattern	Adopt a diet rich in fruits and vegetables, with a reduced amount of saturated fat.	
	The DASH (Dietary Approach to Stop Hypertension) diet, with 2100 kcal/day as originally proposed, is the most used:	
	Fruits (portions/day)	4-5
	Vegetables (portions/day)	4-5
	Milk and dairy products < 1% fat (portions/day)	2-3
	Lean meat, fish and poultry (g/day)	< 180
	Oils and fats (portions/day)	2-3
	Seeds and nuts (portions/week)	4-5
	Added sugars (portions/week)	< 5
	Salt (portion/day)	~ 6 g (3000 mg of sodium)
	Whole grains (portions/day)	6-8
Moderate alcohol consumption	Limit daily alcohol consumption to 1 dose for women and low-weight individuals, and 2 doses for men.	
Physical activity	**For all hypertensives – population recommendation – physical activity practice**	
	Moderate, continuous (1 x 30 min) or cumulative (2 x 15 min or 3 x 10 min) physical activity (similar to walking): at least 30 min/day, 5 to 7 days/week.	
	Aerobic training	
	At least 3 times/week (ideally 5 times/week), minimum of 30 min (ideally 40 to 50 min); Several modalities: walking, running, dancing, swimming; Moderate intensity defined as: higher intensity that still allows talking (no breathlessness), and sensation of mild to moderate tiredness; Maintain training heart rate (THR) between the lower and upper THR calculated as follows: Lower THR = (maximum HR – resting HR) x 0.5 + resting HR*; upper THR = (maximum HR – resting HR) x 0.7+ resting HR* Ideally, the HR used to calculate the intensity of the aerobic training should be determined on a maximum exercise test, with patients on their usual medication. *Maximum HR: obtained either on a maximum exercise test with regular medications, or by calculating maximum HR estimated according to age (220 - age; not to be used for individuals with heart disease or hypertensives on beta-blockers or nondihydropyridine calcium channel blockers). Resting HR: measured after a 5-minute rest, lying down.	
	Resistance training	
	2 - 3 times/week, 8 - 10 exercises for the large muscle groups, prioritizing unilateral execution, when possible; 1 - 3 sets with 10 - 15 repetitions up to moderate fatigue (reducing the movement velocity and avoiding apnea, exhaling during contraction and inhaling when returning to the initial position); Long passive pauses: 90 - 120 s.	

BMI: body mass index; AC: abdominal circumference. Source: Adapted from the 7th Brazilian guideline for arterial hypertension management, 2016.[1]

Table 7 – Clinical situations with indication for or contraindication to specific drugs

Drugs with specific indication	
Clinical situation	Initial therapy indicated
Heart failure	ACEI/ARB, diuretics and BB
AMI, angina pectoris, percutaneous or surgical myocardial revascularization	ACEI/ARB, BB, ASA, statins
Diabetes mellitus	Thiazide diuretics, ACEI/CCB, BB
Chronic renal failure	ACEI/ARB, loop diuretics
Metabolic syndrome	CCB, ACEI/ARB
Aortic aneurysm	BB
Peripheral arterial disease	ACEI, CCB
Pregnancy	Methyldopa, CCB
Contraindicated drugs	
Clinical situation	Contraindicated therapy
Asthma and chronic bronchitis	Non-cardioselective BB
Pregnancy	ACEI, ARB
AV block	BB, nondihydropyridine CCB
Gout	Diuretics
Bilateral stenosis of the renal artery	ACEI, ARB

*ACEI: angiotensin-converting-enzyme inhibitor; ARB: angiotensin-receptor blocker; CCB: calcium-channel blocker; BB: beta-blockers; AMI: acute myocardial infarction; ASA: acetylsalicylic acid; AV: atrioventricular. * ACEI and ARB should not be associated, because of the ONTARGET study. Adapted from*[2,4]

Table 8 – Possible reasons of not achieving proper blood pressure control

- Inadequate adherence to medications, diet, physical activity practice, and consumption of salt, tobacco and alcohol.
- Associated conditions: overweight and obesity, obstructive sleep apnea, chronic pain, blood volume overload, chronic kidney disease, thyroid disease.
- Drug interaction: nonsteroidal anti-inflammatory drugs, corticosteroids, anabolic steroids, sympathomimetic drugs, decongestants, amphetamine, erythropoietin, cyclosporine, tacrolimus, licorice, monoamine oxidase inhibitors, serotonin and norepinephrine reuptake inhibitors.
- Suboptimal therapeutic regimen, low doses of drugs, inappropriate combinations of anti-hypertensive drugs, renal sodium retention (pseudotolerance).
- Secondary hypertension: renovascular disease, primary hyperaldosteronism, pheochromocytoma.

Source: Leung et al.[11]

Table 7 depicts the clinical situations with indication for or contraindication to specific drugs. For chronic kidney disease, ACEI and ARB reduce albuminuria, and thiazide diuretics are used for stages 1 to 3, while loop diuretics, for stages 4 and 5.[2,11-14]

Arterial hypertension in pregnancy

Pregnant women with uncomplicated chronic hypertension should have BP levels lower than 150/100 mmHg, but DBP should not be < 80 mmHg.[1,2,11-14] The use of ACEI and ARB is contraindicated during pregnancy, and atenolol and prazosin should be avoided. Methyldopa, beta-blockers (except atenolol), hydralazine and CCBs (nifedipine, amlodipine and verapamil) can be safely used.[2,11-14]

In chronic gestational hypertension with TOD, BP levels should be maintained under 140/90 mmHg, and the pregnant woman should be referred to a specialist for proper care during delivery and to avoid teratogenicity. Delivery should not be hastened if BP < 160/110 mmHg (with or without anti-hypertensive drugs) up to the 37th week. The fetal growth and amount of amniotic fluid should be monitored with ultrasonography between the 28th and 30th weeks and between the 32nd and 34th weeks, and with umbilical artery Doppler. During delivery, BP levels should be monitored continuously.[1,2,12-14] During the puerperium period, BP levels should be maintained under 140/90 mmHg, preferably with the following drugs, whose use is safe during lactation: hydrochlorothiazide, spironolactone, alpha-methyldopa, propranolol, hydralazine, minoxidil, verapamil, nifedipine, nimodipine, nitrendipine, benazepril, captopril and enalapril.[1,2,12-15]

Preeclampsia (PE) is defined by the presence of SAH after the 20th gestational week, associated with significant proteinuria or presence of headache, blurred vision, abdominal pain, low

Table 9 – Causes of secondary SAH, signs and complementary diagnostic tests

Clinical findings	Diagnostic suspicion	Additional studies
Snoring, daytime sleepiness, MS	OSAHS	Berlin questionnaire, polysomnography or home respiratory polygraphy with at least 5 episodes of apnea and/or hypopnea per sleep hour
RAH and/or hypopotassemia (not necessary) and/or adrenal nodule	Primary hyperaldosteronism (adrenal hyperplasia or adenoma)	Measurements of aldosterone (> 15 ng/dL) and plasma renin activity/concentration; aldosterone/renin > 30. Confirmatory tests (furosemide and captopril). Imaging tests: thin-sliced CT or MRI
Edema, anorexia, fatigue, high creatinine and urea, urine sediment changes	Kidney parenchymal disease	Urinalysis, eGFR calculation, renal US, search for albuminuria/proteinuria
Abdominal murmur, sudden APE, renal function changes due to drugs that block the RAAS	Renovascular disease	Renal Doppler US and/or renogram, angiography via MRI or CT, renal arteriography
Absent or decreased femoral pulses, decreased blood pressure in the lower limbs, chest X ray changes	Coarctation of the aorta	Echocardiogram and/or chest angiography via CT
Weight gain, decreased libido, fatigue, hirsutism, amenorrhea, 'moon face', 'buffalo hump', purple striae, central obesity, hypopotassemia	Cushing's syndrome (hyperplasia, adenoma and excessive production of ACTH)	Salivary cortisol, 24-h urine free cortisol and suppression test: morning cortisol (8h) and 8 hours after administration of dexamethasone (1 mg) at 12PM. MRI
Paroxysmal AH with headache, sweating and palpitations	Pheochromocytoma	Free plasma metanephrines, plasma catecholamines and urine metanephrines. CT and MRI
Fatigue, weight gain, hair loss, DAH, muscle weakness	Hypothyroidism (20%)	TSH and free T4
Intolerance to heat, weight loss, palpitations, exophthalmos, hyperthermia, hyperreflexia, tremors, tachycardia	Hyperthyroidism	TSH and free T4
Renal lithiasis, osteoporosis, depression, lethargy, muscle weakness or spasms, thirst, polyuria	Hyperparathyroidism (hyperplasia or adenoma)	Plasma calcium and PTH
Headache, fatigue, visual disorders, enlarged hands, feet and tongue	Acromegaly	IGF-1 and GH levels at baseline and during oral glucose tolerance test

MS: metabolic syndrome; OSAHS: obstructive sleep apnea-hypopnea syndrome; RAH: resistant arterial hypertension; CT: computed tomography; MRI: magnetic resonance imaging; eGFR: estimated glomerular filtration rate; US: ultrasonography; APE: acute pulmonary edema; RAAS: renin-angiotensin-aldosterone system; ACTH: adrenocorticotropin; AH: arterial hypertension; DAH: diastolic arterial hypertension; TSH: thyroid stimulating hormone; PTH: parathormone; IGF-1: insulin-like growth factor type 1; GH: growth hormone. Source: Malachias et al.[1]

platelet count (< 100,000/mm^3), elevation of liver enzymes (twice the baseline level), kidney impairment (creatinine > 1.1 mg/dL or twice the baseline level), pulmonary edema, visual or cerebral disorders and scotomas. Eclampsia occurs when grand mal seizure associates with PE. The use of magnesium sulfate is recommended to prevent and treat eclampsia, at an attack dose of 4-6 g IV for 10-20 minutes, followed by infusion of 1-3 g/h, usually for 24 hours after the seizure. In case of relapse, 2-4 g IV can be administered. The use of corticosteroids, IV anti-hypertensives (hydralazine, labetalol) and blood volume expansion are recommended. Patients should be admitted to the intensive care unit.[1,2,11-15]

Table 8 lists the reasons for not achieving proper BP control. It is worth noting the importance of ruling pseudoresistance out (WCH).

Secondary arterial hypertension

The prevalence of secondary SAH in the hypertensive population is around 3-5%. The most common cause of secondary SAH is renal parenchymal disease, responsible for 2-5% of the SAH cases. The adrenal causes of SAH and pheochromocytoma occur in less than 1% of all cases of SAH. However, 80% of the patients with Cushing's syndrome have SAH. Physicians must keep a high level of clinical suspicion when managing hypertensives of difficult control. Table 9 lists the clinical findings of the major etiologies of secondary SAH, associating them with the complementary tests that should be used to establish the diagnosis.

Similarly to CNCD, lifelong adherence to the SAH treatment is poor. In the first year, 40% of the patients quit regular treatment, which prevent them from profiting from a reduction in both TOD and cardiovascular events, such as myocardial infarction and stroke. The following factors are related to non-adherence to treatment: adverse effects, number of daily doses and drug tolerance. Fixed drug combinations increase adherence by enabling better individual adequacy, reducing the likelihood of irregular use of daily doses. The involvement of patients and families, as well as a multidisciplinary approach enhance adherence to treatment. The use of interactive apps that increase the participation of patients in BP control is suggested to encourage their persistence and regular medication use.[16]

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Association of Inflammation and Endothelial Dysfunction with Coronary Microvascular Resistance in Patients with Cardiac Syndrome X

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Abstract

Background: Although a proportion of CSX patients have impaired brachial artery flow-mediated dilatation (FMD) in response to hyperemia, suggesting that endothelial dysfunction in these patients may be systemic and not just confined to the coronary circulation; the underlying mechanisms triggering endothelial dysfunction in these patients are still incompletely understood.

Objectives: To assess the association of the index of Microcirculatory Resistance (IMR) with endothelial dysfunction and inflammation in patients with CSX.

Methods: We studied 20 CSX patients and 20 age and gender-matched control subjects. Thermodilution-derived coronary flow reserve (CFR) and IMR were measured using a pressure-temperature sensor-tipped guidewire. Brachial artery FMD was measured using high-resolution, two-dimensional ultrasound images obtained with a Doppler ultrasound device (HDI-ATL 5000, USA) with a 5 MHz to 12 MHz linear-array transducer.

Results: Compared with in control subjects, CFR was significantly lower (2.42 ± 0.78 vs. 3.59 ± 0.79, p < 0.001); IMR was higher (32.2 ± 8.0 vs. 19.5 ± 5.5, p < 0.001); the concentration of hs-CRP and FMD was higher (4.75 ± 1.62 vs. 2.75 ± 1.50; 5.24 ± 2.41 vs. 8.57 ± 2.46, p < 0.001) in CSX patients. The Duke treadmill score (DTS) was correlated positively to CFR and FMD (0.489 and 0.661, p < 0.001), it was negative to IMR and hsCRP (-0.761 and -0.087, p < 0.001) in CSX patients.

Conclusions: The main finding in this study is that the DTS measured in patients with CSX was associated to hsCRP and FMD. Moreover, the independent effects of exercise tolerance can significantly impair FMD and hsCRP in CSX patients; especially it is particularly important to whom where FMD was associated negatively with IMR. (Arq Bras Cardiol. 2017; 109(5):397-403)

Keywords: Acute Coronary Syndrome; Endothelium / physiopathology; Inflammation; Brachial Artery.

Although multiple pathophysiologic abnormalities have been reported in cardiac syndrome X (CSX), generalized endothelial dysfunction and inflammation are accepted as major pathophysiologic mechanisms.[1] It has been proposed that because of endothelial dysfunction, reduced coronary vasodilatation and abnormal arterial constriction comes about in patients with CSX.[2] It was also demonstrated that endothelium-dependent and independent dilatation is impaired in CSX. Masci PG et al[3] have shown that a proportion of CSX patients have impaired brachial artery flow-mediated dilatation (FMD) in response to hyperemia suggesting that endothelial dysfunction in these patients may be systemic and not just confined to the coronary circulation. However, the underlying mechanisms triggering endothelial dysfunction in these patients are still incompletely understood. Studies have shown that impaired brachial artery FMD is significantly associated with elevated hs-CRP concentrations in patients with CSX[4]. These were an important role for inflammation in the modulation of coronary microvascular responses in patients with CSX.[5]

The Index of Microcirculatory Resistance (IMR) is measured at peak hyperemia, thereby eliminating the variability of resting vascular tone and hemodynamics. With recent technological advances, it is now possible to measure pressure while estimating coronary flow with a single pressure-temperature sensor-tipped coronary wire.[6] Therefore, IMR is a readily available, quantitative and reproducible, wire-based method for invasively assessing coronary microvascular function independent of the epicardial artery in the cardiac catheterization laboratory.[7,8]

The aim of the present study was to assess the association of IMR with endothelial dysfunction and inflammation in patients with CSX.

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Methods

Patient population and study protocol

The present prospective study was conducted in the Department of Cardiology, the First Affiliated Hospital of Sun Yat-sen University. We enrolled 20 CSX patients (CSX group) who fulfilled the diagnostic criteria for CSX in the present study as follow:[5] (1) a typical history of exertional angina; (2) a positive exercise treadmill test; and (3) angiographically normal epicardial coronary arteries without minimal irregularities.

This study was our series of research on coronary microvascular dysfunction, and for the first time, we directly demonstrated the relationship of inflammation and endothelial dysfunction in increased microvascular resistance in CSX patients in the small size sample of our previous study.[9]

The control group consisted of 18 age- and gender-matched subjects who were referred for diagnostic coronary angiography due to atypical chest discomfort, with a negative exercise treadmill test and completely normal coronary arteries at angiography.

Subjects with any of the following clinical conditions were excluded from this study: other specific forms of cardiac disease (for example, variant angina, cardiomyopathies, and valvular or congenital heart disease), any regional wall motion abnormalities at resting echocardiogram, ejection fraction less than 50%, atrial fibrillation or left bundle branch block on ECG, uncontrolled hypertension (> 160/100 mmHg) or diabetes mellitus (fasting plasma glucose > 7.0 mmol/L and/or postprandial glucose > 11.0 mmol/L), systemic disorders, and liver or renal insufficiency.

The hospital Ethics Committee approved the study protocol, and each subject gave written informed consent to the study.

Coronary angiography

In all study participants, selective coronary angiography was performed using standard Judkins technique. Coronary arteries were visualized in left and right oblique planes with cranial and caudal angulations. Injection of contrast medium (Iopromide, Ultravist-370; Schering AG, Berlin, Germany) was carried out by an automatic injector, at a speed of 5 ml/s for left coronary artery and 3 ml/s for right coronary artery.

All anti-angina and anti-ischemic medications, except sublingual nitroglycerin, were withheld for at least one week before the examination. All coronary angiograms were analyzed by two experienced independent investigators and only angiograms with visually smooth contours with no wall irregularities were accepted as normal.

Intracoronary thermodilution measurements

After coronary angiography, a coronary pressure wire (PressureWire-4, Radi Medical Systems, Wilmington, Mass.) was calibrated outside the body, equalized to the guiding catheter pressure with the sensor positioned at the ostium of the guiding catheter, and then advanced it until the wire sensor was located in the distal third of the left anterior descending coronary artery, with the transducer distance at 7~10 cm from the guide tip.

With commercially available software (Radi Medical Systems), the shaft of the pressure wire can act as a proximal thermistor by detecting changes in temperature-dependent electrical resistance. The sensor near the tip of the wire simultaneously measures pressure and temperature and can thereby act as a distal thermistor. The transit time of room-temperature saline injected down a coronary artery can be determined using a thermodilution technique.[5] Three injections of saline (3 mL, room temperature) were administered into the coronary artery, and the baseline mean transit time (bTmn) was measured. Intravenous adenosine (140 ug/kg/min) was then administered to induce steady-state maximal hyperemia, then three more injections of saline (3 mL, room temperature) were given, and the hyperemic mean transit time (hTmn) was measured. Simultaneous measurements of mean aortic pressure (Pa, by guiding catheter) and mean distal coronary pressure (P*d*, by pressure wire) were also made in the resting and maximal hyperemic states. CFR was calculated as bTmn divided by hTmn. IMR was calculated as the distal coronary pressure at maximal hyperemia divided by the inverse of hTmn. Fractional flow reserve (FFR) was calculated by the ratio of Pd/Pa at maximal hyperemia.

Measurement of FMD in the brachial artery

Brachial artery FMD was measured using a standardized technique.[10] All subjects were studied in the morning, having abstained from alcohol, caffeine and food for 12 h before the test. The diameter of the artery was measured using high-resolution, two-dimensional ultrasound images obtained with a Doppler ultrasound device (HDI-ATL 5000, USA) with a 5 MHz to 12 MHz linear-array transducer. The right brachial artery was scanned over a longitudinal section, approximately 3 cm to 5 cm above the right elbow. Depth and gain settings were optimized to identify the lumen-to-vessel wall interface. An ECG monitor integrated with the ultrasound machine was also applied. When an optimal image of the brachial artery was obtained, the surface of the skin was marked, and the arm was maintained in the same position throughout the study. Following the measurement of baseline brachial artery diameter, a pneumatic tourniquet placed around the forearm distal to the target artery was inflated to a pressure of 250 mmHg, and inflation was held for 5 min. Reactive hyperemia in the brachial artery was then induced by rapid cuff deflation. A second scan was performed continuously for 60 s before and 120 s after cuff deflation. The ultrasound images were recorded using digital video. The diameter of the brachial artery was measured from the anterior to the posterior interface between the media and adventitia at a fixed distance. The mean diameter was calculated from four cardiac cycles synchronized using the R-wave peaks on the ECG. All measurements were recorded at end-diastole to avoid possible errors resulting from variable arterial compliance. Maximal vasodilation was observed 60 s after cuff release. FMD was calculated as the percentage change in artery diameter from baseline to reactive hyperemia. All of the ultrasonographic assessments were performed by the same blinded radiologist.

Serum high-sensitive C-reactive protein measurements

Blood samples were immediately centrifuged at 2000 g for 10 min, and serum was stored at −80°C for the subsequent analysis of biochemical markers. The level of hsCRP was measured at 550 nm by a particle enhanced immunoturbidimetric assay (Orion Diagnostic, USA) on a Hitachi 7170A (Hitachi, Japan) analyzer. The lower limit of detection for the method is 0.125 mg/L. The interassay coefficient of variations were 0.14% and 2.1% at mean values of 8.20 mg/L and 0.31 mg/L, respectively; the intra-assay coefficients of variation were 0.13% and 2.3% at mean values of 8.21 mg/L and 0.30 mg/L, respectively. An hs-CRP concentration > 3 mg/L was considered abnormal as suggested previously.[11]

Statistical analysis

The statistical analysis was performed using SPSS 13.0 software. Data were expressed as mean ± SD unless otherwise specified. Categorical variables were presented using the absolute number and its proportion. Continuous variables between groups were compared by paired Student's *t* test. Proportions were compared by the Fisher exact test when the expected frequency was < 5, otherwise the chi-square test was applied. If the data followed a normal distribution, two-sample independent Student's T test was used; otherwise, two-sample Wilcoxon Rank Sum Test was used. Pearson's correlation analysis was used to test univariate relations. Significance was considered to be achieved for two-tailed p values < 0.05.

Results

Study population and clinical demographics in two group of patients

Patients' clinical demographics and characteristics for the use of medications in both groups are documented in Table 1. The two groups were similar for age, gender, height, weight, cardiovascular risk factors, blood pressure, heart rate, and left ventricular function, and there were no differences in characteristics for the use of medications between these two groups.

Comparisons of CFR, IMR and FFR between groups

No significant difference was present in exercise duration between groups. Thermodilution-derived IMR could be easily measured in all studied cases. CFR and IMR were significantly lower in CSX group than in Control group, and the FFR values were not different in two groups. The mean values for CFR and IMR was 2.42 ± 0.78 vs 3.59 ± 0.79 and 32.2 ± 8.0 vs 19.5 ± 5.5, whereas FFR (0.95 ± 0.02 and 0.94 ± 0.03) showed no differences (p > 0.05 for all comparisons) (Table 2).

Relationships between hs-CRP, FMD, IMR and DTS

In CSX patients, the DTS was correlated negatively to IMR (r = -0.761, p < 0.001) and positively to FMD (r = -0.661, p = 0.002) with CFR (r = 0.489, p = 0.029), while evaluating correlations of myocardial ischemic threshold with IMR and CFR, we found that exercise duration was correlated

Table 1 – Clinical Demographics in cardiac syndrome X patients and controls

Variable	CSX group (n = 20)	Control group (n = 20)	p Value*
Age, y	53.6 ± 9.8	54.7 ± 10.0	0.727
Male gender, n (%)	5 (25.0%)	6 (30.0%)	0.709
Height, cm	162.0 ± 8.0	161.9 ± 7.5	0.968
Weight, kg	63.2 ± 7.6	62.3 ± 8.3	0.730
Risk factors, n (%)			
Systemic hypertension	10 (50.0%)	7 (35.0%)	0.312
Diabetes mellitus	5 (25.0%)	4 (20.0%)	0.690
Hypercholesterolemia	4 (20.0%)	6 (30.0%)	0.441
Current smokers	2 (10.0%)	3 (15.0%)	0.614
Family history of coronary disease	3 (15.0%)	2 (10.0%)	0.614
Systolic blood pressure, mmHg	127.8 ± 12.2	125.9 ± 12.6	0.622
Diastolic blood pressure, mmHg	73.7 ± 10.2	73.8 ± 6.9	0.971
Rest heart rate, beats/min	70.5 ± 7.5	68.2 ± 8.7	0.376
Left ventricular ejection fraction, %	58.9 ± 7.6	60.3 ± 6.8	0.555
Use of medication, n (%)			
Aspirin	16 (80.0%)	16 (80.0%)	1.000
Statins	12 (60.0%)	9 (45.0%)	0.317
Beta-blockers	11 (55.0%)	7 (35.0%)	0.180
ACE inhibitors and/or ARB	4 (20.0%)	6 (30.0%)	0.441

Values are given as number of patients (%) or mean ± SD. ACE: angiotensin-converting enzyme; ARB: angiotensin II receptor blocker; (*)Mann-Whitney test.

Table 2 – Comparisons of coronary physiology and exercise test results in cardiac syndrome X patients and controls

Variable	CSX group (n = 20)	Control group (n = 20)	p Value*
CFR	2.42 ± 0.78	3.59 ± 0.79	< 0.001
IMR	32.2 ± 8.0	19.5 ± 5.5	< 0.001
FFR	0.95 ± 0.02	0.94 ± 0.03	0.470
Exercise duration (minutes)	7.60 ± 2.30	9.20 ± 2.86	0.059
Net exercise-induced			
ST-segment deviation (millimeters)	1.85 ± 0.75	0.25 ± 0.25	< 0.001
Treadmill angina index	0.45 ± 0.69	0.00 ± 0.00	0.006
Double product (mmHg.bpm)	26051 ± 6177	27366 ± 5247	0.472
DTS	-2.2 ± 7.7	8.1 ± 3.3	< 0.001
Serum hsCRP, mg/L	4.75 ± 1.62	2.75 ± 1.50	< 0.001
Brachial FMD, %	5.24 ± 2.41	8.57 ± 2.46	< 0.001

Values are given as mean ± SD. CFR: coronary flow reserve; IMR: index of microvascular resistance; FFR: fractional flow reserve; DTS: Duke treadmill score; bpm: beats per minute; FMD: flow-mediated dilation; (*)Mann-Whitney test.

positively to FMD (r = 0.448, p = 0.048) with CFR (r = 0.599; p = 0.005) and negatively to IMR (r=−0.604; p = 0.005), however, the DTS and exercise duration was not relevant with hsCRP (r = -0.087, p = 0.716 and r = 0.016, p = 0.948) and FFR (r = -0.309, p = 0.761and r = -0.091, p = 0.703). Importantly, the FMD was associated negatively with IMR (r = -0.869, p < 0.001). Interestedly, double product was significantly correlated to hsCRP (r = -0.502; p = 0.024) and not significantly correlated to FMD (r = 0.328, p = 0.158), CFR (r = 0.429, p = 0.059), IMR (r = -0.399, p = 0.082), and FFR (r = 0.015, p = 0.951) (Table 3).

In control group, the DTS, exercise duration and double product was all not correlated to hsCRP, FMD, CFR, IMR, and FFR.

Discussion

In our past study, we firstly found that coronary microvascular dysfunction in patients with CSX was represented by the increased IMR and the impaired CFR. For the further study, the main finding in this study is that the DTS measured in patients with CSX was associated of hsCRP and FMD. An interesting secondary finding is that double product was significantly correlated to hsCRP. These findings reinforce the importance of inflammation and endothelial dysfunction in the microvascular dysfunction of CSX patients. They also highlight the independent effects of exercise tolerance can significantly impair FMD and associated of hsCRP in CSX patients. Thirdly, it is particularly important to that the FMD was associated negatively with IMR.

IMR is a quantitative, reproducible index that is independent of epicardial coronary disease and specific for the microcirculation. Our previous studies suggested that IMR might be a more superior and reliable index reflecting coronary microcirculatory function. CSX patients are often strongly symptomatic and difficult to manage, which affects microvascular systems and increases vascular tonus. In our past study, coronary microvascular dysfunction in CSX patients was associated with increased IMR, we further documented that the FMD was associated negatively with IMR. It has been suggested that coronary microvascular dysfunction is caused by showed a correlation between coronary microvascular endothelial dysfunction and peripheral vascular dilatation. Our findings also agree with data from Tondi P et al.,[12] who reported FMD in patients who had cardiac syndrome X.

Several studies have demonstrated that endothelial dysfunction and inflammation can be pathophysiological mechanisms of CSX patients, and our results were completely consistent with these opinions.[13,14] Traditional cardiovascular risk factors, insulin resistance,[15] and estrogen deficiency[16] have been reported to be highly prevalent in CSX patients and probably contribute to microcirculatory dysfunction in these patients. Moreover, Ong et al.[4] confirmed a significant correlation between obesity and reduced flow mediated dilatation as well as with hs-CRP concentrations, and they suggested that obesity contributes to low-grade inflammation and impaired FMD in CSX patients. Further studies confirmed that impairment balance between tPA, PAI-1 endothelin-1 and NO, increased sympathetic tonus, abnormal coronary flow reserve, and microvascular spasm were possibly one of the mechanisms of CSX.[17-22] The level of C reactive protein in patients with chest pain and normal coronary arteries correlates with the frequency and duration of chest pain and the extent of ST-segment depression on exercise testing and ambulatory monitoring.[23] Verma et al.[24] reported that recombinant human CRP directly inhibits endothelial progenitor cells differentiation, survival and function at concentrations known to predict adverse vascular outcomes. Therefore, the enhanced extent of inflammation observed in patients with CSX may reduce endothelial progenitor cells levels and function in blood circulation, resulting in attenuated repair capacity of vasculature. Our data supported these findings that hsCRP was significantly correlated to DTS and double product in CSX patients, and it was suggested that exercise tolerance can significantly associated of hsCRP in

Table 3 – Pearson correlation between hs-CRP, FMD, IMR and DTS in cardiac syndrome X patients and controls group

Variable	CSX group (correlation coefficient)			Control group (correlation coefficient)		
	DTS (p value)	exercise duration (p value)	double product (p value)	DTS (p value)	exercise duration (p value)	double product (p value)
CFR	0.489* (0.029)	0.599* (0.005)	0.429 (0.059)	0.241 (0.307)	-0.126 (0.598)	0.229 (0.332)
IMR	-0.761* (0.000)	-0.604* (0.005)	-0.399 (0.082)	0.156 (0.511)	0.053 (0.823)	-0.258 (0.272)
FFR	-0.073 (0.761)	0.091 (0.703)	0.015 (0.951)	0.143 (0.548)	0.252 (0.284)	0.293 (0.210)
hsCRP, mg/L	-0.087 (0.716)	0.016 (0.948)	-0.502* (0.024)	-0.006 (0.980)	-0.072 (0.763)	-0.430 (0.058)
FMD, %	0.661* (0.002)	0.448* (0.048)	0.328 (0.158)	-0.254 (0.279)	-0.092 (0.701)	0.036 (0.880)

*CFR: coronary flow reserve; IMR: index of microvascular resistance; FFR: fractional flow reserve; DTS: Duke treadmill score; bpm: beats per minute; FMD: flow-mediated dilation; (*p < 0.05, Statistical test performed: linear regression analysis and Pearson's correlation coefficient).*

CSX patients. This suggests that the inflammatory damaged vascular endothelial function, further affecting the cardiac microvascular reserve function, influenced the exercise tolerance in CSX patients.

FMD is a noninvasive index of endothelial function and vascular health in humans. During the past decades, a large body of evidence accumulated has indicated that an impaired FMD is detectable in CSX patients, suggesting a generalized abnormality in vascular function.[11] Our data also shows the same result that FMD was associated with IMD and it reflected the microvascular function in CSX patients. Masci et al.,[3] showed that these patients with lower FMD responses had a higher probability of having transient myocardial perfusion defects on thallium-201 single-photon emission computed tomographic. Furthermore, Lekakis et al.[25] suggested that CSX patients had significantly lower brachial artery FMD in response to hyperemia than healthy controls. In addition, it has been demonstrated that coronary microvascular dysfunction as well as peripheral artery endothelial dysfunction in CSX can be associated with elevated CRP concentrations. Teragawa et al.[26] showed, for example, that impaired microvascular coronary responses to intracoronary acetylcholine were significantly more common in patients with chest pain, normal coronary arteriograms and elevated CRP levels compared to patients with normal CRP concentrations.

Study limitations

This is a prospective, observational study designed to demonstrate invasively coronary microvascular dysfunction in CSX patients. The primary limitation was the comparatively small number of patients, but the diagnostic criteria we adopted were well-characterized, including only patients with completely normal coronary angiograms, effort-induced angina pectoris, and positive exercise stress test, which resulted in a relatively low incidence of CSX. Second, we did not exclude subjects with traditional cardiovascular risk factors, such as hypertension, diabetes mellitus, hypercholesterolemia or smoking, which can also influence vascular function and lead to CMD. However, the CSX patients were strictly matched to a control group, with no differences between groups in clinical demographics and cardiovascular risk factors, which gives strength to our findings.

Conclusion

The main finding in this study is that the DTS measured in patients with CSX was associated of hsCRP and FMD. Moreover, the independent effects of exercise tolerance can significantly impair FMD and be associated with hsCRP in CSX patients, especially, it is particularly important to those where FMD was associated negatively with IMR.

Author contributions

Conception and design of the research: Long M, Huang Z, Liao X, Luo C; Acquisition of data: Long M, Huang Z, Luo C; Analysis and interpretation of the data and Statistical analysis: Long M, Zhuang X; Obtaining financing: Long M, Huang Z, Liao X; Writing of the manuscript: Long M, Huang Z, Liao X, Luo C; Critical revision of the manuscript for intellectual content: Long M, Liao X, Luo C; Supervision: Long M, Guo Y, Liao X.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Large Accumulation of Collagen and Increased Activation of Mast Cells in Hearts of Mice with Hyperlipidemia

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Abstract

Background: Hyperlipidemia, which is characterized by an elevation of lipids in the bloodstream, is a major risk factor for cardiac disease.

Objectives: The present study investigated the role of fibrosis in the progression of hyperlipidemia in the mice heart, and whether mast cell activation was associated with the fibrosis process.

Methods: Hyperlipidemia was produced in C57BL/6 mice by feeding them on a high-fat diet for 8 weeks. To assess tissue fibrosis, picrosirius red staining was performed. Hematoxylin & eosin (H&E) staining was performed to identify the histopathological changes in the hearts. Immunohistochemistry was also accomplished to determine the localization of transforming growth factor (TGF)-β and α-smooth muscle actin (α-SMA). Western blotting was performed to analyze the expression of chymase, tryptase, TGF-β, α-SMA and activity of Wnt/β-catenin pathway. At the end, serum total cholesterol (TC) and triglycerides (TG) levels were measured. All the values were expressed as means ± SD, the statistical significance level adopted was 5%.

Results: Hyperlipidemia mice showed significantly increased collagen deposition in the hearts compared with normal mice. In addition, H&E staining showed significant cellular degeneration. Cardiac muscle was arranged in disorder with fracture in mice of the model group. Immunohistochemistry and western blot analysis revealed that expression levels of tryptase, chymase, β-catenin, TGF-β and α-SMA were significantly increased in the hyperlipidemia mice compared with the control group.

Conclusions: The results indicated that mast cell activation might induce cardiac fibrosis by tryptase and chymase in hyperlipidemia, which had a close relationship with the increased activity of TGF-β/Wnt/β-catenin pathway. (Arq Bras Cardiol. 2017; 109(5):404-409)

Keywords: Rats; Hyperlipidemias / physiopathology; Heart; Fibrosis; Leukemia, Mast-Cell.

Introduction

Hyperlipidemia refers to hypercholesterolemia, hypertriglyceridemia and mixed hyperlipidemia, and is a very common biochemical disorder[1] with significantly risk of cardiovascular disease.[2,3] It is reported that the hyperglycemia, another metabolic disorder, has a strong association with cardiac fibrosis that may reduce myocardial compliance and contribute to the pathogenesis of heart failure.[4,5] However, the role of the hyperlipidemia in the development of cardiac fibrosis is poorly understood.

Cardiac fibrosis, which is a common pathologic feature of end-stage heart disease, always results in serious cardiac dysfunction.[6,7] Therefore, potential causes of cardiac fibrosis have to be investigated. Although cardiac fibrosis is an important stage in the progression of heart disease, the mechanisms underlying fibrosis development and progression are still unclear. The process of fibrosis is mechanically characterized by myofibroblast accumulation, collagen deposition, extracellular matrix remodeling, and increased tissue stiffness, so that impairs the organ's function by reducing tissue elasticity and compliance. As reported, the Wnt/β-catenin/TGF-β signaling pathway is a key mediator of fibroblast activation[8,9] and play important role in driving the aberrant synthesis of the extracellular matrix in heart fibrotic diseases.[10,11]

Mast cells have been recognized as important effector cells in tissue fibrosis.[12,13] Potentially mast cell activation and degranulation could result in the release of inflammatory and profibrotic mediators promoting tissue fibrosis.[14] Mast cells express serine proteases: tryptase and chymase, are associated with fibrosis in various diseases.[15,16] As reported, hyperlipidemia could skew the transcriptional regulation of pro-inflammatory factors of myeloid cell, including mast cells, to promote myeloid cell extravasation and atherosclerosis.[17,18] However, little is known about their involvement in cardiac fibrosis in hyperlipidemia mice.

In this study, we aimed to investigate whether hyperlipidemia is associated with cardiac fibrosis. Furthermore, we evaluated the activity of TGF-β/Wnt/β-catenin pathway, the expression levels of tryptase, chymase and α-SMA in hyperlipidemia mice.

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Methods

Declaration of ethics in animals

Eight-week-old C57BL / 6 mice were purchased from the Experimental Animal Center of Dalian Medical University (Dalian, China). Mice were allowed access to water and food ad libitum, but fasted overnight with water available before surgery. All animal experiments were approved by the ethics committee of Dalian Medical University and performed in accordance with the institutional guidelines, and in accordance with the Helsinki Declaration.

Induction of experimental hyperlipidemia

Via random number table, the animals were divided into two experimental groups (n = 8, for convenience) : control group received a normal diet , and the hyperlipidemia (H-lipid) group received high fat diet (HFD, D12451, Research diet, USA) for 8 weeks. Hyperlipidemia was documented by estimating the level of total cholesterol (TC) and triglycerides (TG) in serum using commercially available kits (*Jiancheng Biotech*, Nanjing, China).

Morphological changes

After 8 weeks' high-fat diet, the mice were sacrificed. The hearts of mice were removed, and fixed in 10% (v/v) neutral formalin and processed by standard histological techniques. The hearts were stained with haematoxylin and eosin (H&E) and picrosirius red staining. Then they were examined for the morphological changes. The hearts of mice were also used to determinate expression of protein of chymase, tryptase, TGF-β, β-catenin and α-SMA by western blot analysis and immunohistochemical staining.

Western Blot analysis

According to the manufacturer's instructions, proteins were extracted from mice hearts with protein extraction kit (*KeyGen Biotech*, Nanjing, China). Protein was measured according to the procedure of bicinchoninic acid (BCA) (Solarbio, Beijing, China), with bovine serum albumin as the standard. Proteins (20 μg) were resuspended in electrophoresis sample buffer containing β-mercaptoethanol and separated by electrophoresis on a pre-cast 10% SDS-polyacrylamide gel (Bio-Rad, Hercules, CA), followed by electrotransfer to a PVDF membrane (Millipore, Bedford, MA). Membranes were blocked using 5% non-fat milk in Tris-buffered saline with 0.1% Tween-20 (TBST) for 2 h at 37°C. β-Actin served as loading control. Membranes were incubated overnight at 4°C with a 1:1000 dilution of polyclonal antibody for tryptase, chymase, TGF-β, β-catenin and α-SMA respectively (*Beijing Boisynthesis Biotechnology*, China), and with a 1:1500 dilution of monoclonal antibody for β-actin (Beyotime, China). After subsequent washing with TBST, the blots were then incubated with secondary antibodies. After extensive washing with TBST, membranes were exposed to the enhanced chemiluminescence-plus reagents (ECL) from Beyotime Institute of Biotechnology (Haimen, China) according to the manufacturer's protocol. Emitted light was documented with a BioSpectrum-410 multispectral imaging system with a Chemi HR camera 410(Bio-Rad, Hercules, CA, USA). Protein bands were visualized and photographed under transmitted ultraviolet light. The image was used for semiquantitative measurements based on band densitometry.

Immunohistochemical staining

Histological sections of mice hearts (4 μm thick) were mounted on poly-L-lysine-coated slides. Slides were deparaffinized in xylene and rehydrated in graded alcohols. Sections were pretreated with citrate buffer (0.01 mol/L citric acid, pH 6.0) for 20 min at 95 °C. Then, at room temperature, they were immersed in PBS containing 3% H_2O_2 for 10 min. After exposing them to 10% normal goat serum in PBS for 30 min at room temperature, the tissue sections were incubated at 4°C overnight with rabbit polyclonal anti-α-SMA and TGF-β (dilution 1:100). Then sections were rinsed with PBS, incubated with biotinylated goat anti-rabbit IgG for 20 min at room temperature and treated with 3,30-diaminobenzidine chromogen for 5 min at room temperature. Finally, sections were counterstained with hematoxylin for 6 min.

Collagen quantification

Picrosirius red staining was performed with serially sectioned tissues. Paraffin-embedded tissues were deparaffinized in xylene, rehydrated in graded alcohols and then incubated in 0.1% Sirius Red solution for 1 h at room temperature. Finally, sections were counterstained with hematoxylin for 2 min. The sections were studied under a light microscope at different magnifications

Data analysis

Statistical analysis was computed using SPSS 13.0 software. Group data were expressed as mean ± S.D. Shapiro-Wilk test was used to check the normality of the studied data, and, then, parametric or non-parametric tests were used for the analysis of normal or non-normal data distribution, respectively. Data with normal distribution underwent unpaired Student's sample t-test. In all statistical analyses, Two-sided $p < 0.05$ was considered to indicate a statistically significant result.

Results

Serum biochemistry changed after high-fat diet treatment

Following continuous feeding with high-fat diet for 8 weeks, serum levels of TC and TG in the H-lipid group were significantly higher than the control group (Table 1).

Histological examinations

H&E staining of heart tissues showed that myocardial fibers in control group were in order and their structure was normal. There was no broken fiber, the nucleus of myocardial cell was regular. However, the muscle fiber was arranged in disorder, extensively collapsed and degenerated in hyperlipidemia group (Figure 1).

Table 1 – Serum levels of TC and TG after high-fat diet for 8 weeks. (mmol/l)

Group	TG	TC
Control	0.73 ± 0.12	1.87 ± 0.25
H-lipid	1.21 ± 0.13	3.34 ± 0.33
p value	< 0.05	< 0.05

TC: total cholesterol; TG: triglycerides.

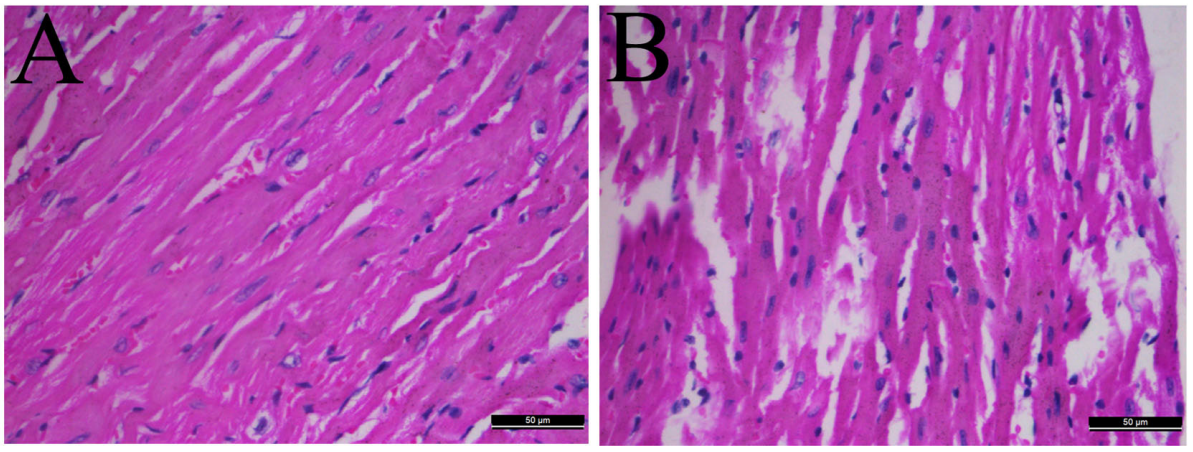

Figure 1 – *Change in heart photomicrographs of hyperlipidemic mice. Hearts were stained with H&E (A-B). Representative sections from hearts of a control mouse (A), hyperlipidemic mouse (B). H&E, magnification × 400.*

Hyperlipidemia increased mast cell chymase and tryptase production

Since mast cell protease play essential role in fibrosis process, their production levels in the case of hyperlipidemia would be our concern. To investigate this, mice heart were immediately removed after sacrifice for western blot analysis. The results of western blot showed that the protein expressions of chymase and tryptase were increased significantly compared with the control group (Figure 2)

Hyperlipidemia promoted activity of TGF-β and WNT/β-catenin pathway.

TGF-β is a pro-fibrotic cytokine that induces proliferation of macrophages and fibroblast through the induction of other growth factors. The Wnt/β-catenin signaling pathway is essential for the fibrosis induced by TGF-β. Western blot analysis of TGF-β and β-catenin showed that increased expression of both proteins were evident in H-lipid group compared to the control group. (Figure 2)

The immunohistochemistry results were similar to the mentioned above. The protein expressions of TGF-β in the mice's hearts of H-lipid group were significantly increased compared with the control group (Figure 3).

Hyperlipidemia induced great collagen accumulation in the heart

Picrosirius red staining suggested significant greater collagen content in the H-lipid group compared with the control groups (Figure 4) moreover, the immunohistochemistry (Figure 5) and western blot (Figure 2) results showed that the expression levels of α-SMA in the heart tissues of the H-lipid group were increased significantly compared with the control group.

