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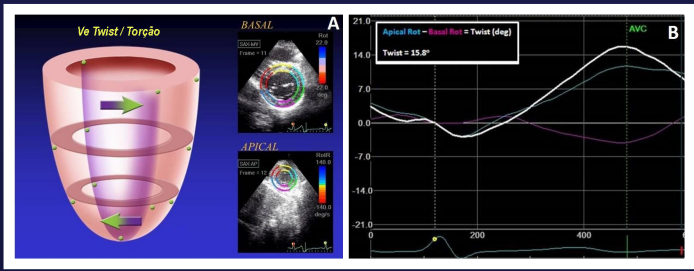

Figure 2 – *A: Representation of LV twist/torsion – clockwise rotation at the base and counterclockwise at the apex (view from the apex). B: Example of LV twist analysis (white line, LV twist; cyan line, apical rotation; pink line, basal rotation). Page. 25*

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Blood Pressure Targets: Will We Reach Definite Figures? I Currently Have Mine

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I was born before the era of evidence-based Medicine. When I was a student, arterial hypertension (AH) used to be diagnosed based on blood pressure (BP) readings greater than 160/95 mm Hg.

Can anyone imagine that now?

Cardiology has advanced a lot in all directions, both in diagnostic and treatment methods. Currently, treatment is based on scientific evidence, and the drug armamentarium available is extremely effective.

But let us go back to AH.

Over the years, we have learned through a large number of studies, initially observational ones, and then intervention ones, that, if on the one side cardiovascular risk increases with BP levels from 115/75 mm Hg on, doubling with every 20-mmHg increase in systolic BP (SBP) and 10-mmHg increase in diastolic BP (DBP), on the other, cardiovascular risk decreases significantly with BP reduction by using the pharmacological treatment offered to hypertensives.[1-5]

There is no definite evidence that non-pharmacological treatment (healthy lifestyle) yields the same results, but that is an obvious universally accepted assumption, although difficult to implement nowadays.[1-5]

Excellent antihypertensive agents have been developed and perfected. The benefits of their use regarding both morbidity and mortality have been proven.[1-5]

There is no doubt about that.

We face, however, some dilemmas, beginning with access to healthcare services and medications, when required. That is crucial, depends on consistent public policies that change the *status quo*; nevertheless, that is not the object of this discussion.[1-5]

Another aspect, concerning treatment itself, is the huge challenge posed by adherence to treatment. Currently, a small number of individuals, aware of their hypertensive condition and of the risks inherent to it, and even having access to the healthcare system, do not adhere to the proposed treatment. This critical issue involves educational measures as the major tool for that behavior change, which is common and universal, but, once again, this is not the object of my observations today.[1-5]

Moving on to what matters!

In the past 40 years, hundreds of studies on the treatment of AH were conducted worldwide, providing us with the opportunity to find that pharmacological therapy modifies the natural history of AH, significantly reducing cardiovascular mortality and morbidity. It is worth noting that this fact is rather due mainly to BP reduction, than to the type of drug used.

Therefore, the epidemiological cycle is closed: BP reduction is effectively beneficial.[1-5]

But how much should it be reduced? To which extent is it safe?

The dilemma of targets!

Most studies showing a reduction in cardiovascular events have been designed to compare BP levels before and after treatment. At first, active drugs were compared to placebo, assessing the changes in BP and its possible benefits. Later, active drugs have been compared to each other, aiming at finding differences between them.

The major focus was not the BP reached, but the difference between initial and final levels.

The best source of evidence in medicine is known to come from randomized controlled clinical trials. Establishing objective targets required the investigation design to have that purpose.

The HOT (*Hypertension Optimal Treatment*) study,[6] published in 1998, was a pioneer in regard to targets, DBP being the reference used. Over 18500 patients aged between 50 and 80 years (mean, 61.5 years), with DBP between 100 and 115 mmHg, were allocated to DBP targets of ≤ 90 mm Hg, ≤ 85 mmHg and ≤ 80 mmHg. As DBP levels lowered, greater reductions in cardiovascular events were observed, the greater benefit occurring in the group of patients whose DBP was reduced to the mean level of 82.6 mm Hg. Reductions to levels below those were observed to be safe, and, among patients with diabetes, the benefits were even higher for the group whose target was DBP ≤ 80 mmHg. That study was a landmark regarding BP targets, and international guidelines have based their recommendation on it for years.[1-4,6]

Over time, there have been hundreds of good quality investigations on drug interventions, involving thousands of patients, and they have continued to support the benefits of BP control; however, the ones assessing targets have been scarce.[1-4,6]

Many might be thinking on some studies aimed at establishing some targets, such as the Italian Cardio-Sis, published in

Keywords

Hypertension; Blood Pressure / physiology; Antihypertensive Agents; Diabetes Mellitus; Blood Pressure Targets.

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2009, that investigated non-diabetic hypertensives over the age of 55 years, and aimed at SBP targets < 130 mmHg or < 140 mmHg. In that study, the intermediate primary outcome 'left ventricular hypertrophy' showed significantly favorable results for lower BP levels, and positive results regarding pre-specified secondary outcomes, which were cardiovascular.[7]

Similarly, in 2008, the CASE-J trial, involving only elderly individuals, was published reporting significant advantages for lower BP levels. In addition, in 2008, the JATOS study, assessing elderly, showed no difference between SBP targets under 140 mm Hg or 160 mm Hg.[8,9]

Until then, all official documents, including our last guideline, assessing what BP levels should be pursued for greater benefits, had worked with BP under 140/90 mmHg for the general population, and under 130/80 mmHg for individuals at high cardiovascular risk, those with cardiovascular disease, diabetes and established kidney disease.[1-4,10,11]

In 2010, the result of the ACCORD study was published.[12] It assessed only patients with diabetes, and tried to define if stricter BP control targets (SBP < 120 mmHg) was advantageous over conventional targets (SBP < 140 mmHg). That study randomized more than 4500 diabetic hypertensives, with a mean age of 62 years, followed up for a mean period of 4.7 years. The primary composite outcome was non-fatal myocardial infarction, non-fatal stroke or cardiovascular death. In addition, several secondary cardiocirculatory outcomes were predefined. The results were null, that is, there was no difference in major, fatal and non-fatal cardiovascular combined events. Regarding secondary outcomes, there was no significant difference, except for fatal and non-fatal stroke, which showed a significant reduction of 41% and 37%, respectively. The group with a more marked BP reduction had more adverse events, but without jeopardizing the end of the study.[12]

That study had a huge repercussion, leading to a new rationale, which, in my opinion, was mistaken.[5] It was implied, almost immediately, that stricter BP targets for diabetic patients would be harmful, and, indirectly there was controversy about lower BP targets for all types of patients.[5]

I assess the ACCORD study findings and interpret its results differently. The primary outcomes were similar, that is, there was no difference.[12] I emphasize the significance of that: there was neither reduction, nor increase in primary events.[12]

On the other hand, but not less important, assessing the secondary outcome 'stroke', we observe that there were benefits for the group with stricter BP control.[12] Who does not want to protect a diabetic patient from a stroke? Just food for thought!

In the sequence of the investigations for more adequate BP levels, by the end of 2015, the SPRINT study was published.[13] Financed by the National Institutes of Health (NIH), without any conflict of interest, the protocol was carefully designed specifically aimed at assessing the more beneficial BP levels in terms of cardiocirculatory outcomes for non-diabetic hypertensives. Individuals aged 50 years or older, with SBP between 130 and 180 mm Hg and at increased cardiovascular risk were selected. For individuals randomized for intensive treatment, a SBP target < 120 mmHg was defined, while for those randomized for standard treatment, a SBP target < 140 mmHg was defined. The primary composite outcome comprised acute myocardial infarction, other acute coronary syndromes, stroke, heart failure and death from cardiovascular causes. The secondary outcomes were defined as the individual components of primary outcome, all-cause death and the addition of primary outcome or all-cause death.[13]

That study randomly assigned 9361 patients, more than 4650 to each treatment type. The mean age was 67.9 years, and the groups were homogeneous in all aspects. In the first year of follow-up, the mean SBP and DBP achieved were, respectively, 121.4 and 68.7 mm Hg in the intensive-treatment group, and 136.2 and 76.3 mm Hg in the standard-treatment group. At the end of the study, the mean SBP levels were 121.5 and 134.6 mm Hg in the intensive-treatment and standard-treatment groups, respectively.[13]

The SPRINT study, planned to last 5 years, was interrupted by the Safety Committee at 3.26 years, because a significant difference in outcomes was observed between the groups on two pre-established occasions for such control. The intensive-treatment group showed a 25%-reduction in primary outcomes, and that difference began to appear from the first year of intervention on. The number of all-cause deaths was also 27% lower in that group, in which there were 43% less deaths from cardiovascular causes. Adverse events were more frequent in that group with stricter BP control, however without significant hindrance to the ongoing research.[13]

That clinical trial was outstanding, had an excellent design, clear objectives and proper development. Its results were categorical. However, as any protocol, it is liable to be challenged, although, in that particular case, most lacked consistency.

One point raised was its early interruption.

But, how not to do that?

It was a matter of safety. How can we not offer the best to our patients? In addition, on a critical review of the study, its results cannot be challenged for that reason. In fact, the trial effectively provided valuable information on the BP targets to be pursued.

A good alternative to randomized clinical trials to confirm scientific evidence is provided by meta-analyses and systematic reviews. However, their results can be challenged, depending on the criteria used for selecting the studies to be assessed and those that should be eventually included.

Regarding BP targets, there is a reasonable number of meta-analyses and systematic reviews on the subject.

Four recent studies on that subject are worth noting: the first dates from 2015, assessing type 2 diabetic patients; two were from 2016, one by Xie et al.[16] and the other by Ettehad et al.,[15] both treating hypertensives in general; and the fourth, published in 2017 in the JACC, approached the same question in elderly patients.[17] All studies showed the benefits of stricter BP control to reduce cardiovascular morbidity and mortality in those patients.[14-17]

In addition, it is worth emphasizing the interesting editorial by Perkovic and Rodgers[18] published in the NEJM in November 2015, concomitantly with the SPRINT

study publication. In that editorial, the authors assessed the ACCORD and SPRINT studies together, identified their similarities and differences, and understood them as complementary, creating a new situation, with an even larger number of patients, and suggested additional results. Those authors reported that, although both studies had the same BP target and similar outcomes, the ACCORD study had a smaller statistical power and primary outcomes less-sensitive to BP changes as compared to those of the SPRINT study (Figure 1). Those authors have not objectively suggested BP levels, but indicated, based on their observations and convictions, that stricter BP targets are welcome, mainly for individuals at higher cardiovascular risk.

At the beginning of 2017, Chobanian published in the JAMA a viewpoint that coincides with my understanding.[19] Its rationale is logical and based on the existing evidence. That author suggested even stricter targets (BP < 120/80 mmHg) for individuals under the age of 50 years. He values DBP for that age group, emphasizing the concept of the importance of DBP for youngsters. He suggested BP levels below 130 mmHg for individuals aged between 50 and 74 years at high cardiovascular risk or with established disease, including those with diabetes, considering the benefits reported in the ACCORD study regarding stroke. Finally, he recommended BP levels <140 mmHg for all patients aged 75 years or older.[19]

Even considering the lack of definitive information on BP levels to be pursued in patients at high cardiovascular risk, inferring that excessively low BP levels can be harmful,[20] those are the figures I have been working with, taking each patient's characteristics into consideration.

Finally, it is worth noting that most individuals maintain BP levels far above any established BP target, and accepting higher BP levels is harmful, and will represent over the years a significant recrudescence of cardiovascular diseases, currently a major cause of morbidity and mortality.

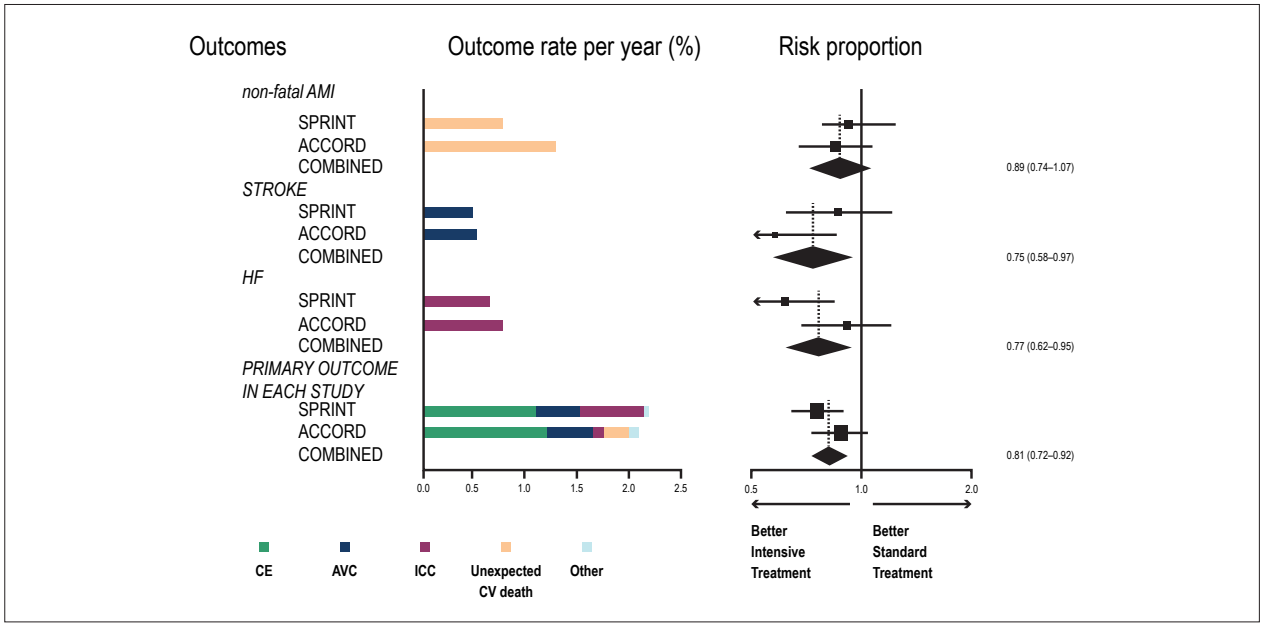

Figure 1 – *Combination of the outcomes of the SPRINT and ACCORD studies, and combined data of both studies. AMI: acute myocardial infarction; CE: coronary events; HF: heart failure; CV: cardiovascular. Adapted from Perkovic e Rodgers.[18]*

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Thromboembolism and Bleeding Risk Scores and Predictors of Cardiac Death in a Population with Atrial Fibrillation

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Abstract

Background: Atrial fibrillation (AF) is a common arrhythmia, with risk of systemic embolism and death. It presents rheumatic etiology in up to 32% of developing countries, whose anticoagulation and evolution data are scarce.

Objectives: to determine the predictors of cardiac death considering the clinical profile, thromboembolism and bleeding scores of patients with AF of a single center, with high prevalence of rheumatic heart disease.

Methods: 302 patients with AF were studied, mean age 58.1 years; 161 women; 96 pts with rheumatic etiology. Patients underwent clinical and laboratory evaluation, measurement of risk scores and the mean follow-up of 12.8 months.

Results: 174 were using warfarin. The averages of the HAS-BLED and ATRIA scores were 1.4 and 1.2, respectively. Percent time in therapeutic range of international normalized ratio was 45.8%. Thirty patients (9.9%) had cardiac death and 41 had some type of bleeding due to warfarin. By univariate analysis, there was statistical significance between cardiac death and permanent AF, blood pressure, systolic dysfunction, R_2CHADS_2, CCS, EHRA and HAS-BLED. There was no association with valvular AF. By multivariate analysis, systemic arterial and pulmonary artery pressures, classification CCS and systolic dysfunction showed statistical significance.

Conclusions: There was no association between cardiac death and valvular AF. Independent predictors of cardiac death were low measures of blood pressure, higher score CCS classification and the presence of systolic ventricular dysfunction. (Arq Bras Cardiol. 2017; 109(1):5-13)

Keywords: Thromboembolism/complications; Hemorrhage; Cardiovascular Diseases/mortality; Atrial Fibrillation/complications.

Introduction

Atrial fibrillation (AF) affects 2% of the population, its prevalence increases with age, reaching the rate of 15% in those with 80 years, and half of the patients with AF present age equal to or greater than 75 years.[1,2] Beyond this epidemiological importance, this arrhythmia is associated with worsening of quality of life and tolerance to efforts, thromboembolic phenomena, hospitalization, heart failure (HF), and double the mortality rate.[1,3-6] AF increases the risk of stroke by 5 times, which also increases with age, with a risk of 1.5% in those between 50 and 59 years of age and 23.5% in the 80-89 age group.[1,3] Among patients with AF rheumatic valve etiology, the risk is 17 times that of the general population and 5 times in relation to patients with non-valvular AF.[7] Due to the risk of thromboembolism, oral anticoagulation is indicated for these patients. However, this therapy has complications, with an annual incidence of bleeding of 2.1 per 100 individuals, resulting in a mortality rate between 13 and 33%.[8,9] Therefore, adequate stratification of the risk of thromboembolism and bleeding is mandatory.

Rheumatic valvulopathy is a disease with a high prevalence in developing countries, yet it is still neglected.[10] In AF, this etiology presents different percentages, from 2.2% in developed countries to 31.5% in those in development.[11] There is scarce current data on the treatment and evolution of patients with AF and rheumatic heart disease.[11-13] Therefore, the objectives of this study are to analyze the clinical profile, thromboembolism and bleeding scores of patients with AF from a single university institution and verify the predictive variables of cardiac mortality.

Methods

It is an observational, longitudinal and prospective study. The population consisted of 302 consecutive patients with AF, who came from the outpatient clinic and the Cardiology nursing ward and who accepted to participate in the study. Patients were selected over a one-year period. The research project was approved by the Ethics and Research Committee of the institution and all patients signed the Informed Consent Term. Patients underwent clinical evaluation, 12-lead electrocardiogram, transthoracic echocardiography, and clinical pathology exams. The diagnosis of AF was made

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by electrocardiogram at the time of symptoms or by virtue of irregular heart rhythm. At the time of inclusion in the study, the following scores were calculated for all patients: *American College of Cardiology* (ACC),[7] HAS-BLED and ATRIA bleeding scores,[14,15] and severity ratings of symptoms and impact in the quality of life of the *Canadian Cardiovascular Society* (CCS)[16] and the *European Heart Rhythm Association* (EHRA),[1] and the Framingham score[17] for predicting stroke (F1) and for predicting death or stroke (F2). On the other hand, the $CHADS_2$, R_2CHADS_2 and CHA_2DS_2-VAS_C[1,14,18] scores were calculated only for non-valvular AF cases. The clinical intercurrences were recorded as thromboembolic events, hemorrhagic events and cardiac death.

For the analysis of the data, the SPSS program (*Statistical Package for Social Science*) version 14.0 was used. The results were expressed in numbers and proportions, for categorical variables, and in measures of central tendency (mean or median) and dispersion (standard deviation) for continuous variables. The Mann-Whitney and chi-square or Fisher tests were used to compare the differences between the continuous and categorical variables, respectively. Survival analysis was performed using the Kaplan-Meier curve, considering the occurrence of cardiac death. Logistic regression analysis was used by the *Stepwise* method, with the dependent variable being the occurrence of cardiac death, considering the variables with $p \leq 0.10$ in the univariate analysis. The level of statistical significance adopted was 5%.

Results

Population characteristics, thromboembolism and bleeding scores

The casuistry consisted of 302 patients who were followed for 12.8 ± 11.8 months (from 15 days to 66 months), of which 161 (53.3%) were female. The mean age was 58.1 ± 15.1 years, ranging from 18 to 92 years. Clinical data, calculated scores and echocardiographic values are shown in Table 1. They had systolic ventricular dysfunction, defined as the ejection fraction lower than 50%, 112 patients (37.1%). Valvular heart disease was of rheumatic etiology in 96 patients. The valvular dysfunction of rheumatic etiology was moderate/important mitral stenosis in 34 patients, moderate/severe mitral regurgitation in 10, double mitral lesion in 13, and 39 patients had been submitted before to the implantation of a prosthesis in the mitral position, being 10 with mechanical prosthesis . Three patients had mitral valve prolapse with moderate/severe insufficiency. Among patients with non-valvular AF were not included patients with valvular morphological alterations.

Clinical pathology examinations at the time of inclusion of the patients showed the following mean values: creatinine of 1.2 ± 1.1 mg/dL (ranging from 0.3 to 11.4), creatinine clearance by the Cockroft formula And Gault of 72.2 ± 36.4 mL/min (between 4.4 and 233.7), serum sodium of 137.4 ± 4.2 (120.0 to 150.0) mmol/L and serum potassium of 4.2 ± 0.6 (1.3 to 6.3) mmol/L.

At the time of inclusion in the study, 174 patients (57.6%) were using warfarin. The HAS-BLED and ATRIA scores presented the mean values of 1.4 ± 1.1 and 1.2 ± 1.5 (median of 1.0), respectively. In 58 patients (19.2%), the HAS-BLED was ≥ 3 and in 14 patients (4.6%) the ATRIA was high risk.

Eighty patients were on antiarrhythmic medication (4 on sotalol, 11 on propafenone and the rest on amiodarone).

Clinical follow-up and survival curves

Patients who presented with electrolytic and metabolic disorders were treated according to their disorders. There was no interference of the researchers regarding the approach and therapies adopted by the attending physicians. For heart rate control in those patients with persistent or permanent AF, beta-blockers, or calcium channel antagonists (verapamil or diltiazem) and/or digoxin were used.

During clinical follow-up of 12.8 ± 11.2 (between 15 days and 66 months), 181 (59.9%) patients used warfarin. The International Normalized Ratio (INR) fraction within the therapeutic range (TTR) was calculated to be 45.8 ± 27.6% (between zero and 100%), and 22.9% of the patients evolved with TTR ≥ 60 %. The mean scores of $CHADS_2$ and CHA_2DS_2-VASC among patients who did not use and those who used warfarin were 1.8 versus 1.6 ($p = 0.22$) and 3.2 versus 2.6 ($p = 0, 12$), respectively.

Thirty patients (9.9%) died from cardiac cause and 41 (22.6%) presented some type of hemorrhage due to the use of warfarin. The causes of cardiac death were: HF in 25 patients (83.3%), sudden cardiac death in 3 (10%) and thrombosis in a mechanical valve prosthesis in 2 (6.6%). Only 6 patients (2%) had a new nonfatal thromboembolic event, and 2 were not in regular use of warfarin. The comparison of the studied variables between the patients with and without cardiac death were shown in Table 2. There was no influence of the use of antiarrhythmic and the evolution to cardiac death (16.7% in antiarrhythmic use had cardiac death and 27.5 % in antiarrhythmic use did not present with cardiac death, $p = 0.14$).

Among patients who died due to heart failure, 24 (80%) had permanent AF ($p = 0.02$ by the log rank test (Mantel-Cox), 95% confidence interval between 39.7 and 47.7). Using the Kaplan-Meier curve and considering as a prognostic basis the occurrence of cardiac death, survival curves were constructed in relation to the following variables: baseline heart disease, presence of systolic ventricular dysfunction and stratification of the score R_2CHADS_2 (low risk: score of 0 and 1, intermediate risk: 2 and 3, high risk: ≥ 4). Twenty-two patients (73.3%) who progressed to cardiac death had dilated myocardiopathy, 5 rheumatic heart valve disease and 3 hypertensive heart diseases. The Mantel-Cox test was applied to compare the curves. In relation to systolic ventricular dysfunction, the odds ratio was 8.1 ($p < 0.0001$) (95% confidence interval: 3.2-20.7). The data are plotted in Figures 1, 2 and 3. There was no difference in survival curves regarding the HAS-BLED, EHRA and CCS classification.

Multivariate analysis

Through the multivariate analysis by *Stepwise* and considering the variables with $p \leq 0.10$ in the univariate analysis associated with cardiac death, the variables systemic arterial pressure (systolic and diastolic), pulmonary artery systolic pressure, CCS classification and systolic ventricular dysfunction were statistically significant (Table 3).

Table 1 – Clinical characteristics and echocardiographic parameters of patients

Variable	Number (proportion and variation)
Femenine gender (%)	161 (53%)
Age (years)	58.1 ± 15.1 (18-92)
BMI (Kg/m^2)	25.1 ± 5.5 (14.9-55.0)
Paroxysmal AF	87 (28.8%)
Persistent AF	45 (14.9%)
Permanent AF	170 (56.2%)
Etiology	
Valvular Heart Disease	99 (32.8%)
Dilated cardiomyopathy	95 (31.5%)
Hypertensive cardiomyopathy	85 (28.1%)
Others (ischemic without ventricular disfunction, congenit, pericarditis constrictive, Brady-Taqui syndrome)	11 (3.6%)
Isolated AF	12 (4.0%)
Previous thromboembolism	62 (20.5%)
HR (bpm)	81 ± 19 (34-180)
SBP (mmHg)	121 ± 22 (60-200)
DBP (mmHg)	75 ± 13 (30-120)
ACC Score	
Low risk	25 (8.6%)
Moderate risk	133 (44.0%)
High risk	143 (47.4%)
$CHADS_2$	1.7 ± 1.1 (0-5)
R_2CHADS_2	2.5 ± 1.7 (0-7)
CHA_2DS_2-VAS_c	2.9 ± 1.8 (0-8)
F1 (%)	11.8 ± 8.8 (4-54)
F2 (%)	29.7 ± 21.4 (7-95)
CCS	2.6 ± 1.1 (0-4)
EHRA	2.7 ± 0.9 (1-4)
LA (mm)	50.7 ± 10.0 (30-84)
LVDD (mm)	55.5 ± 10.4 (33-86)
LVSD (mm)	40.6 ± 12.9 (17-81)
PSAP (mmHg)	43.6 ± 13.8 (10-101)
LVEF (Teicholz)	51.6 ± 17.3 (12-85)

BMI: body mass index; HR: supine heart rate; bpm: beats per minute; SBP: supine systolic blood pressure; DBP: supine diastolic blood pressure; ACC: American College of Cardiology; Framingham score for prediction of stroke (F1) and prediction of death or stroke (F2); CCS: Canadian Cardiovascular Society; EHRA: European Heart Rhythm Association; AE: anteroposterior diameter of the left atrium; LV: left ventricle; LVDD: LV diastolic diameter; LVSD: LV systolic diameter; PSAP: pulmonary artery systolic pressure; EF: ejection fraction.

Discussion

The characteristics of the population of the present study were distinct from records already published[19,20] in relation to age, gender, and mainly regarding the proportion of patients with valvular AF, which was 32.7%. The previous registries were multicentric and performed in developed countries, with only 4.2% of patients with valvar AF, which implied in older age (71.5 and 75 years) and a higher proportion of men (60.1% and 57 %). Due to these factors, the mean of thromboembolism and bleeding scores reported in those registries were also higher, considering the increase in the prevalence of atherosclerosis and blood pressure levels with increasing age. However, the EHRA classification was similar, since 70% of patients in the European registry[19] presented EHRA II or III, as well as the proportion of patients with heart failure between 21.3% and 36% in the cited registries and 31,4% in the present study.