Discussion

Hyperlipidemia is a clinical and metabolic disorder characterized by abnormal elevation of the major circulatory lipid and lipoprotein levels that accounts for approximately 56% cases of cardiovascular diseases worldwide and causes about 4.4 million deaths annually.[19] As reported, the death risk from ischemic heart disease is significantly high in patients with essential hyperlipidemia.[20]

In addition, it was reported that high-calorie diet feeding could induce hyperlipidemia and promoted heart injury and failure in rats.[21] However, there is little knowledge about the mechanisms underlying these biological changes. Cardiac fibrosis is a common pathologic feature of end-stage heart disease. Therefore, as potential causes

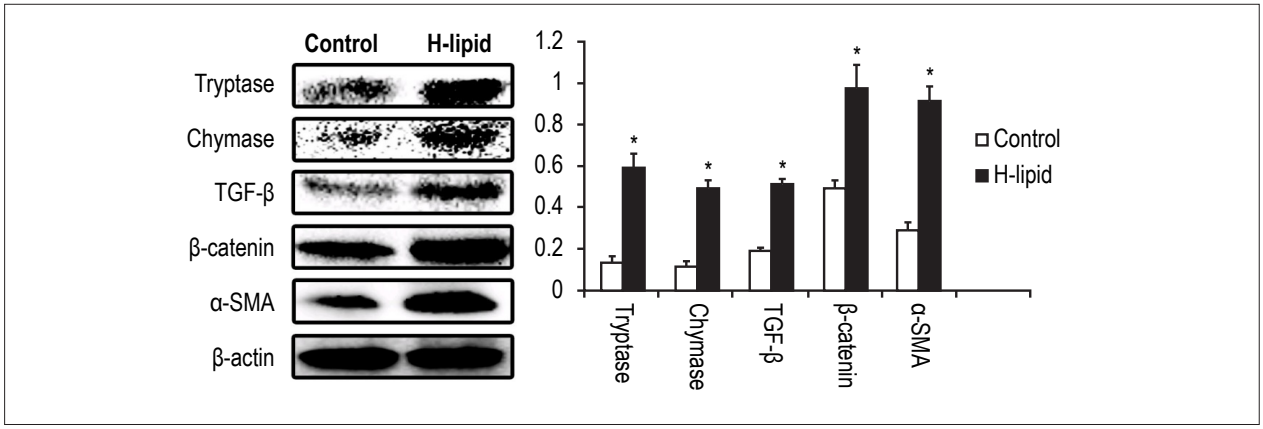

Figure 2 – *Effect of hyperlipidemia on the protein expression of tryptase, chymase, TGF-β, β-catenin and α-SMA in hyperlipidemic mice's hearts. The bar graph shows the relative expression ratio of each protein calculated after normalization by β-actin. Data are expressed as mean± S.D. (*p < 0.05 vs Control group; n = 8)*

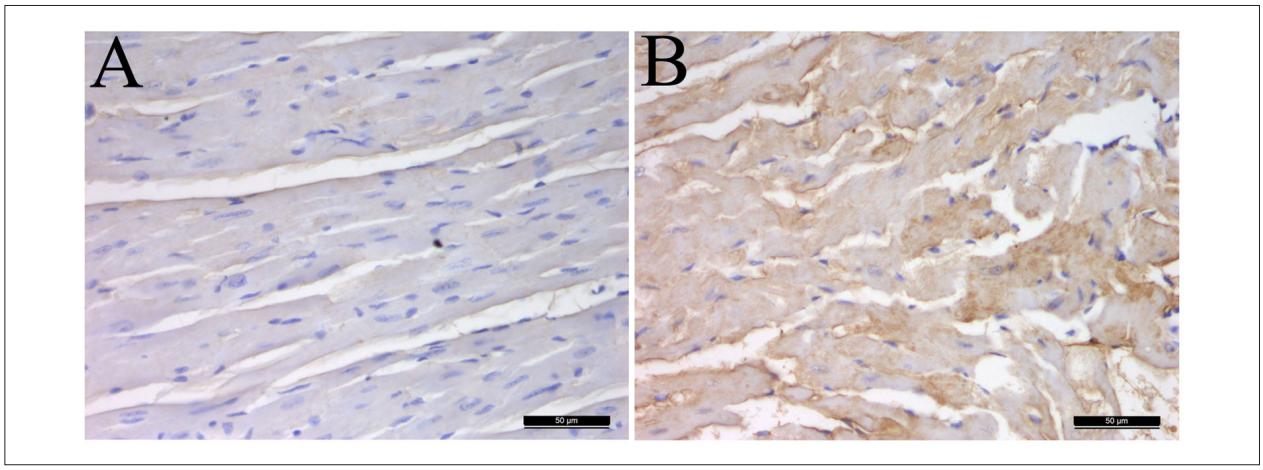

Figure 3 – *Effect of hyperlipidemia on the protein expression of TGF-β in hyperlipidemic mice's hearts. The hearts were immunohistochemical stained (A-B). Representative sections from hearts of a control mouse (A), a hyperlipidemic mouse (B). Immunohistochemical staining, magnification × 400.*

of cardiac fibrosis have to be investigated, we researched whether hyperlipidemia is associated with the cardiac fibrosis progression, contributing to the pathogenesis of heart failure. In the present study, the level of TC and TG in serum was significantly higher in H-lipid group, which indicates that the model of hyperlipidemia mice has been established successfully. Then we investigated the changes in mice heart morphology by H&E staining. The results showed muscular layer of heart arranged in disorder with fracture in mice of the model group. Picrosirius red staining suggested significantly greater collagen content in the H-lipid group compared with the control groups, and western blot showed that the expression level of α-SMA, known as marker of myofibroblast, in H-lipid group was significantly higher than in control group.

Mast cells have been recognized as important effector cells in tissue fibrosis. As reported, mast cells express serine proteases; tryptase and chymase that are associated with fibrosis in various diseases. However, little is known about their involvement in heart disease induced by hyperlipidemia. In our study, the results of western blot showed that the protein expressions of tryptase and chymase in the mice's heart of H-lipid group were increased compared with the control group. To further explore the molecular mechanism of cardiac fibrosis in hyperlipidemia mice, using western blot analysis and immunohistochemical staining, we examined the protein expressions of TGF-β which are intricately linked with the profibrotic effects of mast cells, and the Wnt/β-catenin pathway that is essential for the fibrosis induced by TGF-β. The results of western blot analysis and immunohistochemical staining demonstrated that the protein expressions of TGF-β in the mice's heart of H-lipid group were significantly increased compared with the control group. Moreover, the results of western blot analysis showed that the changed protein expression level of β-catenin was similar to TGF-β.

Conclusions

In summary, as the pathogenesis indicates, the progression of cardiac fibrosis may be induced by hyperlipidemia. Interestingly, heart tissues from hyperlipidemia mice revealed

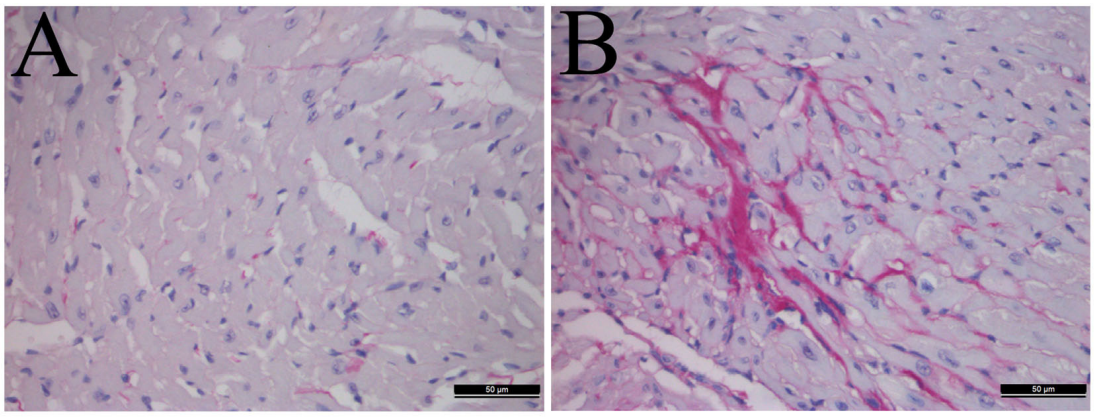

Figure 4 – *Effect of hyperlipidemia on the collagen accumulation in hyperlipidemic mice's hearts. The hearts were picro-sirius red stained (A-B). Representative sections from hearts of a control mouse (A), hyperlipidemic mouse (B). Picro-sirius red staining, magnification ×400.*

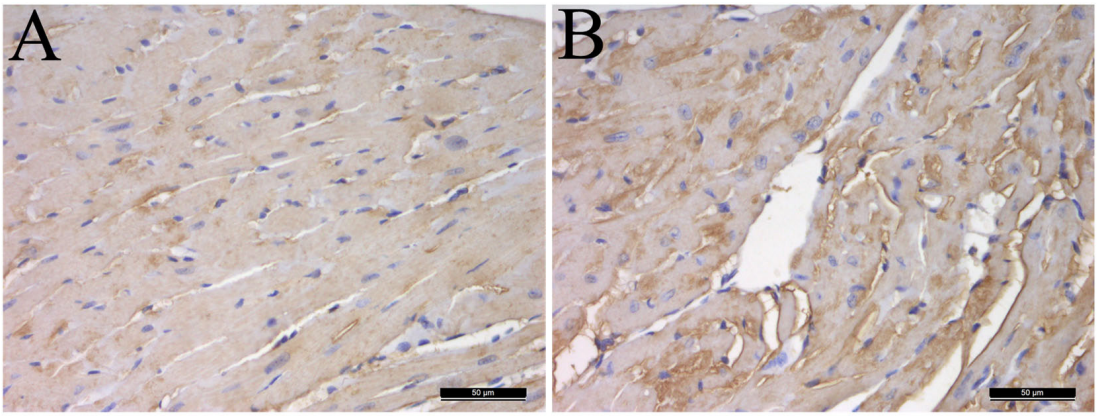

Figure 5 – *Effect of hyperlipidemia on the protein expression of α-SMA in hyperlipidemic mice's hearts. The hearts were immunohistochemical stained (A-C). Representative sections from hearts of a control mouse (A), hyperlipidemic mouse (B). Immunohistochemical staining, magnification × 400.*

increased mast sell activation e upregulated activity of TGF-β/Wnt/β-catenin pathway. The results of this study demonstrated that the mast cells and TGF-β/Wnt/β-catenin pathway were not only very important for the cardiac tissue fibrosis in hyperlipidemia but also a possible target for therapy.

Author contributions

Conception and design of the research and Writing of the manuscript: Cheng Y; Acquisition of data, Analysis and interpretation of the data and Statistical analysis: Cheng Y, Zhu Y, Zhang J, Duan X; Critical revision of the manuscript for intellectual content: Cheng Y, Zhu Y.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

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Evaluation of Left Ventricular Diastolic Function by Echocardiography with Tissue Doppler in Systemic Sclerosis

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Abstract

Background: Systemic sclerosis (SS) is a connective tissue abnormality characterized by fibrosis of the skin and internal organs. Cardiac involvement with consequent myocardial dysfunction in SS is associated with increased morbidity and mortality.

Objective: To investigate the left ventricular (LV) diastolic function in patients with SS and preserved systolic function.

Methods: Patients with SS were evaluated with two-dimensional echocardiography with tissue Doppler for analysis of chamber diameters, LV mass index (LVMI), indexed left atrial volume (iLAV), systolic function of both ventricles, and presence and degree of diastolic dysfunction (DD).

Results: We evaluated 50 patients, divided according to the presence of DD into Group 1 (n = 25; normal diastolic function, E/A ratio ≥ 0.8, deceleration time [DT] > 150 ms and < 200 ms, and septal e' > 8 cm/s) and Group 2 (n = 25; with DD, subdivided into type I DD [E/A < 0.8, DT > 200 ms], type II [E/A ≥ 0.8, septal e' < 8 cm/s, iLAV > 34 mL/m²], and type III [E/A > 2, DT < 150 ms, septal e' < 8 cm/s]). Type I DD was the most frequent (34%), followed by type II DD (16%). LVMI and iLAV were similar in both groups, but septal and lateral e' were reduced only in Group 2. In Group 2, we observed that patients with moderate DD had longer disease duration (p = 0.02).

Conclusion: The prevalence of type I DD was elevated in SS and associated with aging. Disease duration emerged as an important factor in moderate DD. (Arq Bras Cardiol. 2017; 109(5):410-415)

Keywords: Heart Ventricles / function; Echocardiography, Doppler; Sleroderma, Systemic; Ventricular Dysfunction, Left.

Introduction

Systemic sclerosis (SS) is a diffuse connective tissue disease characterized by skin and internal organ fibrosis and thickening, vascular alterations, and eventual ischemic ulcers and visceral abnormalities.[1] The prevalence of SS varies between 7 and 489 individuals per million persons and may vary by gender (more common in women), age (usually emerges between the third and fifth decades of life), and ethnicity (more common in the US and Australia than in Japan or Europe).[2] SS can be clinically subdivided into a limited form of the disease, which only affects the skin of the face, hands, and feet, or a diffuse form, which occurs with thickening of the extremities and abdomen, trunk, and roots of the limbs. The cardiac involvement in SS can be primary (myositis, heart failure, cardiac fibrosis, coronary artery disease, conduction abnormalities, and pericardial disease) or secondary (resulting mainly from pulmonary fibrosis and renal insufficiency).[1-4] Systolic and/or diastolic dysfunction (DD) may be secondary to myocardial fibrosis, left ventricular (LV) hypertrophy, hypertension, renal disease, or respiratory sleep disorder. Echocardiography reveals myocardial disease in 50% to 70% of the cases, but in most patients, cardiac dysfunction is clinically silent until the disease reaches a more advanced stage.[3-6] Techniques derived from two-dimensional echocardiography, such as tissue Doppler, have been used for the evaluation and early detection of ventricular dysfunction in various situations.[5-7] Their use in the study of SS, however, has been limited to studies of small size[8] or with inadequate methodologic definition.[9]

The objective of this study was to evaluate the LV diastolic function by echocardiography associated with tissue Doppler in patients with SS.

Method

The study included patients with SS attending the Rheumatology Outpatient Clinic of the *Hospital das Clínicas* at the Medical School of *Universidade de São Paulo* in the period between November 2010 and December 2011, of both sexes, and older than 18 years. All patients fulfilled the criteria of the American College of Rheumatology (ACR)[10] and, subsequently, the new classification criteria of the ACR and the European League Against Rheumatism (EULAR).[11] We included outpatients with SS without severe visceral manifestations (especially interstitial fibrosis, pulmonary hypertension, cardiomyopathy, or scleroderma renal crisis), as well as without severe comorbidities. All patients had Raynaud's phenomenon.

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The study was approved by the Research Ethics Committee of the *Hospital das Clínicas* and all patients signed an informed consent for participation.

Transthoracic echocardiography

All patients underwent an initial two-dimensional echocardiography with tissue Doppler imaging (Artida, Toshiba, Japan) for a complete evaluation of the ventricular structure and function, with emphasis on an analysis of the diastolic LV function. Based on the guidelines of the American Society of Echocardiography,[12] we obtained measurements of the systolic and diastolic diameters of the LV with the two-dimensional mode to calculate the ejection fraction (Teichholz method). We also obtained the diameters of the aortic root and left atrium from the parasternal long axis view. The calculation of the LV mass was performed using the measurements of the diastolic LV thickness and cavity by the Devereux method[12] and indexed by body surface area. The indexed left atrial volume (iLAV) was obtained from the apical two-chamber and four-chamber views by the modified Simpson method. Valvular alterations were evaluated with the two-dimensional mode, conventional Doppler, and color mapping, with the pulmonary pressure measurement obtained by tricuspid regurgitation and added to the estimate of the right atrial pressure from inferior vena cava.

Diastolic function analysis

Transmitral Doppler measurements were obtained with the Doppler sample volume positioned on the edge of the leaflets in the apical four-chamber view to obtain the E (initial) and A (late) waves, E/A ratio, and E-wave deceleration time (DT). We also obtained tissue Doppler tracings from the apical four-chamber view with the Doppler sample volume positioned in the basal region of the septum and in the lateral mitral annulus ring for analysis of s', e', and a' wave velocity.

The analysis of the diastolic function was performed based on the classification below, following the recommendations of the American Society of Echocardiography:[13]

- Normal Function: iLAV < 34 mL/m^2, E/A 0.8-1.5, E-wave DT > 150 ms and < 200 ms, septal e' wave ≥ 8 cm/s.
- DD type I (mild): E/A < 0.8, E-wave DT > 200 ms, septal e' wave < 8 cm/s.
- DD type II (moderate): iLAV ≥ 34 mL/m^2, E/A 0.8-1.5, E-wave DT between 150-200 ms, septal e' wave < 8 cm/s with an E/e' ratio > 13.
- DD type III (severe): iLAV > 34 mL/m^2, E/A > 2, E-wave DT < 150 ms, septal e' wave < 8 cm/s with an E/e' ratio > 13.

Based on the diastolic function analysis, the patients were divided into two groups: Group 1, with normal diastolic function and Group 2, with DD. The patients were also analyzed in relation to the degree of DD presented. The presence of an inadequate acoustic window and decreased LV ejection fraction (< 50%) were considered exclusion criteria.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess normality. For continuous variables with normal distribution, the data are presented as mean ± standard deviation and for variables without normal distribution, as median and interquartile range (IQR). The categorical variables are presented as absolute numbers and percentages. The groups were compared using two-tailed unpaired Student's *t* test for variables with normal distribution and Wilcoxon test for variables without normal distribution. To assess the degree of DD, we used analysis of variance (ANOVA) and, subsequently, Dunnett's test.

The statistical analyses were performed using the software JMP, version 8 (SAS Institute, Cary, NC, USA). P values < 0.05 were considered statistically significant.

Results

We analyzed 54 patients with SS, three of whom were excluded due to inadequate acoustic window and one due to decreased LV ejection fraction, totaling 50 patients. Most (n = 46) participants were female, and their mean age was 52 ± 11 years. The median disease duration was 9 years (IQR 5-15 years). The minimum and maximum disease durations were 3 and 45 years, respectively. Only three patients had hypertension, and none had diabetes or a history of manifestation of coronary artery disease (Table 1). The echocardiographic data including LV ejection fraction, diameters of the cardiac chambers, pulmonary artery systolic pressure, ventricular mass index, and iLAV were within normal values for this group of patients (Table 2).

The patients presented a very high prevalence of DD, with half of the study sample (25 patients) presenting some degree of DD, of whom the majority (17 patients, 34%) had mild DD (type I) and a lower portion had type II DD (8 patients, 16%), as shown in Figure 1. None of the patients had type III DD.

The groups with and without DD did not differ regarding gender or presence of hypertension. On the other hand, the group with DD was significantly older than the one without DD (58 ± 9 years *versus* 46 ± 10 years, respectively, p < 0,05). The disease duration showed no significant difference between the groups, and the median disease duration was 8 years (IQR 3.5-11.0 years) for patients without DD and 11 years (IQR 6.0-16.5 years) for those with DD (p = 0.178; Wilcoxon test). The LV mass index and the iLAV were also similar in both groups. However, the lateral and septal e' waves were reduced only in patients with DD, resulting in an increased septal E/e' ratio. The lateral E/e' relation did not differ between the groups. The pulmonary pressure was also higher in Group 2 (Table 2).

Table 1 – Patients' clinical data and medications

Variable	
Age (years)	52 ± 11
Female sex	46 (92%)
Disease duration (years) (interquartile range)	9 (5 – 15)
Depression (n, %)	3 (6%)
Hypertension (n, %)	3 (6%)
Pulmonary hypertension (n, %)	6 (12%)
Calcium channel blocker (n, %)	28 (56%)

Table 2 – Echocardiographic data of the total sample of patients and subgroups with and without diastolic dysfunction

Variables	Total n = 50	Without DD n = 25	With DD n = 25	p
Aortic root (mm)	29 ± 3	29 ± 3	29 ± 3	0.631
Left atrium (mm)	36 ± 5	36 ± 4.7	3.7 ± 5.0	0.489
iLAV (cm/m^2)	27 ± 8	24 ± 4.8	29 ± 10	0.417
LV diastolic diameter (mm)	44 ± 5	43 ± 4	44 ± 6	0.06
SW (mm)	9.7 ± 1.2	9.5 ± 1.1	9.9 ± 1.3	0.247
PW (mm)	29 ± 4	9.5 ± 1.1	9.9 ± 1.3	0.768
LV ejection fraction (%)	62 ± 7	63 ± 4	62 ± 3	0.172
LV mass index (g/m^2)	88 ± 28	87 ± 20	90 ± 34	0.950
PASP (mmHg)	30 ± 14	25 ± 7	35 ± 17	0.03
E (cm/s)	84 ± 19	88 ± 17	80 ± 22	0.137
A (cm/s)	79 ± 22	68 ± 13	91 ± 23	0.0001
E/A	1.1 ± 0.3	1.3 ± 0.27	0.9 ± 0.24	0.0001
DT (ms)	188 ± 44	162 ± 25	214 ± 45	< 0.0001
Septal e' (cm/s)	8.5 ± 2.1	10.0 ± 1.6	7.1 ± 1.2	< 0.0001
Lateral e' (cm/s)	11.6 ± 2.7	13.4 ± 2.3	9.9 ± 1.9	< 0.0001
Septal E/e'	10.4 ± 3.6	8.9 ± 2.3	11.9 ± 4.1	0.005
Lateral E/e'	7.7 ± 2.7	8.3 ± 3.3	7.1 ± 1.9	0.189

DD: diastolic dysfunction; iLAV: indexed left atrial volume; LV: left ventricle; SW: septal wall; PW: posterior wall; PASP: pulmonary artery systolic pressure; E: early diastolic filling wave; A: late diastolic filling wave; DT: E-wave deceleration time; e': tissue Doppler early diastolic wave. Unpaired Student's t test for comparison between subgroups with and without diastolic dysfunction.

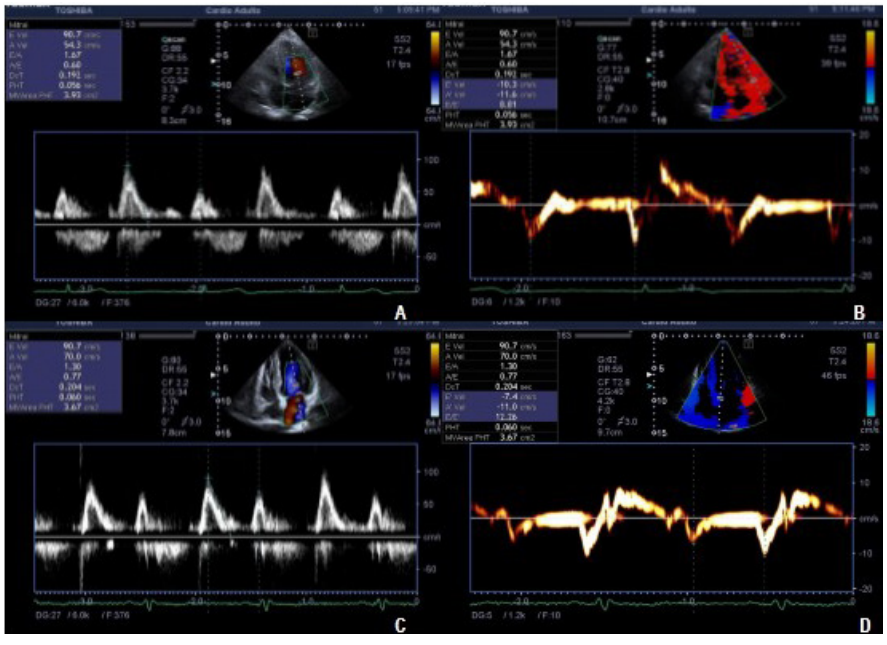

Figure 1 – *A and B: Pulsed Dopper images showing transmitral flow and septal tissue Doppler showing a normal diastolic pattern. C and D: Tissue Doppler images showing a reduced e' wave (7.4 cm/s), compatible with moderate diastolic dysfunction (pseudonormal pattern).*

When we analyzed the subgroups according to the degree of DD, we observed that it was not only age that was related to the presence of DD (Figure 2); for patients with moderate DD (type II), we observed that the duration of the disease was significantly prolonged when compared with patients without DD or with mild DD(p = 0,02). Patients without DD and those with mild DD had disease durations of 9 ± 7 years and 9 ± 4 years, respectively, compared with 18 ± 14 years among patients with moderate DD.

Discussion

Cardiac involvement often occurs in SS and is usually associated with a more reserved prognosis, representing the second most common cause of death after pulmonary involvement.[14] In order to study the presence of early cardiac involvement in SS, the present study assessed the frequency of DD in a group of patients with preserved systolic function. It has been observed that the use of Doppler tissue imaging associated with conventional echocardiography increases the diagnostic accuracy of this method.[6] This was confirmed in our study, which observed the occurrence of DD in half of the patients studied, although this population exhibited preserved systolic function and a low prevalence of other risk factors for DD, such as hypertension, ventricular hypertrophy, or diabetes. Population studies evaluating diastolic function in community-dwelling individuals have observed that DD is mainly associated with increasing age, but is rarely found in the absence of risk factors for diastolic abnormalities, being uncommon even in elderly individuals.[15] Similarly, Kuznetsova et al., in a study evaluating diastolic function in the general population,[16] have shown that in subjects aged between 50-59 years (a similar age range as our cohort), the proportion of total DD reached 42%, and type I DD (abnormal relaxation) was found in about 32% and type II DD in approximately 10% of the population. These values were not very different from those found in our sample of patients with SS; the prevalence of DD was 50%, with 34% of the individuals presenting type I DD, with a prevalence possibly slightly higher for type II DD (16% of the patients with SS). This population study found, in a similar way, an association between DD with age and body mass index, in addition to the presence of comorbidities, such as high blood pressure and increased serum creatinine. It is important to emphasize that in the referred study, almost 70% of the patients with type I DD had hypertension, with this proportion increasing to almost 80% in the subgroup with type II DD. On the other hand, only three patients in our sample had hypertension, and the absence of other comorbidities strongly suggests that DD was associated with SS. It has been reported more recently a higher prevalence of DD in patients with SS when compared with individuals of the same age in the general population. Meune et al. reported the presence of mild DD in 50% of the patients with type I DD (abnormal relaxation) found in a control group.[6] A study conducted by Hinchcliff et al. observed alteration of relaxation in only 23% of the patients with SS, and its presence was associated with increased mortality.[9] We also observed that the degree of DD was mild in most patients, with few cases of moderate DD and no patient with severe DD. These findings suggest that the disease itself evolves more frequently with subtle diastolic changes, prevailing alterations in ventricular relaxation with intracardiac pressures still within normal limits, as demonstrated by the normal E/e' ratio in most cases.

DD may be the result of a primary myocardial involvement in SS[16] or may be secondary to hypertension, LV hypertrophy, pericardial diseases, and coronary disease.[17] In our group of patients, the number of hypertensive patients or patients with other comorbidities associated with DD was not significant, which leads us to believe that the probable cause of this abnormality was the primary cardiac involvement. The presence of a LV mass index within normal values in our sample also corroborates the hypothesis that the evolution of the disease itself is the cause of the cardiac dysfunction, thus rejecting the LV hypertrophy as the cause

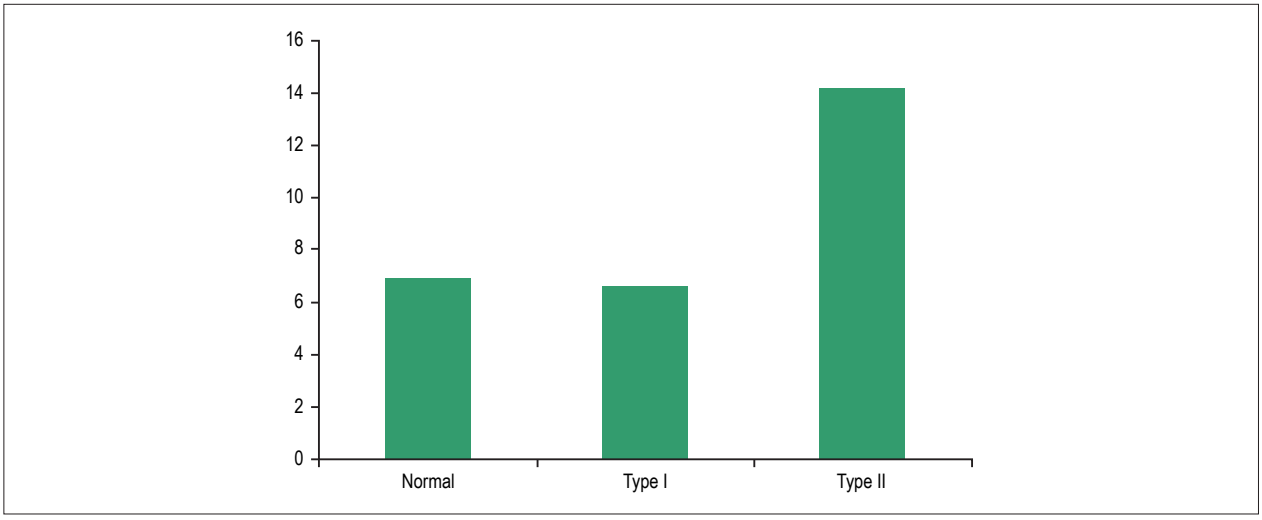

Figure 2 – *Relationship between the degree of diastolic dysfunction and disease duration (p = 0.02). Relationship between the degree of diastolic dysfunction and disease duration in years (p = 0,02)*

of DD. Primary myocardial structural changes (fibrosis) may, in turn, cause DD. Tzelepis et al., using cardiac magnetic resonance imaging,[18] have shown the occurrence of delayed enhancement (compatible with fibrosis) in about 60% of the patients with SS, even in those without evident systolic dysfunction. The presence of DD is clinically important in the evolution of patients with SS, since cardiac involvement in SS is associated with increased mortality.[9,19] In addition, the increased LV diastolic pressures are transmitted to the lungs, with consequent pulmonary hypertension. During exercise, with the increased diastolic pressures, the pulmonary pressure may be even higher, as demonstrated in patients with SS during exercise echocardiography,[20] leading to symptom worsening. It is important to note, however, that the absence of DD during examination does not exclude the presence of myocardial fibrosis.

When we analyzed the factors associated with DD, we observed that the patients were quite similar in regards to clinical characteristics, except in relation to the greater prevalence of DD in older individuals. Several changes in cardiac structure and function derive from the aging process, including a reduced number and increased size of myocytes, with resulting increase in connective tissue.[21,22] In addition, dead myocytes are replaced by collagen, with consequent interstitial fibrosis, making the heart more rigid and less complacent, therefore affecting the diastolic relaxation. When we compared patients with and without DD, we observed that there was no influence of disease duration on DD. However, when we analyzed the subgroups, we observed that in addition to age, there was a significant association between disease duration and moderate DD. The association between myocardial fibrosis and longer disease duration may be related to a greater number of flares of cardiac Raynaud's phenomenon in patients with SS of long duration, since the successive vascular ischemic changes contribute to increased fibrosis. However, none of the patients, even with a longer disease course, presented important DD (restrictive pattern). It is possible that this pattern of more advanced DD may ultimately be found in patients with associated LV systolic dysfunction, but these cases were not evaluated in the present study.

Echocardiographic analysis of the cardiac function is simple and effective, allowing monitoring of the disease and early diagnosis of changes such as DD through the use of conventional Doppler associated with tissue Doppler. As the cardiac involvement is associated with a more severe progression, the demonstration of this involvement by echocardiography could indicate a need for stricter monitoring of this population, in addition to being used as an indication of therapeutic improvement.

As a limitation of this study, the relationship between DD and myocardial fibrosis can only be accepted as hypothetical since the patients did not undergo magnetic resonance imaging to test this association. The study had a cross-sectional design and we still lack long-term prognostic data, which could be associated with the presence of DD in this group of patients.

Conclusion

DD is frequent in patients with SS and normal systolic function. It is characteristically mild and associated with more advanced age. A longer disease duration is associated with a more pronounced DD pattern.

Author contributions

Conception and design of the research: Roque MCF, Arruda AL, Gomes SB, Becker D, Andrade JL, Rodrigues ACT; Acquisition of data: Roque MCF, Sampaio-Barros PD, Arruda AL, Gomes SB, Becker D, Rodrigues ACT; Analysis and interpretation of the data: Roque MCF, Sampaio-Barros PD, Arruda AL, Becker D, Andrade JL, Rodrigues ACT; Statistical analysis: Roque MCF, Rodrigues ACT; Writing of the manuscript: Roque MCF, Sampaio-Barros PD, Gomes SB, Becker D, Andrade JL, Rodrigues ACT; Critical revision of the manuscript for intellectual content: Roque MCF, Sampaio-Barros PD, Arruda AL, Andrade JL, Rodrigues ACT.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

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Electrocardiographic Findings in Brazilian Adults without Heart Disease: ELSA-Brasil

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Abstract

Background: **The electrocardiogram (ECG) is widely used in population-based studies. However, there are few studies on electrocardiographic findings in Latin America and in Brazil. The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) comprised 15,105 participants (35–74 years) from six Brazilian capitals.**

Objectives: **To describe electrocardiographic findings in Brazilian adults without heart disease, stratified by sex, age and race/skin color.**

Methods: **Cross-sectional study with baseline data of 11,094 adults (44.5% men) without heart disease from ELSA-Brasil. The ECGs were recorded with the Burdick Atria 6100 machine and stored at the Pyramis System. ECG analysis was automatically performed using the Glasgow University software. A descriptive analysis of heart rate (HR), P, QRS and T waves' duration, PR and QT intervals, and P, R and T axes was performed. After stratification by sex, race/color and age, the groups were compared by the Wilcoxon and Kruskal-Wallis test at a significance level of 5%. Linear regression models were used to evaluate the behavior of electrocardiographic parameters over age. Major electrocardiographic abnormalities defined by the Minnesota code were manually revised.**

Results: **Medians values of the electrocardiographic parameters were different between men and women: HR 63 vs. 66 bpm, PR 164 vs.158 ms, QT corrected 410 vs. 421 ms, QRS duration 92 vs. 86 ms, P-wave duration 112 vs. 108 ms, P-wave axis 54 vs. 57 degrees, R-wave axis 35 vs. 39 degrees, T-wave axis 39 vs. 45 degrees ($p < 0.001$ for all). The 2nd and the 98th percentiles of each variable were also obtained, and graphs were constructed to illustrate the behavior of the electrocardiographic findings over age of participants stratified by sex and race/skin color.**

Conclusions: **The values for the electrocardiographic measurements herein described can be used as reference for Brazilian adults free of heart disease, stratified by sex. Our results suggest that self-reported race/skin color have no significant influence on electrocardiographic parameters. (Arq Bras Cardiol. 2017; 109(5):416-424)**

Keywords: **Electrocardiography/diagnosis; Adult; Epidemiology Measurements; Healthy People Programs; Cohort Studies.**

Introduction

Electrocardiography (ECG) is a low-cost, widely available test used in cardiovascular assessment.[1] For decades, ECG has been used in large epidemiological studies, in which many of its diagnostic and prognostic utility was defined and confirmed.[2-6] Electrocardiographic findings and their relationship with heart disease (HD) have long been the object of study in white and African-American populations. However, studies in Latin-America, especially in Brazilian population are still scarce. Besides, there are few data available in the medical literature about normal ECG values including ECG measurements, intervals, axes and wave duration for the Brazilian population,[7] particularly for those whose clinical data are available.

The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)[8] is a multicentric, cohort study aimed to prospectively evaluate participants' health and detect determining factors for HD and diabetes. A comprehensive clinical data database of Brazilian adults was constructed from baseline examinations (2008-2010), and these data were correlated with their electrocardiographic parameters.[9] Participants considered free of HD were selected for the present study.

The aim of the present study was to describe the duration of intervals and deflections in participants without HD selected from the ELSA-Brasil study. We aimed to establish normal values for electrocardiographic measurements by sex, age range and race/self-reported skin color in this population.

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Methods

Participants

This study is a descriptive, cross-sectional analysis of data from ELSA-Brasil study, which aims at detecting HD and diabetes determinants in Brazilian adults. ELSA-Brasil study has been conducted in six capitals in Brazil – Belo Horizonte, Porto Alegre, Rio de Janeiro, Salvador, São Paulo e Vitoria – including 15,105 participants, using a methodology described elsewhere.[8-10] ECGs from all participants were obtained during baseline examinations.

Participants with HD, those without race/skin color data (not declared) or of low-prevalent race (mainly of Asian or Indigenous origin), and participants with missing ECG data were excluded. A total of 11,985 participants were included in the study (Figure 1). A prevalent HD was defined as a self-reported history of severe coronary disease (history of acute myocardial infarction or myocardial revascularization), stroke, heart failure or major electrocardiographic changes, according to the Minnesota code (MC).

With respect to patients with systemic arterial hypertension (SAH), all analyses were performed twice – first including data from hypertensive patients' and then excluding these data, in order to evaluate the impact of this comorbidity.

ELSA-Brasil was approved by the Ethics Committee from Universidade Federal de Minas Gerais, number ETIC 186/06, and performed according to the Helsinki declaration. All participants signed the informed consent form.

ELSA-Brasil study protocol

From 2008 to 2010, participants were assessed using a standardized questionnaire, which included questions on cardiovascular system, and underwent anthropometric and physiological assessment including ECG. Risk factors for HD were defined according to national and international guidelines.[11,12] SAH was defined by systolic blood pressure (SBP) ≥ 140 mmHg, diastolic blood pressure (DBP) ≥ 90 mmHg or use of antihypertensive drugs. Diabetes mellitus (DM) was defined by fasting glucose ≥126 mg/dL, postprandial glucose ≥ 200 mg/dL, or glycohemoglobin ≥ 6.5%, in addition to "being treated for DM" or having received the diagnosis of DM. Obesity was defined by a body mass index ≥ 30 Kg/m^2, smoking was defined by "current smoking", i.e., former smokers were not considered for this risk factor.

Race/skin color was assessed by self-report of participants, who answered a multiple-choice question according to the 2008 census in Brazil.[13]

ECG testing

ECG was performed in each center following a previously established protocol,[9] using a Burdick Atria 6100 device calibrated at 10 mm/mV and speed of 25 mm/second. Results were electronically transferred to a reading center located in Belo Horizonte and stored in an electronic database for posterior automated reading by the Glasgow ECG analysis program[14] and codification by the MC.[15-17] Acquisition and analysis of the ECGs are described in a previous publication[8] and included established procedures of quality assurance procedures.

Measurements of PR and QT intervals, P-wave and QRS duration, and P, R and T axes were automatically performed. QT interval was corrected using the Hodges equation. ECGs were classified as presenting major, minor or no abnormalities according to the MC. For 'major' changes we considered: major Q (previous myocardial infarction MC 1-1, 1-2), minor Q plus major changes in ST-T segment (CM 1-3 and CM 4-1 or 4-2 or 5-1 or 5-2), major isolated ST-T abnormalities (CM 4-1 or 4-2 or 5-1 or 5-2), left ventricular hypertrophy associated with changes in ST-T segment (CM 3-1 and CM 4-1 or 4-2 or 5-1 or 5-2), intraventricular conduction defect (left bundle branch block, right bundle branch block, nonspecific intraventricular conduction delay, right bundle branch block associated with blockage of the anterior-superior division of the left bundle branch CM 7-1 or 7-2 or 7-4), Brugada ECG pattern (CM7-9), major QT prolongation (QT ≥ 116%), atrial fibrillation or atrial flutter (CM 8-3), supraventricular tachycardia (CM 8-4-2), atrioventricular conduction defect. (second- and third-degree block, pre-excitement and artificial pacemaker (CM 6-1 or 6-2 or 6-4 or 6-8), asystole and ventricular fibrillation (CM 8-2). Major ECG abnormalities were manually revised by experienced cardiologists for coding quality control and results were published elsewhere.[18]

Statistical analysis

Categorical variables were described as frequencies (percentages). The Shapiro-Wilk test was used to test normality of data distribution; continuous variables with normal distribution were presented as mean and standard deviation, and those without normal distribution were expressed as median and percentiles. For representation of normality values, we used the 2nd and 98th percentiles in place of interquartile range. The percentiles relevant for ECG measures were calculated by age and their progress is shown by smoothed curves, using the loess method.

The Mann-Whitney and the Kruskal-Wallis tests were used for between-group comparisons (sex and race/skin color). The Bonferroni correction was used for multiple comparisons.

The inclusion of hypertensive participants was performed after a sensitivity analysis to evaluate the impact of this variable on the results.

In order to evaluate whether the slopes of the lines in the graph ECG measurements by age were similar between participants, we included interaction terms in liner regression models. 'White' race/skin color was used as reference due to the greatest number of individuals self-reported as so.

The level of significance was set at 5% unless stated otherwise. The analyses were performed using the SPSS version 20 and R version 3.3.0.

Results

Clinical characteristics of participants

Clinical characteristics of participants, stratified by sex and race/skin color, are described in Table 1. In general, there was

Table 1 – Characteristics of participants with electrocardiogram recordings at baseline, without evidence of heart disease (based on clinical history or electrocardiography test) (n = 11,985)

	Men (n = 5,341)			Women (n = 6644)		
Characteristics*	White (n = 2928)	Brown (n = 1672)	Black (n = 741)	White (n = 3577)	Brown (n = 1872)	Black (n = 1195)
Age	52(9)	50(8)	51(8)	52(9)	51(8)	51(8)
Heart rate	65(10)	63(10)	63(9)	67(9)	67(9)	66(9)
Systolic arterial pressure (mmHg)	122(14)	130(17)	130(17)	114(15)	118(16)	122(17)
Diastolic arterial pressure (mmHg)	78(10)	81(11)	81(10)	72(10)	75(10)	77(10)
Body mass index (kg/m²)	27(4.2)	27(4.2)	27(4.3)	26(4.9)	27(4.9)	28(5.5)
Fasting glucose (mg/dl)	114(29)	114(32)	119(40)	105(21)	108(28)	110(29)
LDL-cholesterol (mg/dl)	132(34)	132(37)	134(40)	131(34)	133(34)	129(35)
HDL-cholesterol (mg/dl)	50(11)	50(12)	54(14)	62(15)	61(14)	62(15)
Total cholesterol (mg/dl)	213(42)	214(47)	217(45)	217(40)	218(41)	212(43)
Hypertension (%)	32.7	36.4	45.6	23.8	30.2	43.1
Diabetes (%)	18.7	21.8	26.5	11.6	15.3	22.7
Dyslipidemia (%)	46.6	44.1	43.5	49.5	52.9	47.6
Obesity (%)	19.4	18.7	22.0	20.0	24.4	33.1
Smoking (%)	12.7	15.7	15.5	12.3	11.4	13.5

(*) continuous variables expressed as mean and standard deviation and categorical variables as percentage.

a higher prevalence of SAH, smoking and DM in men than in women, whereas dyslipidemia and obesity were more prevalent among women. In the stratified analysis by self-reported race/skin color, SAH, DM and obesity were more prevalent in "black" race/skin color in both sexes.

Measurement of electrocardiographic intervals and deflections

Significant differences between men and women were found in all electrocardiographic parameters. Heart rate (HR) and QT duration were higher among women, whereas longer P-wave duration, QRS complex and PR interval were found in men (Table 2).

Sensitivity analysis that compared patients with and without SAH revealed no clinically important difference between the groups. Since there were an expressive number of hypertensive patients in the study, we decided to include these individuals in the final analysis. When these patients were excluded from the analyses, the results were quite similar to those obtained from the total study population (supplemental table 1 in apendix).

Effect of race/skin color on electrocardiographic parameters

In the comparison of ECG measurements between races/skin colors, there was a statistically significant difference for most of the outcomes, except for R-wave axis for men and QT and P-wave axis for women. These differences are described in detail in Table 3.

In the graphs of ECG outcome by race/skin color stratified by sex, there was not a wide variation of HR over age in white individuals, whose median HR was slightly higher than that of other races/skin colors (Figure 1). PR interval also showed a slight increase with age, and black race/skin color median line was constantly greater over age than median lines of other races/skin colors in both sexes (Figure 2). QTc interval (QT corrected by the Hodges equation) increased with age and was more prolonged in women than in men in all ages (Figure 3). QRS duration was relatively constant with age, with higher median values in white race/skin color in both sexes (Figure 4). There was also a decrease in R-wave axis with age, with higher median values in white race/skin color at all age ranges, which was more evident in women (Figure 5). The behavior of P-wave duration, P-wave axis and T-wave axis can be analyzed in the Appendix (Figures 1, 2 and 3).

With respect to the slope values (variation of the measurements with age), there was no difference between races/skin colors, except for *pardo* (brown skin color) men, who showed lower HR variation and PR interval duration and greater variation of QRS complex and R-wave axis duration as compared with white race/color.

Discussion

The present study enabled the description of electrocardiographic parameters of Brazilian adults of both sexes without HDs. This is the first publication to describe normal ECG parameters in the Brazilian population. Besides, although previous studies have been performed in many populations,[19-22] most of them included smaller sample sizes, except for the study by Rijnbeek et al.,[22] that included 13,354 participants aged between 16 and 90 years from four population studies in the Netherlands. These studies included apparently healthy subjects defined according to standardized questionnaires. Individuals using medications for HDs and those with risk factors for DM and SAH were excluded. In the present study, we chose not to exclude SAH patients without clear evidence of HD based on

Table 2 – Duration of electrocardiogram intervals and waves in men and women

Measurements*	Men (n = 5341)	Women (n = 6644)	p values (†)
Heart rate (bpm)	63(47 – 86)	66(51 – 87)	< 0.001
P-wave duration (ms)	112(78 – 134)	108(74 – 130)	< 0.001
PR interval (ms)	164(118 – 216)	156(114 – 208)	< 0.001
QRS duration (ms)	92(74 – 114)	86(70 – 106)	< 0.001
QT corrected (Hodges)(ms)	410(379 – 451)	421(389 – 459)	< 0.001
P-wave axis (degrees)	54(–11 – 77)	57(–10 – 78)	< 0.001
R-wave axis (degrees)	36(–43 – 84)	44(–29 – 84)	< 0.001
T-wave axis (degrees)	39(–14 – 77)	46(–07 – 77)	< 0.001

(*) Median and 2nd and 98th percentiles; (†) Mann-Whitney test.