Underutilization of oral anticoagulant is an aspect already reported in the literature, as well as subtherapeutic treatment, with low rates of TTR,[21-23] with improved adherence to therapy over time, as demonstrated by the registries studies.[19,20] Among patients at high risk, with a previous stroke or transient ischemic stroke, about half (ranging from 19% to 81.3%) were not treated with anticoagulants.[22] In the recent published European registry[19] and with a previous history of embolism in 15.5% of patients, 78% were in use of some vitamin K antagonist and 6.1% in the use of new oral anticoagulants, with 13.5% of patients with labile INRs. Accordingly, in the American registry,[20] the mean TTR was 65%, with 17% of patients with INR below the therapeutic range, also showing a greater adherence to the guidelines. In the present study, although about 60% of patients used warfarin at the time of inclusion and during follow-up, one-third of the patients had AF valvular and 20.5% had a history of previous embolism, with TTR lability being observed in 77, 1% of the patients, evidencing an inadequate adherence to the treatment. This underutilization of oral anticoagulation was also verified in a survey conducted in an African country with 25.6% of patients with valvular heart disease and 34.2% with anticoagulant use among eligible patients.[24]

Contemporary data showed an annual mortality rate of 5.8% attributed to AF, reaching up to 8.3%, and that 57.4% of these deaths were of cardiac cause, with 77.3% of them due to HF.[25] In the present study, there was 9.9% of cardiac mortality, with 83% due to heart failure, of which 80% had permanent AF. In addition, survival curves showed higher cardiac mortality among patients with systolic ventricular dysfunction, with a odds ratio of 8.1. This concomitance of AF and HF, with more than half of the patients with AF having HF and more than one third of HF patients with AF,[26] translating, a vicious circle adversely influences the prognosis. Systematic review and meta-analysis have confirmed the association between mortality and systolic dysfunction in patients with AF, compared with that of patients with AF, but without systolic dysfunction, during the 2-year period.[27] In addition, the permanent AF presentation was the most frequent among those with cardiac death in the European multicenter study with a one-year follow-up.[25]

The R_2CHADS_2 score was published in 2013[28] and is calculated by adding 2 more points to $CHADS^2$ for patients with non-valvular AF and creatinine clearance < 60 mL/min to better stratify the risk of thromboembolic events. There are no studies on this score and cardiac mortality in patients with AF. In the study in question, the R_2CHADS_2 score discriminated patients who presented cardiac death, unlike the other $CHADS^2$ and CHA^2DS^2-VAS_C scores. There is influence of age, gender, ethnicity and weight in the estimation of renal function. The Cockroft & Gault[29] formula was developed with a population of 249 patients aged 18 to 92 years, which is the same range as the population of the present study. A study of 925 patients with a mean age of 69 years, ranging from 59 to 75.5 years, comparing the three formulas (Cockroft-Gault, MDRD-4 (Modification of Diet in Renal Disease Study), and CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) demonstrated that the first presented greater accuracy, including comparing groups with EF <40% and ≥40%.[30] Regarding the R_2CHADS_2 score, a recent study[31] with 524 patients with AF demonstrated its utility in predicting stroke also in patients with impaired renal function, compared with the $CHADS^2$ and CHA^2DS^2-VAS_C scores.

Although several variables were associated with cardiac death, the independent predictors of this evolution were systemic arterial pressure, CCS classification, systolic dysfunction, and pulmonary artery systolic blood pressure. A cohort study[32] with patients with AF demonstrated that baseline systolic blood pressure < 120 mmHg was associated with cardiovascular mortality in those with systolic ventricular dysfunction during an average follow-up of 41 months, corroborating the results of the present study. In a systematic review with patients with HF, the highest systolic blood pressure was a favorable prognostic marker.[33]

Regarding the CCS classification, which was validated in terms of the quality of life of patients with AF,[34] its value as an independent variable is due to its graduation, since patients with symptoms of HF secondary to arrhythmia are classified in category 4. On the other side, despite the association between the EHRA score and cardiac death, it was not a predictor of that outcome. This finding was also reported in another cohort of patients with AF, with an association between the score and hospitalization, rather than mortality.[35]

Pulmonary hypertension has been associated with morbidity and mortality, including in those patients with bordering systolic pressure of the pulmonary artery.[36,37] Its most prevalent cause is left ventricular heart disease, with decreased or preserved ejection fraction, and mitral valvopathy. Therefore, this finding in the present study demonstrated what is already reported in the literature.

Limitations of the study

The main limitations of this study are the size of the population and the fact that it is unicentric, which does not reflect the disparities in the approach of these patients between institutions and regions. In addition, the influence of interventions such as arrhythmia reversal or ablation in patients' evolution was not investigated.

Conclusions

In the population with AF and high prevalence of rheumatic valve disease, there was an underutilization of oral anticoagulant, in spite of lower bleeding scores and thromboembolism in relation to those reported in the literature. Survival was lower in those with permanent AF, with dilated cardiomyopathy, and with high-risk R_2CHADS_2. The independent predictors of cardiac death were low measures of systemic arterial pressure, higher CCS scores, presence of systolic ventricular dysfunction, and pulmonary hypertension.

Contribuição dos autores

Conception and design of the research: Silva RMFL. Acquisition of data: Silva RMFL, Silva PA, Lima MC, Sant'Anna LT, Silva TC, Moreira PHV, Gandra RM, Cavalcanti TR, Mourão PHV. Analysis and interpretation of the data: Silva RMFL, Silva PA, Lima MC, Sant'Anna LT, Silva TC, Moreira PHV, Gandra RM, Cavalcanti TR, Mourão PHV. Statistical analysis: Silva RMFL.

Table 2 – Comparison of the means and proportions of the variables among the group of patients who attended without and with cardiac death

Variables	Group without CD (n = 272)	Group with CD (n = 30)	Valor p
Age (Years)	58.7 ± 15.1	53.7 ± 13.8	0.14
Femenine gender	146 (53.6%)	15 (50.0%)	0.59
BMI (Kg/m^2)	25.3 ± 5.3	24.2 ± 6.3	0.13
Permanent AF	146 (53.7%)	24 (80.0%)	0.01
Valvular AF	93 (34.1%)	6 (20.0%)	0.11
HR (bpm)	81.0 ± 19.0	80.3% ± 16.7	0.93
SBP (mmHg)	123.7 ± 20.8	102.0 ± 20.1	< 0.0001
DBP (mmHg)	75.7 ± 13.2	68.1 ± 13.0	0.004
LA (mm)	49.7 ± 9.4	57.9 ± 12.0	0.001
LVDD (mm)	54.7 ± 9.8	64.3 ± 12.3	< 0.0001
LVSD (mm)	39.5 ± 12.2	52.2 ± 14.7	< 0.0001
PASP (mmHg)	42.3 ± 13.3	51.3 ± 12.8	< 0.0001
LVEF (%)	53.2 ± 16.4	37.0 ± 18.4	< 0.0001
Creatinine (mg/dL)	1.2 ± 1.1	1.4 ± 0.6	0.004
Creatinine Clearance (mL/min)	72.8 ± 37.2	57.5 ± 26.5	0.01
Sodium (mmol/L)	137.9 ± 3.9	134.3 ± 4.5	< 0.0001
Potassium (mmol/L)	4.2 ± 0.5	4.1 ± 0.8	0.23
Varfarine use	149 (54.7%)	19 (63.3%)	0.37
TTR	126 (46.3%)	13 (42.3%)	0.66
BP	57 (21.0%)	11 (36.3%)	0.10
CCS	2.5 ± 1.1	3.1 ± 1.0	0.01
EHRA	2.7 ± 0.9	3.2 ± 0.9	0.02
High risk ACC	130 (47.7%)	9 (30.0%)	0.10
$CHADS_2$	1.7 ± 1.2	1.5 ± 0.7	0.60
R_2CHADS_2	2.4 ± 1.7	3.0 ± 1.1	0.02
CHA_2DS_2–VAS_C	2.9 ± 1.8	2.6 ± 1.4	0.59
F1 (%)	12.0 ± 8.6	8.5 ± 5.3	0.03
F2 (%)	30.1 ± 22.0	26.2 ± 14.1	0.90
HAS-BLED	1.4 ± 1.1	1.8 ± 0.9	0.01
ATRIA	1.2 ± 1.6	1.0 ± 1.1	0.68

CD: cardiac death; BMI: body mass index; HR: supine heart rate; Bpm: beats per minute; SBP: supine systolic blood pressure; DBP: supine diastolic blood pressure; Pts: patients; ACC: score of the American College of Cardiology; Framingham score for prediction of stroke (F1) and prediction of death or stroke (F2); CCS: Canadian Cardiovascular Society; EHRA: European Heart Rhythm Association; LA: anteroposterior diameter of the left atrium; LVDD: LV diastolic diameter; LVSD: LV systolic diameter; PSAP: pulmonary artery systolic pressure; EF: ejection fraction; LV: left ventricle; TTR: fraction of the RNI values (international normalized ratio) within the therapeutic range; BP: bleeding patients.

Writing of the manuscript: Silva RMFL. Critical revision of the manuscript for intellectual content: Silva RMFL.

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.

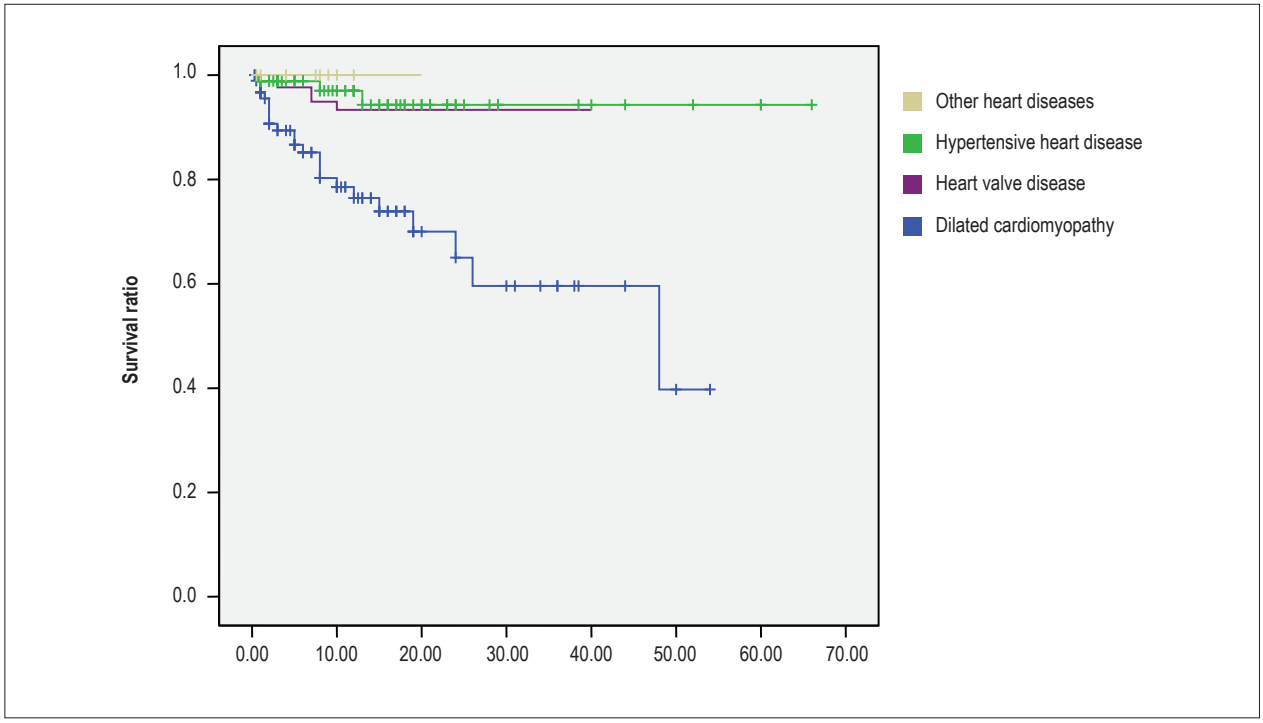
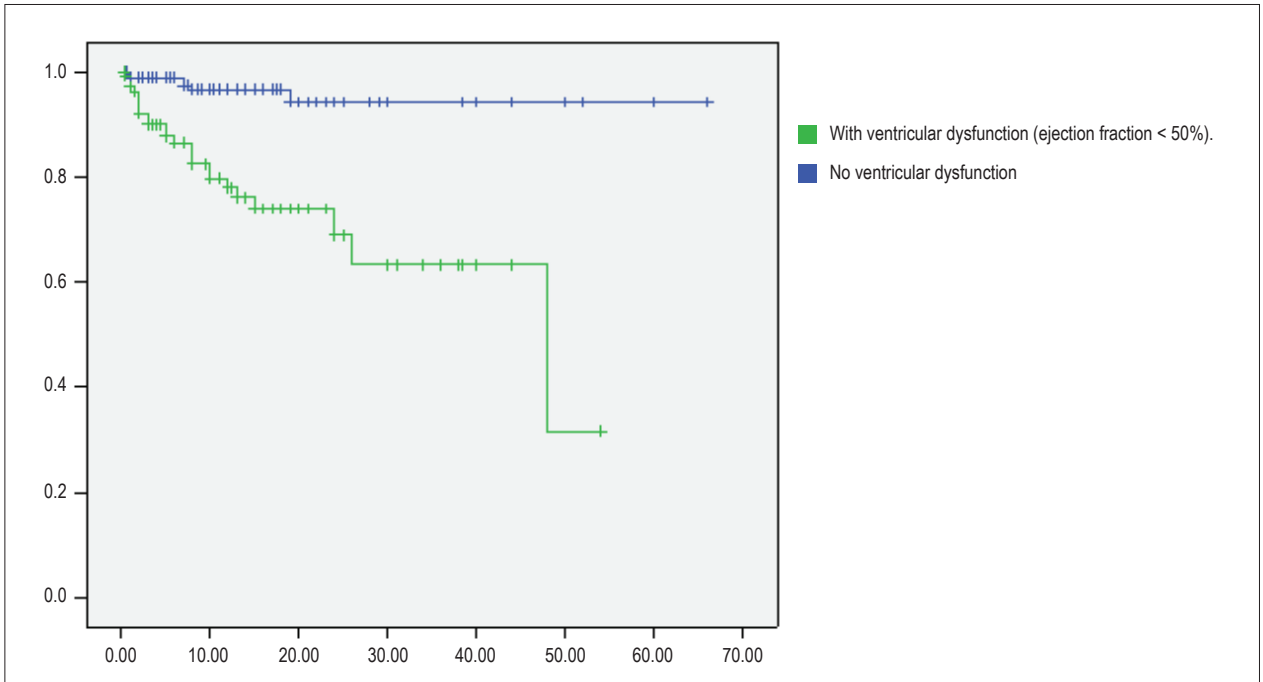


Figure 1 – *p <0.0001 Kaplan-Meier curve of cardiac death free survival of patients in relation to baseline heart disease.*



Time (months)	3	6	10	15	26	48
Cumulative number of patients with ventricular dysfunction and CD	10	13	18	21	23	24
Cumulative survival rate (%)	90.0	86.5	79.6	73.9	63.2	31.6

Figure 2 – *Kaplan-Meier curve and cumulative percentage of cardiac death free survival (CD) of the patients in relation to the presence of systolic ventricular dysfunction.*

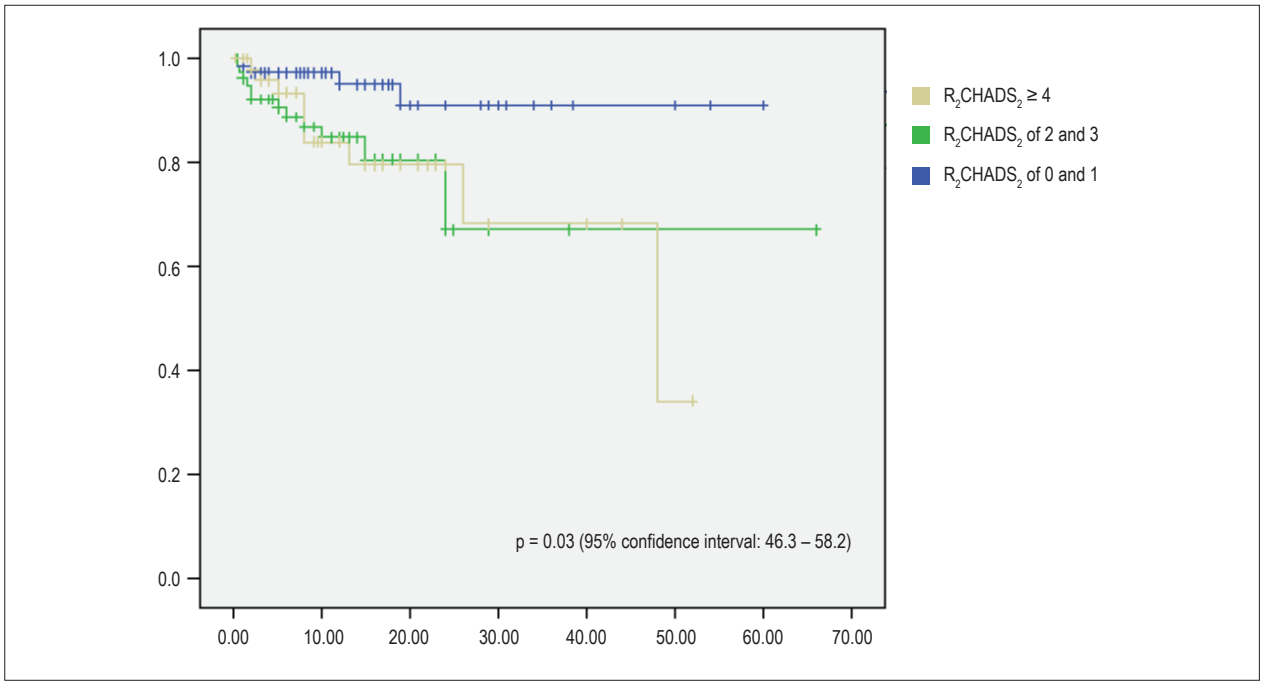


Figure 3 – *Kaplan-Meier curve of free survival of cardiac death (CD) of patients in relation to the stratification of the score R_2CHADS_2.*

Table 3 – Multivariate analysis for the dependent variable cardiac death

Independent variables	Valor p
SBP (mmHg)	0.001
DBP (mmHg)	0.033
CCS Classification	0.002
PSAP (mmHg)	0.006
Systolic Disfunction LV (EF < 0.50)	0.044

SBP: supine systolic blood pressure; DBP: supine diastolic blood pressure; CCS: Canadian Cardiovascular Society; PSAP: pulmonary artery systolic blood pressure; LV: left ventricle.

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Original Article

Late Results of Cox Maze III Procedure in Patients with Atrial Fibrillation Associated with Structural Heart Disease

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Abstract

Background: **Cox-Maze III procedure is one of the surgical techniques used in the surgical treatment of atrial fibrillation (AF).**

Objectives: **To determine late results of Cox-Maze III in terms of maintenance of sinus rhythm, and mortality and stroke rates.**

Methods: **Between January 2006 and January 2013, 93 patients were submitted to the cut-and-sew Cox-Maze III procedure in combination with structural heart disease repair. Heart rhythm was determined by 24-hour Holter monitoring. Procedural success rates were determined by longitudinal methods and recurrence predictors by multivariate Cox regression models.**

Results: **Thirteen patients that obtained hospital discharge alive were excluded due to lost follow-up. The remaining 80 patients were aged 49.9 ± 12 years and 47 (58.7%) of them were female. Involvement of mitral valve and rheumatic heart disease were found in 67 (83.7%) and 63 (78.7%) patients, respectively. Seventy patients (87.5%) had persistent or long-standing persistent AF. Mean follow-up with Holter monitoring was 27.5 months. There were no hospital deaths. Sinus rhythm maintenance rates were 88%, 85.1% and 80.6% at 6 months, 24 months and 36 months, respectively. Predictors of late recurrence of AF were female gender (HR 3.52; 95% CI 1.21–10.25; p = 0.02), coronary artery disease (HR 4.73 95% CI 1.37–16.36; p = 0.01) and greater left atrium diameter (HR 1.05; 95% CI 1.01–1.09; p = 0.02). Actuarial survival was 98.5% at 12, 24 and 48 months and actuarial freedom from stroke was 100%, 100% and 97.5% in the same time frames.**

Conclusions: **The Cox-Maze III procedure, in our experience, is efficacious for sinus rhythm maintenance, with very low late mortality and stroke rates. (Arq Bras Cardiol. 2017; 109(1):14-22)**

Keywords: **Atrial Fibrillation/surgery; Arrhythmias,Cardiac; Mitral Valve; Rheumatic Fever.**

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia in adults, with a close relationship with aging. The prevalence of AF increases from 0.2% in individuals aged 45-54 years to 8% in those aged over 75 years.[1] Among cardiac surgery patients, AF is found in up to 50% of patients undergoing mitral valve surgery and in 1-6% of patients undergoing myocardial revascularization or aortic valve replacement.[2]

Surgical treatment of AF is an alternative, efficient therapeutic approach for long-term maintenance of sinus rhythm. The third version of Cox-Maze procedure (CM III), or traditional Maze, is considered the gold standard surgical procedure for AF.[3] A set of incisions and sutures causes anatomical and functional changes in the atria, allowing the conduction of stimulus from the sinus node to the atrioventricular node and, concomitantly, preventing both maintenance[3] and initiation of AF.[4,5]

CM III procedure leads to high rates of sinus rhythm maintenance[3] and low incidence of late stroke, particularly due to closure of the left atrial appendage.[6] On the other hand, advanced age, increased left atrial dimensions, and long-standing persistent AF have been identified as predictive factors of recurrent AF after the Maze procedure.

Due to technical complexity and assumed increase in morbidity, CM III is performed on a regular basis in relatively few centers nowadays. The use of alternate energy sources and surgical ablation procedures has simplified the traditional procedure, and been increasingly performed worldwide.[7]

The aim of the present study was to assess long-term results of CM III regarding maintenance of sinus rhythm, risk factors for recurrent arrhythmia, late mortality and survival rate free of stroke.

Methods

This study evaluated a cohort of patients with structural heart disease-related AF, who underwent combined CM procedure from January 2006 to January 2013. Surgical treatment consisted of CM III, which was performed as described by Cox et al.[3]

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The study was approved by the Ethics Committee and registered at *Plataforma Brasil* (identification number 20301113.0.0000.0026). Informed consent was obtained from all volunteers and/or caregivers who agreed to participate in the study.

Surgical indications were established based on clinical and surgical criteria, following the Brazilian Society of Cardiology guidelines. Decisions for surgery were made by the same staff, based on the risks of procedure, familiarity and previous experience with the technique, and potential benefits in each case.

Demographical and clinical data, and complementary tests were obtained retrospectively from patients' medical records. Similarly, operative characteristics and post-operative information were collected from electronic and nursing records of the procedure. All data were stored electronically and protected against unauthorized access.

Patients with more than three months of follow-up were invited to participate in the study, by attending a clinical visit with the main investigator (cardiologist) for assessment of cardiac rhythm by 12-lead electrocardiogram and 24-hour Holter monitoring. It is worth pointing out that patients continued their postoperative outpatient treatment provided by the medical staff, and continuation of antiarrhythmic drug therapy was left to the cardiologist's discretion. Occurrence of any recurrent arrhythmia episode was recorded for analysis.

Late AF recurrence was defined according to American cardiology societies' guidelines.[8] Recurrence was defined as the occurrence of AF, atrial flutter, or atrial tachycardia lasting ≥ 30 seconds after a period of at least 3 months surgery. Episodes of early recurrent AF (AF occurring in the period from the day of surgery to the day of hospital discharge) were also verified for prediction of late recurrence.

For assessment of CMIII-related late morbidity, we evaluated the incidence of stroke and mortality.

Surgical technique

All surgeries were performed by the same staff and using the same standard technique.

Surgery began with CMIII procedure, in which a cut was performed into the atrial walls with scissors or electrocautery, followed by continuous suture with polypropylene. Then, resection of left atrial appendage and complete isolation of pulmonary veins were performed, with an ablation line directed toward the mitral valve annulus, an ablation line to the cavo-tricuspid isthmus, a communicating incision from superior to inferior vena cava, and resection of right atrial appendage. After that, if indicated, additional procedures, concomitant to CM III were performed. Patients were then transferred to the post-operative intensive care unit.

Statistical analysis

Categorical variables were expressed as frequencies and percentages. Normally distributed continuous variables were expressed as mean and standard-deviation. Continuous variables with non-normal distribution were expressed as median and interquartile ranges.

Efficacy of surgical treatment was assessed by the rate of maintenance of sinus rhythm at 6, 24 and 36 months. Survival rates free of stroke and death were estimate by Kaplan-Meier curves.

Univariate Cox regression analysis was used for demographic and clinical variables in the assessment of predictors of late AF recurrence. Variables with $p < 0.25$ in the uniariate analyses were included in the multivariate Cox regression analysis. The final multivariate logistic regression model was constructed by excluding the variables from the initial multivariate model, according to their relative importance (which was estimated by the similarity ratio test). The level of significance was set at 0.05, and analyses were performed using the SAS software, version 9.3.

Left atrial diameter was compared with late AF recurrence, and sensitivity and specificity indicators were calculated for the cutoff points and ROC curve. The chi-square test was used to assess the association between early AF recurrence and late recurrence, and to evaluate the impact of the use of arrhythmic drugs on late recurrence.

Results

Ninety-three patients underwent surgical treatment for AF combined with correction of structural heart disease between January 2006 and January 2013. Eighty patients met the inclusion criteria. Thirteen patients who survived the perioperative period were excluded, due to loss to follow up. Mean postoperative follow-up period was 27.5 months (3-89 months).

Baseline (pre-operative) demographic, clinical and echocardiographic data are described in Tables 1, 2 and 3, respectively. Patients were of moderately advanced age (mean of 50 years), and 57 (58.8%) were women. Persistent AF and long-standing persistent AF were the most prevalent conditions, found in 70 (87.5%) patients.

Heart valve disease was identified in 75 (93.8%), and rheumatic mitral valve disease was the most prevalent one, found in 63 (78.8%) patients. Coronary heart disease was diagnosed in 10 (12.5%) patients. There was a considerable increase (mean of 55 mm) in mean left atrial diameter.

Table 4 shows patients' operative data, including the type of surgery performed in combination with the CM III procedure. Sixty-seven (83.7%) patients underwent treatment of mitral valve, either alone or combined with other valve repair procedures. Myocardial revascularization (alone or in combination with valve repair) was found in 6 (7.5%) patients. Five patients underwent atrial septal repair by closure of the interatrial communication (6.3%).

Perioperative results

During hospitalization (perioperative period), 32 (40%) patients had early recurrent AF, atrial flutter or atrial tachycardia. Sixteen patients (20%) had bradyarrhythmias, including atrioventricular block, sinus node dysfunction, sinus bradycardia, and junctional rhythm. Three (3.8%) patients required permanent pacemaker implantation. No patient died in the perioperative period.

Table 1 – Demographic characteristics of patients

Characteristics	N = 80 patients	%
Male sex	33	41.25%
Age	49.94 ±12.06	
Duration of atrial fibrillation (months)*	15 (8-36)	
Paroxystic atrial fibrillation	10	12.5%
Persistent atrial fibrillation	23	28.75%
Long-standing persistent atrial fibrillation	47	58.75%

Values in mean ± standard deviation, or in median (interquartile range).

Table 2 – Clinical characteristics of patients in the preoperative period

Characteristics	N = 80 patients	%
Arterial hypertension	34	42.5%
Diabetes mellitus	6	7.5%
Coronary artery disease	10	12.5%
History of stroke/TIA	10	12.5%
Valve disease	75	93.75%
Rheumatic valve disease	63	78.75%
Congenital heart disease	4	5%
History of cardiac surgery	11	13.75
Medications		
Warfarin	49	61.25%
Amiodarone	21	26.25%
ACEIs/ARBs†	53	66.25%
Beta-blockers	61	76.25%

TIA: transient ischemic accident; ACEI: angiotensin converting enzyme inhibitors; ARBs: angiotensin II receptor blockers.

Table 3 – Echocardiographic characteristics of patients in the preoperative period

Echocardiography	N = 80 patients	%
Ejection fraction (%)	61(56-66)	
LA diameter (mm)	55.66±9.41	
LA volume index (mm/m^2) (n = 54)	61(44-94)	
LV diastolic diameter (mm)	51(45,5-60,5)	
LV systolic diameter (mm)	35(30-43)	
	Mild	64%
Tricuspid regurgitation degree (n = 79)	Moderate	29%
	Increased	7%
PASP (mmHg) /(n = 74)	47.5(40-60)	

** Values indicate Median (Interquartile Interval); † Values indicate Mean (standard deviation); ‡ Left atrial index volume: data obtained for 54 patients; § Degree of regurgitation of the tricuspid valve: data obtained for 79 patients; // PASP: pulmonary artery systolic pressure - data obtained for 74 patients. LA: left atrium; LV: left ventricle.*

Table 4 – Operative data of patients

COX-maze III-combined surgery	N = 80 patients	%
Mitral alone	29	36.25%
Mitral + tricuspid	27	33.75%
MRS alone †	2	2.5%
Mitral + MRS	3	3.75%
Aortic alone	2	2.5%
Aortic + MRS	1	1.25%
Mitral + aortic	3	3.75%
Mitral + aortic + tricuspid	5	6.25%
Others (including congenital	8	10%
Extracorporeal time (minutes) ‡	130 (117-150)*	
Time at postoperative ICU (days) (n = 78)	3 (3-5)*	

**Median and interquartile range; MRS: myocardial revascularization surgery; ICU: intensive care unit.*

On the day of discharge, 66 patients (82.5%) showed sinus rhythm and 14 patients (17.5%) did not. Three (3.8%) were using a pacemaker, and 13 (16.3%) had AF, atrial flutter or atrial tachycardia.

Clinical follow-up after hospital discharge

Rates of sinus rhythm maintenance at 6, 24 and 36 months were 88%, 85.1% and 80.6%, respectively. The number of patients who underwent Holter monitoring and electrocardiogram at these time points were 76, 46 and 31, respectively.