Table 3 – Duration of electrocardiogram intervals and waves by sex and race

			Men		
Measurements *	**White (1) (n = 2928)**	**Brown (2) (n = 1672)**	**Black (3) (n = 741)**	**p values (†)**	**Differences**
Heart rate (bpm)	64(47 – 86)	63(48 – 87)	63(46 – 84)	0.002	1 ≠ (2 = 3)
P-wave duration (ms)	112(78 – 136)	114(78 – 136)	114(80 – 137)	< 0.001	3 ≠ (1 = 2)
PR interval (ms)	164(118 – 216)	164(118 – 219)	166(124 – 225)	0.022	3 ≠ 1
QRS duration (ms)	92(74 – 114)	92(74 – 112)	92(72 – 112)	0.012	1 = 2 = 3
QT corrected (Hodges) (ms)	411(381 – 453)	410(377 – 449)	409(374 – 453)	0.008	2 ≠ 1
P-wave axis (degrees)	54(–10 – 77)	54(–13 – 77)	56(–7 – 79)	< 0.001	3 ≠ (1 = 2)
R-wave axis (degrees)	36(–44 – 83)	35(–42 – 85)	34(–41 – 82)	0.912	
T-wave axis (degrees)	40(–12 – 78)	37(–17 – 77)	34(–24 – 79)	< 0.001	1 ≠ (2 = 3)
			Women		
Measurements *	**White (1) (n = 3577)**	**Brown (2) (n = 1872)**	**Black (3) (n = 1195)**	**p values †**	**Differences**
Heart rate (bpm)	66(51 – 87)	66(50 – 87)	65(49 – 88)	0,019	3 ≠ 1
P-wave duration (ms)	108(72 – 130)	108(74 – 132)	108(74 – 133)	< 0.001	3 ≠ (2 = 1)
PR interval (ms)	156(114 – 208)	158(114 – 210)	160(118 – 216)	< 0.001	3 ≠ (2 = 1)
QRS duration (ms)	86(70 – 106)	86(70 – 106)	84(70 – 104)	< 0.001	1 ≠ (3 = 2)
QT corrected (Hodges) (ms)	421(389 – 459)	421(390 – 460)	420(385 – 462)	0.051	
P-wave axis (degrees)	57(–11 – 78)	56(–8 – 77)	56(–5 – 77)	0.050	
R-wave axis (degrees)	45(–33 – 84)	41(–25 – 83)	38(–24 – 80)	< 0.001	1 ≠ 2 ≠ 3
T-wave axis (degrees)	47(–4 – 77)	45(–16 – 78)	41(–20 – 76)	< 0.001	1 ≠ 2 ≠ 3

(*) Median and 2nd and 98th percentiles (†) p-values calculated by the Mann-Whitney test; when statistically significant (p < 0.05), p-values between race groups were readjusted using the Bonferroni correction method, and considered significant when p < 0.0166.

the assumption that excluding those participants with major electrocardiographic abnormalities (classified by the MC), we would exclude those hypertensive patients with significant electrocardiographic changes caused by SAH (e.g. left bundle branch block, ventricular hypertrophy with repolarization abnormalities). After excluding patients with SAH, sensitivity analysis revealed no clinically significant differences in the electrocardiographic parameters, which corroborated our decision not to exclude these patients from the analyses and gave power to our study.

In the analysis stratified by sex, we observed that QTc was consistently greater in men over different age ranges. The difference in median values was similar (approximately 10 ms) to those described in which different reference values by sex were used.[23] Also, similar to our study, the authors did not report clinically significant differences in the other electrocardiographic parameters between the sexes.

Some differences can be pointed out between our results and others reported in a predominantly Caucasian sample[22]: our sample had lower median HR, and P-wave, PR interval

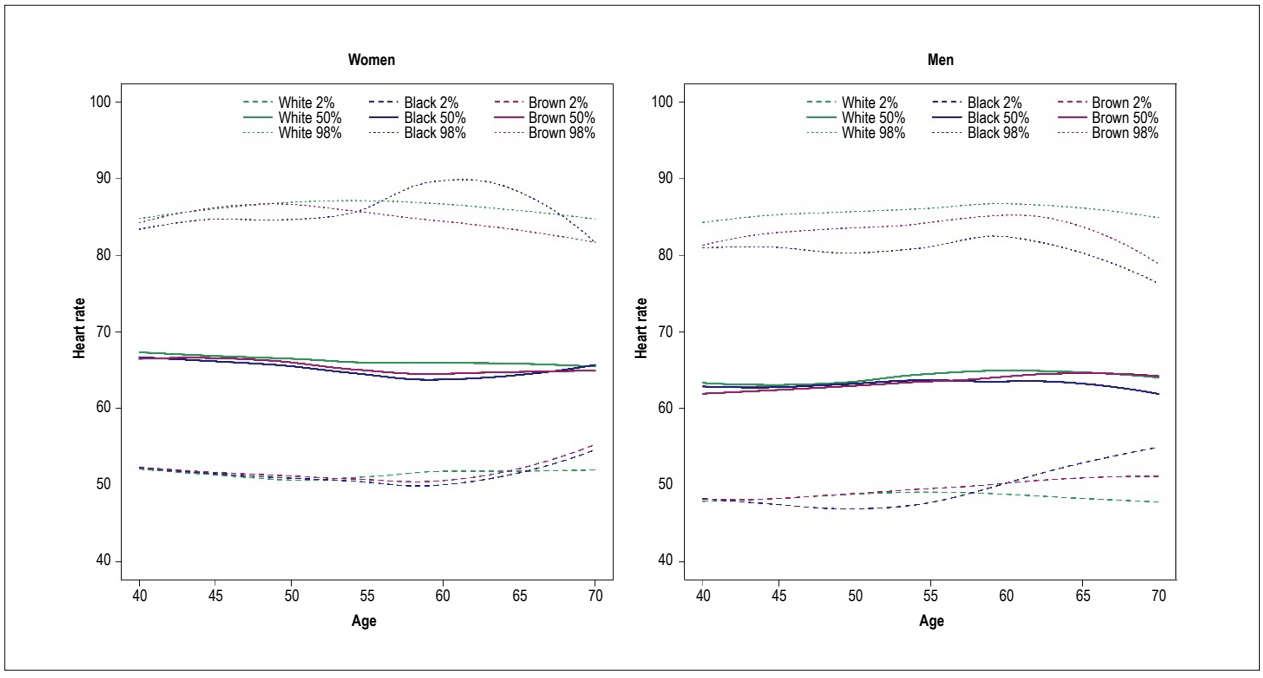

Figure 1 – *Heart rate by age in men and women stratified by self-reported race/skin color. The curves had a negative slope in women and a positive slope in men, with significant difference in pardo (brown race/skin color) men (p = 0.026), who showed lower variation in heart rate with age.*

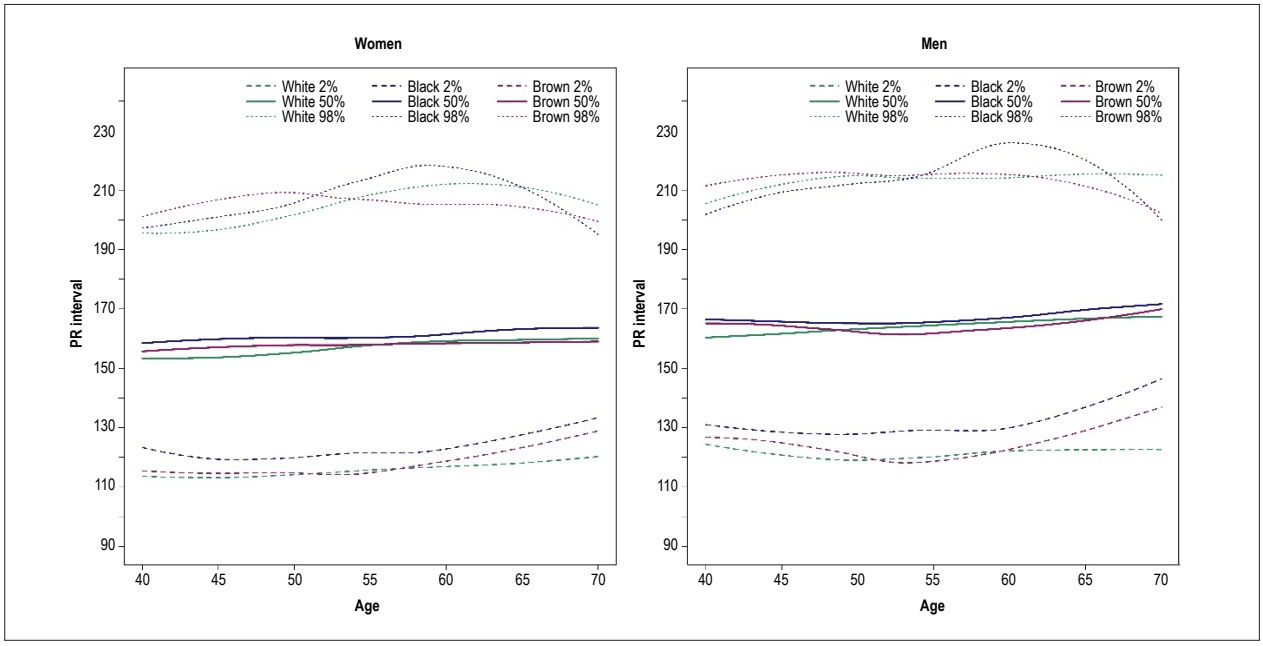

Figure 2 – *Duration of PR interval by age in men and women stratified by self-reported race/skin color. The curves have similar, positive slope, except for pardo (brown race/skin color) men, in which the slope is near zero, tending to negative (p = 0.032).*

and QRS duration. QT corrected by Hodges formula was not significantly different. Nevertheless, these measurements were higher than those reported in a study conducted in India.[21]

On the other hand, there were also similarities between the current study and previous reports. The increase trend of QT corrected and the R-wave axis deviation to the left with age were also reported in populations from different countries, including different races.[18,21]

Despite numerous studies investigating electrocardiographic parameters in different populations, there is still little evidence

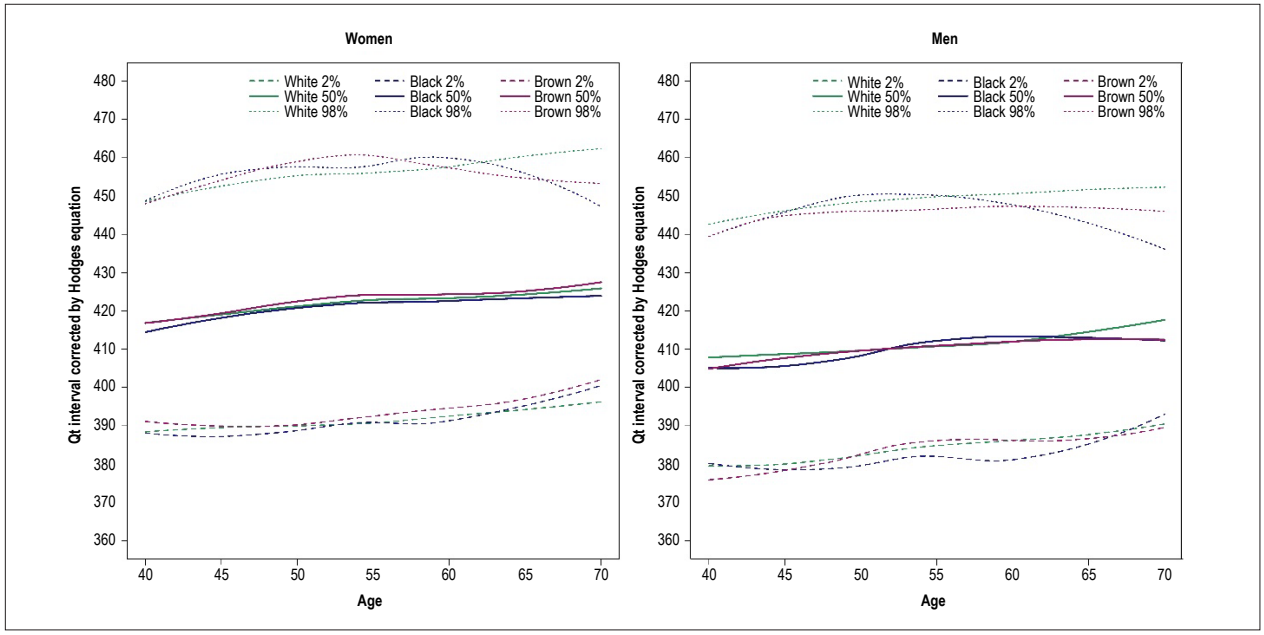

Figure 3 – *Duration of QT interval corrected by Hodges equation by age in men and women stratified by self-reported race/skin color. The slopes of the curves were positive, with no difference between them.*

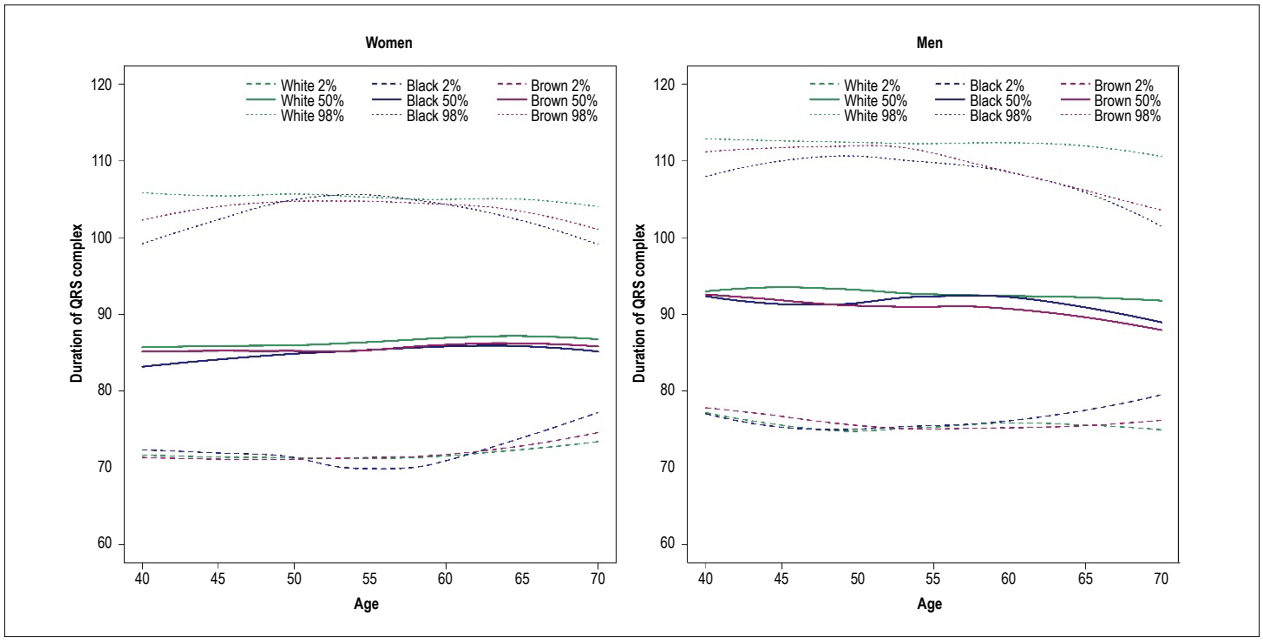

Figure 4 – *Duration of QRS complex by age in men and women stratified by self-reported race/skin color. The slopes were positive in women and negative in men, and significantly greater in pardo (brown race/skin color) men (p = 0.034).*

of the impact of race on these parameters.[19-22] In our study, patients were stratified by race/skin color according to their own reports; only the most prevalent races were included in the analysis and participants who self-reported as "yellow" or "indigenous" were excluded. Although statistically significant differences were found in many parameters between different races/skin colors, the clinical significance of these findings remain questionable. Besides, these differences were of only milliseconds between wave intervals and durations, and there was considerable overlapping of the curves in the graphs.

Among the limitations of our study, we can mention the difficulty in analyzing race/skin color from participants' own reports in such a mixed-race country as Brazil. In this context, distinction between white, *pardo* (brown) and black may be

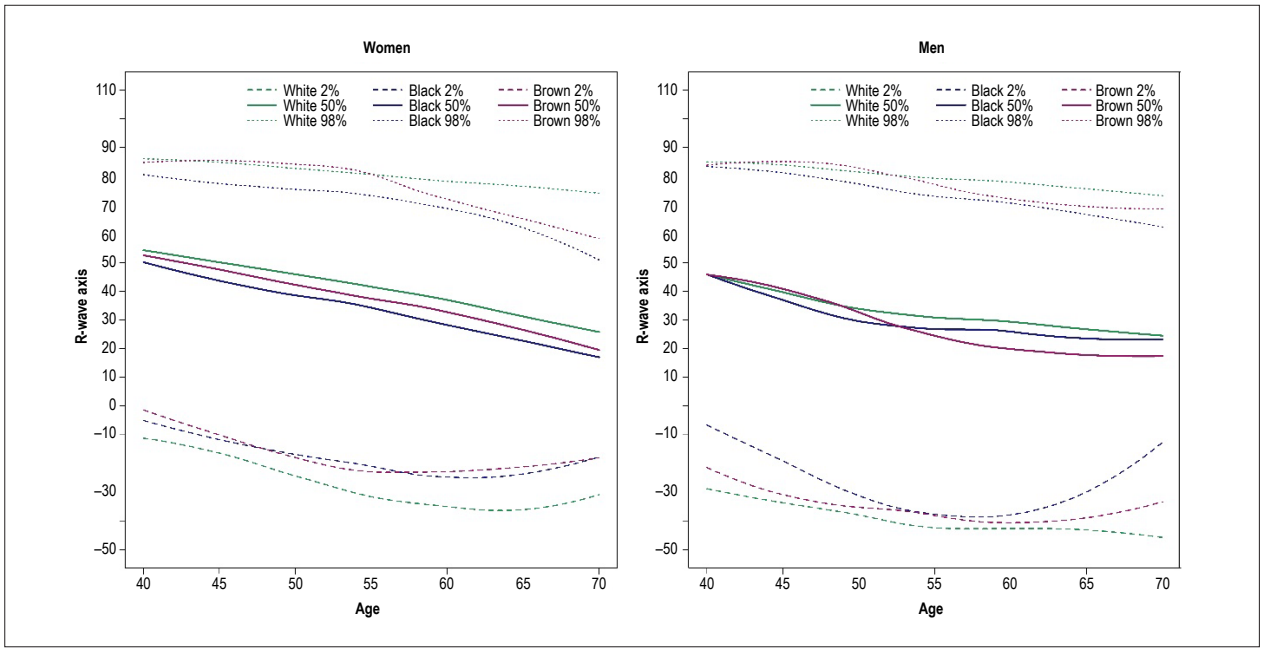

Figure 5 – *R-wave axis by age in men and women stratified by self-reported race/skin color. All curves had a negative slope; a significant difference was found only in pardo (brown race/skin color) men in which a significantly greater slope (p = 0.020) was observed.*

challenging. The decision to maintain hypertensive participants in the analysis of ECGs should be seen with caution, since the possibility that this comorbidity may have affected the results cannot be ruled out.

A strength of this study was the large sample size and the analysis of the relationship between race/skin color and ECG findings. These were obtained using devices of the same brand and model, and a uniform protocol. The clinical variables obtained in a standardized method and a strict quality control enabled a detailed characterization of each participant's health status and clear identification of those free of HD.

From a practical standpoint, our findings tend to corroborate the use of traditional reference values, since they were similar to the results found in this Brazilian population. It is worth mentioning, however, that the interpretation of the PR interval should be viewed with caution, since variation of this parameter within the percentiles considered in the analyses was greater than 200 ms, which is currently considered the cutoff point for first-degree atrioventricular block.[24]

For future perspectives, we highlight the prospective nature of this study, which will make possible the assessment of the electrocardiographic changes in the participants and the effects of aging in this cohort in the outcomes measured. In the current scenario in which physicians try to provide patient-centered care based on patients' needs, our findings will enable the interpretation of ECG in an individualized manner, with possible variations in age- and sex-specific reference values for the Brazilian population. As ECG reading programs and digital ECG devices improve, this scenario may be closer to reality.

Conclusion

This is the first study conducted in Latin America, specifically in Brazil, on the influence of race/skin color on the electrocardiographic parameters. The ECG values here described can be used as reference values for Brazilian adults of both sexes without HD. Our results suggest that self-reported race/skin color had no significant influence on the electrocardiographic parameters.

Author contributions

Conception and design of the research: Pinto Filho MM, Brant LCC, Foppa M, Lotufo PA, Mill JG, Vasconcelo-Silva PR, Almeida MCC, Barreto SM, Ribeiro ALP; Acquisition of data: Pinto Filho MM, Foppa M, Lotufo PA, Mill JG, Vasconcelo-Silva PR, Almeida MCC, Barreto SM, Ribeiro ALP; Analysis and interpretation of the data: Pinto Filho MM, Brant LCC, Padilha-da-Silva JL, Lotufo PA, Ribeiro ALP; Statistical analysis: Pinto Filho MM, Brant LCC, Padilha-da-Silva JL, Ribeiro ALP; Obtaining financing: Lotufo PA, Mill JG, Vasconcelo-Silva PR, Almeida MCC, Barreto SM, Ribeiro ALP; Writing of the manuscript: Pinto Filho MM; Critical revision of the manuscript for intellectual content: Brant LCC, Padilha-da-Silva JL, Foppa M, Lotufo PA, Mill JG, Vasconcelo-Silva PR, Almeida MCC, Barreto SM, Ribeiro ALP.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Original Article

Pericardial Parietal Mesothelial Cells: Source of the Angiotensin-Converting-Enzyme of the Bovine Pericardial Fluid

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Abstract

Background: Angiotensin II (Ang II), the primary effector hormone of the renin-angiotensin system (RAS), acts systemically or locally, being produced by the action of angiotensin-converting-enzyme (ACE) on angiotensin I. Although several tissue RASs, such as cardiac RAS, have been described, little is known about the presence of an RAS in the pericardial fluid and its possible sources. Locally produced Ang II has paracrine and autocrine effects, inducing left ventricular hypertrophy, fibrosis, heart failure and cardiac dysfunction. Because of the difficulties inherent in human pericardial fluid collection, appropriate experimental models are useful to obtain data regarding the characteristics of the pericardial fluid and surrounding tissues.

Objectives: To evidence the presence of constituents of the Ang II production paths in bovine pericardial fluid and parietal pericardium.

Methods: Albumin-free crude extracts of bovine pericardial fluid, immunoprecipitated with anti-ACE antibody, were submitted to electrophoresis (SDS-PAGE) and gels stained with coomassie blue. Duplicates of gels were probed with anti-ACE antibody. In the pericardial membranes, ACE was detected by use of immunofluorescence.

Results: Immunodetection on nitrocellulose membranes showed a 146-KDa ACE isoform in the bovine pericardial fluid. On the pericardial membrane sections, ACE was immunolocalized in the mesothelial layer.

Conclusions: The ACE isoform in the bovine pericardial fluid and parietal pericardium should account at least partially for the production of Ang II in the pericardial space, and should be considered when assessing the cardiac RAS. (Arq Bras Cardiol. 2017; 109(5):425-431)

Keywords: Renin-Angiotensin System; Peptidyl-Dipeptidase A; Pericardial Fluid; Hypertrophy Left Ventricular; Cattle.

Introduction

Cardiovascular diseases are the major cause of morbidity and mortality worldwide.[1] It has been well established that dysregulation or overexpression of the renin-angiotensin system (RAS) leads to several harmful vascular effects, contributing to the pathophysiology of cardiovascular diseases.[2] Angiotensin II (Ang II) is the primary effector hormone of that system, produced by the action of angiotensin-converting-enzyme (ACE) on its substrate, angiotensin I (Ang I).[3-5] Angiotensin II can act systemically or as a tissue factor, locally produced. Tissue Ang II has paracrine and autocrine actions, promoting cell growth, apoptosis, inflammation, oxidative stress and tissue damage, leading to hypertrophy, fibrosis, heart failure and cardiac dysfunction.[6,7] Local tissue[6,8] and intracellular[9] RASs, such as cardiac RAS, have been described, although little is known about the presence of an RAS in the pericardial fluid and its possible sources. Angiotensin II, some growth factors and enzymes have been identified in that fluid.[10] Gomes et al.[11] have shown ACE activity in the human pericardial fluid, and Bechtloff et al.[12] have shown the presence of the protein fraction of ACE in the pericardial fluid of patients with coronary artery disease. However, the source of that enzyme in the pericardial fluid remains unknown.

Because of the difficulties inherent in pericardial fluid collection, the use of appropriate experimental models is essential. The heart is contained inside a fibroserous sac, the pericardial sac, which has an inner layer, the serous pericardium, which delimits the pericardial cavity. Serous pericardium has a visceral membrane, inseparable from the heart, and a parietal membrane, continuous with the visceral one. The pericardial fluid is found inside that cavity.[13,14]

Therefore, characterization in animal models of the pericardial fluid and surrounding tissues, including the source of the macromolecules of that fluid, is essential so that the results can be translated to human beings. This study aimed at collecting evidence in the bovine pericardial parietal membrane and pericardial fluid of the presence of constituents of the Ang II production paths.

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Methods

Collection of bovine pericardial fluid and parietal pericardium

This study used fragments of pericardial parietal membranes, as well as pericardial fluid, of six Nelore cattle (*Bos indicus*, 1758) collected in Delta slaughterhouse (Delta-MG), subject to authorization by the veterinarians in charge. The fragments of pericardial membranes collected were washed and conditioned in saline solution at 4°C. The pericardial fluids, aspirated from the pericardial cavities with 20-mL sterile syringes, were maintained at 4°C and, with the membranes, transported to the laboratory.

Because this study was performed "ex vivo", it required no submission to the Ethics Committee in the Use of Animals (CEUA) UFTM, according to the Inner Regulation of the CEUA/UFTM, article 2, subsection I, §2º.

Processing of pericardial parietal membranes

The fragments of the pericardial parietal membranes were washed in saline solution and dissected in horizontal laminar flow (Labconco, USA), in nutrient DMEM medium, to remove the adipose tissue of the epipericardial layer of the parietal pericardium. Then they were washed in TBS and sliced into fragments of approximately 1.0x0.3 cm, which were embedded in a cryoprotective medium (OCT) and submitted to frozen fixation with liquid nitrogen. After fixation, the fragments were sectioned in a cryostat (Leica Microsystems), and the 2-μm sections obtained were mounted in glass slides, fixed in acetone for 10 minutes, and stored at -20°C.

Pericardial fluid processing

The pericardial fluid was transferred to microcentrifuge tubes and centrifuged at 14000 rpm and 4°C for 10 minutes (Centrifuge 5402, Eppendorf). The supernatants were collected, and the clear ones, with no visual blood contamination, were used, constituting a pericardial fluid pool.

The high concentrations of albumin in the pericardial fluid were reduced by using blue agarose resin (Bio-Rad). Samples of 250 μL of crude extract of pericardial fluid were incubated with 1 mL of blue agarose, balanced with sodium phosphate buffer 0.05 M, pH 6. They were incubated for 2 hours, at room temperature, under agitation. Then, the pericardial fluid extracts were centrifuged at 14000 rpm and 4°C, and the supernatants were collected for further use.

Immunoprecipitation of pericardial fluid

Samples of 500 μL of pericardial fluid, obtained after removing albumin, were incubated with 2.5 μL of anti-ACE antibody (200 μg/mL, Santa Cruz), during the night. Then, 25 μL of CL-4B Sepharose spheres (Amersham Biosciences) conjugated with G protein were added to the samples and incubated for 2 hours. The suspensions were centrifuged for 5 minutes at 14000 rpm. All procedures were performed under agitation at 4°C. The supernatants were discarded and the precipitates collected were diluted with 20 μL of the sample solution.[15] The immunocomplexes obtained were analyzed with SDS polyacrylamide gel electrophoresis (SDS-PAGE).

SDS polyacrylamide gel electrophoresis (SDS-PAGE)

The immunocomplexes obtained were diluted with the sample solution under reducing conditions, at 70°C, centrifuged and applied to gels at 7.5% concentration. The polypeptide bands were separated by use of SDS-PAGE (Mighty Small II SE 260, Amersham Biosciences) at constant 25-mA current. The gels obtained were fixed, stained with coomassie blue and bleached. Duplicates of non-fixed gels were transferred to the nitrocellulose membrane (Invitrogen), in TE 22 (Amersham Biosciences) transfer unit, containing modified Towbin buffer,[16] under agitation, during the night, at 4°C, with a constant 200-mA current. The membranes obtained were stained with Ponceau S to assess the presence of polypeptide fractions, bleached with distilled water, dried in filter paper and submitted to ACE immunodetection.

Immunodetection in nitrocellulose membranes

The nitrocellulose membranes were incubated with 10% skim milk and 2% serum bovine albumin in Tris-buffered saline (TBS), during the night, under agitation, at 4°C, to block nonspecific bindings. Then, that solution was replaced with another containing the primary anti-ACE antibody (200 μg/mL, Santa Cruz), diluted at 1:100, and the membranes were incubated for 2 hours. After incubation with the primary antibody, the membranes were extensively washed with TBS and incubated with the secondary antibody [$F(ab')_2$], rabbit anti-IgG, conjugated with peroxidase (Amersham), diluted at 1:1000, for 2 hours. The membranes were washed again, and the immunoreactive bands were revealed in a solution containing diaminobenzidine (DAB, Dako). The revelation was inactivated in distilled water. All antibodies were diluted in a solution of 1% bovine serum albumin and 0.05% Tween 20 in TBS, the incubation with antibodies being performed at room temperature under agitation. To determine the specificity of the reaction, the membranes were incubated without the primary antibody.

Immunofluorescence in pericardial parietal membranes

The pericardial parietal sections obtained with the cryomicrotome were washed in TBS and incubated with anti-ACE antibody (200 μg/mL, Santa Cruz), for 1 hour, at room temperature, in a dark humid chamber. After incubation with primary antibody, the sections were washed in TBS plus 0.05% Tween 20 several times and incubated with rabbit anti-IgG secondary antibody conjugated with rhodamine (Alexa Fluor Molecular Probes 568). After being extensively washed, the sections mounted with Fluoromount G (Southern Biotech) were observed and documented under a fluorescence microscope Olympus, with a 568-nm wave length. To determine the immunostaining specificity, control sections were incubated without the primary antibody.

Results

ACE detection in the bovine pericardial fluid

When the crude extracts of the pericardial fluid underwent immunoprecipitation with anti-ACE antibody and were analyzed with SDS-PAGE under reducing conditions, a band with molecular mass of approximately 146 kDa, similar to the

ACE mass (Figure 1; arrow), was detected. Of the polypeptide bands observed, the most prominent was the IgG heavy chain, because the antibody was not removed after being added to the pericardial fluid during immunoprecipitation (Figure 1; arrow head). In addition, other thinner bands were noted and could have been co-immunoprecipitated or even not properly removed by washing with buffer. The bovine pericardial fluid ACE immunoprecipitation was confirmed with immunodetection in the nitrocellulose membranes, which evidenced the ACE isoform in the position expected for an enzyme (Figure 2; arrow).

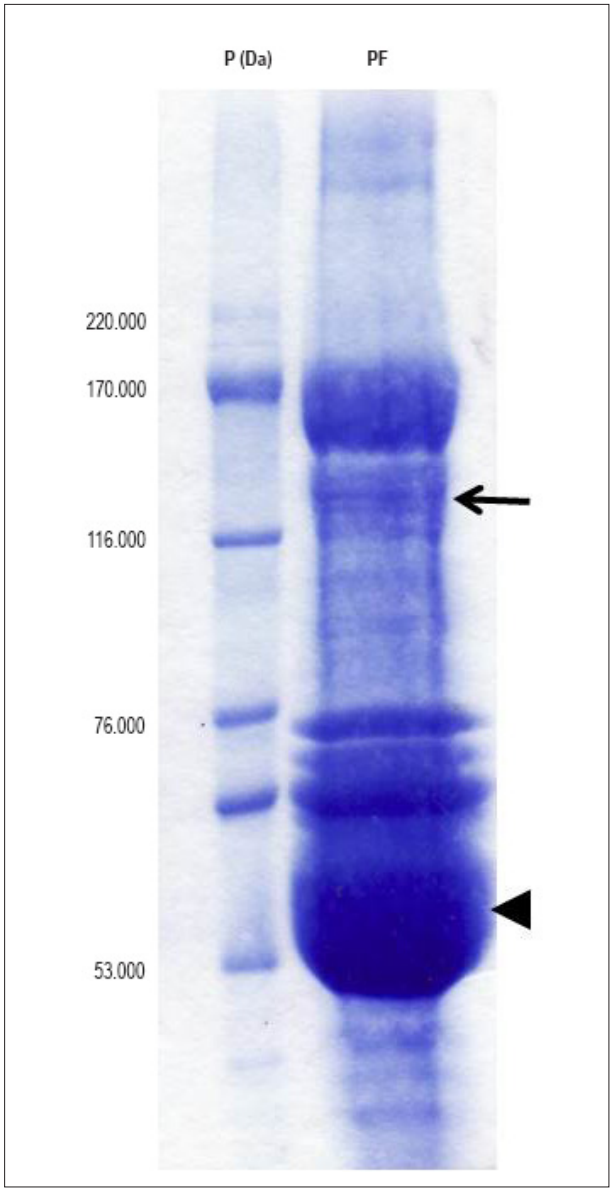

Figure 1 – *SDS-PAGE of samples of bovine pericardial fluid immunoprecipitated with anti-ACE antibody. Representative gel (7.5%), stained with coomassie blue, showing the presence of a band with apparent molecular mass of 146 kDa (arrow), suggestive of an ACE isoform. The arrow head indicates IgG heavy chain. These results are representative of three independent experiments. PF: pericardial fluid; P (Da): patterns of molecular weights.*

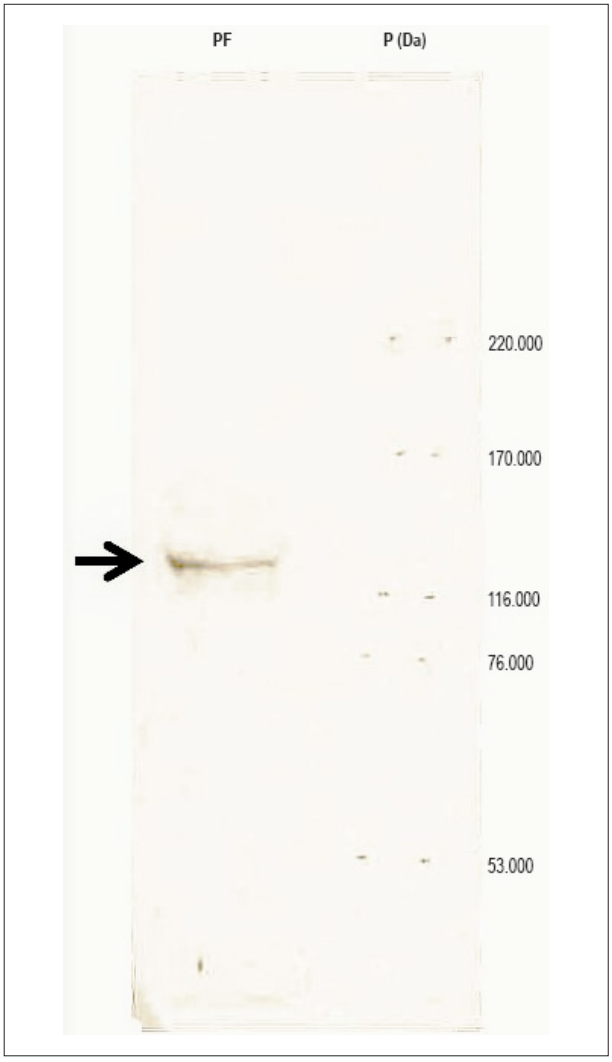

Figure 2 – *Western Blot of samples of bovine pericardial fluid immunoprecipitated with anti-ACE antibody. The polypeptide fractions separated by SDS-PAGE were transferred to the nitrocellulose membranes and probed with anti-ACE antibody. The head indicates the immunomarked ACE isoform. These results are representative of three independent experiments. PF: pericardial fluid; P (Da): patterns of molecular weights.*

Immunolocalization of ACE in the pericardial membrane

The histological sections of the parietal pericardium submitted to ACE detection by use of immunofluorescence showed unequivocal positivity for ACE in the mesothelial cells (Figure 3, right). That positivity neither was continuous in the entire mesothelium nor had the same intensity. Specific fluorescence for ACE was not observed in the fibrous layer of the pericardial membrane, except for the blood vessels, because ACE is expressed in endothelial cells. Negative controls showed no staining. Figure 3 shows the histological section of the parietal pericardium stained with toluidine blue.

Discussion

The present study evidences the presence of an ACE isoform in bovine pericardial fluid, and establishes the ACE

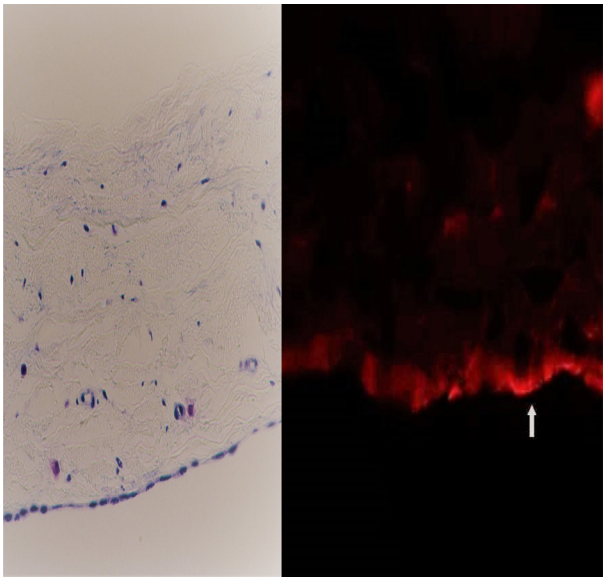

Figure 3 – *Right: image representative of sections of bovine pericardial parietal membranes, cryofixed and submitted to ACE immunodetection. Note the positive mesothelial layer (arrow). Left: histological section of bovine parietal pericardium stained with toluidine blue. Original magnification: 40x.*

location in mesothelial cells of the bovine pericardial parietal membrane, for the first time, indicating that membrane as a possible source of the pericardial fluid ACE.

The RAS, originally characterized as a circulating endocrine system, comprises several enzymatic paths and bioactive components that have several functions.

Currently, there is plenty of evidence of the presence of tissue RASs that influence local cell actions, with intracellular and subcellular components.[17-20] Local RASs have been shown in many tissues/organs, such as heart, kidneys, adrenal glands, blood vessels, pancreas, liver, brain, and adipose tissue.[6,19,21-24] Regarding the cardiac RAS, several of its constituents, such as angiotensinogen, renin, ACE, Ang I and Ang II, and AT1 and AT2 receptors, were detected in different regions of the heart, such as the atria, conduction system, heart valves, coronary arteries and ventricles, being synthesized by different cell types, such as fibroblasts and myocytes.[6,24-26]

The importance of the pericardium and pericardial fluid to control cardiac function has been established in past years. The quiescent nature of the visceral and parietal pericardium has been questioned, resulting in evidence for an important role in the production of substances that could have paracrine actions on the heart. When the human parietal pericardium is compared with that of other species, we observe that bovine parietal pericardium has the closest histological constitution to that of the human species.[27,28] Thus, characterizing the bovine pericardium, mainly the macromolecules and mediators produced by the cells that delimit the pericardial cavity, is paramount to the better understanding of the biology and importance of that membrane and of the pericardial fluid under physiological conditions or in association with any disease.

The pericardial fluid is considered an ultrafiltrate of plasma, added by some components of the myocardial interstitial fluid. Its protein concentration is lower than that of the plasma, but with a relatively high albumin concentration.[14] Substances detected in the human or animal pericardial fluid, such as endothelin-1, beta fibroblast growth factor (bFGF), Ang II, renin, atrial natriuretic factor, vascular endothelial growth factor (VEGF), interleukin-6, and cell adhesion molecules, could act upon the heart.[10,29-31] Modulation of growth and survival of cardiac myocytes,[10] endothelial cells and smooth muscle cells[32,33] are some biological effects of mediators existing in the pericardial fluid of patients with ischemic and non-ischemic cardiac diseases. Limana et al.[34] have shown that, in response to myocardial infarction, epicardial c-kit+ cells reactivate an embryonic program, in which soluble factors of the pericardial fluid play a fundamental role. Thus, the knowledge about that fluid composition has pathophysiological importance and diagnostic significance.[30]

Our results evidence the presence of an ACE isoform in the bovine pericardial fluid, showing the existence of a part of the RAS in the pericardial cavity, probably of local origin. Although the pericardial fluid is a plasma ultrafiltrate and plasma mediators, such as Ang II, can spread to the pericardial fluid with no restriction, the same does not happen with ACE. The structural organization of the mesotelial layer of the pericardium, both parietal and visceral ones, prevent that free circulation. The presence of tight junctions between the mesothelial cells[27,28,35] prevents paracellular transport of macromolecules with molecular mass above 40 KDa.[36] Because the ACE isoform present in the bovine pericardial fluid has molecular mass of approximately 146 KDa, similar to that predicted for human ACE,[5] the paracellular route would not be an access way to the pericardial cavity.

In addition to the above cited factors, which partially support the local synthesis of ACE, ACE localization should be considered. Immunofluorescence evidenced positivity in parietal pericardial mesothelial cells and in the blood vessels of the pericardial membrane. Immunolocalization of ACE in blood vessels was expected, because ACE has ubiquitous distribution in the endothelium.[5,37] However, in mesothelial cells, its immunolocalization is a strong evidence that those cells are the source of pericardial fluid ACE, because: *i*) they have a close anatomical relationship with the pericardial cavity, because they delimit it; *ii*) ACE is an integral protein of the membrane, being, thus, produced by mesothelial cells, with its extracellular domain directed to the pericardial cavity;[5] *iii*) the ability of mesothelial cells to synthesize ACE has been demonstrated by the presence of that enzyme's mRNA in cultured human peritoneal mesothelial cells, by use of RT-PCR;[38] *iv*) they have abundant endoplasmic reticulum and developed Golgi complex,[27,28,35] consistent with the profile of cells capable of active protein synthesis.

Corroborating with those arguments, several studies have shown the metabolic profile of mesothelial cells. Mesothelial cells synthesize and secrete lubricants, such as glycosaminoglycans and surfactant, to prevent friction and the formation of adhesions between the parietal and visceral surfaces.[39] They play a critical role in homeostasis control of the serous membranes in response to injury, inflammation and immunoregulation.[39] In addition, mesothelial cells play a central role in the repair of serous membranes, secretion of inflammatory mediators, chemokines, growth factors and extracellular matrix components. They have different phenotypes, which, depending on their location and activation status, reflect functional differences.[39]

The importance of local RASs has not been totally clarified. Higher concentrations of active cardiovascular mediators in the pericardial fluid than in the plasma raises a question about their origin and possible actions upon the surrounding tissues. The pericardial fluid of patients with coronary artery disease trigger substantial arterial contractions in isolated carotid arteries of rats, which are mediated primarily by ET-1.[40] Our results showed both the presence of an ACE isoform in the pericardial fluid, and, for the first time, the immunolocalization of that protein in parietal pericardial mesothelial cells, suggesting that the parietal pericardial mesothelial layer is one possible source of the pericardial fluid ACE. Thus, the Ang II produced locally could act on its own pericardial mesothelial cells, both parietal and visceral, or even directly on the myocardium, promoting inflammation, oxidative stress and cell death, contributing to cardiac hypertrophy and fibrosis. In addition, it could act directly on the myocardial microcirculation promoting important vasomotor effects. In that context, the pericardial fluid would be an important reservoir of mediators that could modulate the functions of cardiac cells.

The use of experimental models with tissues similar to the human ones, both regarding structural organization and cell constitution, would be more suitable for studying certain human conditions. In addition to structural organization, biochemical and molecular features are fundamental to achieving optimal balance between quantity and quality of the data produced and their relevance for the condition investigated.

The structural features of the bovine pericardial mesotelial layer, similar to the human ones,[27,28] suggest our results can be extended to human pericardial mesothelial cells, which could be the partial source of the human pericardial fluid ACE.[11,12] A better knowledge of both the pericardial fluid constituents and the mesothelial cells in proper animal models could help understanding the paracrine or autocrine effects of mediators produced by the pericardium on the heart.

One limitation of our study was the volume of the pericardial fluid obtained from the animals in the sample. Because of the difficulties inherent in collecting bovine pericardial fluid, the volume was relatively low. Thus, further research is required to clarify how mesothelial cells interact with their local environment and what is their contribution to the production of mediators in the pericardial fluid that can modulate cell actions essential to maintain cardiac homeostasis.

Conclusions

The Ang II present in the bovine pericardial fluid is partially produced by the action of the ACE existing in that fluid, pericardial parietal mesothelial cells being a source of that ACE.