According to the multivariate analysis, predictors of late AF recurrence were female sex (HR 3.52; 95% CI 1.21–10.25; p = 0.02), presence of coronary artery disease (HR 4.73; 95% CI 1.37–16.36; p = 0.01) and increased left atrial diameter (HR 1.05; 95% CI 1.01–1.09; p = 0.02). For every one millimeter increase in left atrial diameter, AF recurrence increased by 5% (Table 5).

Late AF recurrence was found in 20.5% of patients with left atrial diameter ≤ 56 mm, and in 34.3% of those with left atrial diameter > 56 mm. Area under the ROC curve was 0.62 (95%CI 0.48-0.75), with sensitivity of 57% and specificity of 40%.

The impact of early recurrence of AF on late recurrence was evaluated by using the chi-square test (Table 6). No correlation between early and late AF recurrence was observed.

The effect of the use of antiarrhythmic drugs (amiodarone) on late recurrence of AF was analyzed from data of 78 patients (97.5% of total). Twenty-one patients used amiodarone at long-term follow-up; 10 (48%) of them without recurrent AF and 11 (52%) with recurrent AF. The chi-square test revealed an inverse relationship between the use of amiodarone and recurrence of arrhythmia, indicating that there was no protective effect of this medication on late arrhythmia recurrence (Table 7).

Mortality, incidence of stroke, and need for permanent pacemaker implant at late follow-up of CMIII

At long-term follow-up, one (1.25%) death was recorded due to complications of mitral valve surgical repair eight months after the CMIII procedure. Actuarial survival at 12, 24 and 48 months was 98.5%, and the number of exposed patients at these time points was 66, 47 and 18, respectively. Survival rates free of stroke at 12, 24 and 48 months were 100%, 100% and 97.5%. A total of 59 (75.0%) patients used oral anticoagulation at late follow-up, 44 (56.0%) because of prosthetic heart valve.

No patient required implantation of permanent pacemaker at late follow-up.

Discussion

The present study aimed to assess long-term results of the "cut-and-sew" CM III technique in a cohort of patients with AF associated with structural cardiac disease, in terms of maintenance of sinus rhythm, morbidity and mortality, and to determine possible predictors of late AF recurrence.

Success rate of maintenance of sinus rhythm and predictive factors of late recurrence of AF in patients undergoing CM III

Meaningful results were reported by several groups performing the CM III procedure in long-term maintenance of sinus rhythm, due to the consistent nature of the lesion in the atriums and the guarantee of obtaining transmural lesions by this technique.[9]

Stulak et al.[9] reported their experience with the surgical treatment of 1,540 patients with AF at Mayo Clinic, 514 of them undergoing the CM III procedure. In a median follow-up period of 34 months (maximum of 18.5 years), 80% of patients were free from AF and without antiarrhythmic medications. CMIII was superior in maintenance of sinus rhythm as compared with other surgical approaches for AF treatment.

Table 5 – Predictors of late recurrence of atrial fibrillation – crude hazard ratio and adjusted odds ratio for arrhythmia recurrence, by selected demographical and clinical variables

	Hazard ratio – HR (95% CI)			
	Crude	p value	Adjusted	p value
Sex		0.0633		0.0209
Male	1		1	
Female	2.60 (0.95 – 7.13)	0.0633	3.52 (1.21 – 10.25)	0.0209
Amiodarone		0.0892		
No	3.54 (0.82 – 15.24)	0.0892		
Yes	1			
Creatinine	0.14 (0.02 – 0.91)	0.0392		
Coronary artery disease		0.1852		0.0142
No	1		1	
Yes	2.11 (0.70 -6.38)	0.1852	4.73 (1.37 – 16.36)	0.0142
Left atrial diameter	1.04 (0.99 – 1.08)	0.0967	1.05 (1.01 – 1.09)	0.0256

Table 6 – Impact of early recurrence of atrial fibrillation on late recurrence of the disease

Late recurrence	Early recurrence (%)		p value
	Absent	Present	0.4066
Absent	37 (77.08)	22 (68.75)	
Present	11 (22.92)	10 (31.25)	
Total	48 (60.00)	32 (40.00)	

**chi-squared test.*

Table 7 – Association between antiarrhythmics and atrial fibrillation recurrence

Use of antiarrhythmics	Recurrence of AF (%)		Total
	No	Yes	
No	47 (82.46)	10 (17.54)	57
Yes	10 (47.62)	11 (52.38)	21

Chi-squared test = 9.47; p = 0.0021. AF: atrial fibrillation.

Kamata et al.[10] were one of the first authors to investigate the predictors of AF recurrence in the late postoperative period of CMIII. The authors demonstrated that a low "f" wave amplitude (<1mm) and left atrial diameter > 65 mm were inversely related with late sinus rhythm restoration.

In 2005, Gaynor et al.,[11] in a study evaluating the predictors of late AF recurrence after CM surgery, in a mean follow-up of 6 years, demonstrated that a longer duration of preoperative AF was correlated with higher incidence of late AF. In addition, CM III procedure achieved higher success rates compared with other versions of the CM procedure. Left atrial dimensions were not investigated in this study.

Left atrial diameter as a predictor of late AF recurrence after CM surgery was analyzed in a meta-analysis by Sunderland et al.[12] A diameter > 60 mm showed a sensitivity of 100% for AF recurrence, whereas as a diameter < 48.3 mm showed a sensitivity of 100% for reversal of the sinus rhythm. In the study by Gillinov et al.[13] performed at the Cleveland Clinic, the following predictors of AF recurrence after combined CM and mitral valve surgery were reported: longer duration of preoperative AF, larger left atrial diameter, older age, and higher left ventricular mass index. In agreement with these reports, our findings demonstrated that left atrial diameter was an independent predictor of late AF recurrence. However, in contrast with the literature, duration of preoperative AF and the type of AF were not associated with higher postoperative AF recurrence. This may be explained by the small number of patients.

Increased left atrial dimensions and AF with longer duration lead to greater degree of electrical and mechanical remodeling of the left atrium. According to Kottkamp,[14]

the presence of interstitial fibrosis would lead to abnormal conduction and activation of atrial electrical impulse, and increased risk for AF.

In our study, the presence of coronary artery disease was considered a predictive factor of AF recurrence, which may be analyzed under two aspects: first, this data corroborates previous findings suggesting that AF physiopathology in coronary patients is correlated with more advanced stages of myocardial dissease.[15,16] In this case, one may presume that these patients would be more likely to AF recurrence due to the presence of interstitial fibrosis in atrial tissue.

In contrast, in 2003, Damiano et al.[15] already showed excellent results of myocardial revascularization combined with CM III procedure in late efficacy and low mortality index in a group of 47 coronary patients. Other studies have demonstrated this favorable trend of AF surgical repair in coronary disease patients. In fact, current American[17] and Brazilian[18] cardiology societies' guidelines include all types of structural heart diseases as indications for combined treatment of AF by surgical approach.

In our study, female sex was significantly correlated with higher late AF recurrence, which was a distinct characteristic of our study group; or, rather, it may be resulted from the small size of the sample. Further studies are needed to draw conclusions of this relationship between sex and AF recurrence.

We did not find any correlation between early recurrence of AF and late recurrence of arrhythmia. There were 32 cases (40%) of early recurrence of atrial tachyarrhythmias. Other studies, such as those by Gaynor et al.[11] and Gillinov et al.,[13] reported 44% and 38% of early recurrence, respectively. In both studies, there was a high successful rate of sinus rhythm maintenance at long-term.

Up to 3 months after surgical treatment of AF, the substrates and triggers of recurrent atrial tachyarrhythmias may be different from the determinants of baseline arrhythmia and, for this reason recurrences within this period may not be considered a therapeutic failure *per se*.[19]

The presence or not of predictors of late AF recurrence is a valuable information to guide the surgical treatment of AF, especially in those patients with indications of combined surgical heart repair. In this context, back in 1999, Kalil et al[20] proposed that the Maze procedure should be performed in all patients with long-standing, persistent AF, undergoing mitral valve surgery.

According to Pinho-Gomes et al.,[21] surgical repair of AF in patients with rheumatic valve disease would have inferior results, due to the presence of fibrosis and more severe inflammation in these patients. However, this was not observed in the our study, although 78.8% of the patients had rheumatic valve disease. Albrecht et al.,[22] also investigating a group of patients predominantly composed of rheumatic patients undergoing two different surgeries, one of them modified CMIII, also achieved high success rates in the maintenance of sinus rhythm. Also corroborating with our findings, in the study by Abreu-Filho et al.,[23] 70 patients with rheumatic mitral valve disease and long-standing persistent AF were allocated to undergo mitral surgery alone or mitral surgery plus modified CM III. The results showed marked differences in sinus rhythm restoration, with favorable results for the group that underwent surgical correction of AF.

High success rates in sinus rhythm maintenance reported in the main centers may be influenced by insufficient monitoring of cardiac rhythm. In general, the longer the period of electrocardiographic monitoring, the higher the chance of AF recurrence, usually asymptomatic.[24] Based on American and European arrhythmia societies' recommendations,[8] the most effective method to assess the cardiac rhythm is Holter monitoring for up to 7 days.

In most of studies, including in the present one, cardiac rhythm was assessed by electrocardiography in each visit and by at least one 24-hour Holter recording.

Ad et al.[24] compared thee methods for the analysis of cardiac rhythm – electrocardiogram, 24-hour Holter monitoring and long-term (5 days) monitoring. The results revealed higher sensitivity of long-term cardiac monitoring over the other techniques.

In addition to assessing cardiac rhythm, we evaluated the possible effect of antiarrhythmic drugs on AF recurrence. In our study, the use of amiodarone did not provide additional protection to CM III procedure against late AF recurrence. This finding is similar to that reported by Schuetz et al.[25] on surgical treatment of AF using microwave energy ablation. Twelve months after surgery, the authors found no significant difference in AF recurrence between the groups treated and not treated with antiarrhythmic drugs.

Late results of CM surgery in terms of mortality, stroke and pacemaker implantation

There was one late death (1.3%); two (2.5%) patients had ischemic stroke, and no patient required pacemaker implantation.

Due to technical complexity of CM III, the surgery is not performed in some institutions, which may contribute to increased mortality and morbidity rates. The CM IV procedure has been proposed to simplify the original CM III. Weimer et al.[26] showed similar efficacy, shorter operating times and lower complication of CM IV compared with CM III. However, in a population of 212 patients, no difference in 30-day mortality was found between CM III and CM IV, despite the higher frequency of perioperative complications in the CM III group.

Combination of surgical treatment of AF with cardiac surgery causes a minimal increase in mortality and morbidity, with a mortality rate of 4%, in comparison with the rate of 3.3% of cardiac surgery alone.[27]

Although we did not have a control group composed of patients undergoing cardiac surgery alone, the occurrence of one death (1.3%) at long-term follow-up corroborates the literature in the sense that there was no significant effect of CM III on mortality.

An important issue to be discussed is the need of oral anticoagulation in preventing thromboembolic events, taking into consideration restoration and maintenance of sinus rhythm in the postoperative period of CM III. Dr. Cox himself has reported that the technique proposed by his group reduced the incidence of stroke in the perioperative period to less than 1%, and practically eliminated the risk of late stroke.[6]

In our study, the incidence of late stroke of 2.5% is in agreement with the reports of low incidence of cerebrovascular events. However, so far, there has been no strong evidence for interruption of oral anticoagulation in the postoperative period of surgical repair of AF, regardless of maintenance of sinus rhythm.[19] Decision on the suspension of anticoagulation should be individualized.

Another important issue related to CM III surgery is the higher requirement for pacemaker implantation for bradyarrhythmias. According to Gillinov et al.,[28] this may occur in 5-20% of cases. In our study, there were 3 patients (3.8%) in this condition. This may be explained not only by biatrial lesions performed during surgery, but also by an underlying sinus node dysfunction in many patients.[21]

Limitations

The present study has some limitations to be considered. One of them is the retrospective nature of the study, in which all patients who had submitted to CM III in the study period were included in the study. In addition, there was no control group for comparisons of cardiac rhythm in the postoperative period.

Second, assessment of cardiac rhythm was performed only by electrocardiogram and 24-hour Holter monitoring. We did not use other more sensitive methods for detection of AF recurrence, including Holter recordings for up to seven-day, and non-implantable and implantable cardiac monitors available at the market. In addition, success rates may have been overestimated in sinus rhythm maintenance.

Third, we did not analyze any parameter of left atrial contractility after AF surgical repair. It is known that, despite sinus rhythm recovery, a group of patients does not show any improvement of left atrial contractile function, which may result from scars and electrical isolation areas created during the procedure.[19]

Finally, although the low incidence of cerebrovascular events in the late post-operative period in our study was in agreement with previous studies, the possibility that it may have been influenced by the high prevalence of warfarin use (75% of patients) cannot be ruled out.

Conclusions

Information on the late results of CM III with respect to sinus rhythm maintenance and late recurrence of AF contributes to proper indication of the surgery combined with structural heart disease repair. In our population, success rates of late outcomes of the surgery were comparable with those of major centers. In addition, low mortality index and low incidence of stroke were achieved. On the other hand, increased left atrial diameter, coronary heart disease and female sex were shown to be suboptimal predictors of CM III surgery outcomes. The association between sex and CM III outcome needs to be confirmed by further studies.

Author contributions

Conception and design of the research and Statistical analysis: Gomes GG, Kessler IM, Atik FA; Acquisition of data: Gomes GG, Gali WL, Sarabanda AVL, Cunha CR, Atik FA; Analysis and interpretation of the data: Gomes GG, Gali WL, Sarabanda AVL, Kessler IM, Atik FA; Writing of the manuscript: Gomes GG, Atik FA; Critical revision of the manuscript for intellectual content: Sarabanda AVL, Kessler IM, Atik FA.

Potential Conflict of Interest

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Global Longitudinal Strain or Left Ventricular Twist and Torsion? Which Correlates Best with Ejection Fraction?

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Abstract

Background: Estimative of left ventricular ejection fraction (LVEF) is a major indication for echocardiography. Speckle tracking echocardiography (STE) allows analysis of LV contraction mechanics which includes global longitudinal strain (GLS) and twist/torsion, both the most widely used. Direct comparison of correlations between these novel parameters and LVEF has never been done before.

Objective: This study aims to check which one has the highest correlation with LVEF.

Methods: Patients with normal LVEF (> 0,55) and systolic dysfunction (LVEF <0,55) were prospectively enrolled, and underwent echocardiogram with STE analysis. Correlation of variables was performed by linear regression analysis. In addition, correlation among levels of LV systolic impairment was also tested.

Results: A total of 131 patients were included (mean age, 46 ± 14y; 43%, men). LVEF and GLS showed a strong correlation ($r = 0.95$; $r^2 = 0.89$; $p < 0.001$), more evident in groups with LV systolic dysfunction than those with preserved LVEF. Good correlation was also found with global longitudinal strain rate ($r = 0.85$; $r^2 = 0.73$; $p < 0.001$). Comparing to GLS, correlation of LVEF and torsional mechanics was weaker: twist ($r = 0.78$; $r^2 = 0.60$; $p < 0.001$); torsion ($r = 0.75$; $r^2 = 0.56$; $p < 0.001$).

Conclusion: GLS of the left ventricle have highly strong positive correlation with the classical parameter of ejection fraction, especially in cases with LV systolic impairment. Longitudinal strain rate also demonstrated a good correlation. GLS increments analysis of LV systolic function. On the other hand, although being a cornerstone of LV mechanics, twist and torsion have a weaker correlation with LV ejection, comparing to GLS. (Arq Bras Cardiol. 2017; 109(1):23-29)

Keywords: Stroke Volume; Torsion,Mechanical; Strain; Torsion Abnormality; Echocardiography,Doppler; Ventricular Dysfunction, Left.

Introduction

Left ventricle ejection fraction (LVEF) estimation is the major aim of an echocardiographic study and it is usually performed through Teichholz formula or by Simpson´s biplane rule. LVEF reflects myocardial contraction strength and is a longstanding recognized parameter in cardiology, important in a wide range of heart conditions.

STE is a relative new method but has already been extensively validated. By tracking myocardial speckles displacement, frame-by-frame, in an angle-independent way, it allows determination of multiple aspects of LV contraction mechanics such as segmental displacement and velocity, strain and strain rate, rotations, twist/torsion, and its derivatives. Integration of all these parameters comprises a very accurate and sensitive method, which fully characterizes LV systolic function.[1-3] This comprehensive analysis comprises determination of segmental displacement and velocity of wall motion, strain and strain rate, segmental rotations, twist/torsion, and their derivatives. Among all these parameters, global longitudinal strain (GLS) and twist/torsion are currently the most widely used (Figure 1).Torsional dynamics is the essence of LV contraction mechanics.[4-10] Direct comparison of correlations between these novel parameters and LVEF has never been done before. Clinical usefulness of this data rely especially on cases of borderline lower values of LV ejection fraction (0,50-0,55), were exists a possibility of a systolic ventricular dysfunction. This information is crucial and has a major role on patient treatment and prognosis.

In this study we sought to correlate these newer parameters of LV systolic evaluation with LVEF in order to determine which one has the highest correlation with this classical index in echocardiography.

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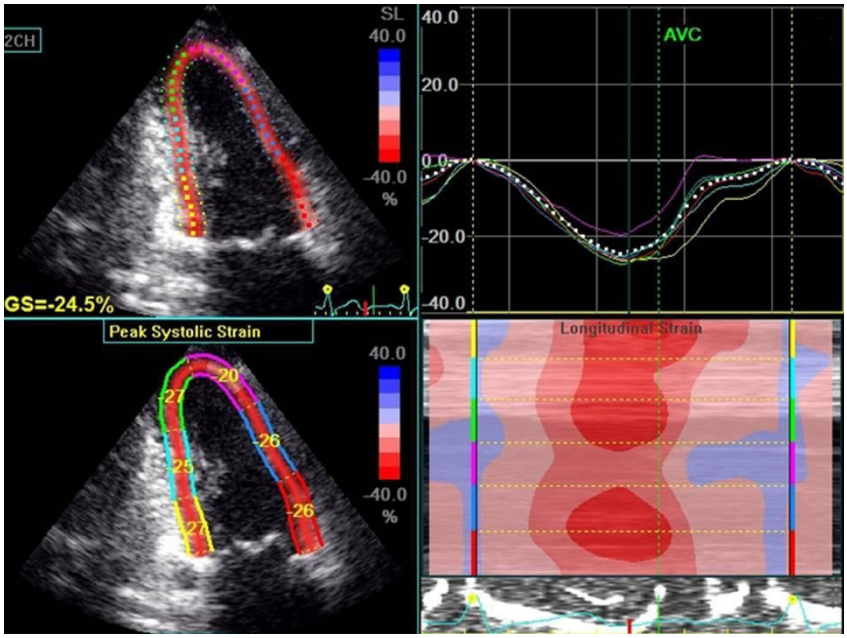

Figure 1 – *Example of global longitudinal strain analysis using speckle tracking echocardiography. GS: global longitudinal strain; AVC: aortic valve closure.*

Methods

Study participants

From January 2010 to August 2013, 135 patients were prospectively recruited to participate in this single center study. Normal volunteers and patients from a general cardiologic outpatient clinic were included. Enrolment of patients comprised all range of LVEF, from normal to severe systolic impairment. Exclusion criteria were the presence of supraventricular arrhythmias (atrial fibrillation or flutter), systemic blood pressure over 180/110 mmHg, history of myocardial infarction or coronary artery disease, pacemaker, significant thyroid disease, end-stage renal failure and patients younger than 18 years-old.

The institutional review board approved the study, and all participants gave informed consent. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Echocardiography and STE imaging acquisition

Echocardiography was performed on commercially available echocardiographic platforms equipped with MS5 probe (GE Vivid 7 and E9, GE Healthcare, Milwaukee, Wis). Comprehensive 2D-Echocardiogram and Doppler evaluation was performed following the recommendations of the American Society of Echocardiography.[11] LVEF was measured by Simpson´s rule. Diastolic function was evaluated by mitral inflow E/A pattern and annular tissue Doppler curves (e´/a´). Valves were assessed by color, pulsed and continuous Doppler.

The echo-STE protocol included acquisition of short axis and apical views. Parasternal short-axis views were obtained at the LV base (mitral valve level) and at the LV apex, close to apical obliteration when there is still a clear visualization of segments. For this apical "cut", in order to avoid quantification bias, we created another new criterion: a clear visual identification of the apex counterclockwise rotation.

Left ventricular twist is the wringing motion of heart around its long axis. It is calculated as the net absolute difference between apical and basal rotations ($LV_{twist} = ROT_{apical} - ROT_{basal}$). Torsion is a normalization of LV twist to the length of LV long axis (LV_{twist}/LV_{lenth}). By widely assumed convention, apical rotation had positive values and basal, negative (Figure 2).[12]

Acquisition of apical views (A3C, A2C and A4C) followed transversal images. Images were acquired at a frame-rate of 40–80 fps. Three consecutive heart cycles were stored.

Speckle tracking analysis was performed offline using a dedicated software (EchoPAC, v. BT10, GE Heathcare). For short axis images[7-12] and for apical 3 anchor points were placed. The software automatically defined the region of interest (ROI) for the entire myocardial layer, which was divided in six color-coded segments (total: 18 segments). Careful attention was especially given to not include myocardial trabecullaes and the pericardium. Adjustments were possible. Following this step, an automatic tracking of myocardial speckles were performed and final results on the quality of this tracking were given for each color-coded segment. If there was a suboptimal tracking of one segment, adjustment was also possible. After accepting this analysis, curves were given for all variables studied and this data exported to a spreadsheet. Global values were defined as the average of segments analyzed.

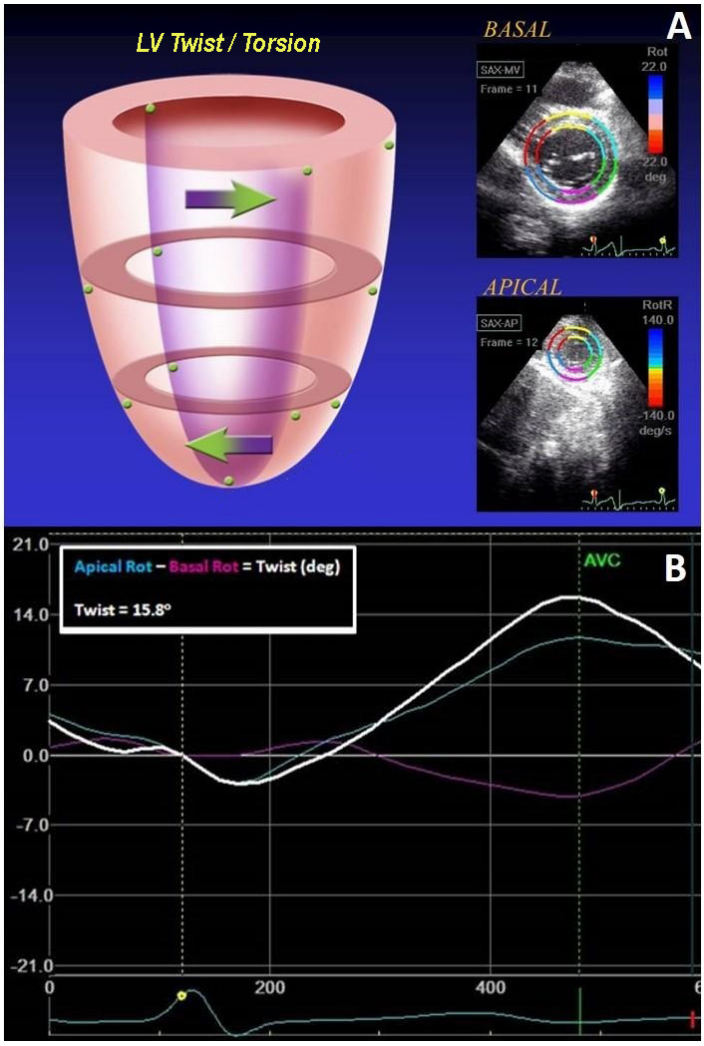

Figure 2 – *A: Representation of LV twist/torsion – clockwise rotation at the base and counterclockwise at the apex (view from the apex). B: Example of LV twist analysis (white line, LV twist; cyan line, apical rotation; pink line, basal rotation).*

Analyzes of correlation was performed using global data and by groups, according to their LVEF: group 1 (LVEF > 0.55), group 2 (LVEF: 0.55-0.30) and group 3 (LVEF < 0.30).

Statistical analysis

Continuous variables are presented as mean ± SD, and categorical variables as numbers and proportions. Kolmogorov-Smirnov test and a histogram analysis were performed to check normality of data distribution. Variables analyzed were assumed to have a normal distribution. Correlation of variables was performed by linear regression analysis with determination of Pearson´s correlation coefficient. Six patients were randomly chosen, three with normal LVEF and three with systolic dysfunction, for analysis of interobserver and intraobserver variability. Two-tailed p values < 0.05 in a confidence interval of 95% were considered statistically significant. Statistics was performed using SPSS 20.0 for Macintosh (SPSS Inc., Chicago, IL).

Results

Among the 135 initially patients enrolled for this study, 4 were excluded because STE analysis was not possible due to poor acoustic images. Therefore, final study population was represented by a total of 131 subjects. The overall feasibility for STE analysis was 97%. Mean age was 46 ± 14 y and 57 (43%) patients were men. A total of 27 (20.6%) individuals had hypertension.

Clinical baseline characteristics are described in table 1. The larger amount of patients was in class I (NYHA) of congestive heart failure functional classification and among all cardiovascular medication routinely prescribed, angiotensin converting enzyme inhibitor, β-blocker and diuretics were the most in use.

Conventional echocardiographic features and data from STE analyzes are shown in table 2. Mean LVEF was 0.52 ± 0.17, ranging from 0.12 to 0.72. Mean values and ranges from

Table 1 – Clinical, demographic and hemodynamic characteristics

Variables	
Age (y)	46 ± 14
Gender M	57 (43%)
Weight (Kg)	70.3 ± 14.4
Height (cm)	165 ± 10
BS (m^2)	1.77 ± 0.21
BMI (kg/m^2)	25.6 ± 3.9
SAH	27 (21%)
DM	6 (5%)
CHF (NYHA) (NYHA)†	
I	38 (29%)
II	20 (15%)
III/IV	3 (2%)
Therapy	
Digital	10 (8%)
ACEi	46 (35%)
βblock	50 (38%)
ARB	14 (11%)
Diuretics	40 (30%)
Aldost. Ant.	31 (24%)
HR (bpm)	69 ± 12
SBP (mmHg)	123 ± 15
DBP (mmHg)	75 ± 11

Continuous variables expressed as mean ± SD. Categorical variables expressed as frequency (proportion). BS: body surface; BMI: body mass index; SAH: systemic arterial hypertension; DM: diabetes mellitus; CHF (NYHA): functional class of congestive heart failure; ACEi: angiotensin converting enzyme inhibitor; β block: beta blocker, ARB: angiotensin II receptor blocker; Ca ++ block: calcium channel blocker; Aldost Ant: aldosterone antagonist; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2 – Echocardiographic variables

Variables	
LA (mm)	37.3 ± 6.2
LVDD (mm)	54.7 ± 10.7
LVSD (mm)	40.8 ± 13.8
LVFS (%)	26.7 ± 10.8
LVEDV (ml)	138.9 ± 66.3
LVESV (ml)	76.2 ± 61.2
LVEF (%)	51.7 ± 17.2
Diastolic Dysfunction	
Normal	71 (54%)
Grade I	40 (30%)
Grade II	14 (11%)
Grade III	1 (1%)
Grade IV	5 (4%)
E wave (m/s)	0.77 ± 0.21
EDT (ms)	214.0 ± 65.9
A wave (m/s)	0.60 ± 0.21
s' (cm/s)	0.06 ± 0.02
e' (cm/s)	0.08 ± 0.03
a' (cm/s)	0.07 ± 0.02
E/e'	12.7 ± 8.3
MR Grade	
Absent/Trivial	77 (59%)
Mild	38 (29%)
Moderate	10 (8%)
Severe	6 (5%)
GLS (%)	-17.64 (± 5.73)
GLSR (1/s)	-1.00 (± 0.27)
Twist (°)	14.91 (± 7.08)
Torsion (°/cm)	1.78 (± 0.91)

Continuous variables expressed as mean ± SD. Categorical variables expressed as frequency (proportion). LA: left atrium; LVDD: left ventricular diastolic diameter; LVSD: left ventricular systolic diameter; LVFS: left ventricular fractional shortening; LVEDV: left ventricle end-diastolic volume; LVESV: left ventricle end-systolic volume; LVEF: left ventricular ejection fraction; E wave: E wave velocity, EDT: E wave deceleration time; A wave: A wave velocity; s': s' wave velocity; e': e' wave velocity; a': a' wave velocity; MR degree: degree of mitral regurgitation.