Author contributions

Conception and design of the research: Sousa Filho IR, Teodoro LGVL, Rodrigues MLP, Gomes RAS; Acquisition of data: Sousa Filho IR, Pereira ICC, Morais LJ, Rodrigues MLP; Analysis and interpretation of the data: Sousa Filho IR, Pereira ICC, Rodrigues MLP; Obtaining financing and Critical revision of the manuscript for intellectual content: Teodoro LGVL, Rodrigues MLP, Gomes RAS; Writing of the manuscript: Sousa Filho IR, Pereira ICC, Morais LJ, Teodoro LGVL, Rodrigues MLP, Gomes RAS.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Cardiac, Metabolic and Molecular Profiles of Sedentary Rats in the Initial Moment of Obesity

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Abstract

Background: **Different types of high-fat and/or high-energy diets have been used to induce obesity in rodents. However, few studies have reported on the effects observed at the initial stage of obesity induced by high-fat feeding on cardiac functional and structural remodelling.**

Objective: **To characterize the initial moment of obesity and investigate both metabolic and cardiac parameters. In addition, the role of Ca^{2+} handling in short-term exposure to obesity was verified.**

Methods: **Thirty-day-old male Wistar rats were randomized into two groups (n = 19 each): control (C; standard diet) and high-fat diet (HF, unsaturated high-fat diet). The initial moment of obesity was defined by weekly measurement of body weight (BW) complemented by adiposity index (AI). Cardiac remodelling was assessed by morphological, histological, echocardiographic and papillary muscle analysis. Ca^{2+} handling proteins were determined by Western Blot.**

Results: **The initial moment of obesity occurred at the 3rd week. Compared with C rats, the HF rats had higher final BW (4%), body fat (20%), AI (14.5%), insulin levels (39.7%), leptin (62.4%) and low-density lipoprotein cholesterol (15.5%) but did not exhibit alterations in systolic blood pressure. Echocardiographic evaluation did not show alterations in cardiac parameters. In the HF group, muscles were observed to increase their +dT/dt (C: 52.6 ± 9.0 g/mm²/s and HF: 68.0 ± 17.0 g/mm²/s; $p < 0.05$). In addition, there was no changes in the cardiac expression of Ca^{2+} handling proteins.**

Conclusion: **The initial moment of obesity promotes alterations to hormonal and lipid profiles without cardiac damage or changes in Ca^{2+} handling. (Arq Bras Cardiol. 2017; 109(5):432-439)**

Keywords: **Rats; Obesity; Diet, Hight-Fat; Cardiac function; Calcium; Adiposity.**

Introduction

Obesity is considered a major syndrome of the XXI century and has reached epidemic proportions worldwide in recent decades.[1,2] According to the World Health Organization, the number of overweight individuals has reached over a billion people, and more than 30% of this population is obese.[3] Obesity is a complex disease, and while some authors have suggested that genetic factors contribute its development,[4] most research emphasizes that major causes of obesity are the so-called exogenous factors, especially the consumption of highly available, palatable food, and lack of exercise.[2,5]

A number of different types of high-fat and/or high-energy diets have been used to induce obesity and mimic human metabolic syndrome in rodents.[6-11] However, few studies have investigated the initial stage of obesity induced by a high-fat diet. Researchers have observed the initial moment of obesity in animals fed a high-fat diet after 4 weeks of treatment.[12-15] However, molecular and cardiac parameters in this initial stage were not presented.

Studies have shown that excess body fat leads to several cardiovascular abnormalities that correlate with the duration and intensity of obesity in humans and in animal models.[16-20] Thus, it becomes necessary to identify the duration and intensity of damage in the early period of the disease. Furthermore, it is important to verify the mechanisms involved in this process, since studies have shown that abnormalities in Ca^{2+} handling may be responsible for the development of cardiac dysfunction in obesity models induced by a high-fat diet.

Due to the lack of studies, our purpose was to characterize the initial moment of obesity and investigate both the metabolic and cardiac parameters in obese rats. In addition, the role of Ca^{2+} handling in short-term exposure to obesity was evaluated.

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Methods

Animal Care

All experiments and procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health and approved by the Botucatu Medical School Ethics Committee (UNESP, Botucatu, SP, Brazil) (approval numberFMB-PE-5/2009).

Thirty-day-old male *Wistar* rats were distributed into two groups: control (C, n = 19) and high-fat diet (HF, n = 19). Group C was fed a standard diet containing 12.3% of its energy from fat, 57.9% from carbohydrate, and 29.8% from protein.The HF animals were fed four high-fat diets (RC Focus 2413, 2414, 2415, and 2416) that differed in their flavouring but not in their micro- and macronutrients. The high-fat diets contained 49.2% of their energy from fat, 28.9% from carbohydrates, and 21.9% from protein as previously described.[17] All rats were housed in individual cages in an environmentally controlled, clean-air room at 23 ± 3°C with a 12-h light/dark cycle (lights on at 6am) and 60 ± 5% relative humidity. After starting the experimental protocol, food consumption (FC), energy intake (EI), feed efficiency (FE), and body weight (BW) were recorded weekly. EI was calculated as follows: EI = average weekly EI multiplied by the caloric value of each diet (C or HF). FE (%) is the ability to convert EI to BW and was determined as the mean BW gain (g)/total calorie intake (kcal) x100.

Characterization of the Initial Moment of Obesity

After starting the experimental protocol, BW was recorded once a weekto characterize the initial moment of obesity. When C and HF groups presented a significant difference in BW, the animals were anesthetized by ketamine injection (50 mg/kg) and xylazine (0.5 mg/kg) intraperitoneal (IP) injection, decapitated, and thoracotomized, and the fat pads were dissected and weighed. The initial moment of obesity was defined by BW (g) measurements that were recorded weekly and complemented by *post-mortem* adiposity index (AI) using the following formula: AI = [body fat (BF)/ BW] x 100. BF (g) was measured from the sum of the individual fat pad weights: epididymal fat + retroperitoneal fat + visceral fat.

Systolic blood pressure

The systolic blood pressure (SBP) of the tail was measured one week before euthanasia with a tail plethysmograph. The animals were warmed in a wooden box at 40°C with heat generated for four minutes to cause vasodilation of the tail artery, and the animals were then transferred to an iron cylindrical support. A sensor was placed in the proximal region of the tail and coupled to an electro-sphygmomanometer (NarcoBioSystem, International Biomedical Inc, TX, USA). The electro-sphygmomanometer was attached to a computer,and SBP was measured using the Biopac software (Biopac Systems Inc., CA, USA).

Insulin tolerance test (ITT)

Blood samples were drawn from the tip of the tail at basal condition and after the intraperitoneal administration of regular insulin (Novolin² R, Novo Nordisk, Bagsvaerd, Denmark) at a dose of 1.5 IU/kg body weight.[21] Blood glucose was then collected at 0 (basal), 5, 10, 15, 20, 25 and 30 min. Glucose levels were determined using a handheld glucometer (Accu-ChekAdvantage; Roche Diagnostics Co., Indianapolis, USA). Insulin resistance was determined from the area under the curve for glucose (AUC; 0-30 minutes).

Echocardiographic evaluation

One week before euthanasia, echocardiographic evaluation was performed using a commercially available echocardiograph (Philips HDI-5000), and left ventricular (LV) structural variables were evaluated as previously described.[22]

Metabolic and hormonal profile

Blood samples were collected, and the serum was separated by centrifugation at 3,000 × g for 15 minutes at 4°C. Glucose, total cholesterol (T-Chol), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and hormones (insulin and leptin) were analysed. Glucose, TChol, HDL and LDL were measured using an automatic enzymatic analysis system (Biochemical analyzer BS-200, Mindray, China). The leptin and insulin levels were determined by enzyme-linked immunosorbent assay methodology using commercial kits (Linco Research Inc., St. Louis, MO, USA).

Morphological and histological analysis

After the initial moment of obesity, the heart weight (HW), HW/final body weight (FBW) ratio, papillary muscle cross-sectional area (CSA) and collagen fraction (n = 14; each group) were recorded.

Papillary Muscle Function

Papillary muscles isolated from the LV were evaluated as previously described.[17]The papillary muscles were evaluated under the baseline condition of 2.5 mM Ca^{2+}, post-rest contraction (PRC) and after elevation of extracellular Ca^{2+} concentration. PRC was studied at an extracellular Ca^{2+} concentration of 0.5 mM, in which the stimulus was paused for 10, 30, and 60 s before restarting the stimulation. Inotropic responses were recorded 5 min after the addition of each dose of extracellular Ca^{2+} (0.5, 1.0, 1.5, 2.0, and 2.5 mM) to the bathing solution.

Western Blot analysis

LV tissue (C; n = 6; HF; n = 6) was analysed by Western Blot to quantify the L-type Ca^{2+} channel, SERCA2A: Sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a) and phospholamban (PLB). expression as previously described.[20] Specific antibodies were obtained against SERCA2 ATPase (ABR, Affinity BioReagents, CO, USA; MA3-910, 1:2,500), PLB (ABR, Affinity BioReagents, CO, USA;MA3-922, 1:500) and L-type Ca^{2+} channel alpha 1C (Sigma-Aldrich, St. Louis, MO;C4980, 1:200). Binding of the primary antibody was detected with peroxidase-conjugated secondary antibodies (rabbit or mouse, depending on the protein). Quantification of blots was performed by Scion Image software (Scion based on NIH image). Targeted bands were normalized to the expression of β-actin by using an antibody (SC81178; 1:1000) obtained from Santa Cruz Biotechnology (CA, USA).

Statistical analysis

The statistical analysis was performed using the Sigma Stat 3.5 software (SYSTAT Software Inc., San Jose, CA, USA). The distribution of the variables was assessed by using the Kolmogorov-Smirnov test for normality, and the results were reported as means ± standard deviation (SD). Comparisons between groups were performed using Student'st-test for independent samples and a repeated-measures two-way analysis of variance (ANOVA) when appropriate. The level of significance considered was 5%.

The sample size (n) was estimated using the equation: $n = [(Z1-\alpha/2 + Z1-\beta) \times r/\Delta)2$, where n is the sample size, Z is the z score, α is the two-sided significance level (0.05; type I error), β is the statistical power (80%; type II error), r is the SD and Δ is the minimal difference between groups.[23] The sample size needed to detect a significant between groups is 10 rats per group; however, we decided to use 19 animals per group for most of the analyses.

Results

BW was similar in the first two weeks of treatment in both groups C and HF (*data not shown*); however, during the third week, BW was greater in group HF than in group C. This moment was characterized as the initial moment of obesity.

Table 1 shows the general characteristics, comorbidities and hormone results from C and HF rats after characterizing the initial moment of obesity (3 weeks). The FBW and weight gain were both higher in HF than in C. The high-fat diet promoted a substantial elevation of epididymal and visceral fat pad weight and BF. However, initial BW and retroperitoneal fat pad weight did not differ between the groups. AI was higher in HF animals (14.5%) compared to C animals. Compared with C group, HF had a lower FC, a greater FE, but a similar EI. Glucose, T-Chol and HDL levels, and SBP were similar between the groups. However, the AUC for glucose obtained in the insulin tolerance test, and LDL, insulin and leptin levels were significantly higher in HF compared to C.

The morphological and histological analyses are presented in Figure 1. Heart weight (Figure 1A), heart/FBW ratio (Figure 1B), myocyte CSA (Figure 1C), and LV collagen fraction (Figure 1D) were similar between the groups.

Echocardiographic evaluation showed that the HF rats did not show a difference in heart rate (HR), left ventricular end-diastolic dimension (LVDD), left ventricular end-systolic dimension (LVSD), posterior wall thickness in diastole (PWTd), relative wall thickness (RWT), left atrium (LA), left ventricular mass (LVM), fractional shortening (FS) endocardial, FS midwall, posterior wall shortening velocity (PWSV), earlydiastolic mitral inflow (E-wave), latediastolic mitral inflow (A-wave), early-to-late diastolic mitral inflow ratio (mitral E/A), E-wave deceleration time (EDT) or isovolumetric relaxation time (IVRT) compared to C rats (Table 2). However, the HF group presented higher aortic diameter (AO) in relation to C. After 3 weeks, the treatment did not promote contractile dysfunction in basal condition or after Ca^{2+} stimulation (Figure 2). However, the results demonstrated that during manoeuvre PRC, an increase in contractile phase was observed after 60 seconds in group HF compared to C, as

Table 1 – General characteristics, comorbidities and hormones

Variables	Groups	
	C (n = 19)	HF (n = 19)
IBW, g	148 ± 12	147 ± 12
FBW, g	290 ± 18	302 ± 22*
WG, g	142 ± 10	155 ± 10*
Epididymal, g	4.6 ± 0.8	5.6 ± 1.2*
Retroperitoneal, g	5.4 ± 1.5	6.4 ± 1.7
Visceral, g	4.1 ± 1.0	4.9 ± 0.9*
BF, g	14.1 ± 3.0	16.9 ± 3.2*
AI, %	4.9 ± 1.0	5.6 ± 0.9*
Glucose, mg/dL	182 ± 27	181 ± 21
AUC, mg/dL/min	2129 ± 193	230 8 ± 218*
T-Chol, mg/dL	63.2 ± 10.4	68.3 ± 6.1
HDL, mg/dL	49.2 ± 7.7	52.9 ± 4.7
LDL, mg/dL	9.0 ± 1.7	10.4 ± 2.4*
SBP, mmHg	127 ± 8	131 ± 14
Insulin, ng/mL	0.83 ± 0.16	1.16 ± 0.28*
Leptin, ng/mL	2.34 ± 0.57	3.80 ± 1.26*

*Values are means ± SD; control (C) and high-fat diet (HF) groups; n: number; IBW: initial body weight; FBW: final body weight; WG: weight gain; BF: body fat; AI: adiposity index; AUC: area under the curve for glucose determined in insulin tolerance test (ITT); T-Chol: total cholesterol; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; SBP: systolic blood pressure; * p < 0.05 vs. C. Student's t-test for independent samples.*

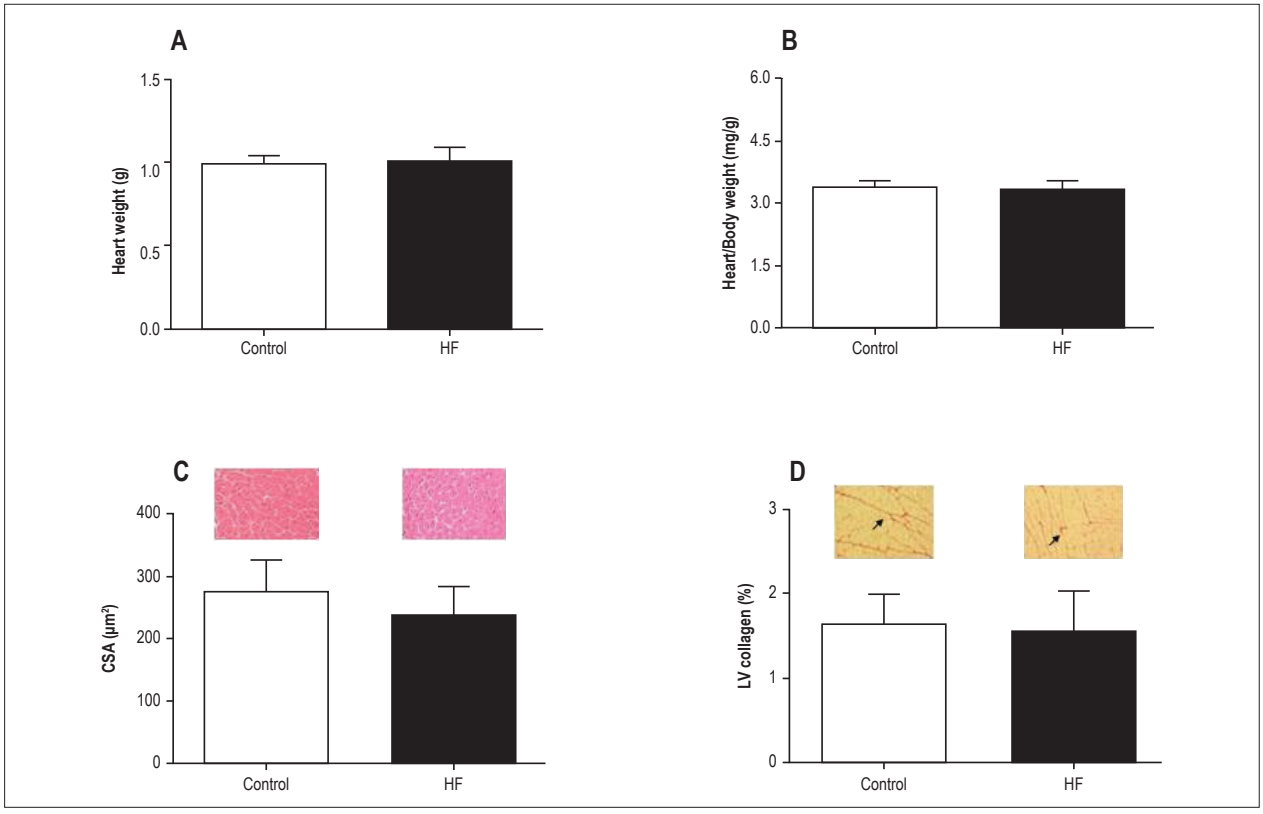

Figure 1 – *Morphological analysis of control (C) versus high-fat diet (HF) rats. A: Heart weight. B: Heart/final body weight ratio. C: Myocyte cross-sectional area (40× magnification lens); representative haematoxylin and eosin-stained left ventricular cross-sections from C and HF rats. D: Interstitial collagen volume fraction of myocardium (20× magnification lens) from C and HF rats; representative picrosirius red-stained left ventricular sections from C and HF rats. Arrows represent the interstitial collagen volume fraction of C and HF. Data presented as the mean ± SD. Student's t test was used for independent samples. There are no differences between groups.*

visualized by positive tension derivative normalized per CSA (+dT/dt) (C: 52.6 ± 9.0 g/mm²/s and HF: 68.0 ± 17.0 g/mm²/s; $p < 0.05$) (Figure 2E). Figure 3 (A-C) shows that 3 weeks of high-fat feeding did not alter the protein levels of the L-type Ca^{2+} channel, SERCA2a or PLB.

Discussion

The determination of the initial moment of obesity is essential to control the duration of experimental protocols because it allows one to accurately assess the influence of exposure to adiposity and, consequently, obesity.[16] Interestingly, little information is available on this process and its cardiovascular consequences.

Some researchers have shown metabolic, cardiac and molecular characteristics only at the end of the experiment,[17,20,24] which precludes analyses regarding the precise onset of disturbances caused by excess adipose tissue and their intensity. A question that arises from the present study is why the experimental studies have not characterized the initial moment of obesity. Thus, the major finding was that there were alterations in the metabolic and lipid profiles at the initial moment of obesity, but without accompanying cardiac damage or changes to Ca^{2+} handling proteins.

High-fat diets are generally accepted as a method of generating a valid rodent model for obesity. According to the data obtained, we developed a valid rodent model for diet-induced obesity with unsaturated fat. After 3 weeks, HF animals showed higher weight gain (16%) and body fat (20%) than C animals. The findings are in agreement with several authors.[18-20,24] In addition, the animal model presented some features of metabolic syndrome, such as central obesity, glucose intolerance and dyslipidemia, but without alterations in SBP, thus demonstrating obesity with its comorbidities. In contrast, previous investigations have observed numerous comorbidities associated with short-term obesity, such as hypertension and diabetes.[25,26]

The initial moment of obesity caused important metabolic abnormalities, such as elevated leptin (62.5%) and insulin (40%) levels in the HF group. According to the literature, leptin is a hormone secreted by adipose tissue and has a direct relationship with the amount of BF.[27] The relationship between leptin levels and fat shows that the amount of leptin is approximately 3 times higher than BF. Furthermore, leptin is able to directly activate nitric oxide production via L-arginine, which is dependent on endothelial integrity[27] and may be a determining factor in the absence of hypertension. In addition, hyperinsulinemia was observed with insulin sensitivity damage, since the glucose AUC was higher in group HF than group C.

The initial moment of obesity did not cause cardiac remodelling, as visualized by histological and echocardiographic analysis. Dhanasekaran et al.[28] reported

Table 2 – Echocardiography data

Variables	Groups	
	C (n = 10)	HF (n= 10)
HR, bpm	341 ± 40	316 ± 22
LVDD, mm	7.85 ± 0.53	7.98 ± 0.37
LVSD, mm	3.66 ± 0.63	3.70 ± 0.44
PWTd, mm	1.47 ± 0.06	1.44 ± 0.07
RWT	0.19 ± 0.02	0.18 ± 0.01
AO, mm	3.39 ± 0.14	3.53 ± 0.13*
LA, mm	5.27 ± 0.32	5.39 ± 0.31
LVM, g	0.81 ± 0.06	0.83 ± 0.10
FS endocardial, %	53.6 ± 5.7	53.6 ± 4.5
FS midwall, %	33.0 ± 3.0	33.9 ± 3.7
PWSV, mm/s	40.38 ± 5.49	38.79 ± 3.05
E-Wave (cm/s)	94.5 ± 13.6	87.4 ± 3.9
A-Wave (cm/s)	67.4 ± 18.7	60.2 ± 4.7
Mitral E/A	1.4 ± 0.4	1.5 ± 0.2
EDT, ms	42.0 ± 4.8	43.8 ± 4.9
IVRT, ms	20.4 ± 3.1	20.1 ± 3.8

*Data presented as the mean ± standard deviation. C: control and HF: high-fat diet groups; n: number; HR: heart rate; LVDD: left ventricular end-diastolic dimension; LVSD: left ventricular end-systolic dimension; PWTd: posterior wall thickness in diastole; RWT: relative wall thickness; AO: aortic diameter; LA: left atrium; LVM: left ventricle mass; FS endocardial: fractional shortening; FS midwall: fractional shortening; PWSV: posterior wall shortening velocity;early (E-wave) and late (A-wave) diastolic mitral inflow; E/A: early-to-late diastolic mitral inflow ratio; EDT: E-wave deceleration time; IVRT: isovolumetric relaxation time; *p < 0.05 versus C (control). Student's t test for independent samples.*

that insulin resistance induced by obesity with associated hyperinsulinemia could promote cardiac remodelling via the growth-promoting properties of insulin or by attenuating the anti-apoptotic signalling of the phosphatidylinositol 3' –kinase/protein kinase B pathway.[29] Studies in mice with (functional) leptin deficiency have suggested that the cardiac hypertrophy developing in states of chronic hyperleptinaemia may result from an inability to transduce anti-hypertrophic and/or cardioprotective effects of the adipokine.[29]

Nonetheless, obesity is still considered a risk factor in the development of cardiovascular disorders, despite the called "obesity paradox". A number of rodent models of obesity have been studied in terms of cardiovascular adaptations.[12,15,24] Diet-induced obese rats exhibit many of the hemodynamic alterations associated with human obesity, but there is no evidence to date that these animals will develop severe cardiac depression. In the current study, echocardiographic and papillary muscle analysis showed that there was no change in cardiac parameters in both groups. The absence of functional changes may be due to the short term of the exposure to HF diet.

According to the results, short-term exposure to HF diet promoted specific changes at contraction phase after the PRC manoeuvre, indicating absence of myocardial function impairment. This result could be related to Ca handling changes; however, our results show that there was no change in the levels of Ca^{2+} L-type channels, PLB or SERCA2a protein, suggesting that the kinetic properties of calcium are preserved in the onset of obesity. Furthermore, the post-translational modifications known to affect the activity of these proteins, such as phosphorylation and glycosylation, were not investigated in the present study.

Study limitations

The study did not investigate post-translational modifications known to affect the activity of proteins, such as phosphorylation and glycosylation, which could consolidate the absence of alterations in the expression of proteins involved in the intracellular calcium handling at the initial moment of obesity.

Conclusion

The initial moment of obesity promotes alterations to hormonal and lipid profiles without cardiac damage and changes to Ca^{2+} handling in a rat model of unsaturated high-fat diet-induced obesity. Taken together, these findings could be relevant to human pathology and enable the verification and prevention of disturbances in the early period of obesity.

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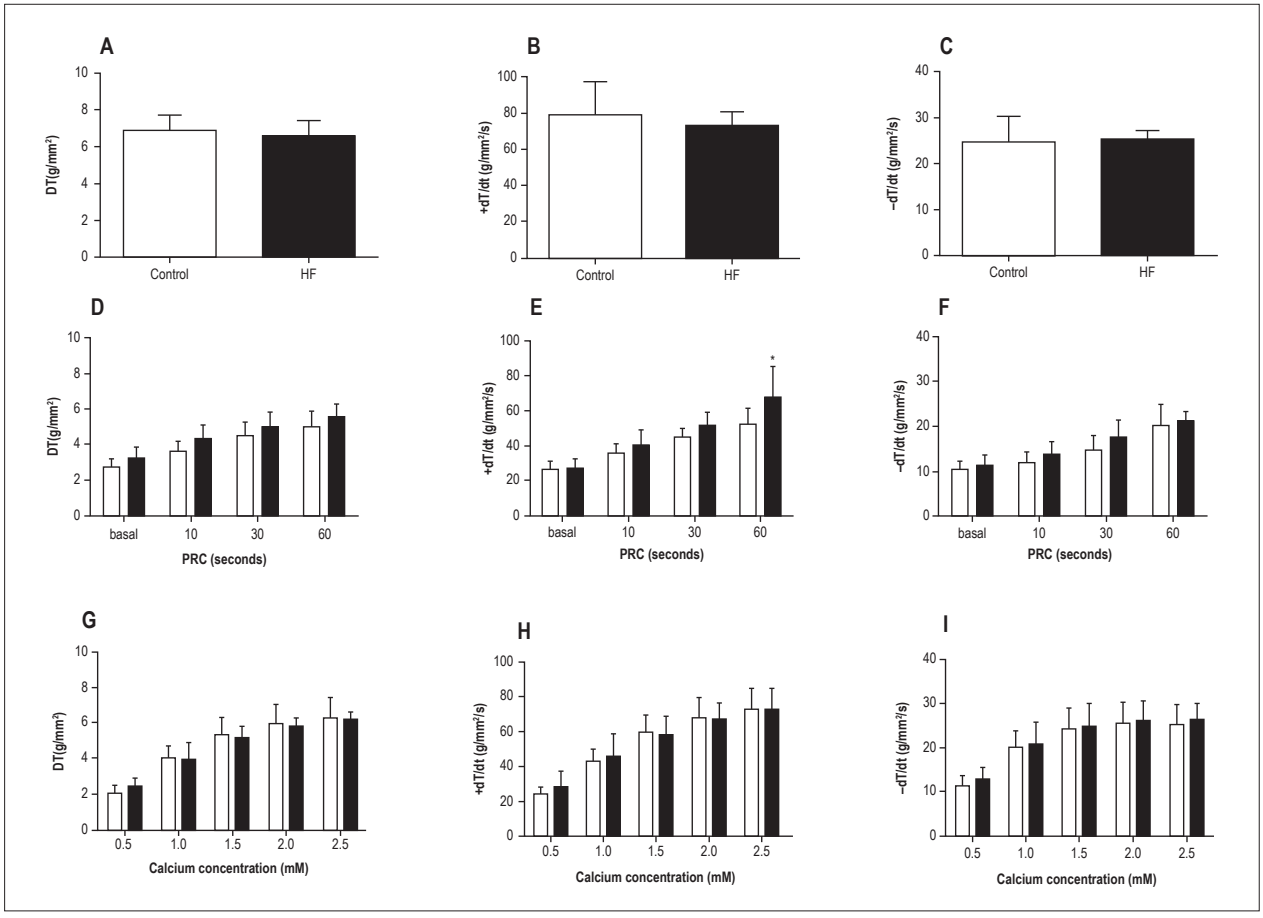

Figure 2 – *Basal condition (A, B and C), post-rest contraction (D, E and F) and effects of increasing extracellular Ca^{2+} concentration (G, H and I) in papillary muscles of control (C) and high-fat diet (HF) rats (white bars = C; black bars = HF; n = 19 in each condition). Maximum developed tension normalized percross-sectional area (DT) [g/mm²], negative (-dT/dt[g/mm²/s]) and positive (+dT/dt[g/mm²/s]) tension derivatives normalized per cross-sectional area. Data presented as the means ± SD. *p < 0.05 versus C. Student's t-test for independent samples (A, B and C) and repeated-measures two-way ANOVA (D, E, F, G, H and I); Student-Newman-Keuls post-hoc test.*

Author contributions

Conception and design of the research: Jacobsen BB, Cicogna AC, Leopoldo APL, Leopoldo AS; Acquisition of data: Jacobsen BB, Cordeiro JP, Campos DHS, Nascimento AF, Sugizaki MM, Cicogna AC; Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content: Jacobsen BB, Cordeiro JP, Campos DHS, Nascimento AF, Sugizaki MM, Cicogna AC, Padovani CR, Leopoldo APL, Leopoldo AS; Statistical analysis: Cicogna AC, Padovani CR; Obtaining financing: Cicogna AC, Leopoldo APL, Leopoldo AS; Writing of the manuscript: Jacobsen BB, Sugizaki MM, Cicogna AC, Padovani CR, Leopoldo APL, Leopoldo AS.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

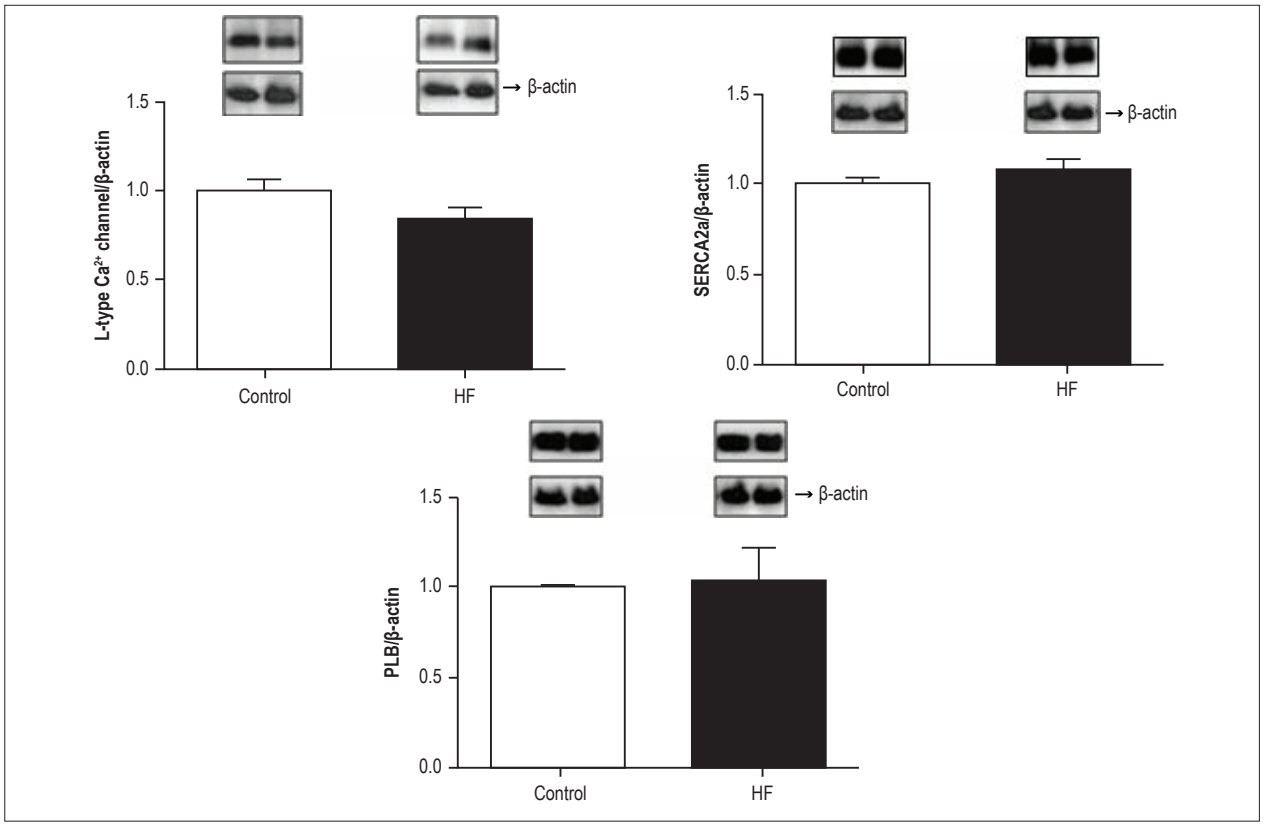

Figure 3 – *Cardiac protein expression by Western Blot. The data are means ± SD (n = 6 in each group); control (C) and high-fat diet (HF); A: L-type Ca^{2+} channel; B: Sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a) and C: phospholamban (PLB). Student's t-test for independent samples.*

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First results of the Brazilian Registry of Percutaneous Left Atrial Appendage Closure

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Abstract

Background: Left atrial appendage closure (LAAC) is an effective alternative to oral anticoagulation (OA) for the prevention of stroke in patients with non-valvular atrial fibrillation (NVAF).

Objective: To present the immediate results and late outcomes of patients submitted to LAAC and included in the Brazilian Registry of Percutaneous Left Atrial Appendage Closure.

Methods: 91 patients with NVAF, high stroke risk (CHA_2DS_2VASc score = 4.5 ± 1.5) and restrictions to OAC (HAS-BLED score = 3.6 ± 1.0) underwent 92 LAAC procedures using either the Amplatzer cardiac plug or the Watchman device in 11 centers in Brazil, between late 2010 and mid 2016.

Results: Ninety-six devices were used (1.04 device/procedure, including an additional non-dedicated device), with a procedural success rate of 97.8%. Associated procedures were performed in 8.7% of the patients. Complete LAAC was obtained in 93.3% of the successful cases. In cases of incomplete closure, no residual leak was larger than 2.5 mm. One patient needed simultaneous implantation of 2 devices. There were 7 periprocedural major (5 pericardial effusions requiring pericardiocentesis, 1 non-dedicated device embolization and 1 coronary air embolism without sequelae) and 4 minor complications. After 128.6 patient-years of follow-up there were 3 deaths unrelated to the procedure, 2 major bleedings (one of them in a patient with an unsuccessful LAAC), thrombus formation over the device in 2 cases (both resolved after resuming OAC for 3 months) and 2 strokes (2.2%).

Conclusions: In this multicenter, real world registry, that included patients with NVAF and high thromboembolic and bleeding risks, LAAC effectively prevented stroke and bleeding when compared to the expected rates based on CHA_2DS_2VASc and HASBLED scores for this population. Complications rate of the procedure was acceptable considering the beginning of the learning curve of most of the involved operators. (Arq Bras Cardiol. 2017; 109(5):440-447)

Keywords: Atrial Fibrillation; Septal Occluder Devices; Atrial Appendage; Stroke; Cardiovascular Surgical Procedures; Medical Records.

Introduction

Although still significantly underdiagnosed,[1] atrial fibrillation (AF) is a public health issue with major socio-economic impact, and its relative incidence has constantly grown over the years.[2] One of the greatest risks of this arrhythmia is left atrial thrombus formation, which occurs in 10% of patients with AF (even when acute), and it is associated with a 3.5 times elevated risk for stroke, reaching average annual rates of 5%.[3-5] In order to prevent this devastating complication, the Guidelines recommend oral anticoagulation (OAC) with vitamin K antagonists or one of the new oral anticoagulants (NOACs) as Class I for the treatment of patients with non-valvular atrial fibrillation (NVAF) and at high risk for stroke, defined by the CHA_2DS_2-VASc score.[6] In spite of being quite effective, these drugs depend on treatment adherence and, more importantly, their use is associated with high risk of bleeding.[7,8]

As a "local therapy" that does not depend on adherence and reduces the risk of bleeding, left atrial appendage closure (LAAC) proved to be an effective alternative to OAC for the prevention of stroke in patients with non-valvular atrial fibrillation (NVAF), with lower bleeding risk.[9] In a recent meta-analysis, including about 88000 patients, LAAC has also shown to be superior to placebo and to double antiplatelet therapy and comparable to the NOACs in the prevention of mortality and stroke or systemic embolism in these patients, with a similar bleeding risk.[10]

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In spite of its great therapeutic potential and a vertiginous growth of its indication and application in other countries, the LAAC procedure is still little known and little used in Brazil, with scarce data in the national literature. This article aims to report the results of the largest Brazilian multicenter registry of LAAC.

Methods

Ninety-one consecutive patients with permanent or paroxysmal NVAF, with high stroke risk and restrictions to OAC, underwent 92 LAAC procedures between 2010 and 2016 in 11 Brazilian centers. All patients that underwent LAAC in these centers were included, and the data related to the procedures and to the follow-up of patients were collected prospectively and analyzed retrospectively.

A preoperative evaluation with transesophageal echocardiography (TEE) was performed in all patients. Patients with LAA thrombus or LAA anatomy deemed unfavorable to intervention (landing zone < 13 mm or > 30 mm or LA depth < 10 mm) were excluded. For the eligible patients, the OACs were suspended when in use, 3-5 days pre-procedure. All the interventions were guided simultaneously by angiography and intraoperative TEE, and one of the 2 devices available in the Brazilian market (Figure 1) was implanted: the Amplatzer Cardiac Plug (ACP, St. Jude Medical, St. Paul, MN), available since 2010, and the Watchman (Boston Scientific, Marlborough, MA), available since mid-2015. Both devices and their respective implant techniques have been described previously in detail.[9,11]

Procedural success was defined as effective implantation of the occluder device in the LAA, without periprosthetic residual flow larger than 5 mm, according to evaluation of the intraoperative TEE. Major adverse events were defined as the occurrence of death, stroke, systemic embolization, device embolization, acute myocardial infarction, pericardial effusion with cardiac tamponade or bleeding with the need for transfusion, data collected and reported during both hospitalization and follow-up.

The follow-up considered the practice of each investigator, but it included at least one clinical visit in every center and one control TEE carried out from three months after the procedure, searching for the detection and quantification of periprosthetic residual flow or thrombus formation over the prosthesis. In case there is no finding or adverse event, the last follow-up available was considered in the analysis.

Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics v.20 software. Data for categorical variables were presented as frequencies and proportion. Continuous variables with normal distribution were described by mean ± standard deviation and compared through Student's t-test for paired samples. Other quantitative variables were described by median, first quartile and third quartile. The condition of normality was evaluated using the Kolmogorov-Smirnov test. Values of $p < 0.05$ were statistically significant.

Results

The clinical characteristics of patients are detailed in Table 1. Ninety-one patients (males 59.3%, mean age = 73.1 ± 10.1 years) with NVAF (62.6% permanent, 37.4% paroxysmal) and at high risk for systemic embolism (CHA_2DS_2-VASc score = 4.5 ± 1.5, 49.5% with previous stroke) and for bleeding (HAS-BLED score = 3.6 ± 1.0, 61.5% with previous bleeding episodes while on OAC – Table 2) were treated. Major indications for LAAC were important previous bleeding episodes (mainly gastrointestinal or neurological) or labile INR (Figure 2). Sixty-eight percent of patients were deemed ineligible for OAC by their clinicians, whether with vitamin K antagonists or one of the NOACs.

Procedure-related data are presented in Table 3. Forty-five percent of the 92 interventions were performed with the aid of a proctor. An ACP was implanted in 94.6% of cases, and a Watchman device in 5.4% (Figure 3). A total of 96 occluder devices was used in 92 procedures (1.04 device/procedure). Prosthesis implantation was successful in 97.8% of cases. The procedure was aborted in two patients due to short LAA depth (< 10 mm) in one of the patients, and to an oversized landing zone (> 30 mm) in another, both characteristics underestimated in the

Table 1 – Clinical Characteristics of Patients (n = 91)

Variable	Result*
Age (years)	73.1 ± 10.1
65-75	27 (29.7)
>75	47 (51.6)
Male	54 (59.3)
Atrial Fibrillation	
Permanent	57 (62.6)
Paroxysmal	34 (37.4)
LVEF (%)	58.2 ± 13.4
$CHADS_2$ score	3.1 ± 1.3
CHA_2DS_2-VASc score	4.5 ± 1.5
HAS-BLED score	3.6 ± 1.0
Ineligible for OA	62 (68.1)
Congestive cardiac failure	28 (30.8)
High blood pressure	78 (85.7)
Diabetes	33 (36.3)
Previous CVA	45 (49.5)
Peripheral vascular disease	22 (24.2)
Renal and liver dysfunction	21 (23.1)
Previous bleeding	56 (61.5)
Labil INR	27 (29.7)
Drugs or alcohol	21 (23.1)

** Mean ± standard deviation or frequency (percentage). LVEF: left ventricular ejection fraction; OA: oral anticoagulation; CVA: cerebrovascular accident; INR: international normalized ratio.*

Table 2 – patients distribution according to $CHADS_2$, CHA_2DS_2-VASc and HAS-BLED scores (n = 91)

Score	$CHADS_2$	CHA_2DS_2-VASc	HAS-BLED
	n (%)	n (%)	n (%)
0	0	0	0
1	10 (11.0)	1 (1.1)	1 (1.1)
2	21 (23.0)	9 (9.9)	14 (15.4)
3	27 (29.7)	13 (14.3)	27 (29.7)
4	18 (19.8)	26 (28.6)	32 (35.2)
5	12 (13.2)	19 (20.8)	16 (17.5)
6	3 (3.3)	15 (16.5)	1 (1.1)
7	n/a	6 (6.6)	0
8	n/a	1 (1.1)	n/a
9	n/a	1 (1.1)	n/a
mean ± SD	3.1 ± 1.3	4.5 ± 1.5	3.6 ± 1.0

SD: standard deviation; n/a: not applicable.

Figure 1 – *Watchman device (left) and Amplatzer Cardiac Plug (right).*

initial TEE. Due to an incomplete closure of the LAA following the implantation of an ACP 16 mm, one patient received an additional non-dedicated device (a septal occluder of 25 mm in diameter), with good initial results. However, a control fluoroscopy performed after 4 days revealed embolization of the device to the aortic arch. The prosthesis was removed percutaneously, and a second ACP 28 mm was successfully implanted over the initial ACP 16 mm, which led to complete closure of the LAA.

The average diameter of the implanted prosthesis was 24.2 ± 3.8 mm, corresponding to the mean left atrial appendage dimensions of 20.4 ± 4.3 mm derived from TEE and 20.9 ± 4.1 mm from angiography (p = 0.012 between the diagnostic methods). Thus, the average oversizing of the implanted device was 21.5 ± 13% based on the TEE measurement and 18.1 ± 9.1% according to the angiography. The prosthesis sizes most frequently used were 24 and 26 mm (Figure 4), and the first selected device was effectively implanted in 95.6% of successful cases. Concomitant procedures (coronary angioplasty, closure of an atrial septal defect or patent foramen ovale) were performed along with LAAC in 8.7% of cases. Average fluoroscopy time was 16.7 ± 8.7 minutes and a mean contrast volume of 157.5 ± 81.8 ml was used per procedure. The absence of periprosthetic residual flow was verified in 93.3% of successful cases and, among the residual leaks detected, none was larger than 2.5 mm.

There were 7 periprocedural major adverse events: 5 cases of cardiac tamponade [3 of them late (24h – 5 days after intervention); 4 of 5 were treated with pericardiocentesis, however the other required surgical drainage], the non-dedicated device embolization mentioned above, and a coronary air embolism without sequelae. Minor complications occurred in 4 patients (4.4%): one pericarditis (post-tamponade), one discrete pericardial effusion without clinical repercussions, one case of post-procedural pulmonary congestion and one arteriovenous fistula. After a median length of stay in hospital of two days, all the patients but 2 (one considering the assistant clinician's preferences, the other for presenting one ulcerated plaque in the aorta) were discharged with the prescription of acetylsalicylic acid and clopidogrel, without OAC.

Clinical follow-up was obtained in 97.8% of patients – 2 patients were lost to follow-up. After a period of 128.6 patient-years (median = 346 days and interquartile range of 195 to 985 days), there were three deaths unrelated to the procedure. There were two episodes of major bleeding: one of them in a patient with unsuccessful LAAC, which continued on warfarin therapy; the other was a gastrointestinal bleeding in a patient on dual antiplatelet

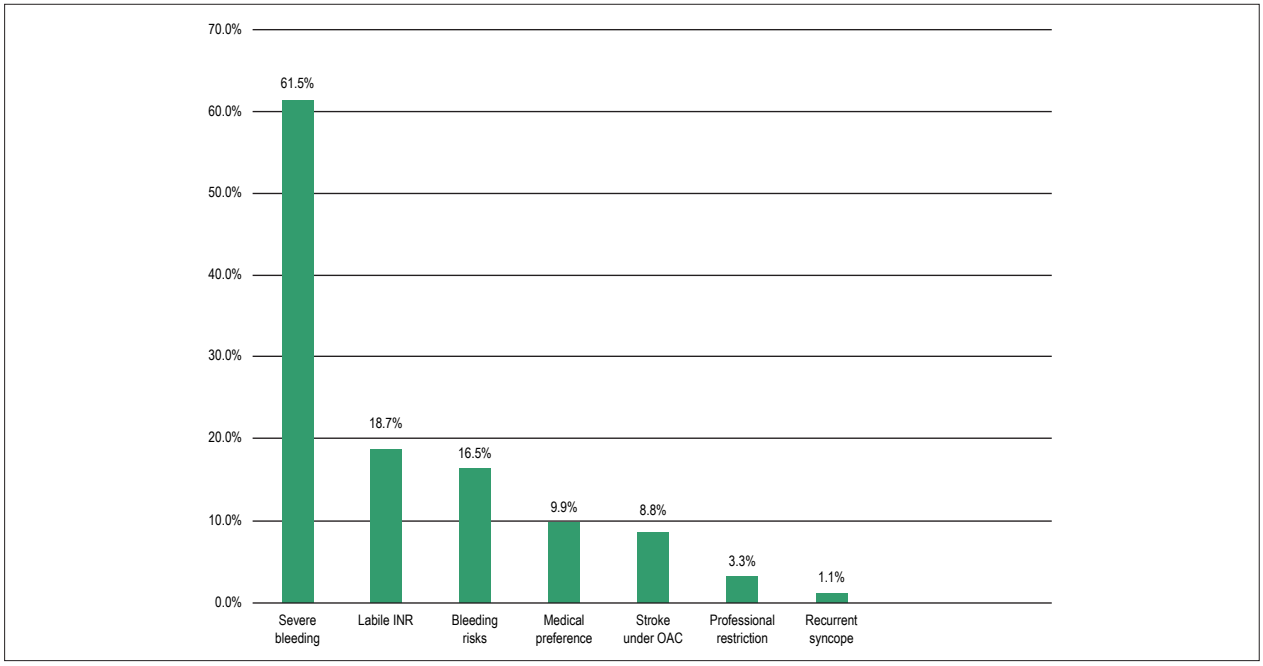

Figure 2 – *Contraindications to oral anticoagulation*. INR: international normalized ratio; OAC: oral anticoagulation. * the same patient may have multiple contraindications.*

aggregation therapy. Periprosthetic residual flow (all less than 2.5 mm) persisted in 5 of the 6 patients in whom they were originally detected, none of them with clinical consequences. No late development of residual flow was detected. In 2 patients, thrombus formation was detected over the device, both treated successfully after resuming OAC for three months. Only two patients (2.2%) had ischemic stroke at follow-up: one after six months, and the other 9 months after the intervention.

Discussion

The basis for the hypothesis that systemic embolism can be prevented by closure of the LAA was the demonstration that, in patients with NVAF, more than 90% of atrial thrombi originate in this structure.[12] After the initial experience with the PLAATO device[13] and with the use of non-dedicated Amplatzer occluders,[14] more than 3500 patients were included in 2 randomized and several observational studies with the Watchman device,[9,15-17] whose results led to the approval of the device by the Food and Drug Administration (FDA) in 2015. Several unicenter and multicenter registries with the ACP device and its last generation, Amulet, were also published, the biggest of them including more than 1000 patients.[11,18-23] Because of the favorable results of the intervention, the European Guidelines for the Management of Atrial Fibrillation validated the LAAC, in 2012, as a therapeutic strategy for patients with NVAF at a high stroke risk with a recommendation class IIb and a level of evidence B.[24] Surprisingly, this level of recommendation did not evolve in the guidelines upgrade, published in 2016.[25] The current Guidelines of the American College of Cardiology / American Heart Association / Heart Rhythm Society for the management of patients with atrial fibrillation, published in 2014,[6] do not yet include recommendations on indications for LAAC. However, considering the technical developments of the procedure, the recent FDA approval of the WATCHMAN device and, especially, the last favorable results from the PROTECT AF trial, which showed a significant reduction in mortality compared with OAC in the late follow-up,[26] the use of LAAC in clinical practice has expanded significantly in the USA, and it is anticipated that these guidelines recommendations will be updated soon.[27] Published in 2016, and in accordance with this new body of information, the II Brazilian Guidelines for Atrial Fibrillation recognize LAAC as a valid alternative to OAC, with a class IIa recommendation, both for patients at high risk for thromboembolic phenomena and with contraindication for oral anticoagulants (level of evidence B), and for those with cardioembolic ischemic stroke despite correct use of oral anticoagulants (level of evidence C).[28]

One of the biggest limitations of the PROTECT-AF trial, a reference study on LAAC, was the unexpected complication rate of 7.7% associated with the implantation of the Watchman filter device.[9] With the ACP device, national and international registries show that complication rates vary between 3.8% and 7.3%.[11,19,22] Although within this range, the rate of complications in the Brazilian Registry is relatively high, probably as a reflex of the beginning of the learning curve of most operators with both prostheses. A review of the literature shows, however, that continued experience with the intervention decreases the complication rate of the procedure to as low as 2.8%.[17]

The Brazilian Registry of Left Atrial Appendage Closure treated the population with the highest risk profile for systemic embolism and bleeding, compared to all registries and trials available in the literature. $CHADS_2$ and CHA_2DS_2-VASc average scores of 3.1 and 4.5 are equal or higher than those related to the study populations in the PROTECT-AF,[9] PREVAIL,[16] Ewolution[17] trials and in the

Table 3 – Periprocedural data (n = 92)

Variable	Result*
Access	
Transseptal	85 (92.4)
PFO/IC	7 (7.6)
LAA diameter (implant zone)	
Angiography (mm)	20.9 ± 4.2
TEE (mm)	20.4 ± 4.3
Device oversizing	
Angiography (%)	18.1 ± 9.1
TEE (%)	21.5 ± 13.0
Implanted device (n)	
ACP	87 (94.6)
Watchman	5 (5.4)
Non-dedicated device	1 (1.1)
Devices used per procedure	1.04
Sucess	90 (97.8)
Complete occlusion of the LAA	84 (93.3**)
Associated intervention	
PFO occlusion	4 (4.4)
IC occlusion	2 (2.2)
Coronary angioplasty	2 (2.2)
Major adverse events	
Procedure-related death	0
CVA	0
Coronary air embolism	1 (1.1)
TIA	0
Embolization of dedicated device	1 (1.1)
Acute myocardial infarction	0
Cardiac tamponade	
Acute	2 (2.2)
Late (> 24h)	3 (3.3)
Major bleeding	0

**Mean ± Standard deviation (percentage); ** Considering successful cases. PFO: patent foramen ovale; IC: interatrial communication; LAA: left atrial appendage; TEE: transesophageal echocardiography; ACP: Amplatzer Cardiac Plug; CVA: cerebrovascular accident; TIA: transient ischemic attack.*

multicenter experience with the ACP[23] (2.2 and 3.5, 2.6 and 4.0, 2.8 and 4.5 and 2.8 and 4.5 respectively – Figure 5). Nonetheless, the annual stroke rate during the follow-up was notably low (1.7% - 2 events/128.6 patient-years, a reduction of 68.5% compared to the 5.4% annual rate estimated by the CHA_2DS_2-VASc score). This rate is between the 1.6% demonstrated in the meta-analysis, which includes the Watchman trials[29] and the 1.8% demonstrated by Tzikas et al.[23] with the ACP trial, and confirms the efficacy of the intervention in our population.

Due to the underutilization and to the discontinuity of treatment, both reaching rates of up to 40%,[29] OAC reaches only a fraction of its therapeutic potential. For adherent patients, the risk of major bleeds remains significant. In spite of a best-use profile, the administration of NOACs is still associated with the occurrence of major bleeding in 2-3% of patients/year, even in those at low risk.[7] The older the patient, the higher the rates and the severity of bleeding. A recent study with 32000 American veterans aged over 74 years and with AF treated with warfarin showed a hospitalization incidence due to traumatic intracranial hemorrhage of 4.8/1000 patient-years, and 6.2/1000 patient-years, when multiple events per patient are included.[30] In this sense, the Brazilian Multicenter Registry showed that the bleeding rate was reduced by 77% compared to the expected rates based on the HAS-BLED score (1.7 versus 7.4 events/100 patient-years). This is especially significant considering that 83.5% of patients had a high bleeding risk with a HAS-BLED score ≥ 3 – also the worst risk profile compared to other studies available in the literature (Table 2 and Figure 5). If we consider only the patients effectively treated with LAAC, this rate is even lower, since one of the bleedings occurred in one of the patients whose intervention was unsuccessful, and this patient was treated with OAC.

Although thrombus formation at the atrial sides both of the Watchman device and of the ACP has been reported in 2 – 5% of cases, thromboembolic stroke rates secondary to this cause are very low (0.3 – 0.7%), and in general thrombus resolution is obtained after resuming OAC for short periods of time (< 3 months).[31] This was also the case for the 2 patients in this Registry in which thrombus over the device was detected in the follow-up. Periprosthetic residual flow, found in 6 patients immediately after the intervention and which persisted in 5 of them at the follow-up, is also frequently described with both prostheses, but does not seem to have clinical significance if it is less than 5mm,[32,33] which was also the case in all 6 patients.

The clinical benefits of LAAC increase when patients with higher CHA_2DS_2-VASc and HAS-BLED scores are treated, and they become more evident over time, due to the interruption of cumulative bleeding risk associated with continuous anticoagulation therapy.[34] In addition to the reduction of objective stroke and bleeding rates, however, patients submitted to LAAC also experience a more subjective, but significant, quality of life improvement, especially due to the reduction of minor bleedings and to the lack of need for frequent monitoring, interactions with food and drugs and lifestyle restrictions associated with OACs.[35] These factors, although less measurable, must also be taken into account when the risk-benefit ratio of the intervention is calculated.

Conclusion

In conclusion, LAAC has proven to be effective in a real-world population with high-risk AF for reducing significantly the annual stroke and bleeding rates when compared to the expected rates based on CHA_2DS_2-VASc and HAS-BLED scores. The complication rates of the procedure must be weighed against the risks, discomforts and limitations associated with continuous and uninterrupted exposure to OAC.

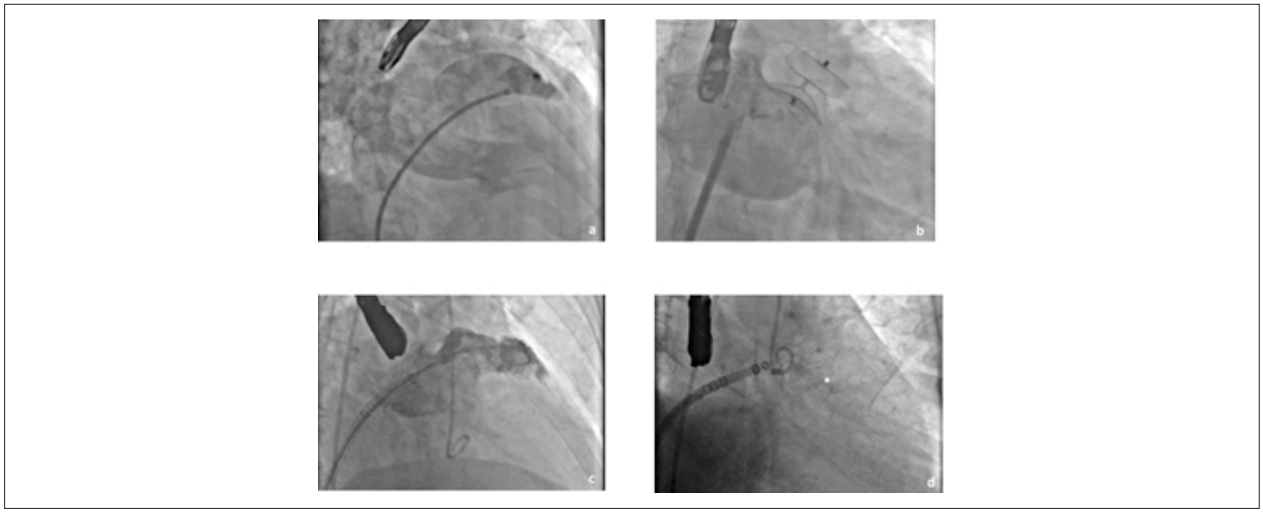

Figure 3 – *Implantation of the Amplatzer Cardiac Plug (ACP) and Watchman devices. 3a and 3c) left atrial appendage angiographies, pre-occlusion; 3b) Post-implantation, ACP device; 3d) Post-implantation, Watchman device (*).*

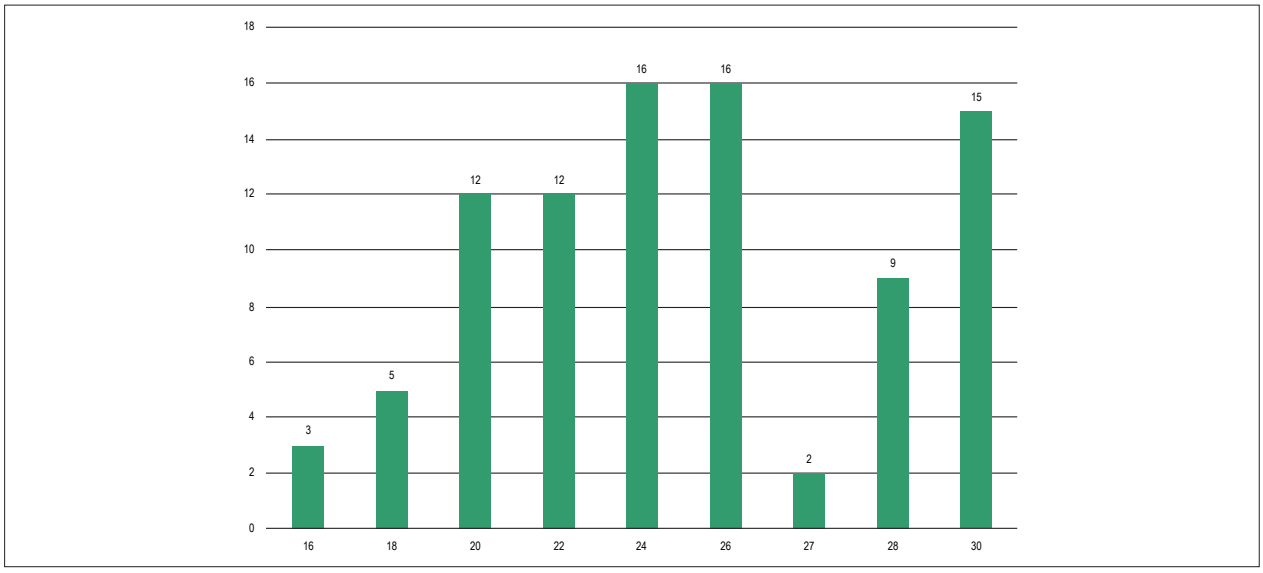

Figure 4 – *Distribution of the sizes of the implanted devices (mm). Data are expressed in number of devices.*

Limitations

This study has several limitations. As an inherent limitation to a non-randomized study, there is no control group, and the comparison of event rates was based on rates predicted by scores. As in every observational study, there may be flaws in patient selection. However, the Registry was designed in order to include all the patients who were candidate for the procedure (intention to treat), reflecting a real-world practice. Although the data have been prospectively collected, this is a retrospective analysis, without independent monitoring, or a core lab analyses. Especially due to reimbursement difficulties in Brazil, basically all centers included in this Registry are centers with low volume of LAAC and, thus, the learning curve of the operators is flattened, which has a direct impact on complication rates. The follow-up included more than 95% of patients treated, but not all of them. And, finally, all the data collected were spontaneously reported by investigators, without independent adjudication.

Author contributions

Conception and design of the research and Analysis and interpretation of the data: Guérios EE, Chamié F; Acquisition of data and Critical revision of the manuscript for intellectual content: Guérios EE, Chamié F, Montenegro M, Saad EB, Brito Junior FS, Caramori PA, Simões LC, Oliveira FRA, Giuliano LC, Tavares CMF; Statistical analysis, Obtaining financin and Writing of the manuscript: Guérios EE.

Potential Conflict of Interest

Guérios EE is proctor for St. Jude Medical / Abbott for percutaneous left atrial appendage closure.

Figure 5 – *Comparison between mean $CHADS_2$ (5a) and CHA_2DS_2-VASc (5b) scores and proportion of patients with HAS-BLED score ≥ 3 (5c) in the populations studied in the Brazilian Registry of Percutaneous Left Atrial Appendage Closure vs other registries and trials.*

Sources of Funding

There were no external funding sources for this study.

Study Association

This study is not associated with any thesis or dissertation work.

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Endostatin a Potential Biomarker for Heart Failure with Preserved Ejection Fraction

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Abstract

Background: Endostatin is a circulating endogenous angiogenesis inhibitor preventing neovascularization. Previous studies demonstrated the prognostic value of Endostatin among patients with heart failure with reduced ejection fraction (HFrEF). However, the role of Endostatin among patients with heart failure with preserved ejection fraction (HFpEF) remains unclear.

Objective: This study aimed to investigate the association between serum Endostatin levels, natriuretic peptide levels and the severity of left ventricular diastolic dysfunction and the diagnosis of HFpEF.

Methods: Endostatin serum concentrations were measured in 301 patients comprising 77 HFpEF patients, 169 patients with asymptomatic left ventricular diastolic dysfunction (ALVDD), and 55 controls with normal cardiac function.

Results: Endostatin serum levels were significantly elevated in patients with HFpEF (median/interquartile range 179.0 [159-220]) and ALVDD (163.8 [145.4-191.3]) compared to controls (149.1 [130.6-176.9]), $p < 0.001$ and $p = 0.004$, respectively) and significant correlated with N-terminal pro B-type natriuretic peptide (NT-proBNP).

Conclusions: This hypothesis-generating pilot study gives first evidence that Endostatin correlates with the severity of diastolic dysfunction and may become a novel biomarker for HFpEF. We hypothesize a rise in Endostatin levels may reflect inhibition of adaptive angiogenesis and adverse cardiac remodeling. (Arq Bras Cardiol. 2017; 109(5):448-456)

Keywords: Heart Failure; Endostatins; Natriuretic Peptides; Biomarkers; Stroke Volume.

Introduction

The patient population affected by heart failure (HF) is growing in a constant manner. This is because of an aging society, western lifestyle and improved acute clinical care (e.g. after myocardial infarction).[1] Although, the treatment of chronic conditions improved over the last decades, mortality and morbidity rates in this patient population are amongst the highest for western healthcare systems.[2] In the United States (US) HF is the leading cause for hospitalization for patients older > 65 years of age.[3] In 2030 the direct costs for heart failure will reach 70 billion US$ in the US alone.[4] Half of the patients affected by HF present with a diastolic dysfunction and a preserved ejection fraction (HFpEF), with this proportion increasing.[5] Clinical data proves that those patients suffering from a reduced ejection fraction (HFrEF) show better outcomes compared to HFpEF patients.[6-8] A reason might be that no therapy has been shown to improve outcomes in HFpEF.[9] Current therapeutic options including fluid management, blood pressure control and physical exerciseto relief patients' symptoms. A major drawback regarding the development of new therapies for HFpEF, is the absence of clear diagnostic criteria.[10] This makes the definition of patient populations for clinical studies difficult. At present, the diagnosis is solely based echocardiography. Especially, the separation between HFpEF and HFrEF is even more challenging and misleading in patients with newly diagnosed HF.[11] Therefore new strategies for disease phenotyping in HF are urgently needed. New biomarkers may achieve better disease phenotyping.[12] Although, many reports have been published on HF biomarkers over the last decades, the impact on clinical decision making is still limited.[13] BNP/NTproBNP demonstrated high clinical utility to identify patients at high risk for heart failure hospitalization and death. However, in this context these markers for clinical studies are only applicable in relatively stable patients and not in terminal HF patients. Furthermore, the use of BNP/NTproBNP in clinical practice to optimize therapy with drugs, which are known to improve patient`s outcome is suitable.[14] However, BNP/NTproBNP is not accepted as surrogate endpoint and can only exploratorily be used as endpoint in clinical trials. The appraisal of clinical utility of BNP/NTproBNP manifests in the current guidelines for the management of heart failure.[15] A number of publications

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propose Endostatin, a potent angiogenesis inhibitor, known mostly from oncology, as a potential new HF biomarker candidate.[16-18] Most importantly Gouya et al. reported in a prospective observational cohort study in 151 HF patients, a correlation between elevated circulating Endostatin levels and mortality. Furthermore, this study showed a clear association between Endostatin levels and progressing diastolic dysfunction, the key characteristic of HFpEF.[19] This is why we hypothesize that Endostatin could potentially be a biomarker suitable to diagnose and disease phenotype HFpEF patients. In the present study, we aimed to investigate the sole role of Endostatin as a biomarker for HFpEF and diastolic dysfunction.

Methods

The study protocol was approved by the Ethics Committee of the Private University of Witten/Herdecke, Germany (project n°. 91/08) and conducted in accordance with the Declaration of Helsinki. Signed written informed consent was obtained from all patients.

Study population

Participants of the prospective observational cohort study were patients contacting the HELIOS Klinikum Wuppertal Heart Center (Wuppertal, Germany) for elective coronary angiography or diagnostic work-up of heart failure. Patients with a stable or suspected coronary artery disease (CAD) and/or a diagnostic workup of CHF were included in the study. The exclusion criteria were: left ventricular ejection fraction (EF) < 50%, known CAD with progressive chest pain within the last month, coronary angioplasty or myocardial infarction within 6 weeks, hypertrophic cardiomyopathy, moderate-to-severe valvular heart disease, uncontrolled hypertension, atrial fibrillation or other severe arrhythmias, serum-creatinine > 2,0 mg/dl. Patients selected for the control group had to have no history or symptoms of CHF, a normal ejection fraction > 55%, a ratio of the early diastolic transmitral velocity (E) and the early diastolic tissue Doppler velocity (E´) of < 8, and normal NTproBNP values. A total of 301 patients were recruited and assigned to three groups based on echocardiographic diagnostic criteria as recommended by the European Society of Cardiology.[20] The control group consisted of 55 patients (29 males) with normal diastolic function (DF). The group with asymptomatic left ventricular diastolic dysfunction (ALVDD) contained 169 patients (95 males) with E medial < 8 cm/s, E/E' medial ratio 8-15 and NT-proBNP levels < 220 pg/ml. The group with HFpEF comprised 77 patients (46 females, 31 males) displaying ALVDD Grad II - III with E/E´ratio > 15, NT-proBNP levels > 220 pg/mL and current or previous signs or symptoms of heart failure.

Echocardiography

Echocardiography was performed using a standard ultrasound system (Vivid 7, General Electric, Milwaukee, Wisconsin). A complete transthoracic study was performed including 2D, M-mode, spectral and colour Doppler techniques following current recommendations and guidelines.[21,22] The left atrium volume index (LAVI) was calculated using the biplane area-length method. Left ventricular EF was measured by means of the modified biplane Simpson's method.[23] Left ventricular mass index (LVMi) was computed with the Devereux formula indexed to the body surface.[22] HFpEF was defined in accordance with the EAE/ASE recommendation, based on the assessment of left ventricular diastolic function.[24] Primary measurements included mitral inflow peak early (E-wave) and late (A-wave) diastolic filling velocities as well as systolic (S) and early diastolic (E') mitral annular velocities whereat in each case three consecutive beats were measured and averaged. Conventional transmitral flow was measured with Pulse-waved Doppler (PW). PW tissue Doppler imaging (DTI) was performed at the junction of the septal and lateral mitral annulus in the apical 4-chamber view. Based on primary measurements E/A and E/E' ratios were calculated.

Laboratory analysis

Peripheral venous whole blood samples were taken after 5 minutes at rest for routine laboratory testing (OGTT, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, creatine, leucocytes, hemoglobin, creatin kinase, TSH, hsCRP, GOT, GPT). Blood was drawn into pyrogen-free tubes without any additives, centrifuged at room temperature, aliquoted and stored at –80°C. All laboratory analysis were outsourced to Roche Diagnostics (Penzberg, Germany) and performed on blinded samples. For analysis of plasma NT-proBNP the Elecsys 2010 NT-proBNP assay (Roche Diagnostics, Mannheim, Germany) was used. For measurement of Endostatin the ELISA assay of R&D Systems (Minneapolis, MN USA) was used. All assays were performed according to manufacturer's recommendations.

Statistical analysis

All analyses were performed using SPSS statistical software (SPSS 19.0, Chicago, IL, USA). The data are presented as median with $25^{th}/75^{th}$ percentiles (interquartile range) for continuous variables or as absolute numbers and corresponding percentages for categorical variables unless otherwise specified. Log transformed values were used for analysis as appropriate. A p-value < 0.05 was considered statistically significant. We used the Kolmogorov-Smirnov test as appropriate to test for normal distribution. The Mann-Whitney U-test was used to analyze differences between the medians of two groups and the Kruskal-Wallis test to test the equality of medians among more than two distinct groups. Fisher's Test was used for the comparison of two sets of binary variables and the χ^2 test to evaluate differences in proportions in more than 2 sets of categorical variables. Endostatin and NT-proBNP levels were compared across subjects with normal diastolic function, mild ALVDD and HFpEF by the Mann-Whitney U-test, and Jonckheere-Terpstra test. Spearman rank correlation was used to identify variables associated with Endostatin. A multivariable model was included to predict the presence of HFpEF and included the following covariates: Endostatin, age, gender, diabetes, hypertension, coronary artery disease and body mass index. Due to the exploratory nature of this study, there is no minimum required sample size.

Results

Study population characteristics

Table 1 provides an overview of the clinical characteristics of all 301 patients included in our study. The three groups showed comparable diastolic blood pressure, resting heart rate and history of myocardial infarction and stroke. Patients with mild ALVDD or HFpEF were older, more obese, had a higher systolic blood pressure on average and showed a higher prevalence of comorbidities including CAD and coronary artery bypass graft, as well as cardiovascular disease risk. In addition, treatments varied across groups.

Endostatin and diastolic function

Table 2 summarizes the laboratory data and echocardiographic function parameter stratified by the study groups HFpEF vs. ALVDD vs. controls. Levels of Endostatin were 179.0 [159-220] ng/mL in HFpEF, 163.8 [145.4-191.3] ng/mL in ALVDD and 149 [130.6 - 176.9] ng/mL in the control group, respectively (Figure 1A). Serum levels of Endostatin were significantly higher in patients with HFpEF ($p < 0.001$) and mild ALVDD ($p = 0.001$; Table 2) compared to individuals from the control group. Furthermore, Endostatin serum concentration was elevated in patients with mild ALVDD compared to asymptomatic controls with normal diastolic and systolic function ($p = 0,004$). In addition, there was a significant association between increasing Endostatin quartiles and higher NT-pro-BNP levels. No clinically relevant differences were observed in the clinical routine laboratory assessments. In multivariable analysis included the covariates Endostatin, age, gender, diabetes, hypertension, coronary artery disease and body mass index, age ($p < 0.001$) and Endostatin ($p = 0.008$ were independently associated with HFpEF

Association of Endostatin levels with cardiac structure and function

Increasing quartiles of Endostatin were significantly associated with structural changes of the heart like the extent of LV- hypertrophy and left atrial enlargement, reflecting adverse cardiac remodeling. Moreover, increasing quartiles of Endostatin were significantly associated with worsening diastolic function measured by tissue Doppler imaging (E', E/E') (table 3). Thus, patients within the highest quartiles

Table 1 – Baseline characteristics of the study population. Values are median (25-75interquartile range) or absolute numbers and percentage (%)

Clinical variables (median/interquartile range or %)	Studied patient groups			p value
	Control (n = 55)	mild ALVDD (n = 169)	HFpEF (n = 77)	
Age (years)	54 (48-61)	66 (58-71)	73 (68-77)	< 0.001*
BMI (kg/m^2)	25.5 (24.1-29.1)	27.8 (25.6-32.3)	27.5 (25.7-32.0)	0.001*
Waist circumference (cm)	98 (86-107)	102 (94-114)	102 (98-111)	0.002*
Hip circumference (cm)	98 (94-103)	103 (96-111)	105 (98-114)	0.003*
Systolic BP (mmHg)	125 (110-136)	134 (127-140)	136 (130-140)	0.001*
Diastolic BP (mmHg)	80 (70-80)	80 (76-84)	80 (72-84)	0.12
Resting HR (beats/min)	70 (68-76)	72 (69-76)	70 (65-76)	0.51
CAD, n (%)	21 (38,2)	99 (58,6)	49 (63,6)	0.009*
CABG, n (%)	1 (2.0)	5 (3.0)	9 (11.7)	0.007*
PCI, n (%)	15 (27.3)	75 (44.4)	33 (42.9)	0.074
History of MI, n (%)	8 (14.5)	36 (21.3)	17 (22.1)	0.501
History of stroke, n (%)	1 (2.0)	5 (3.0)	3 (3.9)	0.824
Cardiovascular risk factors				
Treated hypertension, n (%)	38 (69.1)	148 (88.1)	73 (96.1)	< 0.001*
Diabetes mellitus, n (%)	4 (7.3)	45 (26.6)	22 (28,6)	< 0.001*
Medication				
ACE inhibitor, n (%)	26 (47.3)	110 (65.1)	42 (54.5)	0.042*
AT1 receptor blocker, n (%)	6 (10.9)	17 (10.1)	23 (29.9)	< 0.001*
Diuretics, n (%)	8 (14.5)	45 (26.6)	36 (49.8)	< 0.001*
Ca^2 blocker, n (%)	6 (10.9)	23 (13.6)	21 (27.3)	0.013*
B-blocker, n (%)	28 (50.9)	103 (60.9)	57 (74.0)	0.021*

**statistically significant (p < 0.05). BMI: body mass index; BP: blood pressure; HR: heart rate; CAD: coronary artery disease; CABG: coronary artery bypass graft; PCI: percutaneous coronary intervention; MI: myocardial infarction; DF: diastolic function; ALVDD: left ventricular diastolic dysfunction; HFpEF: heart failure with preserved ejection fraction; NS: non-significant. The Mann-Whitney U-test was used to analyze differences between the medians of two groups and the Kruskal-Wallis test to test the equality of medians among more than two distinct groups.*

Table 2 – Laboratory data and echocardiographic parameters. (25-75interquartile range) or absolute numbers and percentage (%) X^2 test was used as appropriate

Clinical variables	Studied patient groups			p value
	Control (n = 55)	mild ALVDD (n = 169)	HFpEF (n = 77)	
Biomarker				
Endostatin (ng/ml)	149.1 (130.6-176.9)	163.8 (145.4-191.3)	179.0 (159-220)	< 0.001*
NT-pro-BNP (pg/ml)	90.1 (45.8-129.2)	86.7 (43.7-170.7)	343.6 (151.7-703.4)	< 0.001*
Routine parameter				
Total cholesterol (mg/dl)	189 (163-228)	193 (171-221)	191 (170-210)	NS
LDL-cholesterol (mg/dl)	107 (89-135)	109 (89-135)	109 (86-129)	NS
HDL-cholesterol (mg/dl)	53 (46-64)	50 (38-62)	48 (41-61)	NS
Triclyceride (mg/dl)	119 (83-185)	142 (100-206)	131 (104-189)	NS
Lp (a) (mg/dl)	8 (5-27)	18 (6-39)	15 (6-52)	NS
TSH (mU/l)	1.20 (0.94-2.09)	1.42 (0.824-2.08)	1.315 (0.80-1.90)	NS
Creatinine (mg/dl)	0.8 (0.7-0.9)	0.9 (0.7-0.9)	0.9 (0.75-1.10)	NS
hsCRP	0.1 (0.1-0.3)	0.3 (0.1-0.6)	0.3 (0.2-0.69)	0.005*
Glucose	89 (84-97)	100 (91-111)	97 (89-103)	0.020*
Hb (mg/dl)	14.3 (13.3-15.1)	14.1 (13.2-15.0)	13.6 (12.5-14.5)	0.004*
CK (U/l)	76 (58-105)	78 (60-114)	72 (55-104)	NS
SGOT (U/l)	25 (21-31)	25 (21-31)	26 (21-32)	NS
LV geometry				
IVS (mm)	10 (9-11)	12 (10-13)	12 (11-14)	< 0.001*
PLW (mm)	10 (9-11)	12 (10-13)	12 (11-14)	< 0.001*
LVEDD(mm)	44 (42-47)	44 (39-48)	45 (41-50)	NS
LVESD (mm)	30 (28-34)	29 (25-34)	31 (27-36)	NS
LVMi (g/m²)	72 (62-84)	81 (67-102)	91 (77-119)	< 0.001*
Systolic function				
EF (%)	68 (62-72)	67 (61-71)	67 (63-73)	NS
S_{max} (cm/s)	7.2 (6.3-8.0)	6.3 (5.7-7.5)	6.1 (5.4-6.7)	< 0.001*
Diastolic function				
LA-Index (ml/m²)	25.4 (21.8-28.7)	29.8 (25.7-33.3)	39.3 (36.7-49.1)	< 0.001*
E (cm/s)	60 (60-80)	60 (50-70)	80 (70-90)	< 0.001*
A (cm/s)	60 (50-70)	80 (70-90)	80 (70-90)	< 0.001*
E/A ratio	1.14 (0.68-1.25)	0.75 (0.67-0.86)	1.11 (0.85-1.25)	< 0.001*
E´septal (cm/s)	8.4 (7.3-9.4)	5.9 (5.2-6.8)	5.4 (4.6-6.3)	< 0.001*
E´lateral (cm/s)	10.7 (9.5-13.0)	8.2 (6.9-9.5)	6.9 (5.6-8.4)	< 0.001*
Average E´(cm/s)	9.8 (8.6-11.0)	7.2 (6.1-8.1)	6.2 (5.2-7.2)	< 0.001*
E/E´septal ratio	8.0 (6.9-9.0)	10.2 (8.3-11.9)	15.1 (12.5-17.1)	< 0.001*
E/E´average ratio	7.0 (6.0-7.7)	8.4 (6.8-10.1)	13.3 (11.1-14.8)	< 0.001*

**statistically significant (p < 0.05); NT-proBNP: N-terminal fragment of the prohormone B-type natriuretic peptide; LDL: low density lipoprotein; HDL: high density lipoprotein; Lp (a): lipoprotein (a); TSH: thyroid stimulating hormone; hsCRP: high sensitive C-reactive protein; Hb: hemoglobin; CK: creatinkinase; SGOT: serum glutamic oxaloacetic transaminase; IVS: interventricular septum; PLW: posterior lateral wall; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; EF: ejection fraction; LA: left atrial; E: early diastolic transmitral velocity; A: late diastolic transmitral velocity; E´: early diastolic tissue Doppler velocity; DF: diastolic function; LVDD: left ventricular diastolic dysfunction; HFpEF: heart failure with preserved ejection fraction; NS: non-significant. The Mann-Whitney U-test was used to analyze differences between the medians of two groups and the Kruskal-Wallis test to test the equality of medians among more than two distinct groups.*

Table 3 – Echocardiographic parameters stratified according to serum Endostatin quartiles. Values are median (interquartile range) or n (%). X^2 test was used as appropriate

Parameter	Endostatin 1rd quartile	Endostatin 2nd quartile	Endostatin 3rd quartile	Endostatin 4th quartile	p value
LV geometry					
IVS (mm)	11 (9-12)	12 (11-13)	12 (11-13)	12 10-14)	0.032*
PLW (mm)	11 (10-13)	11 (10-13)	12 (10-13)	12 (10-14)	NS
LVEDD (mm)	44 (41-47)	43 (40-47)	47 (40-49)	45 (40-48)	NS
LEVSD (mm)	30 (27-34)	29 (26-32)	31 (27-35)	29 (24-37)	NS
LVMi (g/m²)	76.4 (61.6-100.4)	74.2 (66.2-97.8)	87.8 (72.9-100.3)	94.5 (70.8-117.1)	0.024*
Systolic function					
Ejection fraction (%)	65 (60-70)	68 (63-72)	67 (61-71)	69 (63-74)	0.029*
S_{max} (cm/s)	6.6 (5.8-7.7)	6.6 (5.8-7.7)	6.4 (5.6-7.2)	6.1 (5.3-7.0)	0.005*
Diastolic function					
LA-Index (ml/m²)	28.6 (23.8-35.3)	31.2 (25.7-35.2)	29.9 (25.7-36.9)	33.4 (27.9-38.8)	0.023*
E (cm/s)	60 (50-75)	60 (50-70)	70 (50-80)	70 (60-80)	NS
A (cm/s)	70 (60-80)	70 (60-80)	80 (70-90)	80 (70-95)	< 0.001*
E/A ratio	0.9 (0.7-1.2)	0.9 (0.7-1.1)	0.8 (0.7-1.0)	0.8 (0.7-1.1)	NS
E´septal (cm/s)	6.9 (5.6-8.0)	6.0 (5.3-7.3)	6.3 (5.3-7.3)	5.6 (4.9-6.2)	< 0.001*
E´lateral (cm/s)	9.1 (7.1-10.7)	8.6 (7.0-10.2)	8.3 (7.0-10.2)	7.5 (6.3-8.9)	0.001*
Average E´	7.9 (6.8-9.3)	7.4 (6.3-8.5)	7.6 (6.2-8.3)	6.4 (5.5-7.5	< 0.001*
E/E´septal ratio	8.8 (7.5-11.4)	10.3 (8.3-12.5)	10.8 (8.3-13.0)	12.1 (9.8-15.8)	< 0.001*
E/E´average ratio	7.5 (6.5-9.8)	8.5 (7.1—10.5)	8.9 (7.1-11.5)	10.5 (8.4-13.1)	< 0.001*
Laboratory					
NT-pro-BNP (pg/ml)	81.40 (45.1-137.3)	93.25 (43.70-211.6)	104.6 (52.8-179.7)	218.2 (100.35-516.15)	< 0.001*

**statistically significant (p < 0.05). IVS: interventricular septum; PLW: posterior lateral wall; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; EF: ejection fraction; LA: left atrial; E: early diastolic transmitral velocity; A: late diastolic transmitral velocity; E´: early diastolic tissue Doppler velocity; NT-proBNP: N-terminal fragment of the prohormone B-type natriuretic peptide; DF: diastolic function; LVDD: left ventricular diastolic dysfunction; NS: non-significant, HFpEF, heart failure with preserved ejection fraction. The Mann-Whitney U-test was used to analyze differences between the medians of two groups and the Kruskal-Wallis test to test the equality of medians among more than two distinct groups.*

of Endostatin serum levels showed more advanced cardiac remodeling (LV hypertrophy and left atrial enlargement) as well as more severe diastolic function abnormalities reflecting increasing left ventricular filling pressures (Figure 1B). Consistently, there was a significant positive moderate correlation between Endostatin and NT-proBNP levels ($r = 0.32$, $p < 0.001$; Figure 1C).

Discussion

We hypothesized that circulating Endostatin levels are altered in patients with ALVDD and HFpEF. Furthermore, elevated levels are associated with the presence and severity of diastolic function abnormalities. To verify the hypothesis we performed a clinical observational study including 301 patients, which were assigned based on their echocardiographic characteristics to three different groups. To our knowledge, this is the first published report linking increased circulating Endostatin levels to the presence and severity of diastolic function abnormalities and HFpEF in a well phenotyped cohort of patients with normal systolic function. In the present study, Endostatin showed a graded increase from controls over ALVDD to HFpEF. Furthermore, higher Endostatin levels were significantly associated with established markers of structural cardiac abnormalities including the LAVi and increased LV mass as well as functional abnormalities like E/E' ratio. Particularly, an increased LAVi without concomitant mitral valve disease reflects a chronic remodeling process typical for HFpEF.[25] Consistently, we found that elevated Endostatin levels were associated with elevated NT-proBNP levels, a well-recognized prognostic marker and indicator of elevated ventricular filling pressures among patients, independent from LVEF.[26]

Endostatin, a 20-kDa proteolytic fragment from the C-terminal domain of collagen XVIII, was shown to have an inhibitory effect on tumor growth working as an antiangiogenic growth factor.[27] Endostatin plays a role in the local balance of angiogenesis and shows potent anti-angiogenic activity by inhibiting proliferation and migration of endothelial cells in addition to inducing endothelial cell apoptosis.[27] Endostatin is produced by the proteolytic cleavage of the C-terminal domain of collagen XVIII, a component of the extracellular matrix. The precise mechanism of conversion from collagen XVIII to Endostatin has not yet been fully elucidated.[28,29] Recent studies of

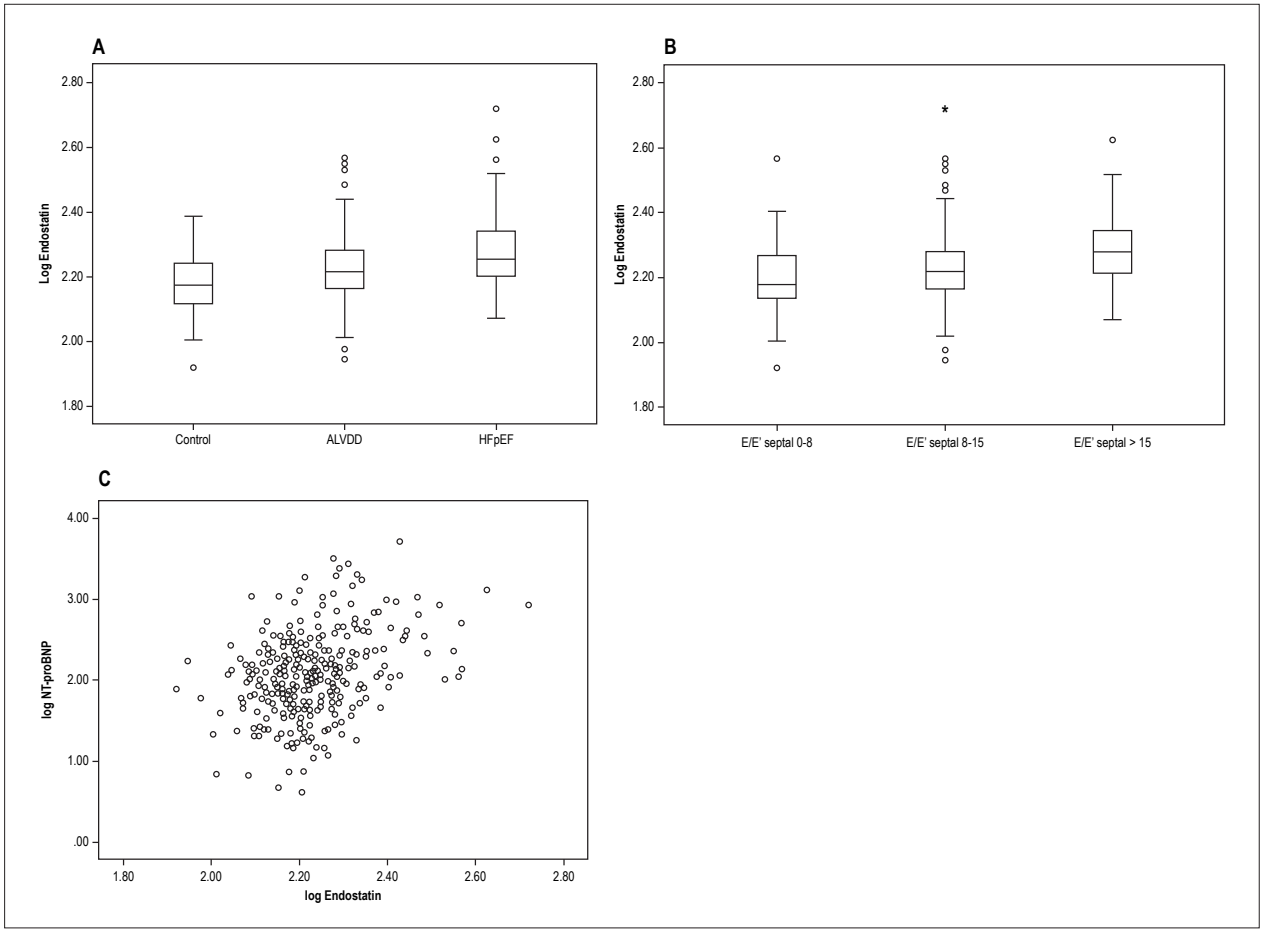

Figure 1 – *(A) The boxplot graphics show serum Endostatin levels for the ALVDD, HFpEF patients and the control group. (B)The correlation between Endostatin levels and the E/E' ratio as surrogate for increased left ventricular filling pressures. (C) The logarithmic dot blot displays the correlation of Endostatin serum levels with NT-proBNP.*

patients with coronary artery disease (CAD) demonstrate that Endostatin protein levels correlate significantly with reduced angiogenesis and poorly developed cardiac collateral vasculature.[18,30]

The results from our study fit well to the pathophysiological model used to explain the development of HFpEF. In general, HFpEF is a complex disease involving an interplay of various factors. There is the hypothesis that a failure of oxygen delivery to the cardiomycytes triggers a pro-angiogenic response in patients suffering from heart failure.[31-33] Nonetheless, angiogenic and antiangiogenic growth factors often co-exist in tissues with angiogenesis.[34] Thus, the status of endothelial cells and endothelial function is determined by a balance between these positive and negative factors on angiogenesis, and the balance may in inappropriate shifted towards antiangiogenic factors in patients with HF. It was shown that the role of microvascular dysfunction and microvascular inflammation is especial for patients with the diagnosis of HFpEF.[5,35,36] A new pathophysiologic model presented by Redfield et al.[8] points from pro-inflammatory coexisting conditions to systemic endothelial inflammation and impaired oxygen delivery.[8] Global ventricular performance is highly dependent on oxygen supply and thus myocardial perfusion, and an essential component of myocardial perfusion during ventricular hypertrophy is the myocyte–microvascular balance and the myocyte/capillary ratio. In cardiac autopsy specimens, it has recently been shown that microvascular rarefaction is a downstream phenomenon in HFpEF.[37] Furthermore, Kitzman et al.[38] has demonstrated that HFpEF patients display significant abnormalities in the skeletal muscle as well as an abnormal capillary-to-fiber ratio, probably building the basis for severe exercise intolerance in HFpEF patients.[38] In addition, Gouya et al.[19] have shown in a relatively small HFrEF study population that high levels of serum Endostatin were associated with all-cause mortality and concluded that the effect of increased angiogenesis is HF may be blunted by an overspill of anti-angiogenic factors such as Endostatin.[19] Thus, we hypothesize that similar pathophysiological concepts may be involved in patients with HFpEF, were a high proportion of patients has a coincidence of coronary artery disease and diabetes, both damaging the endothelial structure.[39] This was also true for our patient population as shown in table 1. Endostatin could be moderator of the microvascular effects seen in these patients.[40]

Several limitations of this study must be acknowledged. The observational nature of the present study prohibits definitive determination of cause and effect relationships. Second, the present study was a single-center experience

with a relatively small number of subjects. Third, longitudinal follow-up data were not available to test associations between the Endostatin serum levels and clinical outcomes. Moreover, we enrolled consecutive patients referred for elective coronary angiography and echocardiography, which may not represent a general population cohort without evidence or suspicious for cardiovascular diseases.

Further studies should include more patients from a broader population and capture longitudinal data including information about hospitalization and mortality.

Conclusion

In this exploratory hypothesis-generating study, we provide first evidence that Endostatin correlates with the presence and severity of diastolic dysfunction and HFpEF and may become a novel biomarker for the diagnosis and stratification of HFpEF. Increased Endostatin levels may reflect deterioration of diastolic function caused by adverse remodeling. Further prospective studies are needed to determine the causal relationship as well as the diagnostic and prognostic value of Endostatin in HFpEF and the potential role as a therapeutic target.