STE data are as follow: GLS, 17.64% ± 5.73 (3.47 –26.46); GLSRs, 1.00 s^{-1} ± 0.27 (0.39 – 1.58); Twist, 14.90° ± 7.08 (-9.54 – 31.60); Torsion, 1.78 °/cm ± 0.91 (-1.03 – 4.05).

A very strong correlation was identified between LVEF and GLS (r = 0.95; r^2 = 0.89; p < 0.001) (Figure 3). Correlation between LVEF and GLSRs was also good (r = 0.85; r^2 = 0.73; p < 0.001). On the other hand, comparing to these longitudinal parameters, correlation of LVEF and torsional mechanics was weaker: twist (r = 0.78; r^2 = 0.60; p < 0.001); torsion (r = 0.75; r^2 = 0.56; p < 0.001).

Analyzes of correlations according to levels of systolic impairment data is provided in table 3. Correlation was stronger between GLS and LVEF in groups with systolic mild/moderate dysfunction (r = – 0.88; p < 0.001) and severe (r = – 0.82; p < 0.001). On the other hand, this correlation was very weak in cases with preserved LV contraction (r = 0.40; p < 0.001).

Intraobserver and interobserver variabilities

Interobserver and intraobserver variabilities for longitudinal parameters were 6%, and 5%, respectively, with lower variability for longitudinal strain (3 and 4%).

For the variables obtained from short axis view, including twist and torsion, interobserver variability was 23%. Torsion had the highest (38%). Intraobserver variability was 19%. Basal rotation had the highest (32%).

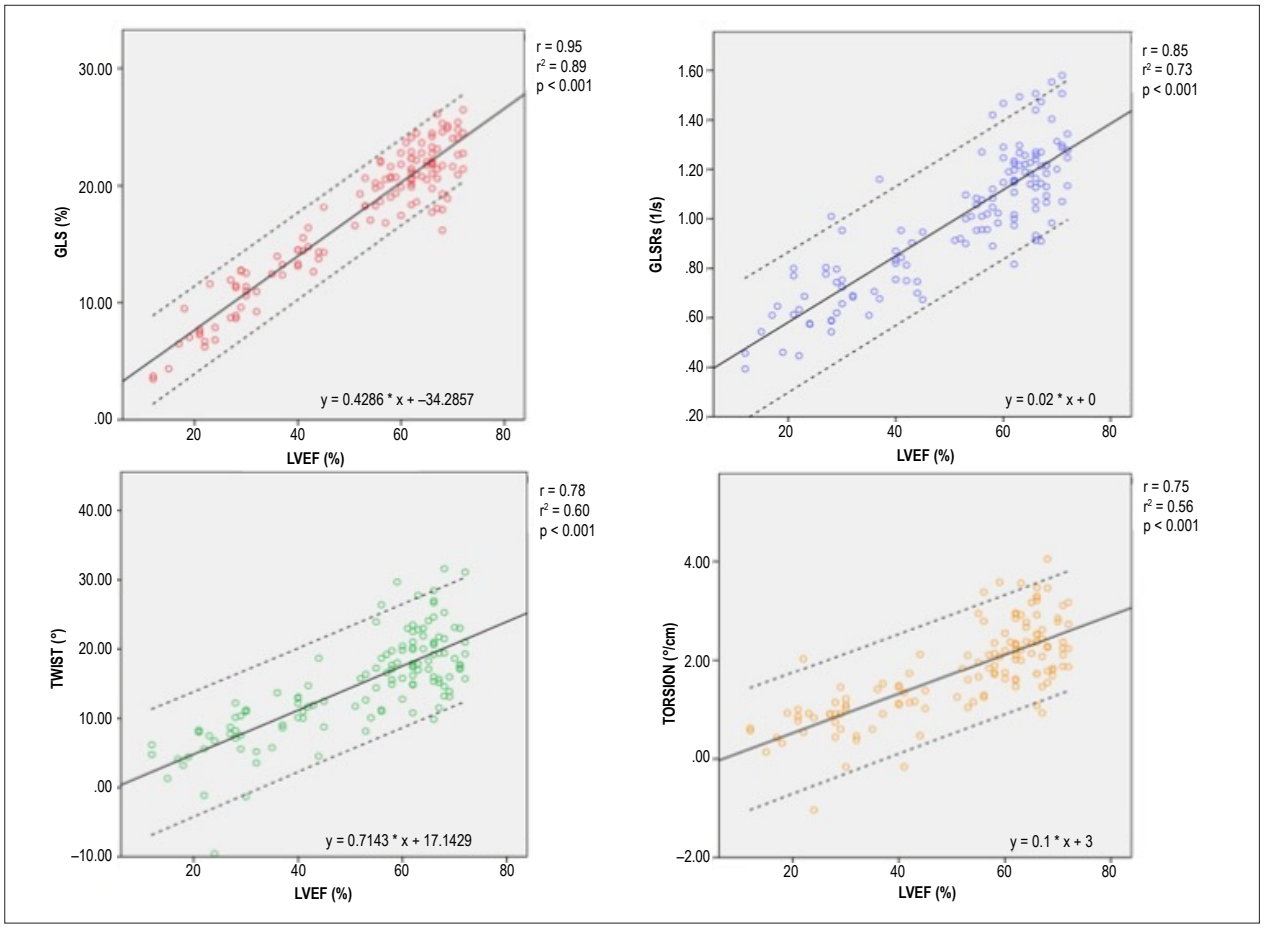

Figure 3 – *Correlation of different LV contraction parameters with LVEF (p < 0.001 for all correlations). GLS and GLSRs are displayed in absolute values. LVEF: left ventricle ejection fraction; GLS: global longitudinal strain; GLSRs: systolic global longitudinal strain rate.*

Table 3 – Correlation between LVEF and parameters of LV contraction mechanics, according to levels of systolic impairment. Pearson's coefficient (r)

	LVEF > 0.55	LVEF 0.55 – 0.30	LVEF < 0.30
LVEF X GLS	– 0.40 *	– 0.88 *	– 0.82 *
LVEF X GLSRs	– 0.36 $^{\Delta}$	– 0.57 $^{¥}$	– 0.55 $^{\Phi}$
LVEF X Twist	0.13 $^{\Phi}$	0.44 $^{¥}$	0.34 $^{\Phi}$
LVEF X Torsion	0.14 $^{\Phi}$	0.45 $^{¥}$	0.23 $^{\Phi}$

*LVEF: Left ventricle ejection fraction; GLS: Global longitudinal strain; GLSRs: Systolic longitudinal strain rate. * p < 0.001; $^{\Delta}$ p = 0.02; $^{¥}$ p = 0.03; $^{\Phi}$ p = NS*

Discussion

In this study we sought to correlate these newer parameters of systolic evaluation with LVEF, in order to determine which one has the highest correlation with this classical index in echocardiography. Our major interest was in GLS and LV twist/torsion correlations, as they are the most used.

Our results showed a very strong correlation between LVEF and GLS. Such correlation has already been demonstrated experimentally by Weideman et al.[13] and in previous clinical studies by Reant et al.,[14] Hayat et al.[15] and Kleijn et al.[16] These authors also found this good correlation, especially with global area strain measurement using tridimensional speckle tracking echocardiography (r = 0.81–0.91). Goo-Yeong Cho et al.[17] tested GLS and circumferential strain as surrogates of LVEF as prognostic tool for cardiac adverse events in patients with acute heart failure. Both of them were independent prognostic predictors of death and readmission for heart failure.[17]

Our aim was also to seek correlation with other parameters of LV torsional mechanics, twist and torsion. We also demonstrated a good correlation with LVEF, but not as strong as we found with GLS. An explanation for this fact may reside on tridimensional motion of myocardial segments. As 2D-STE misses one

orientation of this movement, accuracy of tracking myocardial speckle decreases, possibly affecting these values. This is more significant on LV short-axis, where circumferential and radial measurements are made. Out-of-plane longitudinal movement is missed and has a reasonable impact on tracking, sometimes appearing as noise. On the other hand, LV circumferential and rotation movement does not have a substantial impact on longitudinal axis slightly affecting the tracking.[18]

Clinical aspects

Results raised from this study have a clinical and practical significance, especially in cases of LVEF estimated in its lower normal limits (LVEF 0,50–0,55). In such cases, GLS may help to objectively define LV contraction strength. Lower values of GLS in a setting of a normal LVEF may represent an ejection fraction overestimation or a possible decrease in myocardial deformation, a step just before a future global LV contraction reduction. In addition, GLS analysis is relatively easy to be performed, taking only a few minutes during a conventional echocardiogram and adds a sensitive and objective parameter to left ventricle systolic function evaluation.

Finally, despite having a worse correlation with LVEF, LV twist and torsion are still good sensitive parameters that can add an objective characterization of myocardial global systolic function.

Limitations

Notwithstanding the fact that STE method was extensively validated, it is an evolving technique, and improvements, such as on tracking accuracy, are still needed. Additionally, this accuracy is also highly dependent on image quality. Suboptimal resolution can produce a negative impact on final results.

In this study, we used 2D-STE precluding the analysis of tridimensional myocardial segments movement. The lack of analysis of one out plane movement may have had some impact on final result. Currently, 3D-STE may overcome this drawback.[19]

The subjectivity of echocardiography can bring biases of quantification. This is exemplified when referring to the "cutting" level of the LV in its short axis. Anatomical landmarks were followed to try to standardize levels, such as mitral valve to the basal level and the papillary muscles to the medium level. However, for the apical segment, there is no anatomical marker and small variations on the level of image acquisition can lead to distorted values. In order to preclude this fact, we set another new criterion: a visual identification of, at least, a tendency of rotation of the apex (differentiating from LV middle level).

Conclusions

GLS of the left ventricle have highly strong positive correlation with the classical parameter of ejection fraction, especially in cases with LV systolic impairment. Longitudinal strain rate also demonstrated a good correlation. Clinical usefulness of this data rely especially on cases of borderline lower values of LV ejection fraction (0,50–0,55), were exists a possibility of a systolic ventricular dysfunction. GLS increments analysis of LV systolic function. On the other hand, although being a cornerstone of LV mechanics, twist and torsion have a weaker correlation with LV ejection, comparing to GLS.

Author contributions

Conception and design of the research, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Lima MSM, Villarraga HR, Mathias Junior W, Tsutsui JM; Acquisition of data: Lima MSM, Abduch MCD, Lima MF, Cruz CBBV, Sbano JCN; Analysis and interpretation of the data and Obtaining funding: Lima MSM, Tsutsui JM; Statistical analysis: Lima MSM, Villarraga HR.

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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Impact of the Use of Different Diagnostic Criteria in the Prevalence of Dyslipidemia in Pregnant Women

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Abstract

Background: There is a physiologic elevation of total cholesterol (TC) and triglycerides (TG) during pregnancy. Some authors define dyslipidemia (DLP) in pregnant women when TC, LDL and TG concentrations are above the 95th percentile (p95%) and HDL concentration is below the 5th percentile (P5%) for gestational age (GA).

Objective: To compare the prevalence of DLP in pregnant women using percentiles criteria with the V Brazilian Guidelines on Dyslipidemia and the association with maternal and fetal outcomes.

Results: Pregnant women with high-risk conditions, aged 18-50 years, and at least one lipid profile during pregnancy was classified as the presence of DLP by two diagnostic criteria. Clinical and laboratorial data of mothers and newborns were evaluated.

Conclusion: 433 pregnant women aged 32.9 ± 6.5 years were studied. Most (54.6%) had lipid profile collected during third trimester. The prevalence of any lipid abnormalities according to the criteria of the National Guidelines was 83.8%: TC ≥ 200 mg/dL was found in 49.9%; LDL ≥ 160 mg/dL, in 14.3%, HDL ≤ 50 mg/dL in 44.4% and TG ≥ 150 mg/dL in 65.3%. Any changes of lipid according to percentiles criteria was found in 19.6%: elevation above the P95% for TC was found in 0.7%; for LDL, 1.7%; for TG 6.4% and HDL lower than the P5% in 13%. The frequency of comorbidity: hypertension, diabetes, smoking, obesity and preeclampsia was similar among pregnant women when DLP was compared by both criteria.

Conclusion: The prevalence of DLP during pregnancy varies significantly depending on the criteria used, however none demonstrated superiority in association with comorbidities. (Arq Bras Cardiol. 2017; 109(1):30-38)

Keywords: Dyslipidemias / diagnosis; Pregnancy / high-risk; Pregnancy Complications; Lipids; Prevalence.

Introdutcion

Lipids and lipoproteins change in gestation due to interactions between genetic, energetic and hormonal factors. Gestational hyperlipidemia is physiological and results from increased insulin resistance, lipoprotein synthesis and lipolysis in adipose tissue that mobilize fats to serve as an energetic substrate for fetal growth.[1-4]

The majority of pregnant women presents an increase in triglycerides (TG) in the third trimester, high density lipoproteins (HDL) in the second half of gestation, and progressive increasing of intermediate and low density lipoproteins (IDL) (LDL) over the trimesters.[5] In the last trimester, total cholesterol (TC) may increase by 25 to 50% and TG by 200 to 400%.[6-7]

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Dyslipidemia (DPL) in pregnancy is characterized by TG and TC concentrations above the 95th percentile and HDLs below the 5th percentile[8], and this definition differs from that used for adults according to the *Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults* (NCEP)[9] used in the V Brazilian Directive. Several researchers evaluated the lipids in gestation using the criterion of percentiles[5;10-14] and reference values per quarter were established.[2]

Gestational hyperlipidemia is associated with metabolic morbidities such as obesity[15-16] and gestational diabetes[1;6] and is a risk factor for acute pancreatitis[17], preeclampsia[3,15,18,19] and preterm birth.[18-20] Hypertriglyceridemia at the end of gestation is associated with the development of DLP in the postpartum decades[2;8] and the offspring is at greater risk of being born large for gestational age[21] and having atherosclerosis in adult life.[22-25]

Although pregnancy is recognized as a potential cause of DLP,[26] the lipid profile is not part of the routine obstetric exams.[27] The lack of consensus regarding reference values per trimester, lack of standardization of the diagnostic criteria, lack of determination of risk groups and of evidences demonstrating the impact of DLP treatment on pregnancy limit the accomplishment of screening.