Author contributions

Conception and design of the research: Barroso MC, Dinh W; Acquisition of data: Barroso MC, Gülker JE, Dinh W; Analysis and interpretation of the data: Barroso MC, Boehme P, Kramer F, Gülker JE,Mondritzki T, Koehler T, Karoff M, Dinh W; Statistical analysis: Dinh W; Writing of the manuscript: Barroso MC, Boehme P, Dinh W; Critical revision of the manuscript for intellectual content: Boehme P, Kramer F, Mondritzki T, Koehler T, Gülker JE, Karoff M, Dinh W.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

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Original Article

Importance of Clinical and Laboratory Findings in the Diagnosis and Surgical Prognosis of Patients with Constrictive Pericarditis

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Abstract

Background: **International studies have reported the value of the clinical profile and laboratory findings in the diagnosis of constrictive pericarditis. However, Brazilian population data are scarce.**

Objective: **To assess the clinical characteristics, sensitivity of imaging tests and factors related to the death of patients with constrictive pericarditis undergoing pericardiectomy.**

Methods: **Patients with constrictive pericarditis surgically confirmed were retrospectively assessed regarding their clinical and laboratory variables. Two methods were used: transthoracic echocardiography and cardiac magnetic resonance imaging. Mortality predictors were determined by use of univariate analysis with Cox proportional hazards model and hazard ratio. All tests were two-tailed, and an alpha error ≤ 5% was considered statically significant.**

Results: **We studied 84 patients (mean age, 44 ± 17.9 years; 67% male). Signs and symptoms of predominantly right heart failure were present with jugular venous distention, edema and ascites in 89%, 89% and 62% of the cases, respectively. Idiopathic etiology was present in 69.1%, followed by tuberculosis (21%). Despite the advanced heart failure degree, low BNP levels (median, 157 pg/mL) were found. The diagnostic sensitivities for constriction of echocardiography and magnetic resonance imaging were 53.6% and 95.9%, respectively. There were 9 deaths (10.7%), and the risk factors were: anemia, BNP and C reactive protein levels, pulmonary hypertension >55 mm Hg, and atrial fibrillation.**

Conclusions: **Magnetic resonance imaging had better diagnostic sensitivity. Clinical, laboratory and imaging markers were associated with death. (Arq Bras Cardiol. 2017; 109(5):457-465)**

Keywords: **Pericarditis, Constrictive/surgery; Diagnosis, Prognosis; Diagnostic Imaging; Pericardiectomy.**

Introduction

Constrictive pericarditis (CP) results from loss of pericardial elasticity, which generates a restrictive syndrome. It is an infrequent cause of heart failure (HF), poorly diagnosed because of its peculiar pathophysiology.[1-3] The inelastic pericardium prevents ventricular filling, usually leading to signs and symptoms of right HF, which can be mistaken for other causes of HF and liver diseases, hindering the patient's outcome; because CP is a potentially curable disease, the sooner it is treated, the better the prognosis.[4,5]

Previous studies have reported the clinical profile and the value of imaging tests for diagnosing CP.[6-8] However, data on the Brazilian population are scarce. This study was aimed at assessing the clinical characteristics, sensitivity of laboratory tests and factors related to death in a case series of patients with CP undergoing pericardiectomy in a period of 13 years at a tertiary referral center.

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Method

Population

We studied retrospectively 95 patients diagnosed with CP and submitted to pericardiectomy from January 2002 to August 2015. Of those, 11 patients were excluded due to incomplete medical records, leaving 84 to the final analysis.

The following clinical data were collected: age, sex, duration of symptoms, hospital length of stay, ascites, edema, pericardial knock, jugular venous distension, paradoxical pulse, Kussmaul sign, and atrial fibrillation (AF)]. Pericardial knock was characterized as a rough protodiastolic sound, coinciding with the rapid Y descent. The following laboratory tests were performed: hemoglobin, brain natriuretic peptide (BNP) and C reactive protein (CRP). Chemiluminescence immunoassay was used to measure BNP (Bayer ADVIA Centaur Bayer Diagnostics, São Paulo, SP, Brazil).

Preoperative cardiac echocardiography and magnetic resonance imaging suggested the diagnosis of CP in the presence of at least two findings compatible with constriction:

pericardial thickening; septal bounce (paradoxical movement); inspiratory reduction in the mitral E wave (> 25%); and inferior vena cava dilatation. When more than one test was available, we chose the oldest.

Tuberculous pericarditis was diagnosed by use of pericardial biopsy, evidencing caseous granuloma or positive polymerase chain reaction for the bacillus. Post-radiation and post-surgical CP were defined in the presence of previous mediastinal radiotherapy and cardiac surgery, respectively. Idiopathic CP was defined when patients did not fit in any cited group.

Survival was obtained in the medical records or via telephone contact.

Pericardiectomy

Pericardiectomy was performed via median sternotomy in all cases, without cardiopulmonary bypass. Total pericardiectomy was defined as the excision of the anterior pericardium up to the phrenic nerves and the diaphragmatic surface. The visceral and parietal pericardium was removed whenever technically possible.

Statistical analysis

Initially descriptive analysis was performed. Measures of central trend and dispersion were presented as means and standard deviations or medians and minimum and maximum values, according to distributions. Qualitative variables were reported as absolute frequency and percentages. Chi-square or Fisher exact tests were performed to assess the differences between the groups regarding categorical variables.

All quantitative variables were tested to assess if they had a normal distribution by use of the Shapiro-Wilk test, and visually, by use of quantile-quantile graphs (Q-Q plot) or histograms. Logistic regression was used in the presence of dichotomous outcomes.

Predictors of mortality were determined by use of univariate analysis with Cox proportional hazards model. Hazard ratio (HR) and respective 95% confidence intervals (CI) and alpha values (p) were presented. Because of the small number of patients assessed in this study, multiple analysis to investigate the factors independently associated with death could not be performed.

All tests were two-tailed, an alpha error ≤ 5% was considered statically significant, and 95%CI was used in the analysis.

Data were entered in the Microsoft Excel 365 software, and analyzed in the STATA statistical program, version 13.0 (StataCorp LP, College Station, Texas, USA).

Results

This study assessed 84 patients, with a mean age of 44 ± 17.9 years, and 77% of the male sex. On admission, all had symptoms of HF, with predominance of jugular venous distension (89%), edema (89%) and ascites (62%). The median duration of those symptoms was 24 months, ranging from 1 to 1460 days (Table 1). The mean hospital length of stay was 10 days, and at the intensive care unit, 3 days. The etiologies of CP were as follows: idiopathic (69.1%); tuberculous (21.4%); post cardiac surgery (5.9%); systemic inflammatory disease (2.4%); and post radiotherapy (1.2%).

Despite the advanced degree of HF, we found low levels of BNP (median, 157 [14-692] pg/mL) and high inflammatory activity (CRP: median, 7.40 [0.72-142]). On chest X-ray, pericardial calcification was identified in 19 patients (22.6%) (Table 1).

Table 1 – Distribution of patients with constrictive pericarditis according to clinicals and laboratory characteristics

Parameter	Value
Male sex (%)	65 (77,4%)
Age (years)	
mean (SD)	44.4 (± 18)
median [min – max]	44.5 [14 – 86]
Symptom duration (months)*	
median [min – max]	24 [1 –1460]
BNP (pg/dL)**	
median [min – max]	157 [14–692]
CRP (mg/dL)***	
median [min – max]	7.40 [0.72 – 142]
Hemoglobin (g/dL)	
median [min – max]	13 [8.0 – 17.4]
Creatinine (mg/dL)	
mean (dp)	1.06 (± 0.24)
EuroScore	
median [min – max]	0.91 [0.56 – 17.68]
Urgent surgery	23
ICU LOS (days)	
median [min – max]	3.00 (0 – 80)
Hospital LOS (days)	10 [0 – 91]
Atrial fibrillation (%)	29 (34.5%)
NYHA III-IV (%)	66 (78.5%)
Jugular venous distention	75 (89%)
Pericardial knock	17 (20%)
Paradoxical pulse	11 (13%)
Edema	75 (89%)
Ascites	52 (62%)
Kussmaul sign	12 (14%)
Pleural effusion	37 (44%)
Calcification on chest X-ray	19 (22%)
Lethality	9 (10.7%)

Missing data: (*)27; (**)20; (***)17
BNP: brain natriuretic peptide; CRP: C reactive protein; SD: standard-deviation; ICU: intensive care unit; LOS: length of stay.

Table 2 – Echocardiographic variables

Echocardiographic variables	n (%)
Suggestive of CP	45 (54.2)
Non-suggestive of CP	38 (45.8)
Pericardial thickening	59 (70.2)
Respiratory variation in mitral and tricuspid flow	38 (45.2)
PASP > 55	8 (9.5)
Ejection fraction (%)	
median [min – max]	60 (32 – 80)
Stroke volume (mL)	
median [min – max]	34 (10 – 118)
Diastolic volume (mL)	
median [min – max]	88 (8 – 173)
LVSD (mm)	
median [min – max]	29 (18 – 50)
LVDD (mm)	
median [min – max]	45 (31 – 59)
Left atrium (mm)	
median [min – max]	43.5 (30 – 73)
Aortic sinus (mm)	
median [min – max]	30 (18 – 42)
Ventricular septum (mm)	
median [min – max]	8 (6 – 13)
PW (mm)	
median [min – max]	8 (6 – 12)

CP: constrictive pericarditis; PASP: pulmonary artery systolic pressure; LVSD: left ventricular systolic diameter; LVDD: left ventricular diastolic diameter; PW: posterior wall.

Of the echocardiographies performed, only 45 (53.6%) suggested CP, with pericardial thickening in 59 (70.2%), and respiratory variation in mitral and tricuspid flows in 38 (45.2%), indicating diastolic restriction (Table 2). Regarding cardiac resonance imaging, 73 patients underwent the exam, and 70 (95.9%) showed signs suggestive of CP. Pericardial thickening (pericardial thickness > 4 mm) was shown in 66 (90.4%) exams, the most common changes being aortocaval dilatation (70 – 95.9%) and septal bouncing (paradoxical movement of the ventricular septum, 64 – 87.6%) (Table 3).

Survival analysis

The mean hospital length of stay was 14.6 days (standard deviation of 13.2 days) and the median hospital length of stay, 10 days [0 - 91 days]. Of the 84 patients assessed, 9 died (lethality: 10.7%), 6 (66.6%) of whom in the first 30 postoperative days (early death), and the other 3, after that period.

The most frequent cause of early death was cardiogenic shock (5 patients – 55.5%), and only one patient had mixed cardiogenic and septic shock. Regarding the three deaths occurring after the 30th postoperative day, one was preceded by mixed cardiogenic and septic shock, and the other two, by cardiogenic shock.

Early death showed no association with any of the variables studied (Table 4).

Death showed a statistically significant association with: AF; laboratory markers (preoperative hemoglobin, CRP and BNP); and echocardiographic marker (pulmonary hypertension > 55 mm Hg). The other variables in this sample showed no statistically significant association with death in CP (Tables 4 and 5).

Thus, in patients with CP, death was associated with the presence of AF, lower hemoglobin levels (< 13 g/dL), higher CRP (≥ 7.4 mg/dL) and BNP (≥ 157 pg/mL) levels, and pulmonary artery systolic pressure (PASP) > 55 mm Hg. Their respective HR are shown in Tables 5 and 6.

Discussion

Despite the advances in complementary methods, in many patients, the correct diagnosis of pericardial constriction is established late, with a mean time elapsed between symptom onset and diagnosis of 24 months.

Constrictive pericarditis can simulate a large variety of clinical and cardiological syndromes, such as liver cirrhosis, myocardial failure and restrictive cardiomyopathies.[9] The clinical findings are those of HF, with anasarca and ascites predominating over lower limb edema. Nonspecific symptoms include fatigue, anorexia, nausea, dyspepsia and weight loss. Thus, correct diagnosis requires a high index of clinical suspicion by the cardiologist.

The clinical findings reported in a case series of the Mayo Clinic were: HF (67%); chest pain (8%); abdominal symptoms (6%); restrictive symptoms (5%); atrial arrhythmias (4%); and severe liver disease (4%). In 6% of the cases, there was low cardiac output, repetitive pleural effusion and syncope. The duration of symptoms prior to surgery was also long, of 11.7 months, ranging from 3 days to 30 years.[6]

In our case series, the major symptom was biventricular NYHA functional class III-IV HF (78%), with predominance of right HF, and ascites. The patients had cardiac cachexia, increased jugular venous pulse and Kussmaul sign. Pericardial knock was an infrequent finding, observed in 20% of our cases; however, when present, it suggested CP. On auscultation, the pericardial knock is characterized by a rough, sharp, protodiastolic sound that results from the vibration of the ventricular wall during the rapid filling phase, coinciding with the rapid Y descent. It can be mistaken for the third cardiac sound, which, however, is usually lower pitched. One of the patients was being assessed for liver transplantation; however, the increase in jugular venous pulse with rapid Y descent and pericardial knock suggested the correct diagnosis of pericardial constriction. A significant number of patients (34%) had AF, probably due to the long course of their disease.

Tuberculosis, collagenosis, neoplasms and previous cardiac surgery are some etiologies of CP, which is most commonly idiopathic or secondary to viral pericarditis. In developed

Table 3 – Cardiac magnetic resonance imaging variables

Variables	N°	%
Magnetic resonance imaging		
Not performed	11	13.1
Suggestive of CP	70	95.8
Non-suggestive of CP	3	4.1
Pericardial enhancement	14	19.2
Myocardial enhancement	9	12.3
Septal bouncing	64	87.6
Aortocaval dilatation	70	95.9
Left atrial enlargement	58	79.5
Left ventricular function (%)		
median [min – max]	60	(25 – 82)
Pericardial thickening (mm)		
median [min – max]	5	(0 – 20)

CP: constrictive pericarditis.

Table 4 – Descriptive statistics of quantitative variables of patients with constrictive pericarditis according to the occurrence of death after pericardiectomy

Variable	DEATH		p+
	NO (n = 75)	YES (n = 9)	
	median [min – max]	median [min – max]	
Age	43 [14 – 86]	48 [23 – 69]	0.84
Symptom duration (days)	24 [2 – 1460]	12 [1 – 96]	0.485
Postop. hospital LOS	10 [3 – 91]	6 [0 – 40]	0.3
ICU LOS	3 [2 – 80]	2.5 [0 – 8]	0.464
Preop. hemoglobin	13.3 [8 – 17.4]	11.9 [9.6 – 13]	0.017
Creatinine	1.06 [0.62 – 1.75]	1.0 [0.77 – 1.58]	0.815
C reactive protein	5.54 [0.72 – 142]	32.2 [5.76 – 83.8]	0.022
BNP	143 [14 – 468]	432 [160 – 692]	0.001
EuroScore II	0.88 [0.56 – 17.68]	1.67 [0.79 – 6.0]	0.214
Ejection fraction	0.60 [0.32 – 0.80]	0.60 [0.48 – 0.70]	0.581
Stroke volume	35 [10 – 118]	32 [20 – 35]	0.691
Diastolic volume	88 [8 – 173]	83.5 [51 – 108]	0.444
LVSD	29 [18 – 50]	29 [24 – 32]	0.449
LVDD	45 [31 – 59]	44 [35 – 48]	0.401
PW	8 [6 – 12]	8 [7 – 11]	0.912
Septum	8 [6 – 13]	8 [7 – 13]	0.482
LA	44.5 [30 – 73]	42 [41 – 65]	0.409
Aortic sinus	30 [18 – 42]	31 [27 – 37]	0.293

(+) Logistic regression. Postop: postoperative; LOS: length of stay; ICU: intensive care unit; Preop: preoperative; BNP: brain natriuretic peptide; LVSD: left ventricular systolic diameter; LVDD: left ventricular diastolic diameter; PW: posterior wall; LA: left atrial diameter.

countries, the most frequent etiologies are post-cardiac surgery and post-radiation. Tuberculosis continues to be a frequent etiology in developing countries or in immunosuppressed patients.[1,10]

The etiologies found in our study were as follows: idiopathic (69%), tuberculosis (21%), post-cardiac surgery (6%), systemic inflammatory disease (2%), and post-radiation (1%). In the case series of the Mayo Clinic, the etiological diagnosis

Table 5 – Deaths of patients diagnosed with constrictive pericarditis according to demographic, clinical and laboratory characteristics.

Variables	Total	deaths (lethality)	HR	95%CI	p§
Early death*					
yes	–	6 (66.6%)	–	–	
Sex					**0.98**
male	65	7 (10.7%)			
female	19	2 (10.5%)	0.98	0.20 – 4.75	
Age (years)					**0.484**
< 44	41	3 (7.13%)			
≥ 44	43	6 (13.9%)	1.93	0.48 – 7.75	
Etiology					**0.626**
Idiopathic	58	7(12.06%)			
Tuberculosis	18	1 (5.55%)			
Post-cardiac surgery	5	1 (20%)			
Radiotherapy	1	0			
Systemic disease	2	0			
Atrial fibrillation					**0.05**
yes	29	6 (20.6%)	3.87	1.01 – 16.23	
no	55	3 (5.4%)			
NYHA ≥ 2					**1.00**
no	16	2 (12.5%)			
yes	68	7 (10.29%)	0.93	0.19 – 4.51	
Total symptom duration (days)†					**0.356**
< 24	28	5 (17.8%)			
≥ 24	29	4 (13.7%)	0.51	0.12 – 2.13	
ICU LOS ≥ 3 days‡					0.454
no	43	5 (11.6%)			
yes	28	3 (10.7%)	0.91	0.70 – 1.16	
Postop. hospital LOS ≥ 10 days					**0.503**
no	37	5 (13.5%)			
yes	47	4 (10.8%)	0.54	0.14 – 2.05	
PASP > 55 mm Hg					**0.046**
no	60	5 (8.3%)			
yes	8	3 (37.5%)	4.5	1.38 – 24.2	
Preop. hemoglobin					**0.003**
< 13	33	8 (24.2%)			
≥ 13	33	0	0	–	
C reactive protein					**0.0**
< 7.4	26	1 (3.8%)			
≥ 7.4	26	7 (87.3%)	8.5	1.04 – 69.33	
BNP					**0.011**
< 157	32	0			
≥ 157	32	7 (21.8%)	NC		

CI: confidence interval; ICU: intensive care unit; LOS: length of stay; Postop: postoperative; PASP: pulmonary artery systolic pressure; Preop: preoperative; BNP: brain natriuretic peptide. * Early death (≤ 30 postoperative days); † 57 available data; ‡ 63 available data; § Fisher exact test; NC: could not be calculated.

Table 6 – Variables associated with death

Variable	Late death (n = 3)	Early death (n = 6)	p+
	median [min-max]	median [min-max]	
Age	23 [48 – 69]	23 [51 – 61]	0.884
Symptom duration (days)	24 [12 – 96]	12 [1 – 24]	0.366
Postop. hospital LOS	58 [40 – 71]	1.5 [1 – 23]	0.175
Preop. hemoglobin	9.6 [11.8 – 12.6]	12 [10.2 – 13]	0.507
BNP	683 [160 – 692]	431 [321 – 465]	0.469
Creatinine	1.1 [1.04 – 1.36]	0.95 [0.77 – 1.58]	0.48
CRP	74.5 [5.76 – 74.8]	29.4 [18.58 – 83.9]	0.491

(+) Logistic regression. Postop: postoperative; Preop: preoperative; BNP: brain natriuretic peptide; CRP: C reactive protein.

was established in 73% of the 135 patients studied, the major being as follows: post-cardiac surgery (18%), pericarditis (16%), and post-radiation (13%). Other less frequently reported etiologies were rheumatological diseases: rheumatoid arthritis, polymyalgia, myopericarditis, psoriatic arthritis, and Still's disease. In that case series, the infectious causes were infrequent: one case of fungal pericarditis and two of tuberculosis.[6]

Physiological and structural data can be obtained by use of echocardiography, and cardiac tomography and resonance. Recent advances in diagnostic methods have provided complementary assessment in patients with diagnostic suspicion of constriction.[8,11-13]

Echocardiography is the first non-invasive test that can help when CP is suspected, allowing the differential diagnosis with forms of right ventricular failure, such as restrictive cardiomyopathy, pulmonary hypertension, and mitral valve disease.[14] The major findings include pericardial thickening, better visualized on transesophageal echocardiogram, abnormal movement of the ventricular septum, dilatation and lack of inspiratory collapse of the inferior vena cava, respiratory variation in mitral (> 25%) and tricuspid (> 40%) flows, normal or increased velocity of E' waves, and biatrial enlargement.

In our case series, only 54% of the cases had an echocardiogram suggestive of pericardial constriction. It is worth noting that 30% of the patients had no pericardial thickening, despite surgical confirmation. Thus, the diagnosis of CP and indication for pericardiectomy should not be postponed in the absence of pericardial thickening or when there is no other signal of pericardial constriction.[6,15] It is worth noting that, in the absence of echocardiographic signs suggestive of CP, the diagnosis of CP is delayed. Many services lack more sophisticated complementary exams, such as resonance and tomography. The presence of signs and symptoms of right HF should raise the suspicion of restrictive syndrome. Regarding the topographic and etiological diagnosis of restrictive syndromes, we should consider endocardial, myocardial and, especially among us, pericardial affections. Thus, when the echocardiogram is inconclusive, other complementary exams should be performed to confirm the diagnosis of pericardial constriction, such as cardiac tomography and resonance. The later the diagnosis, the worse the prognosis.

Tomography and resonance provide a fair anatomical assessment of the pericardium and cardiac chambers. In addition, resonance imaging provides the functional assessment of diastolic restriction signs. In our study, resonance imaging suggested CP in 96% of the patients.

The pericardium has a fibrous structure with T1 and T2 hypointense characteristic as compared to the myocardium. A pericardial thickness of 2 mm is considered normal. Pericardial thickening of 4 mm suggests CP, and that of 6 mm has high specificity for the diagnosis of constriction. Approximately 10% to 20% of the patients undergoing surgery have normal pericardial thickness, which, thus, does not rule out the diagnosis. In such cases, there is visceral pericardial inflammation (epicarditis), pericardiectomy being often curative. Talreja et al.,[16] in a retrospective study of 26 patients diagnosed with CP and normal pericardial thickness (< 2 mm), have found the following etiologies: cardiac surgery (42%), chest radiation (19%), post-myocardial infarction (12%), and idiopathic (12%).

In most cases, resonance imaging provides the presumptive diagnosis of CP. In addition to pericardial thickening, other characteristics provided by that exam, such as atypical movement of the ventricular septum and inferior vena cava dilatation, should be valued.

In only 4% of the cases, the diagnosis was complemented with catheterization and chest tomography. Thus, even with important clinical suspicion and no confirmation by complementary exams, we indicate exploratory thoracotomy, because, if the diagnosis is not performed, a poor outcome is highly likely.

Another finding in our case series was low BNP levels (median, 157 pg/mL), despite the advanced functional class HF. That could be explained by diastolic restriction and lower parietal tension, determining a lower stimulus to BNP release. In previous studies, CP had lower BNP levels as compared to those of other restrictive syndromes, such as restrictive cardiomyopathy, and that measurement could be useful for the differential diagnosis with other restrictive syndromes.[17-19] Thus, in patients with predominantly right HF and normal or mildly elevated BNP levels, CP should be considered in the differential diagnosis.

Asymptomatic patients should undergo periodical assessment of liver function, functional capacity and jugular venous pressure. The clinical treatment consists in anti-inflammatory drugs, corticosteroids, diuretics and antibiotics. The treatment for tuberculosis should begin prior to surgery and continue after that. Diuretics should be used to reduce, but not eliminate, jugular venous pressure, ascites and edema, because patients need preload to maintain cardiac output.

Pericardiectomy is the definite treatment for pericardial constriction in symptomatic patients. Increased jugular venous pressure, need for diuretics, evidence of liver failure and reduced functional capacity are indicative of surgery. All our patients were symptomatic and had a long disease course, having thus indication for surgery.

Nine patients (10.7%) died, six in the early postoperative period (within 30 days from surgery), and three, later. Most deaths were due to low cardiac output after pericardiectomy, and two deaths were secondary to cardiogenic and septic shock. In our case series, the following factors were related to death: laboratory markers (anemia, increased inflammatory activity: CRP and BNP levels); clinical marker (AF); and imaging (pulmonary artery hypertension).

The possible mechanism that relates increased mortality and BNP levels has not been totally clarified. We can assume that elevations in that biomarker reflect factors that influence survival, such as myocardial dysfunction, ischemia and increased filling pressures.

Despite the 10.7% mortality, the EuroScore was low. However, the EuroScore study did not contemplate patients undergoing pericardiectomy. We believe, thus, that other perioperative risk scores should be used in patients undergoing pericardiectomy.

Constrictive pericarditis has a variable prognosis with in-hospital mortality ranging from 5% to 16%.[20,21]

Tokuda et al.[22] have assessed 346 patients submitted to pericardiectomy and found a 10% mortality rate, with the following predictors of worse prognosis: functional class IV; kidney failure; previous cardiac surgery; and use of cardiopulmonary bypass. Peset et al.[21] have reported a 16% mortality rate, 80% being due to right HF and low cardiac output. Bashi et al.,[23] however, have reported a reduction in mortality from 16%, in the 1954-73 period, to 11%, in the 1974-86 period, attributed to improvement in postoperative care. In the last period, however, the patients had more advanced disease.

In the study of the Mayo Clinic, with 135 patients, there were 39 deaths (29%), 26 of which occurred after hospital discharge. The 5- and 10-year survivals were 71% and 52%, respectively. The survival determinants were: age, previous radiotherapy, functional class, and sodium concentration. Signs and symptoms of functional class II-IV HF were detected in approximately 31% of the patients at some point of their disease course (mean time, 7 months after surgery). The late predictors of HF were age, radiation and ascites.[6]

Similarly to our results, Bertog et al.[20] have found increased pulmonary artery pressure as an adverse factor, which was attributed to the severity of the concomitant pericardial constriction, myocardial dysfunction or associated pulmonary pathologies.

The ejection fraction can decrease in the postoperative period and can require months to normalize; during that period, the use of digoxin, diuretics and vasodilators can be clinically useful. Other determinants of the prognosis depend on the extent of myocardial atrophy and fibrosis, and on the degree of calcification and adhesion between the epicardium and myocardium, which hinders surgical debridement.

Constrictive pericarditis is curable when early diagnosed and has a good postoperative outcome. Its better understanding and identification will enable us to reduce its high morbidity and mortality rates.

Conclusions

Constrictive pericarditis manifests with signs and symptoms of biventricular HF, with predominance of right HF, and slightly elevated BNP levels.

The diagnosis is late, with median of 24 months between symptom onset and the correct diagnosis.

Magnetic resonance imaging has better sensitivity for the diagnosis as compared to echocardiography.

Clinical markers (AF), laboratory measures (hemoglobin < 13 g/dL, CRP ≥ 7.4 mg/dL, BNP ≥ 157 pg/mL) and imaging (PASP > 55 mm Hg) were associated with mortality.

Limitations

The sample consisted mainly of patients with CP of idiopathic etiology, from one single cardiology tertiary center, which can represent bias of selection, and limit the external validity of the results.

Because echocardiography was performed by one single examiner, the reproducibility of the results, that is, intra- and inter-observer variability, could not be assessed. However, the examiner was experienced and blind to the other results.

Author contributions

Conception and design of the research: Fernandes F, Melo DTP, Ramires FJA, Mady C; Acquisition of data: Fernandes F, Melo DTP, Dias RR, Tonini M, Fernandes VS, Rochitte CE; Analysis and interpretation of Melo DTP the data, Obtaining financing and Critical revision of the manuscript for intellectual content: Fernandes F, Melo DTP, Mady C; Statistical analysis: Melo DTP, Moreira CHV; Writing of the manuscript: Fernandes F, Mady C.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Comparison of Secondary Prevention Status between Percutaneous Coronary Intervention and Coronary Artery Bypass Patients

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Abstract

Background: Data are scarce regarding disparities in cardiovascular risk factor management between patients treated with percutaneous coronary intervention (PCI) and those treated with coronary artery bypass grafting (CABG).

Objective: Whether the goal achievement rates of cardiovascular risk factors were different between PCI and CABG patients.

Methods: We retrospectively reviewed the data retrieved from a clinical record database of patients admitted to Beijing Anzhen Hospital between January 1, 2014, and December 31, 2014, who underwent PCI or CABG.

Results: Compared with the CABG group, low-density lipoprotein cholesterol (LDL-C) < 1.8 mmol/L (28.6% vs. 24.7%; p < 0.01), LDL-C < 2.07 mmol/L (43.5% vs. 39.4%; p < 0.01) and blood pressure (BP) < 140/90 mm Hg (85.6% vs. 77.7%; p < 0.01) goal achievement rates were significantly higher in the PCI group. Compared with patients ≥ 60 years old: patients < 60 years old had better BP < 140/90 mm Hg goal achievement rates (87.7% vs. 84.4%; p < 0.01) in the PCI group, and better fasting blood-glucose (FBG) < 7 mmol/L (79.4% vs.72.0%; p < 0.01) and HbA1c < 7% (79.4% vs. 70.1%; p < 0.01) goal achievement rates in the CABG group. Compared with females: males had better LDL-C < 2.07 mmol/L (24.7% vs. 28.5%; p < 0.01), FBG < 7 mmol/L (71.8% vs.75.2%; p < 0.01) and HbA1c < 7% (70.4% vs. 74.1%; p < 0.01) goal achievement rates in the PCI group.

Conclusion: Patients in the PCI group were generally more likely than those in the CABG group to achieve LDL-C < 1.8 mmol/L and BP goals. The control of cardiovascular risk factors differed between patients ≥ 60 years old and < 60 years old. Female patients were less likely to achieve LDL-C, FBG and HbA1c goals. (Arq Bras Cardiol. 2017; 109(5):466-474)

Keywords: Percutaneous Coronary Intervention; Coronary Artery Bypass; Myocardial Revascularization; Risk Factors.

Introduction

In China, the number of patients who undergo coronary revascularization increases with cardiovascular disease outbreaks. Percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG) are two major coronary revascularization procedures. Although PCI and CABG have saved plenty of lives, they do not prevent the progression of arterial atherosclerosis, and major events and secondary revascularization rates remain high in patients 5 years later.[1] Taking secondary preventive drugs is important for those patients.[2,3]

Roughly 14,000 patients underwent CABG or PCI in Beijing Anzhen Hospital every year. However, in practice, we found that cardiovascular physicians and cardiothoracic surgeons have different concerns with respect to long-term prognosis, which might influence the prescription of secondary preventive drugs and further leads to unbalanced control of coronary artery disease (CAD)-related risk factors, such as LDL-C, blood pressure (BP), fasting blood-glucose (FBG), and hemoglobin A1c (HbA1c), in PCI and CABG patients. In addition, previous studies have reported that the control of cardiovascular risk factors was different in different age groups and different sex. We hypothesized that patients who have undergone CABG would be less likely than patients who have undergone PCI to achieve lipid, FBG, HbA1c and BP goals. We assessed the goal attainment and clinical outcomes in PCI and CABG patients, and the goal achievement rates in patients ≥ 60 years old and < 60 years old, females and males.

Methods

Source population

This retrospective study enrolled 14,230 patients who underwent PCI (n = 9,866) or CABG (n = 4,364) in Beijing Anzhen Hospital between January 1, 2014, and December 31, 2014. The index date was that of the revascularization procedure. We excluded patients (n = 7,707) aged < 18 years with a history of coronary revascularization, malignant tumor, multiple organ dysfunction syndrome, or organ transplantation, without complete demographic data, without continued drug prescription record, or whose second lipid

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level values were not available after the index date. A total of 6,523 patients were ultimately included in the analysis and matched by propensity score.

Data collection

Clinical information was retrieved from computerized clinical records, and relevant clinical data were extracted up to December 31, 2015, the start of the data collection period. We obtained the following data: age; sex; history of present illness; comorbidities (hypertension, diabetes, stroke, peripheral vascular disease, chronic kidney disease); cardiovascular disease-related risk factors (smoking, drinking, obesity); coronary artery lesions (SYNTAX score); lipid, BP, FBG and HbA1c levels before discharge and during follow-up. Date of cardiac death, recurrent acute coronary syndrome (ACS), stroke, non-fatal acute myocardial infarction (AMI), and revascularization were also collected for the patient outcome analysis. Composite endpoints were defined as cardiac death, recurrent ACS, and stroke. Recurrent ACS was defined as recurrent non-fatal AMI and unstable angina. Lipid, BP, FBG and HbA1c levels before discharge were defined as lipid, BP, FBG and HbA1c levels before the coronary revascularization procedure, while lipid, BP, FBG and HbA1c levels during follow-up were defined as the most recently lipid, BP, FBG and HbA1c levels (at least 3 months after discharge) if there was no endpoint event, and lipid, BP, FBG and HbA1c levels during re-hospitalization if there was an endpoint event. Hypertension, diabetes, dyslipidemia, stroke, peripheral vascular disease, chronic kidney disease, alcohol heavy drinking, and obesity were defined as published previously.[4] The follow-up period of each individual from the discharge date until December 31, 2015, was also calculated. Lipid goal attainment was defined as an LDL-C < 1.8 mmol/L (70 mg/dL) and non-high-density lipoprotein cholesterol (HDL-C) < 2.6 mmol/L (100 mg/dL),[5] or LDL-C < 2.07 mmol/L (80 mg/dL) and non-HDL-C < 2.8 mmol/L (110 mg/dL).[6] The FBG goal attainment was defined as FBG < 7.0 mmol/L; HbA1c < 7%. Blood pressure goal attainment was defined as BP < 140/90 mm Hg.[7]

This study was approved by the Beijing Anzhen Hospital Ethics Committee.

Statistical methods

Propensity scores were estimated using a multiple logistic regression analysis. PCI and CABG patients were 1:1 matched using the nearest neighbor matching method. Continuous variables with normal distribution were presented as mean ± standard deviation, and those with non-normal distribution were presented as median and interquartile range. Categorical variables were depicted as absolute numbers and percentages. K-S test was used to verify the normality of the data. Continuous variables were compared using the Wilcoxon signed rank test or a paired *t* test, and categorical variables were compared using the chi-square test. Kaplan-Meier survival curves were used to compare the cumulative incidence of composite endpoint events. Cox regression analysis was performed to evaluate the influence of baseline covariates on composite outcomes. The log-rank test was performed before Cox regression. Variables with P values ≤ 0.10 were candidates for the multivariate model. Covariates included in Cox regression analysis were as follows: age, sex, hypertension, diabetes mellitus, dyslipidemia, smoking, stroke, peripheral artery disease, chronic kidney disease, body mass index (BMI), left ventricular ejection fraction (EF), SYNTAX score, and achievement of LDL-C, FBG, HbA1c and BP goals. All analyses were performed with SPSS (version 22.0; IBM, Armonk, NY, USA). All tests were two-tailed, and P values < 0.05 were considered statistically significant.

Results

Baseline characteristics

A total of 6,523 (PCI = 4,728; CABG = 1,795) patients were enrolled in the study. Compared to patients in the PCI group, those in the CABG group were older and more likely to have a history of diabetes and stroke; less likely to have a history of hypertension, and dyslipidemia; and presented lower BMI, HDL-C level and left ventricular EF, and higher SYNTAX score. A total of 1,790 matched patient pairs were created after propensity-score matching was performed for the entire population. The baseline characteristics did not differ significantly between the PCI and CABG groups after the propensity-score matching (Table 1).

LDL-C, FBG, HbA1c, and BP goal attainment rates in total and propensity matched PCI and CABG patients

Compared with the CABG group, LDL-C < 1.8 mmol/L, LDL-C < 2.07 mmol/L and BP < 140/90 mmHg goal achievement rates in the PCI group were significantly higher in the unmatched patients after discharge. The FBG and HbA1C target attainment rates did not differ significantly between the two groups after discharge (Table 2). In propensity matched patients, LDL-C < 1.8 mmol/L, LDL-C < 2.07 mmol/L and BP < 140/90 mmHg goal achievement rates in the PCI group were significantly higher than in the CABG group. The FBG and HbA1c goal achievement rates were not significantly different between the two groups (Table 2).

Clinical outcomes

In unmatched patients, composite endpoint rates were significantly higher in the PCI group than in the CABG group (Table 4). The median follow-up duration was 10.99 months. In propensity matched patients, composite endpoint rates were not significantly different between the two groups (Figure 1, Table 4). Recurrent ACS rates were significantly higher in the PCI group than in the CABG group in both matched and unmatched patients (Table 4). Stroke incidence was significantly higher in the CABG group than in the PCI group (Table 4). On multivariable Cox regression analysis, LDL-C < 1.8 mmol/L and HbA1c < 7% were independent predictors of composite endpoints in the unmatched overall, PCI, and CABG patients, hazard ratio were reduced in those patients who achieved goals (Table 3). To determine whether the composite endpoint rates in the matched patients according to PCI and CABG were consistent, we applied subgroup analysis. Compared with patients in the PCI group, patients in the CABG group had better clinical outcome regarding diabetes and obesity, and patients ≥ 60 years old subgroups (Figure 2).

Table 1 – Baseline characteristics of patients in PCI and CABG groups

	Total population			Propensity-matched population		
	PCI n = 4728	CABG n = 1795	p value	PCI n = 1790	CABG n = 1790	p value
Age (years)	58.9 ± 10.2	61.9 ± 9.0	< 0.01	62.0 ± 9.9	61.9 ± 9.0	0.68
Sex (male)	3499(74.0)	1353(75.4)	0.26	1369(76.5)	1349(75.4)	0.43
Hypertension	2394(61.2)	1073(59.8)	< 0.01	1068(59.6)	1072(59.9)	0.87
Diabetes	1461(30.9)	634(35.3)	0.001	632(35.3)	630(35.5)	0.94
Dyslipidemia	3749(79.3)	1361(75.8)	0.002	1360(76.0)	1348(75.3)	0.64
Stroke	265(5.6)	169(9.4)	< 0.01	150(8.4)	168(9.4)	0.29
PVD	52(1.1)	23(1.3)	0.54	27(1.5)	23(1.3)	0.57
CKD	33(0.7)	9(0.5)	0.38	7(0.4)	9(0.5)	0.62
Smoking	2392(50.6)	863(48.1)	0.07	848(47.4)	863(48.2)	0.62
BMI	26.5 ± 3.4	25.4 ± 2.9	< 0.01	25.3 ± 3.3	25.4 ± 2.9	0.57
LVEF (%)	62.2 ± 8.3	60.4 ± 9.0	< 0.01	60.7 ± 9.1	60.5 ± 9.0	0.50
SYNTAX score	23.4 ± 9.3	28.1 ± 10.1	< 0.01	27.8 ± 9.3	28.0 ± 10.2	0.19
Lipid levels before discharge (mmol/L)						
TC	4.58 ± 0.9	4.57 ± 1.1	0.87	4.56 ± 1.0	4.57 ± 1.1	0.82
TG	1.87 ± 1.2	1.83 ± 1.1	0.24	1.83 ± 1.1	1.83 ± 1.1	0.99
LDL-C	2.86 ± 0.8	2.88 ± 0.9	0.52	2.87 ± 0.8	2.88 ± 0.9	0.75
HDL-C	1.00 ± 0.2	0.97 ± 0.2	< 0.01	0.97 ± 0.2	0.97 ± 0.2	0.56
FBG (mmol/L) and HbA1c (%) levels before discharge						
FBG	6.08 ± 1.7	5.77 ± 1.5	0.07	5.91 ± 1.6	5.77 ± 1.5	0.36
HbA1c	5.93 ± 1.1	5.78 ± 1.1	0.17	5.80 ± 1.1	5.78 ± 1.1	0.64
Blood pressure (mmHg) before discharge						
SBP	127.65 ± 15.4	124.04 ± 17.7	0.13	124.5 ± 16.3	124.04 ± 17.7	0.91
DBP	76.54 ± 11.3	75.35 ± 10.6	0.04	75.04 ± 10.3	75.35 ± 10.6	0.32

Values are presented as mean±standard deviation and median with interquartile range or n (%); PCI: percutaneous coronary intervention; CABG: coronary artery bypass grafting; PVD: peripheral vascular disease; CKD: chronic kidney disease; BMI: body mass index; LVEF: left ventricular ejection fraction; TC: total cholesterol; TG: triglyceride; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; FBG: fasting blood-glucose; HbA1c: hemoglobin A1C; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2 – LDL-c, FBG, HbA1c, and BP goal achievement rates in PCI and CABG groups

	Total population			Propensity-matched population		
Risk factor goals	**PCI**	**CABG**	**p**	**PCI**	**CABG**	**p**
LDL-c <1.8 mmol/L[a]	1352(28.6)	443(24.7)	0.002	522(29.2)	442(24.7)	0.003
LDL-c < 2.07 mmol/L[b]	2055(43.5)	708(39.4)	0.003	787(44.0)	707(39.5)	0.007
FBG < 7 mmol/L[c]	3498(74.2)	1342(74.8)	0.492	1361(76.0)	1342 (75.0)	0.46
HbA1c < 7%[c]	3456(73.1)	1321(73.6)	0.686	1349(75.4)	1319(73.7)	0.25
BP < 140/80 mmHgd	4049(85.6)	1394(77.7)	0.000	1525(85.2)	1391(77.7)	0.000

Values are presented as n (%); a, Chinese guidelines on prevention and treatment of dyslipidemia in adults, 2007; b, ESC/EAS guidelines for the management of dyslipidaemias, 2011; c, Chinese guidelines on type 2 diabetes prevention and treatment, 2013; d, Chinese guidelines on prevention and treatment of hypertension, 2011. PCI: percutaneous coronary intervention; CABG: coronary artery bypass grafting; LDL-C: low density lipoprotein cholesterol; FBG: fasting blood-glucose; HbA1c: hemoglobin A1C; BP: blood pressure.

Table 3 – Independent predictors of composite endpoints in PCI and CABG groups

	Overall			ICP			CRM		
Variables	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Sex[a]	0.298	(1.08-1.68)	0.008	0.251	(1.01-1.64)	0.043	0.414	(0.87-2.64)	0.144
PCI vs CABG	0.821	(1.81-2.85)	0.000						
Smoking[a]	1.692	(1.29-2.72)	0.000	1.783	(1.43-3.13)	0.000	1.113	(0.98-1.81)	0.754
LDL-c < 1.8	-2.197	(0.07-0.17)	0.000	-2.329	(0.06-0.16)	0.000	-1.023	(0.09-0.45)	0.000
HbA1c < 7%	-0.363	(0.58-0.85)	0.000	-0.403	(0.54-0.82)	0.000	-0.392	(0.53-0.88)	0.000
EF < 40%[b]	-0.241	(0.52-1.19)	0.252	-0.101	(0.56-1.47)	0.686	-0.825	(0.20-0.95)	0.037
Dyslipidemia[c]	1.164	(0.96-1.45)	0.120	1.256	(1.03-1.63)	0.030	1.09	(0.59-1.43)	0.679
BP < 140/80 mmHg	-0.475	(0.32-0.49)	0.000	-0.432	(0.37-0.50)	0.000	-0.129	(0.39-1.76)	0.788

Values are presented as n (%); CI: confidence interval; HR: Hazard ratio; a: sex and smoke were significant predictors in overall and PCI-treated patients; b: EF > 40% was a significant predictor in CABG-treated patients; c: dyslipidemia was a significant predictor in PCI-treated patients. PCI: percutaneous coronary intervention; CABG: coronary artery bypass grafting; LDL-C: low density lipoprotein cholesterol; HbA1c: hemoglobin A1C; EF: ejection fraction; BP: blood pressure.

Table 4 – Clinical outcomes in PCI and CABG groups

	Total population				Propensity-matched population			
	PCI	CABG	HR(95% CI)	p	PCI	CABG	HR(95% CI)	p
Composite endpoints	424(9.0)	101(5.6)	1.652(1.32.2.07)	0.000	126(7.0)	101(5.6)	1.27 (0.97.1.66)	0.09
Recurrence ACS	389(8.2)	80(4.5)	5.935(4.619.7.626)	0.000	116(6.5)	80(4.5)	1.48(1.11.1.99)	< 0.01
Stroke	29(0.6)	19(1.1)	1.535(0.858.2.748)	0.146	8(6.4)	19(1.1)	0.42(0.18.0.96)	0.03
Cardiac death	6(0.1)	2(0.1)	3.007(0.606.14.917)	0.157	2(0.1)	2(0.1)	1.00(0.14.7.11)	1.00

Values are presented as n (%). Composite end points included recurrent ACS, stroke and cardiac death. ACS: acute coronary syndrome. PCI: percutaneous coronary intervention.

LDL-C, FBG, HbA1c, and BP goal attainment rates in unmatched patients with different ages

In unmatched overall and PCI patients, compared with patients ≥ 60 years old: patients < 60 years old had better BP < 140/90 mm Hg goal achievement rates and worse LDL-C < 2.07 mmol/L goal achievement rates. The LDL-C < 1.8 mmol/L, FBG < 7 mmol/L, and HbA1c < 7% goal achievement rates were not significantly different. In unmatched CABG patients, compared with patients ≥ 60 years old: patients < 60 years old had better FBG < 7 mmol/L, HbA1c < 7%, BP < 140/90 mm Hg goal achievement rates, the LDL-C < 1.8 mmol/L and LDL-C < 2.07 mmol/L goal achievement rates were not significantly different between the two groups (Table 5).

LDL-C, FBG, HbA1c, and BP goal attainment rates in unmatched patients of different sexes

In unmatched overall and PCI patients, compared with females: males had better LDL-C < 1.8 mmol/L, FBG < 7 mmol/L, and HbA1c < 7% goal achievement rates. The LDL-C < 2.07 mmol/L and BP < 140/90 mmHg goal achievement rates were not significantly different. Those goal achievement rates were not significantly different in CABG patients between females and males (Table 5).

LDL-C, FBG, HbA1c, and BP goal attainment rates in unmatched patients with different ages and different sexes

In unmatched patients ≥ 60 years old, compared with females, males had better LDL-C < 1.8 mmol/L, FBG < 7 mmol/L, and HbA1c < 7% goal achievement rates. The LDL-C < 2.07 mmol/L and BP < 140/90 mmHg goal achievement rates were not significantly different. Those goal achievement rates were not significantly different in patients < 60 years old between females and males (Table 6).

Discussion

PCI and CABG techniques were developed rapidly in the late 90s in China. The surgical volume of PCI was increased by 30%-50% per year, and up to 567583 in 2015, forefront in the world. With the improvement of surgical techniques, mortality of CABG was reduced greatly, and was acceptable by an increasing number of patients. Although PCI and CABG successfully saved plenty of lives, how to decrease the incidence of revascularization is a major problem at present. Therefore, the emphasis of secondary prevention is particularly important after PCI and CABG.

In the present study, our major findings are as follows: (a) in overall and the propensity score-matched patients, lipid

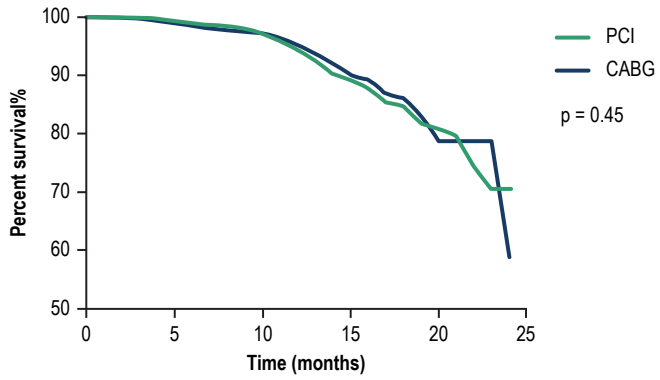

Figure 1 – *Kaplan-Meier cumulative events for composite endpoint. Composite endpoint events (cardiac death/recurrent acute coronary syndrome/stroke) rate were not significantly different between percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG) patients.*

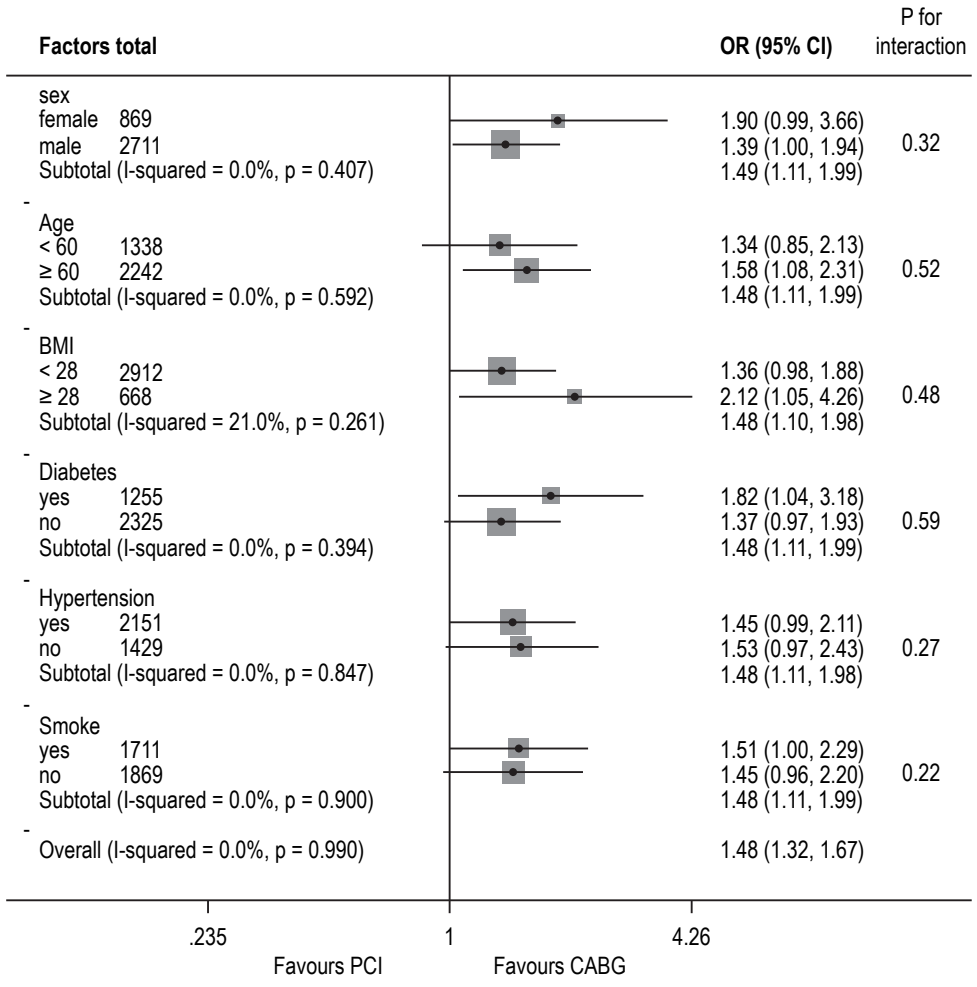

Figure 2 – *Comparative unadjusted hazard ratios of recurrent ACS for subgroups in propensity-matched populations of the percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG) groups. CI: confidence interval; BMI: body mass index; ACS: acute coronary syndrome.*

Table 5 – LDL-c, FBG, HbA1c, and BP goal achievement rates in different age and sex

	Overall			PCI			CABG		
LDL-c, FBG, HbA1c, and BP goal achievement rates in patients who < 60 and ≥ 60 year old									
Risk factor goals	**< 60**	**≥ 60**	**p**	**< 60**	**≥ 60**	**p**	**< 60**	**≥ 60**	**p**
LDL-c < 1,8 mmol/L[a]	640(26.3)	1155(28.3)	0.079	474(27.0)	878(29.6) ††	0.056	166(24.4)	277(24.8)	0.859
LDL-c < 2,07 mmol/L[b]	967(39.7)	1796(44.0)	0.001	703(40.0)	1352(45.5) ††	0.001	264(38.9)	444(39.8)	0.704
FBG < 7 mmol/L[c]	1817(74.5)	3023(74.0)	0.608	1278(72.8)	2219(74.7)	0.138	539(79.4) ‡‡	804(72.0)	0.001
HbA1c < 7%[c]	1805(72.9)	2972(72.7)	0.240	1266(72.0)	2190(73.7) †	0.196	539(79.4) ‡‡	782(70.1)	0.001
BP < 140/80 mmHgd	2093(85.9)	3350(82.0)	0.000	1541(87.7) *	2508(84.4) ††	0.002	552(81.3)	842(75.4)	0.004
LDL-c, FBG, HbA1c, and BP goal achievement rates in female and male									
Risk factor goals	**Female**	**Male**	**p**	**Female**	**Male**	**p**	**Female**	**Male**	**p**
LDL-c < 1,8 mmol/L[a]	399(24.7)	1396(28.5)	0.003	306(25.5)	1046(29.6)	0.006	93(22.2)	350(25.4)	0.188
LDL-c < 2,07 mmol/L[b]	661(40.9)	2102(42.8)	0.165	502(41.9)	1553(44.0)	0.197	159(38.0)	549(39.9)	0.502
FBG < 7 mmol/L[c]	1152(71.8)	3689(75.2)	0.002	851(71.0)	2647(75.0)	0.006	301(72.0)	1042(75.7)	0.131
HbA1c < 7%[c]	1139(70.4)	3638(74.1)	0.003	832(69.4)	2624(74.4)	0.001	307(73.4)	1014(73.6)	0.937
BP < 140/80 mmHg[d]	1330(82.3)	4113(83.8)	0.137	1019(85.0)	3030(85.9)	0.457	311(74.4)	1083(78.6)	0.068

*Values are presented as n (%); a, Chinese guidelines on prevention and treatment of dyslipidemia in adults, 2007; b, ESC/EAS guidelines for the management of dyslipidemia, 2011; c, Chinese guidelines on type 2 diabetes prevention and treatment, 2013; d, Chinese guidelines on prevention and treatment of hypertension, 2011; *: in patients who < 60, compared with CABG group, p < 0.01; ††: in patients who ≥ 60, compared with CABG group, p < 0.01; †: in patients who ≥ 60, compared with CABG group, p < 0.05; ‡‡: in patients who < 60, compared with PCI group, p < 0.01. PCI: percutaneous coronary intervention; CABG: coronary artery bypass grafting; LDL-C: low density lipoprotein cholesterol; FBG: fasting blood-glucose; HbA1c: hemoglobin A1C; BP: blood pressure.*

Table 6 – LDL-c, FBG, HbA1c, and BP goal achievement rates between different sex in patients < 60 years old and patients ≥ 60 years old

Risk factor goals	< 60			≥ 60		
	Female	**Male**	**p**	**Female**	**Male**	**p**
LDL-c < 1,8 mmol/L[a]	81(24.5)	559(26.5)	0.426	318(24.7)	837(29.9)	0.001
LDL-c < 2,07 mmol/L[b]	120(36.3)	847(40.2)	0.171	541(42.1)	1255(44.8)	0.100
FBG < 7 mmol/L[c]	239(72.2)	1578(74.9)	0.290	913(71.0)	2111(75.4)	0.003
HbA1c < 7%[c]	232(70.1)	1573(74.7)	0.076	907(70.5)	2065(73.8)	0.032
BP < 140/80 mmHg[d]	277(83.7)	1816(86.2)	0.217	1053(81.9)	2297(82.0)	0.905

Values are presented as n (%). LDL-C: low density lipoprotein cholesterol; FBG: fasting blood-glucose; HbA1c: hemoglobin A1C; BP: blood pressure

and BP goal attainment rates were different between PCI and CABG patients; however, LDL-C, FBG, HbA1c and BP goal attainment rates were not optimistic in either group, (b) the LDL-C and BP goal achievement rates in the PCI group, and the FBG and HbA1c goal achievement rates in the CABG group were different between patients < 60 years old and those ≥ 60 years old; (c) the LDL-C, FBG and HbA1c goal achievement rates were significantly lower in females in the PCI group, as well as in patients ≥ 60 years old.

LDL-C and BP goal achievement rates in the PCI group were significantly higher than in the CABG group, and a possible reason might be the difference in medication use and adherence. Hlatky et. al have observed that medication possession ratios of secondary preventive drugs were significantly lower in CABG patients than in PCI patients, and the use of statins was significantly lower in CABG patients than in PCI patients.[8] Possible reasons for such disparities might be as follows: (a) in our hospital, some patients after CABG were taken care of by surgeons, treatment strategies differed between cardiologists and cardiothoracic surgeons. Cardiologists followed guidelines and had better performance in using preventive drugs than cardiothoracic surgeons, while cardiothoracic surgeons usually pay more attention to whether the surgery was successful, postoperative complications and wound repair situations rather than secondary prevention drug prescription and health education before discharge;[4] (b) some other patients might be followed up by cardiologists after CABG in the outpatient clinic, however, cardiologists

may have been trained to consider CABG as a more effective or complete treatment, leading to the neglect of long-term secondary prevention; and (c) patients might feel that a CABG is the definitive treatment for their CAD and that medications are no longer necessary, making them less likely to visit doctors in outpatient clinics and take useful suggestions from them.[9] The FBG and HbA1c goal achievement rates were low and were not significantly different between the PCI and the CABG group. Only almost less than one third of all diabetic patients achieved their FBG and HbA1c goals. Hypoglycemic drugs do not belong to the optimal medical therapy (OMT) drugs, sometimes the cardiologists just focused on the OMT treatment and ignored the FBG control; another reason might be that diabetic patients were recommended to go to endocrinology outpatient clinics by cardiologists and cardiothoracic surgeons, but these patients were always less likely to visit another outpatient clinic since they thought they already had one.

In spite of the disparities between PCI and CABG patients in cardiovascular risk factor control, the achievement rates of LDL-C, FBG, HbA1c and BP goals remain low in the PCI group. Possible reason might be that interventional cardiologists are usually more conditioned to consider dual antiplatelet therapy (DAPT) issues and sometimes ignore the use of other secondary prevention drugs.

In our study, composite endpoints were significantly higher in the un-matched PCI group than in the CABG group. This was consistent with previous studies which suggested that patients who underwent CABG had better clinical outcomes than those who underwent PCI.[10,11] In the propensity-matched patients, although the recurrent ACS rate was significantly higher in the PCI group, composite endpoints were not significantly different between the two groups. In our multivariate Cox regression analysis, sex, smoking, and achieved LDL-c, HbA1c, and BP goals were independent predictors for composite endpoints in PCI patients, while EF>40%, achieved LDL-c, and BP goals were independent predictors for composite endpoints in CABG patients. The LDL-c and BP goal achievement rates were significantly higher in the PCI group, the HbA1c target attainment rate, although not significantly different, was better in the propensity matched PCI group. The results suggested that secondary prevention was important in reducing post-revascularization events. In the propensity matched subgroup analysis, patients with diabetes, obesity, and ≥ 60 years old had better clinical outcome in the CABG group, in accordance with former studies.[12-14]

The LDL-C < 2.07 mmol/L goal attainment rate of ACS patients in the DYSIS-China study was 29.7%. In our study, it was significantly improved, but remain very low in PCI and CABG patients. Baseline LDL-C levels were reported to be lower in Chinese ACS patients than in western countries' ACS patients in previous studies.[15,16] LDL-C was recommended to be lower than 2.07 mmol/L in Chinese lipid management guideline. Whether the target LDL-C should be in accordance with that of western countries lipid management guidelines (LDL-C < 1.8 mmol/L) remains controversial. Lee et al. have observed that, compared with LDL-C < 2.6 mmol/L, an LDL-C < 1.8 mmol/L did not improve survival in ACS patients.[17] However, in our study, achieving the LDL-C < 1.8 mmol/L goal was an independent predictor of decreased composite endpoint risk,[18] which suggests that the LDL-C goal of Chinese lipid management guideline in the future should be consistent with that of western countries.

In the PCI group, BP goal achievement rate was higher in patients < 60 years old than those ≥ 60 years old, the FBG and HbA1c goal achievement rates were higher in patients < 60 years old in the CABG group. The results were consistent with those of previous studies that older patients always underuse the recommended secondary preventive drugs and always had bad adherence to those drugs,[19] which further lead to worse risk factor target attainment. However, the LDL-C goal achievement was much better in patients ≥ 60 years old. The result differed from most of the former studies, but was consistent with that of Rajendran et al.,[20] who found that older patients more often achieved lipid target than younger patients. The results may suggested that the clinicians in our hospital are realizing the importance of statin treatment with each passing day. Hogh et al.,[21] have discovered that age-related differences in using secondary prevention drugs have been reduced or even eliminated, which suggested that the disparities in risk factor target attainment will also be eliminated over time. Why the risk factor target attainment was inconsistent between PCI and CABG in different age groups remains unclear, but the results suggested that we should pay more attention to older patients in secondary prevention.

Females were considered to be less likely to achieved their cardiovascular risk factor targets since they were less likely to take secondary preventive drugs due to many reasons. For example, the lowering estrogen levels, higher adverse events and poor adherence might have influence on drug use.[22] However, in the study by Jankowski et al.,[23] they have found that the frequency of achieving recommended goals in secondary prevention were not sex-related. In our study, the LDL-C, FBG, and HbA1c goal achievement rates were significantly higher in males than in females in the PCI group and in patients ≥ 60 years old. The result suggested that we should pay attention to older women during the secondary prevention process and make sure they are given the optimal treatment.

Limitations of the study

Our study had several limitations. Firstly, it was a single-center observational study performed at a major cardiovascular hospital in China, and the clinical strategies of physicians and surgeons may differ from those of other hospitals in China. Secondly, although propensity score matching was performed to adjust for potential confounding factors in PCI and CABG patients, initial selection bias and unmeasured variables exist.

Conclusion

Our research showed that there are disparities between PCI and CABG patients in CAD-related risk factor target attainment. Secondary prevention is critical in reducing post-revascularization endpoints. The risk factor target attainment also differed between patients ≥ 60 years old and < 60 years old, females and males, which suggested that cardiologists and cardiothoracic surgeons

should pay more attention to those special patients and make correct clinical decisions in the secondary prevention process, which can further ensure those patients have a better prognosis and greater clinical benefits.

Author contributions

Conception and design of the research and Acquisition of data: Xia-qing Gao, Yan-fang Li, Zhi-li Jiang; Analysis and interpretation of the data and Writing of the manuscript: Xia-qing Gao; Statistical analysis: Xia-qing Gao, Zhi-li Jiang; Obtaining financing and Critical revision of the manuscript for intellectual content: Yan-fang Li.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

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Phytosterols in the Treatment of Hypercholesterolemia and Prevention of Cardiovascular Diseases

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Abstract

Phytosterols are bioactive compounds found in foods of plant origin, which can be divided into plant sterols and plant stanols. Clinical studies consistently indicate that the intake of phytosterols (2 g/day) is associated with a significant reduction (8-10%) in levels of low-density lipoprotein cholesterol (LDL-cholesterol). Thus, several guidelines recommend the intake of 2 g/day of plant sterols and/or stanols in order to reduce LDL-cholesterol levels. As the typical western diet contains only about 300 mg/day of phytosterols, foods enriched with phytosterols are usually used to achieve the recommended intake. Although phytosterols decrease LDL-cholesterol levels, there is no evidence that they reduce the risk of cardiovascular diseases; on the contrary, some studies suggest an increased risk of atherosclerosis with increasing serum levels of phytosterols. This review aims to address the evidence available in the literature on the relationship between phytosterols and risk of cardiovascular disease.

Introduction

Cardiovascular diseases (CVD) remain the main cause of mortality worldwide, accounting for about 30% of all deaths.[1] During the last two decades, deaths due to CVD decreased in developed countries but increased sharply in low- and middle-income countries.[1,2]

Atherosclerosis is the main pathological process that leads to the development of CVD, including acute myocardial infarction (AMI), heart failure, and stroke.[3] Early identification of risk factors for CVD is fundamental for the prevention of the onset and/or progression of atherosclerosis. The main risk factors for atherosclerosis include smoking, hypertension, diabetes mellitus, advanced age, family history of CVD, and dyslipidemia.[4,5]

The fundamental role of dyslipidemia – especially hypercholesterolemia – in the development of CVD has already been confirmed.[4] Through a wide range of plasma cholesterol concentrations, there is a strong positive linear correlation between risk of CVD and levels of total cholesterol and low-density lipoprotein cholesterol (LDL-cholesterol) levels. Additionally, it has been demonstrated that a reduction in LDL-cholesterol levels reduces the risk of CVD.[4,6,7]

There is evidence that elevated serum LDL-cholesterol levels may cause atherosclerotic CVD independently of other risk factors.[8] Dyslipidemia can be considered a primary risk factor for atherosclerotic CVD and may be a prerequisite for atherosclerosis, occurring before the participation of other risk factors.[5] The increase in serum LDL-cholesterol concentrations appears to be necessary for atherogenesis. LDL comprises more than 75% of the atherogenic lipoproteins, with the remaining including cholesterol-rich remnants of lipoproteins rich in triglycerides (chylomicrons and very–low-density lipoproteins; VLDL). When LDL infiltrates into the arterial wall, it initiates and promotes atherosclerosis.[8]

According to the World Health Organization (WHO), 39% of adults (> 25 years) worldwide have increased total cholesterol concentrations (> 190 mg/dL). The prevalence is greater in Europe (54%), followed by the Americas (48%).[9]

The treatment of hypercholesterolemia must include nonpharmacological measures, which are recommended for all patients, as well as the use of pharmacological therapy that may be indicated in specific situations.[10] The drugs currently available for the treatment of hypercholesterolemia include statins (hydroxy-methylglutaryl-coenzyme A [HMG-CoA] reductase inhibitors), ezetimibe (a selective inhibitor of cholesterol absorption), and resins or bile acid sequesters. Statins should be used as the first choice due to their powerful effect on LDL-cholesterol reduction (25-55%) and because they are the most study-validated drugs for reduction of cardiovascular events. Ezetimibe has a moderate effect on LDL-cholesterol reduction (15-25%). Resins may be associated with statins when the target LDL-cholesterol is not achieved despite the use of statins, leading to a reduction of 30% in LDL-cholesterol levels.[4,8,10]

Nonpharmacological treatment of dyslipidemia must include changes in dietary habits and physical activity, in addition to weight loss, when indicated.[10] In the nutritional approach, intake of saturated fatty acids and trans fatty acids must be limited as well as the intake of cholesterol, in addition to increasing the intake of soluble fiber. The consumption of phytosterols is also indicated for the treatment of hypercholesterolemia, according to several guidelines and consensuses of different societies worldwide.[4,5,8,10-12] There is consistent evidence that the intake of phytosterols (2 g/day) is associated with a significant reduction in LDL-cholesterol (8 - 10%).[11] However, there are no data indicating that the consumption of phytosterols may reduce the risk of CVD. On the contrary, some studies suggest that the concomitant elevation in plasma concentration of phytosterols can increase the risk of development of atherosclerosis.[13,14]

Keywords

Cardiovascular Diseases; Phytosterols; Atherosclerosis; Cholesterol, LDL.

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This review aims at addressing the evidence available in the literature on the relationship between phytosterols and risk of CVD.

Phytosterols

Definition, classification, and food sources

The term "phytosterols" is used to describe plant sterols and their saturated derivatives, plant stanols.[15,16] Phytosterols are bioactive compounds found naturally in foods of plant origin and present a chemical structure similar to that of cholesterol,[17] which is found only in foods of animal origin. More than 250 phytosterols have already been identified.[15] The plant sterols more commonly found in the diet are beta-sitosterol, campesterol, and stigmasterol. In regards to plant stanols, beta-sitostanol, and campestanol are the two most common types.[18]

Food sources of phytosterols include vegetable oils, mainly corn (909 mg/100 mL), sunflower (411 mg/100 mL), soybean (320 mg/100 mL), and olive (300 mg/100 mL); oleaginous fruits such as almonds (183 mg/100 g); cereals like wheat germ (344 mg/100 g), and wheat bran (200 mg/100 g); in addition to fruits and vegetables, such as passion fruit (44 mg/100 g), orange (24 mg/100 g), and cauliflower (40 mg/100 g).[18] A typical western diet contains approximately 300 mg of sterols and 30 mg of plant stanols,[17] while vegetarian diets can achieve a higher content (300 - 500 mg/day).[18] This amount of phytosterols present in a regular diet is considered small to achieve the recommended daily intake of phytosterols able to present therapeutic effects on LDL-cholesterol reduction (~2 g/day), and it is generally required the consumption of foods enriched with phytosterols or, alternatively, the use of supplements of phytosterols.[16,18] In Brazil, some processed foods enriched with phytosterols are available, including margarine, yogurt, and milk.

Mechanism of action on cholesterol

The main mechanism by which phytosterols lower LDL-cholesterol levels is through a reduction (30 - 50%) in the intestinal absorption of cholesterol.[15,16] This reduction may be mediated by some mechanisms, in particular, the competition with cholesterol by the solubilization in mixed micelles in the intestinal lumen, reducing the amount of cholesterol available for absorption.[15,19] Other proposed mechanisms include: (1) modification in the expression of genes that encode the proteins that carry sterols, such as the Niemann-Pick C1-like 1 (NPC1-L1) protein, reducing the transport of cholesterol to the enterocyte, or ATP-binding cassette transporters (ABCG5 and ABCG8), promoting the efflux of cholesterol from the enterocytes to the intestinal lumen; (2) reduced rate of cholesterol esterification in the enterocyte; and (3) increased removal of cholesterol from the body through the transintestinal cholesterol excretion (TICE).[17] In response to a decrease in the absorption of dietary cholesterol, hepatic synthesis of cholesterol seems to increase, but the increase in hepatic release of cholesterol is not sufficient to compensate for the lower absorption of dietary cholesterol.[16]

Intestinal absorption of plant sterols (< 2%) and stanols (< 0.2%) is much lower than that of cholesterol (~50%).[17] As a result of the low absorption and efficient biliary excretion after hepatic uptake, circulating levels of phytosterols are very low, ranging from 0.3 to 1.0 mg/dL for plant sterols and 0.002 to 0.012 mg/dL for plant stanols. The distribution of phytosterols through the main classes of lipoproteins is similar to that of cholesterol; therefore, they circulate mainly in LDL particles (65 - 70%).[11]

Cholesterol-lowering effect of phytosterols

Since the late 1950s, numerous studies have consistently indicated that foods enriched with phytosterols reduce the concentrations of LDL-cholesterol.[20-24] Table 1 shows some randomized, placebo-controlled clinical trials published since 2010 that evaluated the effects of foods enriched with phytosterols on the cholesterolemia. The Table demonstrates that the dose is around 2 - 4 g/day and the reduction in LDL-cholesterol is ~10%. For example, the study by Párraga-Martínez et al.[25] evaluated the intake of 2 g/day of plant stanols during 12 months and observed an 11% reduction in LDL-cholesterol levels. The study of Vásquez-Trespalacios & Romero-Palacio (2014)[26] evaluated the intake of 4 g/day of plant stanols and observed after 4 weeks a 10.3% decrease in LDL-cholesterol levels. Among the studies listed in Table 1, the study that used the highest dose of phytosterols (8.8 g/day of plant stanols) was the one conducted by Gylling et al. (2010),[27] which showed a 17.1% reduction in LDL-cholesterol levels.

Recent meta-analyses confirmed the cholesterol-lowering effect of phytosterols, in addition to comparing the effects of sterols with those of stanols, and evaluating the dose-response relationship.[21-24] The dose-response relationship presented a slight variation among the meta-analyses, and there is still no consensus. A meta-analysis conducted by Ras et al.[24] included 124 studies with a mean phytosterol dose of 2.1 g/day (range 0.2 to 9.0 g/day). The intake of 0.6 - 3.3 g/day was associated with a gradual decrease in the concentration of LDL-cholesterol of 6 - 12%. Plant sterols and stanols had comparable effects. The studies with doses exceeding 4 g/day were not grouped together, because they were few and had large dose variations. The authors concluded that the LDL-cholesterol reducing effect of both plant sterols and stanols increased until an intake of approximately 3 g/day, with a mean effect of 12%.[24] A meta-analysis conducted by Talati et al.[22] compared the effects of plant sterols and stanols on LDL-cholesterol and also observed no significant differences.

The data indicating the cholesterol-lowering effect of foods enriched with phytosterols derive from intervention studies with good methodological quality, conducted with a relatively large number of participants, and the results are generally consistent.[28] According to several international scientific societies, the regular use of 2 g/day of phytosterols under supervision can be recommended for a 10% LDL-cholesterol reduction.[4,5,8,10-12]

Leaving the scenario of fortified foods, some recent studies have evaluated the effects of phytosterols in tablets or capsules,[29-32] with the objective of evaluating whether this form of supplementation (considered more practical by many authors) would also be effective in lowering cholesterol.

Table 1 – Randomized clinical trials evaluating the effects of supplementation of phytosterols on cholesterolemia

Authors (year)	n	Type of phytosterol (food/supplement)	Dose of phytosterol (g/day)	Duration	↓ LDL-cholesterol (%)	P value (*versus* control group)
Studies with foods enriched with phytosterols						
Gylling et al. (2010)[27]	49 individuals with mild-to-moderate hypercholesterolemia	Stanols (spread and drink)	8.8	10 weeks	17.1	0.01
Gylling et al. (2013)[39]	92 asymptomatic individuals (not using lipid-lowering drugs)	Stanols (spread)	3	6 months	10.2	0.001
Buyuktuncer et al. (2013)[40]	70 individuals with mild-to-moderate hypercholesterolemia	Stanols (yogurt)	1.9	4 weeks	6.3	0.005
Vásquez-Trespalacios & Romero-Palacio (2014)[26]	40 individuals with moderate hypercholesterolemia	Stanols (yogurt)	4	4 weeks	10.3	< 0.01
Ras et al. (2015)[41]	240 individuals with hypercholesterolemia	Sterols (*spread*)	3	12 weeks	6.7	< 0.05
Párraga-Martinez et al. (2015)[25]	182 adults with hypercholesterolemia	Stanols (yogurt)	2	12 months	11	0.01
Studies with capsules or tablets						
Maki et al. (2012)[32]	32 subjects with primary hypercholesterolemia	Sterols/Stanols (pill)	1.8	6 weeks	4.9	< 0.05
Maki et al. (2013)[29]	28 subjects with primary hypercholesterolemia	Sterols/Stanols (softgel capsule)	1.8	6 weeks	9.2	< 0.001
Ottestad et al. (2013)[30]	41 individuals with total cholesterol 180 - 300 mg/dL	Sterols/Stanols (softgel capsule)	2	4 weeks	2.7	0.32
McKenney et al. (2014)[31]	30 adults with familial hypercholesterolemia	Sterols/Stanols (softgel capsule)	1.8	6 weeks	4.3	< 0.01

LDL-cholesterol: low-density lipoprotein cholesterol.

The study by Maki et al.[29] used softgel capsules, providing 1.8 g/day of esterified sterols/stanols in conjunction with lifestyle changes recommended by the National Cholesterol Education Program (NCEP) for 6 weeks and observed a 9.2% reduction in LDL-cholesterol levels. A significant reduction in LDL-cholesterol levels was also observed in studies conducted by Maki et al.[32] and McKenney et al..[31] However, the study conducted by Ottestad et al.[30] did not observe a significant reduction in LDL-cholesterol levels. A recent meta-analysis[33] including eight studies published from 1992 to 2013 with a duration of 4 - 6 weeks and doses of phytosterols between 1 to 3 g/day in tablets or capsules observed a significant reduction in LDL-cholesterol (on average 12 mg/dL), which was similar to that observed with food enriched with phytosterols. Therefore, despite the lack of consensus, most studies indicate that the use of phytosterols in tablets or capsules can be effective in reducing LDL-cholesterol levels.

There is evidence that the consumption of phytosterols in association with lipid-lowering therapy is able to promote a further reduction in serum cholesterol levels. These benefits have been observed in association with statins[34-36] and also with ezetimibe.[37,38] The meta-analysis developed by Han et al.[36] included 15 randomized clinical trials that evaluated the effect of diets enriched with phytosterols in patients using statins. The phytosterols in combination with statins, compared with statins alone, produced a significant reduction of 12 mg/dL in LDL-cholesterol levels.

Phytosterols and cardiovascular disease

The direct relationship between intake of foods enriched with phytosterols and CVD risk has not been investigated in randomized clinical trials. It has been estimated that it would be necessary to follow up at least 33,000 individuals for about 10 years for a proper assessment of the effects of phytosterols on hard endpoints, hindering the viability of such studies.[42]

By inference based on the cardioprotective efficacy of other cholesterol-lowering interventions, some authors consider that phytosterols may reduce cardiovascular risk; however, this statement should not be performed until studies proving this fact are available.[43]

– Serum levels of phytosterols

The speculation in regards to a potential deleterious effect of phytosterols has been largely motivated by the fact that phytosterolemia (also known as sitosterolemia), a rare autosomal recessive disease, is characterized by a 50-fold increased circulating concentration of plant sterols and may be associated

with early atherosclerosis. However, the consumption of foods enriched with phytosterols is associated with a much lower increase (around twice) in circulating plant sterols.[44]

Several studies have evaluated the association between plasma phytosterol concentration and CVD; however, the results are conflicting. Some studies have found a positive association between serum levels of phytosterols (or the relationship phytosterols/cholesterol) and risk of CVD;[13,14] while others did not observe any association or even found an inverse association.[45-47] Genser et al.[48] published a systematic review and meta-analysis based on 17 studies involving 11,182 individuals and found no evidence of an association between serum concentration of phytosterols and development of CVD. The authors of this meta-analysis attributed the great divergence in the results of the studies to the different designs of studies and adjustments for potential confounding variables. They suggest that biases can occur if the investigators fail to make appropriate adjustments, mainly for serum levels of lipoproteins, in particular for LDL-cholesterol.[48] Another possibility is the fact that circulating phytosterols alone do not increase the risk of CVD but are rather only markers of cholesterol absorption.[49]

– Intermediate markers of cardiovascular risk

Due to the absence of studies evaluating cardiovascular outcomes, the investigation of intermediate risk markers for CVD represents an acceptable and viable form to evaluate the relationship between phytosterols and cardiovascular risk. Currently, there are available studies assessing endothelial dysfunction, arterial stiffness, diameter of the retinal vessels, and inflammation of low degree.[39,41,50-54]

In a study conducted by Gylling et al.,[39] the consumption of plant stanols (3 g/day) for 6 months showed beneficial effects on arterial stiffness, especially in men. In addition, endothelial function, assessed by peripheral arterial tonometry, improved with a reduction in LDL-cholesterol and non–high-density lipoprotein (non-HDL) cholesterol. A study conducted by Heggen et al.[52] tested the effects of two margarines enriched with plant sterols (2 g/day) from two different vegetable oils during 4 weeks. The authors observed a significant reduction in E-selectin and plasminogen activator inhibitor 1 (PAI-1) with the ingestion of only one of the margarines. In this study, there was no significant reduction in vascular cellular adhesion molecule-1 (VCAM-1) and tumor necrosis factor alpha (TNF-α) with any of the margarines, and also no association was observed between LDL-cholesterol reduction and changes in E-selectin and total PAI-1.[52]

On the other hand, supplementation with 3 g/day of plant sterols for 12 weeks in 240 subjects with hypercholesterolemia did not result in beneficial effects on arterial stiffness and endothelial function.[41,54] In this study, the endothelial function was assessed by flow-mediated dilation[41] and circulating biomarkers: intercellular adhesion molecule-1 (ICAM-1), VCAM-1, and E-selectin.[54]

In two crossover clinical trials including children with familial hypercholesterolemia, the consumption of phytosterols during 4 weeks failed to improve endothelial function assessed by flow-mediated dilation, although it induced a significant reduction in LDL-cholesterol levels.[50,51]

A randomized clinical trial conducted by Kelly et al.[53] evaluated the effects of consumption over the long term (85 weeks) of plant sterols and stanols on the diameter of retinal vessels (microcirculation). The study included three groups of patients who consumed margarine enriched with plant sterols (2.5 g/day), margarine enriched with plant stanols (2.5 g/day), and margarine without phytosterols. There were no significant changes in venular diameter in the three groups, but the changes in serum concentrations of campesterol (a type of plant sterol) were positively associated with the changes in venular diameter, regardless of LDL-cholesterol level ($r = 0.39$, $p = 0.03$).

In regards to inflammation, there was no significant reduction in any of the markers evaluated in the study conducted by Ras et al.[54]: C-reactive protein (CRP), serum amyloid A, interleukin (IL)-6, IL-8, TNF-α, and ICAM-1. In a systematic review and meta-analysis recently published, Rocha et al.[55] evaluated the effect of consumption of phytosterols on inflammatory markers, particular on CRP. The study included 20 randomized clinical trials ($n = 1,308$) involving foods enriched with phytosterols as active treatment. The reduction in CRP concentration with the consumption of phytosterols was 0.10 mg/dL, which did not reach statistical significance.[55]

The results of these studies evaluating the effects of phytosterols on intermediaries markers of cardiovascular risk observed no consistent beneficial effects. Thus, there is no current evidence that the use of phytosterols may reduce the risk of CVD by acting on these markers.

– Intake of oxidized phytosterols

Recent publications have alerted to another potential deleterious effect of phytosterols: the intake of oxidized phytosterols. Plant sterols (but not stanols, because they are saturated) may oxidate, forming oxidized phytosterols and, similarly to what is observed with cholesterol oxidation, these substances are believed to be atherogenic.[56] However, in studies with humans, there is still no consensus on whether the intake of foods enriched with sterols is able to increase the serum concentration of oxidized phytosterols. For example, in the clinical randomized, crossover trial conducted by Baumgartner et al. (2013),[57] 43 healthy individuals consumed during 4 weeks margarine enriched with sterols (3 g/day), margarine enriched with stanols (3 g/day), and a control margarine. The consumption of margarine enriched with sterols did not increase the serum concentration of oxidized phytosterols.[57] Another study by the same group[58] investigated the effects of intake of sterols on the concentration of oxidized phytosterols during the postprandial period. In this study, the individuals consumed a drink containing none or 3 g of plant sterols or stanols. Blood samples were collected for up to 8 h, and 4 hours later, the individuals received a second drink (without sterols or stanols). The concentration of oxidized phytosterols increased significantly after consumption of the meal with sterols in comparison with the meal with stanols and the control meal. This increase was only observed after the consumption of the second drink and the authors concluded that it is still unclear whether the increase in oxidized phytosterols in the postprandial period is due to absorption or

endogenous formation. Therefore, until the present moment, there is no consensus on the role of oxidized phytosterols in the development of CVD.

Phytosterols and liposoluble vitamins

Considering that phytosterols reduce the intestinal absorption of cholesterol, it is reasonable to imagine that these substances may also reduce the absorption of liposoluble vitamins and antioxidants. The serum levels of vitamins A, D, and K1 are generally not affected by the consumption of phytosterols.[59] However, some studies suggest that phytosterols may promote a modest reduction in plasma concentration of carotenoids (mainly β-carotene, α-carotene, and lycopene)[27,60] and tocopherols,[61] but other studies have not observed this fact.[62,63] A recently published meta-analysis evaluated the effects of phytosterol consumption in plasma concentrations of liposoluble vitamins and carotenoids. It included 41 randomized clinical trials (n = 3,306) with a mean phytosterol intake of 2.5 g/day. In the analyses adjusted for total cholesterol, there was a significant reduction in the concentration of hydrocarbon carotenoids (β-carotene, α-carotene, and lycopene) and some oxygenated carotenoids (zeaxanthin and cryptoxanthin). In contrast, there was no significant reduction in the concentration of tocopherol, vitamin D, or retinol. A very important finding of this meta-analysis was that the concentration of these substances remained within the normal range, giving no indication that the observed reductions could have negative health implications.[64] Noakes et al.[65] have demonstrated that it is possible to avoid reductions in plasma carotenoid concentrations during the consumption of phytosterols through an increase in daily consumption of carotenoid-rich fruits and vegetables.

Is the consumption of phytosterols safe?

There are still no available studies with long-term follow-up ensuring the safety of regular consumption of products enriched with phytosterols, as highlighted in recent publications of the European Society of Cardiology / European Atherosclerosis Society[12] and the American Heart Association / American College of Cardiology.[66] However, based on the absence of adverse effects in short-term studies and experimental studies, several authors consider that the consumption of phytosterols is safe and may be indicated for lowering cholesterol, including in association with drug therapy.[11,59,63,67,68] Furthermore, different scientific societies recommended the use of phytosterols in the treatment of hypercholesterolemia.[4,5,8,10-12,28] It is important to note that phytosterol supplementation is contraindicated in the rare patients presenting phytosterolemia (or sitosterolemia).[16,67]

The addition of phytosterols to industrialized food as an ingredient to reduce cholesterol has already been approved by several regulatory agencies around the world, including Health Canada, U.S. Food and Drug Administration (FDA), European Food and Safety Authority (EFSA), Food Standards Australia New Zealand (FSANZ),[16] and National Health Surveillance Agency (ANVISA) in Brazil.[69]

According to ANVISA, foods enriched with phytosterols should display the following label information: "Phytosterols help reduce the absorption of cholesterol. Their consumption must be associated with a balanced diet and a healthy lifestyle." ANVISA determines that to display this information, the portion of the product ready for consumption should provide at least 0.8 g of free phytosterols. In addition, the label of these products should include phrases such as: "The product is not suitable for children younger than 5 years, and pregnant or nursing women."[69]

Final considerations

The European Atherosclerosis Society recently published a consensus on phytosterols that concluded that based on the reducing effect of LDL-cholesterol and absence of adverse signs, the consumption of foods enriched with phytosterols may be considered: (1) in individuals with hypercholesterolemia presenting intermediate or low cardiovascular risk without indication of pharmacotherapy, (2) as an adjunct to pharmacological therapy in patients with high and very high cardiovascular risk who fail to achieve the goals of LDL-cholesterol with statins or are intolerant to statins, and (3) in adults and children (> 6 years) with familial hypercholesterolemia, along with lifestyle changes and drug therapy.[11]

Most guidelines and consensus on the treatment of dyslipidemia and/or prevention of CVD recommend the intake of phytosterols in the amount of approximately 2 g/day with the goal of reducing LDL-cholesterol by approximately 10%, in association with lifestyle changes.[4,5,8,10-12,28]

Currently, the knowledge about the relationship between consumption of phytosterols and risk of CVD is incomplete. The available evidence does not confirm that phytosterols may confer cardiovascular protection and also does not show deleterious effects. Further studies are needed, especially with long-term supplementation of phytosterols.

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Limitations in the Diagnosis of Noncompaction Cardiomyopathy by Echocardiography

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Noncompacted myocardium (NCM) was first reported by Grant in 1926 as a heterogeneous myocardial disorder characterized by prominent ventricular trabeculation, intratrabecular recesses, and bilayered myocardium composed by a compacted and a noncompacted layer.[1,2] It can occur isolated or associated with other cardiomyopathies, complex syndromes, metabolic disorders and congenital heart diseases, such as Ebstein's anomaly, left ventricular (LV) or right ventricular outflow tract obstruction, bicuspid aortic valve, cyanotic congenital heart diseases, and coronary artery anomalies. Although NCM usually affects the left ventricle, it can also affect both ventricles or the right ventricle alone.[3]

The etiology of LV noncompaction is uncertain, and several etiological bases have been implicated. It is believed to be due to pathogenic mechanisms resulting in a failure in the final phase of myocardial morphogenesis, or myocardial compaction. Increasing evidence has supported a genetic base by identifying mutation in the genes that encode sarcomeric, cytoskeletal and nuclear membrane proteins.[4-6]

Although considered rare by some authors, NCM incidence and prevalence are uncertain. Ritter et al.[7] have reported a 0.05% prevalence in all echocardiographic exams of a large institution. Patients with heart failure (HF) have been reported to have a 4% prevalence of NCM.[8]

Currently, it is controversial whether NCM is a distinct cardiomyopathy or a morphological characteristic shared by different heart diseases. Thus, while the World Health Organization/International Society and Federation of Cardiology considers NCM an unclassified cardiomyopathy, the American Heart Association considers it a primary genetic cardiomyopathy.[9,10] The most recent classification of cardiomyopathies proposed by the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases considers NCM an unclassified familial cardiomyopathy.[11]

The clinical presentation can occur at any age, being highly variable. Patients can be asymptomatic or have symptoms of severe HF, associated or not with lethal arrhythmias, sudden cardiac death and thromboembolic events.[12]

Many asymptomatic patients are identified incidentally by an echocardiography performed for assessment of cardiac murmur or for familial screening after identifying an index case.[12]

Symptoms of HF occur in more than half of the patients with NCM, LV dysfunction being reported in up to 84% of them. In addition, arrhythmias are common: atrial fibrillation can affect 25% of adult patients, and ventricular tachyarrhythmias, up to 47% of patients. The occurrence of thromboembolic events, such as stroke, transient ischemic attack, pulmonary embolism and mesenteric ischemia, ranges from 0 to 38%, according to studies published.[3,13,14]

Electrocardiographic abnormalities can be present in up to 90% of patients, being, however, unspecific. The most common findings include intraventricular conduction delay, LV hypertrophy, ventricular repolarization changes, and Wolff-Parkinson-White syndrome.[3,13,14]

The increasing advancement in imaging techniques, in addition to the increasing application of genetic tests for the diagnosis of NCM, significantly impacts on the understanding of the mechanisms involved in the NCM genesis and its clinical treatment.

Of the cardiac imaging techniques, echocardiography and cardiac magnetic resonance imaging (CMR) are the major diagnostic tools. Because of its large availability and easy access, in addition to no need for contrast agents, no radiation exposure, and mainly its low cost as compared to CMR, echocardiography is the first choice and most commonly used method for the diagnosis of NCM.[8,12-14]

Usually the diagnosis of NCM should be considered in the presence of a bilayered myocardium composed by one thinner epicardial layer and one thick endocardial layer with prominent trabeculations and deep intraventricular recesses. The trabeculations are mainly identified on two-dimensional (2D) mode, but can be evidenced on one-dimensional or M mode. Color Doppler imaging shows blood flow in those recesses in continuity with the left ventricle.[8,12-15]

Different echocardiographic criteria have been used to diagnose NCM, and the main ones used in clinical practice are as follows (Table 1):

1. Jenni et al.[16] consider for the diagnosis of NCM the presence of two myocardial layers, a thin compacted one (compacted myocardium - CM), and another thicker, noncompacted layer (NCM), with deep

Keywords

Cardiomyopathies; Heart Failure; Diagnostic Imaging / trends; Echocardiography.

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Table 1 – Criteria proposed for the diagnosis of noncompacted myocardium.[6,13]

Criterion	Chin et al.[2]	Jenni et al.[16]	Stöllberger et al.[17]
Number of patients	8	7	104
Phase of the cardiac cycle	End-diastole	End-systole	Trabeculations assessed at end-diastole; NCM and CM assessed at end-systole
View	Short axis parasternal views and/or apical views	Short axis parasternal	Conventional and modified views
NCM/CM ratio	-	>2	-

endomyocardial recesses filled with blood flow on color Doppler, in the absence of other cardiac abnormalities. A NCM/CM ratio > 2 is considered diagnostic. The measures should be acquired at end-systole on short axis parasternal view[16] (Figure 1).

2. Chin et al.[2] define NCM as the presence of excessively prominent ventricular trabeculations and progressively increased total thickness of the myocardial wall from the mitral valve and towards the apical region, characterized by CM/(NCM + CM) ≤ 0.5, assessed at end-diastole on short-axis parasternal views and/or apical views[17] (Figure 2).
3. Stöllberger et al.[17] define NCM as the presence of three or more trabeculations along the LV endocardial borders, different from the papillary muscles, false tendons and aberrant muscle bands, which move synchronized with the CM. In that study, the trabeculations were better visualized at end-diastole, while the bilayered myocardium was better assessed at end-systole[18] (Figure 1).
4. Recently, Paterick et al.[13] (Wisconsin) have proposed the diagnosis of NCM as a ratio NCM/CM > 2, with measures taken at end-diastole on short-axis parasternal view. This criterion requires clinical validation[13] (Figure 1).

Critical analysis

The diagnostic criteria described by Jenni et al.[16] and Chin et al.[2] are based on the measurement of NCM and CM thicknesses. However, the criteria differ regarding the cardiac cycle point at which the measurements should be taken. Chin et al.[2] propose the measurements of NCM and CM thickness be performed at end-diastole, while Jenni et al. propose them at end-systole.[16]

The criteria proposed by Jenni et al.[16] used an NCM/CM ratio > 2 at end-systole, generating higher specificity and lower sensitivity as compared to the criteria by Chin et al.,[2] who use the CM/(NCM + CM) ratio ≤ 0.5. However, the increase in sensitivity is due to a decrease in specificity, as compared to the criteria by Jenni et al.[16]

A recent study has assessed the accuracy of the echocardiographic criteria described by Chin et al.,[2] Jenni et al[16] and Stöllberger et al.[17] for the diagnosis of NCM in patients with HF as compared to a control group of normal individuals. The size and the number of the trabeculations identified on apical view at end-diastole were assessed, as were the measurements of the NCM layer thickness on short-axis parasternal view at end-systole. Only concordant cases assessed by two reviewers were considered positive.[18]

In that study, the percentages of patients meeting the diagnostic criteria for NCM were as follows: Chin et al.[2] criteria, 79%; Jenni et al.[16] criteria, 64%; and Stöllberger et al.[17] criteria, 53%. In that study, the Chin et al. criteria had higher sensitivity, however with a higher percentage of false-positive diagnoses. The correlation between the three echocardiographic criteria applied was weak, with only 30% of patients meeting all three criteria. All individuals of the control group had preserved ventricular dimensions and systolic function. Five control group individuals (4 black and 1 white) met at least one criterion for the diagnosis of NCM. This result emphasizes the limitation of the echocardiographic criteria to diagnose NCM, particularly in black individuals, leading to an excessive diagnosis of NCM.[18] In that study, if the control group was formed by individuals with HF, those results might have been even more discrepant.

The criterion proposed by Paterick et al.[13] showed good correlation with the CMR findings, and, according to those authors, that criterion provided more accurate measurements of NCM and CM layer thickness. However, those criteria have not been validated, requiring additional confirmation and comparison with other populations with cardiac structural disease before they are adopted as a feasible diagnostic option.[13]

Despite the increasingly frequent diagnosis of NCM, the echocardiographic criteria applied for that purpose are based on studies with limited numbers of patients and different methodologies. The point of the cardiac cycle at which the measurements of NCM and CM thickness are taken influences directly the relationship between the two layers assessed. Myocardial thickness is maximal at systole, and minimal at diastole, which directly affects the ratio between NCM and CM. In addition, the echocardiographic view on which those measurements are taken should be considered. Most criteria suggest that the measurements be taken on short-axis parasternal view; however, in daily clinical practice, measurements are more often taken on apical 4- and 2-chamber views. Finally, there is no consensus on the ratio between NCM and CM to be adopted as the diagnostic criterion, because of the lack of uniformity accepted for diagnosis.

In addition, some studies have shown a considerable number of young athletes meeting the NCM diagnostic criteria, emphasizing the lack of specificity of the current diagnostic criteria when applied to highly-trained athletes.[19]

Although infrequent, NCM has been reported in the right ventricle. However, in such cases, the diagnostic criteria are even more restricted as compared to those applied to the

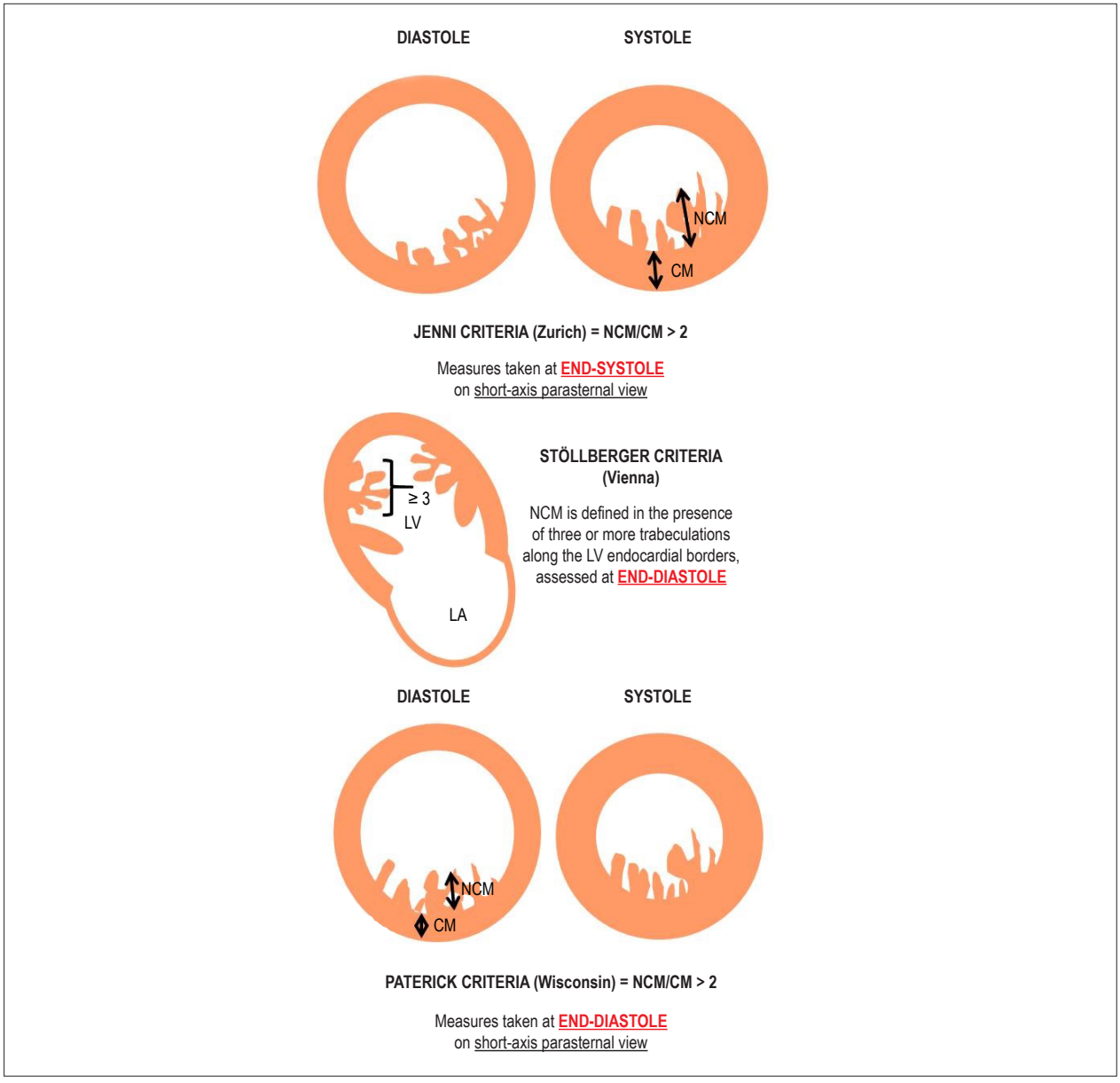

Figure 1 – *Criteria proposed for the diagnosis of noncompacted myocardium. NCM: noncompacted myocardium; CM: compacted myocardium; LV: left ventricle; LA: left atrium.*

left ventricle on echocardiography, because of the limitation of the right ventricle echocardiographic analysis due to its complex geometry, which cannot be contemplated on only one echocardiographic view.[20,21]

Thus, despite the increasing knowledge on NCM by echocardiography professionals, the diagnostic bases of the criteria applied are frail. Therefore, studies with a larger number of patients diagnosed with NCM are required, in addition to uniformization of the views used for the measurements, and the identification of the most suitable point in the cardiac cycle for that purpose. Such studies should ideally compare healthy individuals and patients with HF, because some "normal" patients can meet the echocardiographic criteria for the diagnosis of NCM, with no apparent clinical finding and benign prognosis, considering that the prevalence of NCM seems higher in patients with HF. In addition, the definition of the diagnostic criteria should take into consideration the particularities of specific populations, such as highly-trained athletes and black individuals, as well as the right ventricular morphological characteristics.

Conclusions

Echocardiography is the first choice and most commonly used cardiac imaging method for the diagnosis of NCM. Higher knowledge and understanding of NCM is the first

Figure 2 – *Criteria proposed by Chin for the diagnosis of noncompacted myocardium. NCM: noncompacted myocardium; CM: compacted myocardium; LV: left ventricle; LA: left atrium.*

step to increase diagnostic accuracy in echocardiography laboratories. However, the echocardiographic criteria used so far for that purpose are highly varied and have been based on studies with a reduced number of patients.

Advanced techniques, such as three-dimensional echocardiography, use of contrast agents to better define the endocardial borders, mainly in the apical region of patients with limited acoustic window, as well the analysis of myocardial strain by use of speckle tracking, are promising methods, with potential to increase the diagnostic accuracy of echocardiography in patients with NCM. The use of such techniques in clinical practice has increased in past years; however, the improvement of imaging methods requires study and constant redefinition of the echocardiographic criteria for the diagnosis of NCM.[22-25]

The high prevalence of NCM in low-risk populations, such as athletes and normal black individuals, suggests that the increase in LV trabeculations and recesses can represent a pattern of response to the chronic increment of preload. Thus, because of the current limitations for the diagnosis of NCM, integration of clinical and electrocardiographic assessments, as well as a multimodality approach with echocardiography and CMR, is suggested.[26-28]

In addition to the multimodality imaging approach, future perspectives include changes that suit different ethnicities and functional assessment based on multicenter and international collaboration, incorporating genetic data for a more accurate diagnosis of NCM.[26-28]

Author contributions

Conception and design of the research: Hotta VT;Writing of the manuscript: Hotta VT, Tendolo SC; Critical revision of the manuscript for intellectual content: Hotta VT, Rodrigues ACT,

Fernandes F, Mady C; Perform clinical follow-up of patients with NCM: Nastari L.

Potential Conflict of Interest

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Study Association

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Case 6/2017 - Extensive Giant Left Coronary Artery Aneurysm Due to Kawasaki Vasculitis in Asymptomatic 48-Year-Old Man

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Clinical data: Three months ago, there were retrosternal pain, fatigue and tachycardia (170 bpm) for atrial flutter reversed with amiodarone. Thoracic tomography revealed a giant aneurysm of the left coronary artery. Corrected atrial septal defect at 3 years of age. He has active life with moderate sport. Corrected cryptorchidism at 12 years of age with impairment of fertility.

Physical examination: good general condition, eupneic, acyanotic, normal pulses on the 4 limbs. Weight: 88 Kgs, H: 172 cm, *upper extremity blood pressure*: 130/80 mmHg, HR: 60 bpm. Aorta not palpated at the suprasternal notch.

Precordium: non-palpable ictus cordis, without systolic impulses. Hypofonetic heart sounds, without heart murmurs. Unpalpable liver and clean lungs.

Additional Examinations

Electrocardiogram: sinus rhythm, without cavitary overloads, complete right bundle branch block and 1st degree atrioventricular block. PR: 0.22, QRS: 0.109 with complexes rSr´ in V1 and RS in V6; AP = + 0°, AQRS = + 220°, AT = + 66°.

Chest X-ray: normal cardiac area (cardiothoracic index = 0.50) and linear vascular image with increased density bordering the ventricular arch (Figure 1A).

Echocardiogram: normal cardiac chambers except for discrete left atrial enlargement, normal biventricular function. Dilatation of the left coronary artery corresponding to the circumflex artery at the atrioventricular junction in the anterolateral wall of the left ventricle, measuring 40 mm. Aorta = 34 mm, LA = 46, RV = 25, LV = 47, septum = posterior wall = 10 mm, LVEF = 68%.

Holter: Sinus rhythm, heart rate = 56 to 100, mean = 72 bpm. Polymorphic ventricular extrasystoles, bigemy, frequent, especially at dawn and morning. 1st-degree atrioventricular block, PR = 0.26, alternating with normal AV conduction. Absence of changes in ventricular repolarization and symptoms.

Keywords

Left Coronary Artery Aneurysm; Kawasaky Disease; Coronary Artery Disease.

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Coronary angiography: right coronary occluded at the origin, with intracoronary collateral circulation. Trunk of the left coronary artery with large aneurysmal dilatation and parietal irregularities with whirling flow. Anterior descending artery exhibits occlusion at the origin and distal opacification by ipsilateral collaterals. Circumflex artery exhibits large ectasia and parietal irregularities. It emits ipsilateral collateral circulation to anterior descending and right coronary. Left ventriculography exhibits preserved diastolic volume and discrete anteromedial hypokinesia, with competent mitral valve (Figure 1B, C, D).

Computed tomography of the thorax shows a large elongated sacculation of vascular origin in the subaortic region, next to the topography of the circumflex artery, 10.0 cm on the largest axis (Figure 1E).

Clinical Diagnosis: Extensive giant aneurysm of the left coronary artery from the trunk to the middle third of the circumflex with total obstruction of the anterior descending and right coronary arteries.

Clinical Reasoning: In asymptomatic patient with previous correction of simple cardiac defect (ASD at 3 years of age), recent clinical features of atrial flutter and complete right bundle branch block did not imply the existence of coronary pathology with exaggerated dilation of the coronary arteries without ischemia and/or ventricular dysfunction. This diagnosis was established by imaging tests, particularly by chest angiotomography and coronary angiography. The chest radiograph, if better analyzed, could have opened the diagnostic suspicion and the echocardiogram emphasized the diagnosis.

Differential diagnosis: the presumptive cause of the unusual aneurysm of the coronary arteries, due to its extension and magnitude, was oriented to a previous arteritis process. Given the concomitant presence of clear obstructions, especially of the right coronary artery, the immediate assumption was that of Kawasaki syndrome, which occurs in the infantile age and progresses to significant alterations of risk still in the child as myocardial infarction, rupture of aneurisms and sudden death. The evolution in adulthood is rare, but possible because the aneurysm, even sharp, can evolve silently without causing harm, as noted. Other causes of arteritis refer to Takayasu syndrome, connective tissue diseases (polyarteritis nodosa, lupus and scleroderma), atherosclerosis, and infections such as syphilis.

Conduct: There was a preventive surgical indication due to the magnitude of the coronary alterations. The aneurysm of the left coronary trunk was 45 mm in diameter with organized thrombus in the interior. It was done

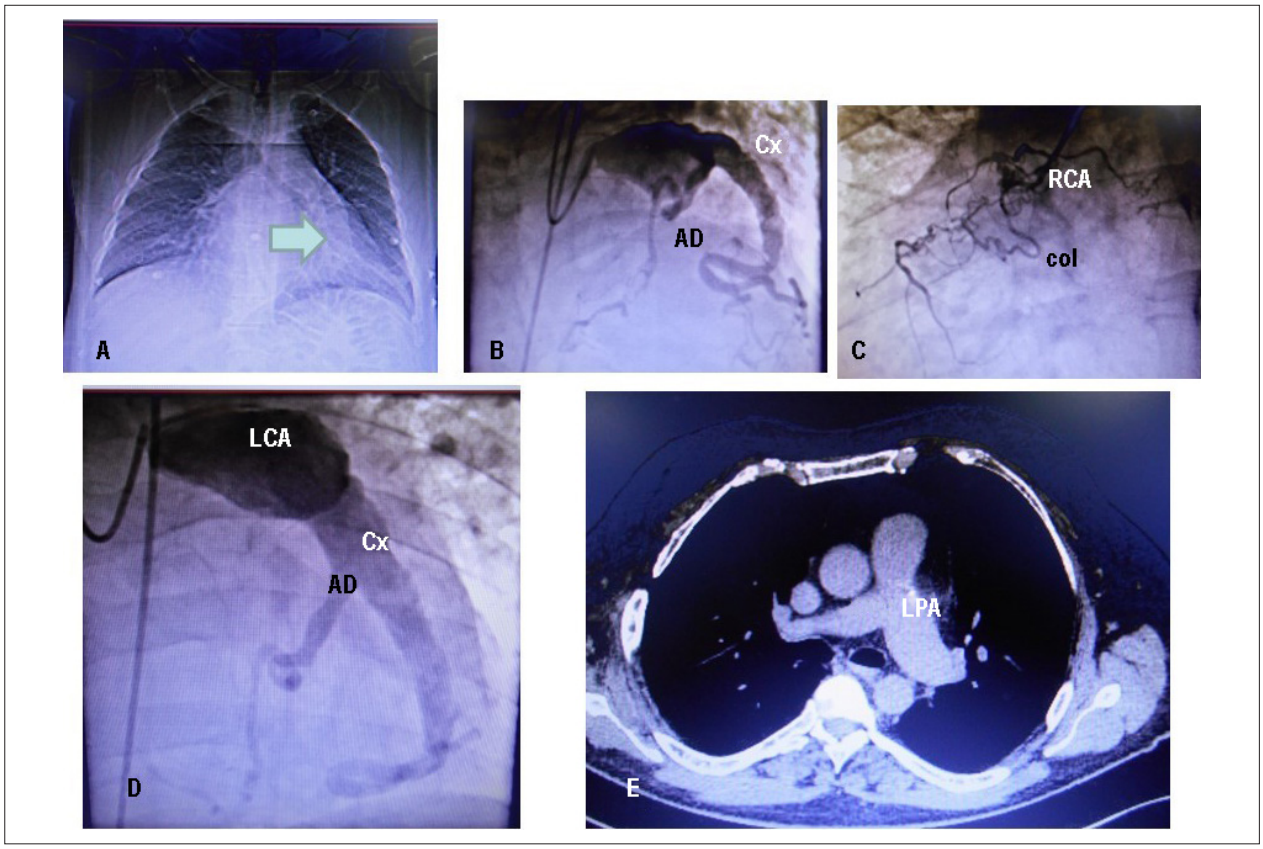

Figure 1 – *Chest X-ray in PA highlights normal cardiac area and pulmonary vascular tissue. Left ventricular border shows a dense rectilinear image that corresponds to the left coronary artery aneurysm (arrow). Coronary angiography points out the aneurysm of the trunk of the left coronary artery and the circumflex artery (B and D), the obstruction of the anterior descending artery (B and D) and the right coronary artery (C). It is observed filling of this artery, with total obstruction, from the left coronary and the distal part of the AD. Chest tomography shows dilatation of the left pulmonary artery, and normal caliber of ascending and descending aorta (E).*

thrombectomy and interposition of 10 mm dacron tube in its proximal and distal stumps. Bypass from the aorta to the anterior descending artery, extracted from the left thigh (rare site of whole vein, since the others presented inflammatory tissue without perviability, including the left mammary artery). Prolonged surgery (5:40 pm ECC and 151-minute ischemia) with the exclusion of the left marginal that emerged from the large aneurysm, caused cardiogenic shock with ECMO continuity for 11 days, 22-day intra-aortic balloon and impaired ventricular function, but with progressive improvement of 34 to 58%, without enlargement of cardiac cavities, but inferior and lateral akinesia. Histological study of the coronary artery fragment revealed thick arterial wall with fibrosis, calcification, epithelioid granulomas with giant multinucleated cells, characteristics of Kawasaki vasculitis.

Comments: Anatomical normality after correction of coronary aneurysms brought relief despite a myocardial ischemic process due to the interruption of emergent vessels. In the literature, 28 cases of coronary aneurysms in adults were reported in a period of 49 years,[1] and most of them (68%) were operated by aneurysm ligation and coronary artery bypass grafts, with good progression in the majority (95%). There have been reports of percutaneous intervention in localized aneurysms[2] of less than 10 mm, as well as cases in clinical treatment with anticoagulants, but with an unfavorable outcome (62.5%).

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Combined Mitral and Aortic Valvar Bioprosthesis Transcatheter Transapical Implant: First Description in Brazil

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Introduction

From the earliest days of the heart surgery, the valves, when the reparation was not possible, were replaced by prosthetics using cardiopulmonary bypass. The good results of these procedures are well known. In the recent years, minimally invasive alternatives have been developed, aiming to make possible the treatment of individuals under high risk of complications and death caused by the conventional procedure. In 2002, the first transcatheter valvar implantation was made, which revolutionized the treatment of severe aortic stenosis. The equipment, techniques and skills have progressively evolved since then. More recently, from 2009, transcatheter mitral valvar implants for treatment of the prosthesis dysfunction (valve-in-valve) started to be performed. Currently, the transcatheter valvar implantation is one of the fields of greater development in cardiology.

Case Report

A male patient aged 72 was admitted to the emergency room with congestive heart failure of progressive worsening. He presented a history of rheumatic fever, coronary artery disease, atrial fibrillation, chronic renal failure (creatinine clearance of 58 mL/min/1,72 m^2) and amaurosis secondary to the macular degeneration of the retina. He underwent two previous cardiac surgical procedures: mitral valvuloplasty and coronary artery bypass grafting in 1993 and mitral valve replacement by bioprosthesis and new myocardial revascularization in 1998. There was a great technical difficulty in the last procedure, due to multiple adhesions.

Upon physical examination at the entrance, he presented BP = 120/70 mmHg, HR = 60 bpm, irregular. Cardiac auscultation: hyperphonetic first heart sound, crescendo/decrescendo systolic murmur 5+/6+ in an aortic focus and holosystolic regurgitating murmur 3+/6+ in a mitral focus. The rest of the physical examination went without particularities.

An echocardiography was performed, which showed a left atrium of 74 mm, diastolic diameter of the left ventricle of 52 mm and systolic diameter of 32 mm, thickness of the septum and posterior wall of 15 mm, systolic pulmonary artery pressure of 53 mmHg and ejection fraction of 77%. The mitral bioprosthesis was calcified with average LA/LV gradient of 13 mmHg, area of 1.7 cm^2 and important reflux. The aortic valve was calcified, with important stenosis (peak transvalvar gradient of 104 mmHg and average of 62 mmHg, valve area of 0.67 cm^2) and discreet reflux.

The cineangiocoronariography identified saphenous vein graft to the occluded right coronary artery, mammary artery to the anterior pervious descending, and multiple lesions in the native beds of the right coronary and left anterior descending arteries.

The thoracic angiotomography showed valve calcium score of 3580 agatston and important coronary atheromatosis and ascending aorta (Figure 1).

There were no acute compensating factors for heart failure, except for congestion attributed to the aortic and mitral valvar disease. The surgical risk by EuroSCORE II was of 13.23%. Due to the high surgical risk and technical difficulties reported in the last surgery, a discussion was held by the "Heart Team", and they opted for the valvar double percutaneous treatment through transapical transcatheter implant and approach of the coronary lesions retrospectively, prioritizing the resolution of the hemodynamic condition of the patient.

On September 2015, the implantation of the aortic bioprosthesis and, after, of the mitral bioprosthesis were performed, both Inovare-Braile, numbers 28 and 30, respectively (Figure 2). The procedure occurred without complications. On the third post-operatory day, piperacillin and tazobactam were started, for the treatment of nosocomial pneumonia. He presented plateletopenia (up to 30,000/mm^3), worsening of the acute renal insufficiency and moderate pericardial effusion, quickly reversed with the use of corticosteroids and usual clinical measures. Following clinical stabilization, the patient was discharged. The three-dimensional echocardiography after the discharge presented well-placed prosthesis, without significant periprosthetic reflux in the valve in the aortic position, peak LA/LV gradient 24 mmHg and average of 14 mmHg. On the mitral position, a moderate periprosthetic reflux was observed, average LA/LV gradient of 9mmHg, mitral area 1.5 cm^2, SPAP 46 mmHg. There was clinical improvement for functional class II.

Discussion

The dysfunction of the valvar bioprosthesis may be secondary to the degeneration of the leaflets due to wear, calcification or rupture, as well as the formation of *pannus* (host tissue), thrombus or perivalvular leak. The durability is smaller when in the mitral position, in young individuals,

Keywords

Transcatheter Aortic Valve Replacement; Heart Valve Prosthesis Implantation; Heart Valve Prosthesis.

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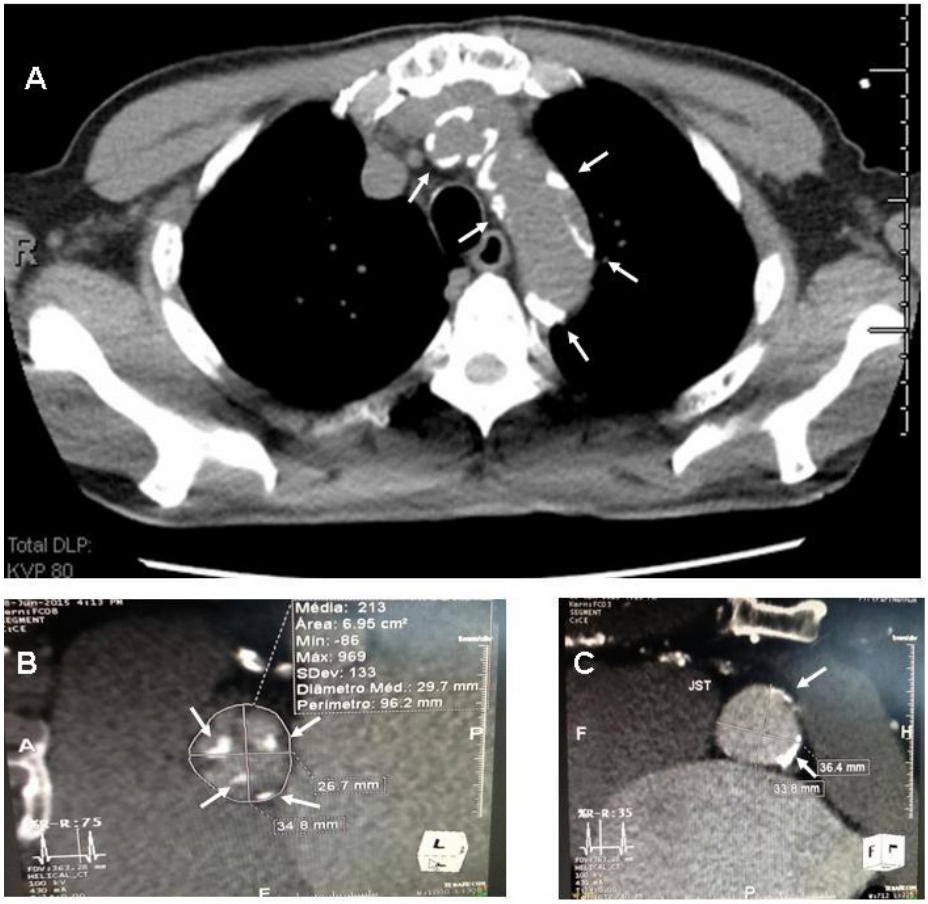

Figure 1 – *Computed thoracic tomography showing intense calcification of the ascending aorta (A), aortic valve (B) and sinotubular junction (C). Measurements of the valve diameter (A) used to define the size of the prosthesis and of the sinotubular junction (B).*

in the presence of prosthetic mismatch, renal failure and hyperparathyroidism.[1,2]

The increase in the use of biological prosthesis, associated to the increase of survival of operated individuals, have made surgical rapprochement increasingly common. The valve replacement surgery is, to this moment, the procedure of choice in cases of graft dysfunction, and it is associated to higher morbidity and mortality in relation to the first intervention. The technical difficulties of the surgical rapprochement may imply in longer period of cardiopulmonary bypass, need for transfusional support, and diaphragmatic paralysis by phrenic nerve injury, prolonged vasoplegia, aorto-coronary graft injury and increased risk of death.[1,2]

The predictors of higher risk of complication are: advanced age, cognitive dysfunction, peripheral vascular disease, chronic lung disease, renal failure, functional class IV heart failure by the New York Heart Association (NYHA), ventricular dysfunction, combined procedures, number of rapprochement, mitral valve replacement, emergency surgery, shock in the preoperative period, stent thrombosis, the presence of endocarditis and paravalvular abscess.[1,2]

The most used cardiac surgical risk scores in the daily practice are the STS and the EuroSCORE II. The former does not contemplate the risk calculation for double valvar replacement, as in the case at hand. The risk estimated by the EuroSCORE II was of 13.23%, indicating high risk of death.

One of the alternatives to the conventional aortic valvar surgery for individuals of high surgical risk is the transcatheter implant, known as Transcatheter Aortic Valve Implantation (TAVI), which is being performed since 2002.[3] Since then, over 50,000 devices have been implanted throughout the world, with clinical outcomes that are similar to the surgery for patients with high or prohibitive surgical risk.[1] The bioprosthesis may be expandable by balloon or self-expanding, both dependent on the presence of aortic valve calcification to prevent its displacement, however, with higher risk of perivalvar regurgitation in cases of extreme calcification.

The two most common pathways for the TAVI are transfemoral and transapical – a technique started in 2006, with access through minithoracotomy without the need for extracorporeal circulation. Although the transapical technique is more invasive, the advantages over the femoral

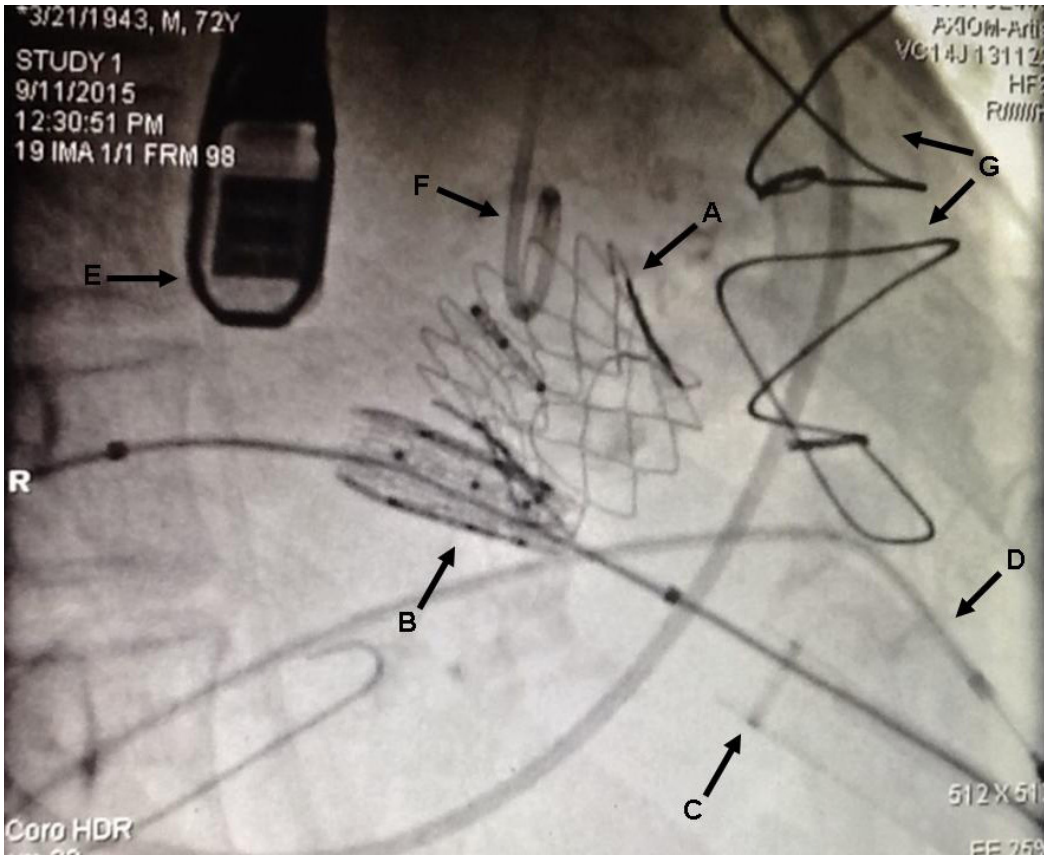

Figure 2 – *Radioscopy image showing Inovare-Braile bioprosthesis in aortic position no. 28 already implanted (A) and in mitral position no. 30, immediately after the implant (B). The sheath with guidewire for implantation of the mitral prosthesis (C), transvenous pacemaker electrode (D), transducer of the transesophageal echocardiography (E), pigtail catheter in the ascending aorta (F) and prior sternotomy wires (G) are observed.*

pathway are: greater ease of valve implantation due to the proximity of the valve annulus of the cardiac apex, less manipulation of the aorta and peripheral arterial system reducing vascular complications and stroke.[1,4] There is no impediment to the use of this technique in patients with previous myocardial revascularization.

The progress in the techniques and materials for transcatheter valve implantation in native valves allowed the strategy to be adapted for the treatment of dysfunction of biological prostheses, a technique called valve-in-valve. The aortic valve-in-valve procedures were the first to be performed, expanding the use of equipment and skills idealized for the TAVI. Since then, procedures with balloon-expansible and self-expansible prosthesis have been performed. Soon after, the method was expanded for mitral, pulmonary and tricuspid interventions.[1,5,6]

The first mitral valve-in-valve procedures were performed in 2009, initially, with balloon-expansible prosthesis and, after, also with self-expansible prosthesis. From 2011, implants over the post annuloplasty valve annulus began to be performed, known as valve-in-ring. Most recently, from 2014, the mitral transcatheter interventions on native valves began.[1,7]

Lastly, combined transcatheter procedures have been reported in the last few years.[8-10] It is a treatment that requires further investigation, exclusively proposed in cases where the conventional surgical procedure is prohibitive. We suggest a multidisciplinary discussion with a "Heart Team" for each patient, aiming to design the best type of intervention individually and cautiously.

Conclusion

There are few reports of combined intervention in disorders of aortic and mitral valves.[8-10] This case was the first performed in Brazil with the implantation of the Inovare Braile national prosthesis, showing the huge potential for future interventions in selected patients.

Author contributions

Conception and design of the research, Obtaining funding and Critical revision of the manuscript for intellectual content: Sampaio RO, Paixão MR, Fonseca JHAP, Tarasoutchi F; Acquisition of data and Analysis and interpretation of the data: Sampaio RO, Paixão MR, Miranda TT, Veronese ET, Fonseca JHAP; Statistical

analysis: Sampaio RO, Paixão MR; Writing of the manuscript: Sampaio RO, Paixão MR, Miranda TT, Tarasoutchi F.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

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Study Association

This study is not associated with any thesis or dissertation work.

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Transcatheter Aortic Valve Implantation with Embolic Protection System in a Patient with Left Ventricle Apical Thrombus

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A 68-year-old woman was admitted in our acute cardiac care unit due to cardiogenic shock. The transthoracic echocardiography (TTE) showed severe aortic stenosis, severe left ventricle (LV) systolic dysfunction (ejection fraction 20%) and a large apical thrombus (Figure 1A-B). We performed an emergent percutaneous aortic balloon valvuloplasty (Figure 1C). During the procedure, the coronary angiography revealed no epicardial coronary disease (Figure 1D). Despite some mild clinical and hemodynamic improvement (mean gradient reduced from 40 to 30 mmHg), she remained in New York Heart Association (NYHA) class IV.

The case was discussed by our heart team and she was considered to be at high operative risk (Society of Thoracic Surgery score 12%; EUROSCORE II 15%). Therefore, we have decided to implant a transcatheter aortic valve (TAVI) using an embolic protection system. Aortic annulus sizing was performed intra-procedure using transoesophageal echocardiography, which also showed the apical thrombus (Figure 1E). Firstly, the Sentinel Cerebral Protection System (Claret Medical, Inc) was deployed through right radial access (Figure 1F). Afterwards, a 26 mm Edwards Sapien 3 TAV (Edwards Lifesciences Corporation) was implanted by transfemoral approach (Figure 1G). The procedure went without complications and the patient showed remarkable clinical and hemodynamic improvement, being discharged 11 days after TAVI, medicated with warfarin. In the one-year follow-up, the patient was in NYHA class I, TTE showed normally functioning TAV, improvement of the LV function (40%) and no evidence of apical thrombus (Figure 1I).

Keywords

Heart Valve Prosthesis Implantation; Embolic Protection Devices; Shock, Cardiogenic.

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Author contributions

Conception and design of the research and Acquisition of data: Almeida JG, Ferreira S, Caeiro D; Analysis and interpretation of the data: Almeida JG, Ferreira S; Writing of the manuscript: Almeida JG; Critical revision of the manuscript for intellectual content: Almeida JG, Ferreira S, Caeiro D, Ribeiro J; Supervision: Ribeiro J, Ribeiro VG.

Potential Conflict of Interest

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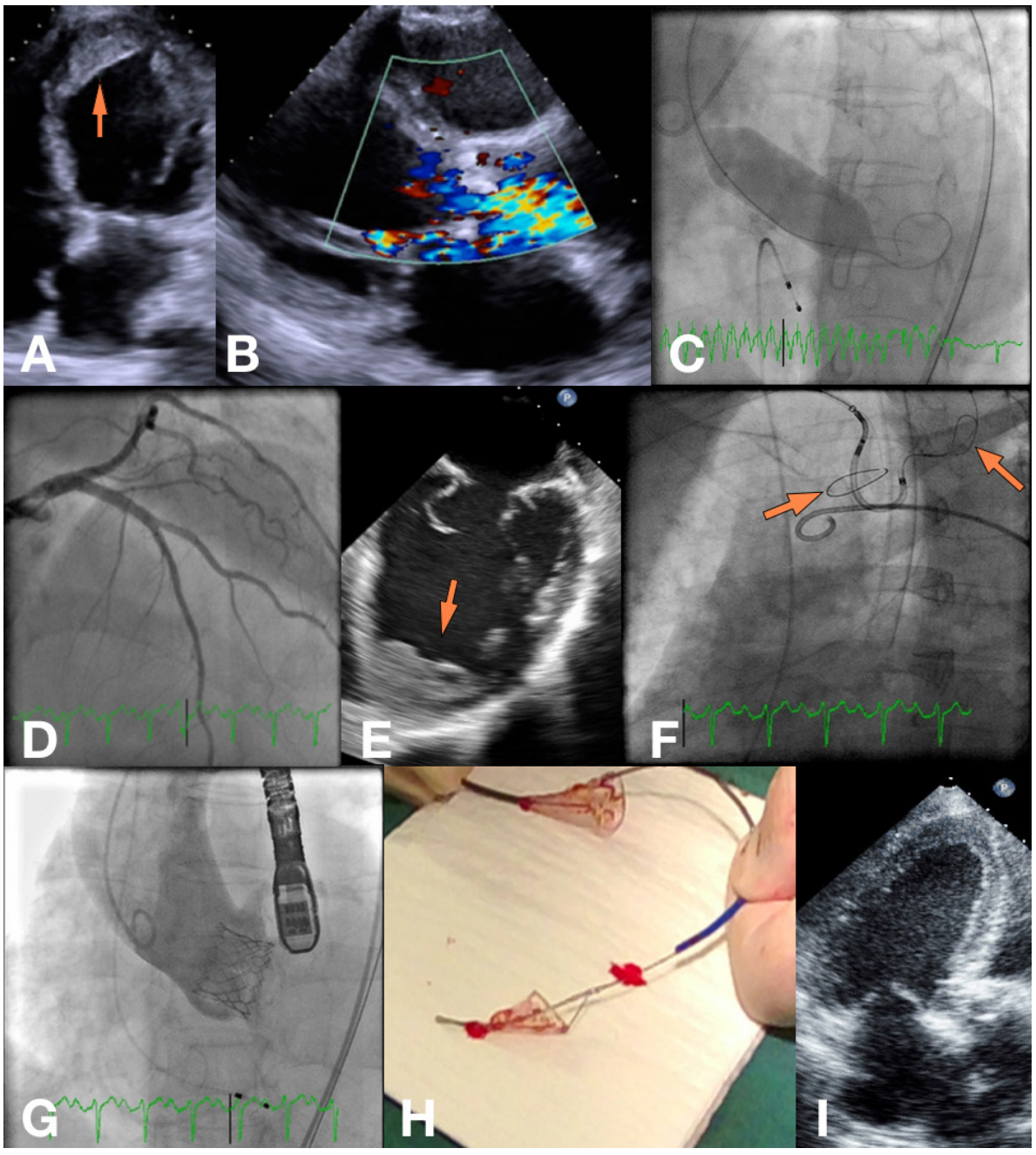

Figure 1 – *A) Four-chamber view from the admission TTE (arrow: apical thrombus); B) Colour Doppler showing turbulent flow through the aortic valve in parasternal long-axis view; C) Percutaneous aortic balloon valvuloplasty; D) Left coronary angiography; E) TEE showing the large apical thrombus (arrow); F) Embolic protection system deployment (arrows: filters); G) Angiography after TAV implantation; H) Embolic filters with particulate debris; I) Three-chamber view from a TTE, 3-months after the procedure. TTE: transthoracic echocardiography; TEE: transoesophageal echocardiography; TAV: transcatheter aortic valve.*

Association between SYNTAX Score and Coronary Collateral Circulation

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Dear Editor,

I have read the article entitled "Which Coronary Lesions Are More Prone to Cause Acute Myocardial Infarction?" by Sen et al. with great interest, recently published in journal. The investigators reported that more than 70% of the patients with acute myocardial infarction (MI) had coronary collateral circulation (CCC) with Rentrop scores of 1-3 during primary coronary angiography. This shows that most cases of acute MI in our study originated from underlying high-grade stenosis, challenging the common believe. Higher serum triglycerides levels, greater mean platelet volume, and increased white blood cell and neutrophil counts were independently associated with impaired development of collateral vessels.[1]

Synergy between percutaneous coronary intervention with Taxus and cardiac surgery (SYNTAX) score is the angiographic scoring system and is widely used to evaluate the severity and complexity of coronary aretry disease.[2] SYNTAX score (SS) predicts not only possible peri-procedural difficulties but also indicates the pattern of atheroma including length, thrombosis, and calcification of the lesion.[3] Association between multi-vessel disease and CCC has been reported by several studies.[4,5] Börekçi et al.[4] reported that higher SS in patients with poor CCC. Cetin et al.[5] observed that in the poor CCC group, SS were significantly higher compared to good CCC group.

In this context, considering association between SS and CCC, correlation of this study's result with SS may shed light on further studies.

Keywords

Myocardial Infarction; Coronary Circulation / physiopathology; Coronary Angiography; Coronary Artery Disease; Probability.

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Letter to the Editor

Reply

Dear editor

I have read the letter to the editor about my study entitled "Which Coronary Lesions Are More Prone to Cause Acute Myocardial Infarction?" with great interest. The author proposed that high Syntax score might be correlated with poor coronary collateral development.

Coronary collateral vessels serve as conduit between proximal site of occluded coronary artery and its distal parts.[1] The most important triggered effect of the collateral vessel development is the shear stress due to occlusion. 20-25% of normal humans have coronary collateral circulation but it is weak and small and it cannot be seen during coronary angiography. Pressure gradient after occlusion or severe stenosis of a coronary artery lead to endotelial stimulation and arteriogenesis and also enlargement of preexisting collateral vessels.[2]

In the study performed by Borekci et al.[3] they found that patients with poorly developed collateral coronary circulation had higher SYNTAX scores compared with the well-developed coronary collateral group. However, multivariate analysis revealed that there was no relationship between SYNTAX score and coronary collateral flow. So far, there has not been a specific study addressing this issue. In our study, we did not calculate the SYNTAX score because it was not the aim of the study. I think that other specific trials addressing this issue are needed to determine if there is any relationship between the SYNTAX score and the coronary circulation or not. In addition, the possible mechanism of this relationship has to be explained.

Taner Sen

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In the "Atualização da Diretriz Brasileira de Dislipidemias e Prevenção da Aterosclerose - 2017", published as a supplement to the *Arquivos Brasileiros de Cardiologia* [Arq Bras Cardiol 2017; 109(2Supl.1):1-76], the following corrections should be considered:

– On page 35, first paragraph, the following sentence and respective references (311 and 312) were added: "PCSK-9 inhibitors may be considered either combined with other lipid-lowering agents or isolated in statin-intolerant patients, when the targets proposed for cardiovascular risk are not met.[311,312]"

– Ref. 311: Kastelein JJ, Ginsberg HN, Langslet G, et al. ODYSSEY FH I and FH II: 78 week results with alirocumab treatment in 735 patients with heterozygous familial hypercholesterolaemia. Eur Heart J. 2015;36: 2996-3003. Ref. 312: Raal FJ, Stein EA, Dufour R, Turner T, Civeira F, Burgess L, Langslet G, Scott R, Olsson AG, Sullivan D, Hovingh GK, Cariou B, Gouni-Berthold I, Somaratne R, Bridges I, Scott R, Wasserman SM, Gaudet D; RUTHERFORD-2 Investigators. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. Lancet. 2015 Jan 24;385(9965):331-40. Both references were added to the list of references, and hence the references previously numbered as 311-315 have been changed to 313-317 both in the text and in the reference list.

– On page 35, section 9.2.2.4, first paragraph, the part of the sentence "for the treatment of FH, and can be used for homozygous, as long as the defect has not been caused by a "negative receptor"" was replaced by "for FH treatment. The drug evolocumab was tested in HoFH in the Tesla B study, with an additional LDL-c reduction by 21.3%, and is not effective in homozygous forms of the disease in which the receptor is negative or nul.[318]", with inclusion of the reference 318.

– Ref. 318: Raal FJ, Honarpour N, Blom DJ, Hovingh GK, Xu F, Scott R, Wasserman SM, Stein EA; TESLA Investigators. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. Lancet. 2015 Jan 24;385(9965):341-50. The reference 318 was added to the list of references, and hence the references previously numbered as 316-554 have been changed to 319-557 both in the text and in the reference list.

– On page 38, section 9.2.4.2, please consider "Tangier", starting with capital letter.

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