In a population of high-risk pregnant women, we compared the prevalence of DLP defined by the criteria of the V Brazilian Dyslipidemia Directive with the specific criteria for pregnancy. We also evaluated the agreement between the criteria and the relationship between maternal lipid profile and maternal-fetal outcomes.

Methods

Study population

The population of the study was pregnant with an age between 18 and 50 years old, accompanied at the outpatient clinic of endocrine diseases during the gestation of the Maternity Professor José Maria de Magalhães Neto (MPJMMN) of Santa Casa da Bahia between April 2010 and March 2014. Those who had at least one lipid profile evaluation during gestation analyzed in a single laboratory and delivery in the MPJMMN. Demographic, clinical, obstetric, laboratorial, and labor data were obtained from medical records. The study was approved by the ethics and local research committee.

Measurement of lipids

Blood samples were collected after a fast of 12-hour. Plasma concentrations of total cholesterol, HDLc and TG were determined by the automated colorimetric enzymatic method and LDL cholesterol was measured by the automated kinetic method. In pregnant women with more than one lipidogram collected during pregnancy, the first examination was considered for analysis. Less than half had more than one lipidogram in gestation. They made, respectively, two, three and four lipidograms during pregnancy: 109 (25.2%), 31 (7.2%) and 6 (1.4%). The gestational age (GA) at weeks at the time of blood collection was obtained from estimates of gestational age during the first ultrasound (USG). In patients in whom the first USG was not available, GA was estimated at the date of collection of the lipidogram by the date of the last menstrual period.

Definition of dyslipidemia in pregnancy

The prevalence of dyslipidemia was evaluated considering two definitions: 1. "Percentile criteria" when there was elevation of TC, LDL-c and TG concentrations above the 95th percentile and HDL-c levels below the 5th percentile for gestational age.[5] Normal values in pregnant women in the first, second and third gestational trimesters were obtained from the study of Piechota W and col[2] and are available in table 1. 2. "Criteria of the V Brazilian Dyslipidemia Guideline" when TC, LDL and TG were, respectively, higher than 200 mg/dl, 160 mg/dl and 150 mg/dl, HDL-c, lower than 50 mg/dl.[28] It was characterized as a carrier of dyslipidemia in pregnant women with at least one lipid fraction being altered.

Maternal weight gain

Weight gain by the pregnant woman was categorized as below, in or above the target as recommended by the *Institute of Medicine*,[29] which guides different weight gain intervals according to pregestational BMI.

Neonatal outcomes

Neonates were classified as small for gestational age (SGA) when birth weight was below the 10th percentile and large for gestational age (LGA) when the weight was above the 90th percentile for gestational age and gender. The reference used was the Brazilian population of Pedreira et al.[30] (2011).

Prematurity was defined as gestational age at birth between 24 and 36 weeks and 06 days of gestation. Preterm neonates were classified according to severity of prematurity in: preterm (< 31 weeks), preterm (31-33 weeks and 6 days) and preterm (4 to 36 weeks and 6 days) . Gestation dating was established through the first USG and somatic Capurro. In cases in which the first USG was not available (0.81%), GA was estimated at birth by LMP (Last Menstrual Period) and somatic Capurro.

Statistical analysis

The data were analyzed to characterize the distribution normality by the Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean ± standard deviation and, for non-normal distributions, as median and interquartile range. Categorical variables were reported in absolute and percentage values. The subjects were categorized as having dyslipidemia for each of the two criteria and the statistical differences between continuous variables were obtained by means of the unpaired Student t test when the variables had normal distribution or by the *Mann-Whitney* U when they had a non-parametric distribution. The association between laboratory data of lipids and fractions, whose distribution was non-normal, with clinical variables: gestational age at birth, newborn weight and maternal weight gain were investigated using *Spearman*'s correlation tests. The concordance between the two criteria defining dyslipidemia was evaluated by the Kappa index. ROC curves were created, two in which the test variable was the percentile dyslipidemia criterion (curves A and B) and two (curves C and D) with the guideline criterion to determine the accuracy in predicting dichotomous weight outcomes of the

Table 1 – Percentiles 95 for TC, LDLc and TG and 5 for HDLc in mg/dl according to Piechota and Staszekski[2]

Period	TC (mg/dl)	LDLc (mg/dl)	HDLc (mg/dl)	TG (mg/dl)
Out of pregnancy	251	167	34	171
1° Quarter	277	186	35	175
2º Quarter	319	217	42	254
3º Quarter	380	250	40	414

TC: total cholesterol; LDL: low density lipoproteins; HDL: high density lipoproteins; TG: triglycerides.

neonate above the 90% percentile and gestational age at birth of 37 weeks or less. We calculated the area under the curve and the 95% confidence interval. Value of $p < 0.05$ was considered statistically significant. All analyzes were performed in the SPSS version 13 program.

Results

A total of 433 pregnant women with a mean age of 32.9 ± 6.4 years and mean gestational age of 24.8 ± 7.6 weeks were evaluated. The main reason for referral to the outpatient clinic for endocrine diseases during pregnancy was diabetes, which represented 84.8% of the cases, thyroid diseases accounted for 6.9% of referrals and 43.2% were hypertensive. The clinical and demographic characteristics of the population are shown in table 2.

Dyslipidemia due to elevation of TC, LDLc and TG or reduction of HDLc was 4,3 times more frequent when dyslipidemia was considered by the V Dyslipidemia Guideline criterion in relation to percentile criterion (83.8 vs 19.6%). The 85 cases of dyslipidemia identified by the percentile criterion were among the 363 cases identified by the V guideline criterion. The most commonly found lipidic alteration was the reduction of HDLc, according to the criterion of percentiles and elevation of triglycerides, according to the criterion of V guideline (table 3). In addition, there was an increase in the frequency of any of the lipid changes with the progression of the quarters (figures 1 and 2).

Table 2 – Clinical and demographic characteristics of the general population categorized according to the presence of any lipidic alteration according to the percentiles criteria and the Brazilian V guideline (n = 433, results expressed as mean, standard deviation, median and interquartile range)

	General n = 433	Dyslipidemia Percentile criteria n = 85	Dyslipidemia Guideline criteria n = 363	No data	p
Age§	32,9 ± 6,5 33,1 (28,5–37,9)	31,9 ± 6,4 32,0 (27,6–37,5)	32,9 ± 6,4 33,1 (28,7–37,9)	0	0,1
First time mother(%)	24,7%	31,0%	25,7%	44	0,19
N by quarter (1°, 2° e 3°)	23/173/237 5,4/40,0/54,6%	4/34/47 4,7/40,0/55,3%	14/130/219 3,9/35,8/60,3%	0	
BMI pre-gestacional§ (kg/m2)	30,1 ± 6,5 29,7 (25,4–34,2)	29,0 ± 6,9 27,4 (23,4–32,5)	30,3 ± 6,5 30,1 (25,9–34,3)	108	0,09
Previous SAH (%)	167 (43,2%)	27 (38,6%)	141 (43,4%)	46	0,41
DM (%)	323 (84,8%)	57 (82,6%)	275 (85,7%)	52	0,39
Previous DLP (%)	80 (21,0%)	19 (27,9%)	69 (21,6%)	52	0,1
Smoking(%)	9 (2,4%)	3 (4,4%)	8 (2,5%)	51	0,25
Previous preeclampsia (%)	42 (14,0%)	7(14,9%)	36 (1,4%)	133	0,86
Previous preterm birth (%)	89 (20,6%)	18 (21,2%)	70 (19,3%)	0	0,58
Delivery mode (vaginal)	152 (35,1%)	31 (36,5%)	133 (36,6%)	0	0,92
TC (mg/dl) ¶	204,0 ± 83,1 199,0 (169,0–229,0)	212,8 ± 167,9 190,0 (151,0–235,0)	211,2 ± 88,2 206,1 (175,0–235,3)	6	0,005
LDLc (mg/dl) ¶	109,7 ± 42,8 105,0 (81,2–131,0)	115,2 ± 61,1 104,0 (71,0–151,0)	114,9 ± 43,6 111,0 (85,0–136,0)	22	0,22
HDLc (mg/dl) ¶	55,2 ± 15,1 54,0 (45,0–63,2)	40,9 ± 13,0 39,0 (35,0–45,8)	53,7 ± 15,4 51,0 (44,0–62,8)	11	< 0,0001
TG (mg/dl) ¶	199,9 ± 176 176,0 (136,0–229,0)	296,6 ± 664,2 224,0 (152,0–272,0)	215,8 ± 327,8 191,0 (152,0–237,0)	12	0,002
GA in childbirth (weeks) ¶	37,5 ± 3,0 38,0 (37,0–39,0)	37,4 ± 2,6 38,0 (37,0–39,0)	37,6 ± 2,9 38,0 (37,0–39,0)	0	0,15
Newborn weight (g) ¶	3187 ± 852 3325 (2794–3677)	3183 ± 792 3335 (2785–3695)	3195 ± 806 3325 (2812–3686)	0	0,94
Glycated hemoglobin (g/dl) ¶	6,3 ± 1,7 6,0 (5,3–7,2)	6,7 ± 1,9 6,5 (5,4–7,9)	6,3 ± 1,6 6,0 (5,3–7,1)	141	0,09
Weight gain (kg) §	8,8 ± 8,3 8,6 (3,7–13,3)	7,9 ± 8,2 8,3 (1,1–14,2)	8,6 ± 8,2 8,7 (3,6–13,6)	136	0,46
BMI adequacy B/I/A*	112/85/97 38,1/28,9/33	22/12/16 44,0/24,0/32%	92/69/84 37,6/28,2/34,3	139	

BMI: body mass index; hypertension: systemic arterial hypertension; DM: diabetes mellitus; DLP: dyslipidemia; GA: gestational age at delivery. TC: total cholesterol; LDL: low density lipoproteins; HDL: high density lipoproteins; TG: triglycerides. Adequacy of weight according to Institutes of Medicine (IOM): B low, I ideal and A high. For the p values: the groups were compared according to the presence of dyslipidemia considering the two criteria, when the distribution was normal, the Student's t test was used; When the non-parametric distribution was used, the Mann Whitney U test was used.*

Table 3 – Prevalence of dyslipidemia according to the two criteria (n = 433)

Cholesterol and fractions	Percentile criteria General prevalence n (%) Prevalence per quarter n (%)	Guideline criteria General prevalence n (%) Prevalence per quarter n (%)	No data
TC	3 (0,7) 0 (0); 2 (66,7); 1 (33,3)	213 (49,9) 4 (1,9); 67 (31,5);142 (66,7)	6
LDL	7 (1,7) 1(14,3); 1(14,3); 5(71,4)	40 (11) 0 (0); 13 (32,5); 27 (67,5)	22
HDL	55 (13) 1 (1,8); 18 (32,7); 36 (65,5)	161 (44,4) 12 (7,5); 51 (31,5); 98 (60,9)	11
TG	27 (6,4) 2 (7,4); 18 (66,7); 7 (25,9)	275 (65,3) 3 (1,1); 94 (34,2); 178 (64,7)	12
Any lipidic alteration	85 (19,6) 4 (4,7); 34 (40); 47 (55,3)	363 (83,8) 14 (3,9); 130 (35,8); 219 (60,3)	22
All lipidic alteration	0 (0)	13 (3,6) 0(0); 2(15,4); 13(84,6)	22

TC: total cholesterol; LDL: low density lipoproteins; HDL: high density lipoproteins; TG: triglycerides.

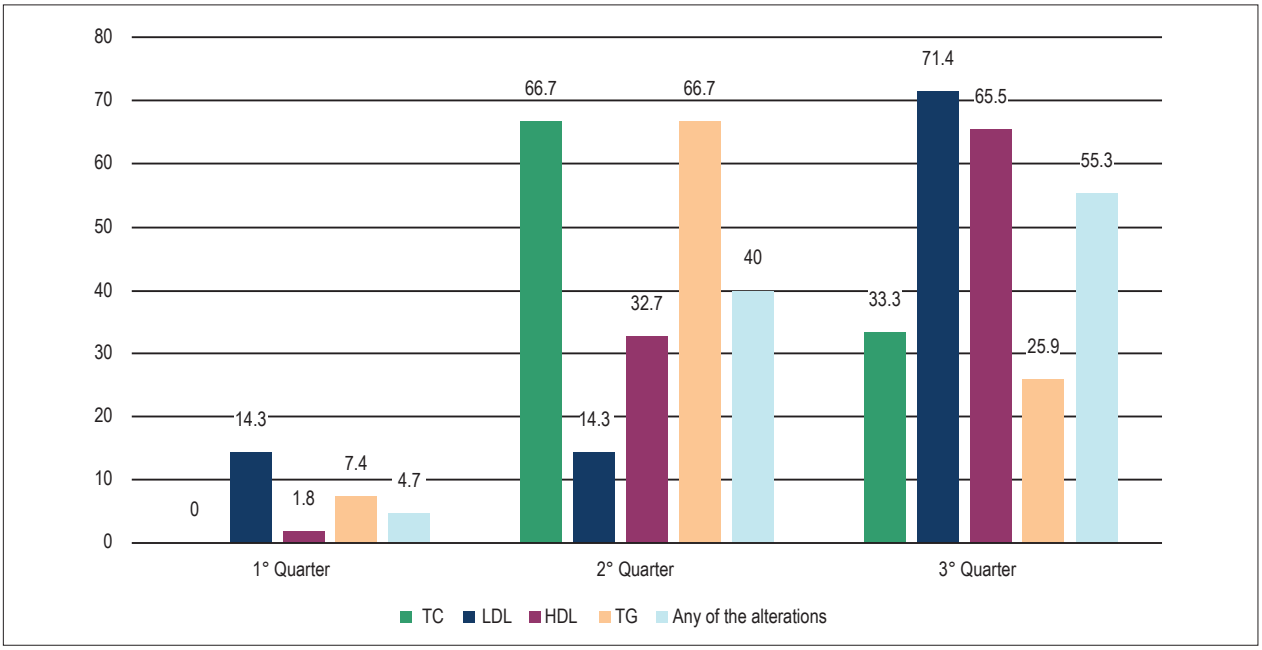

Figure 1 – *Prevalence of dyslipidemia according to percentile criteria. TC: total cholesterol; LDL: low density lipoproteins; HDL: high density lipoproteins; TG: triglycerides.*

The frequency of comorbidities SAH, DM, smoking, obesity and previous preeclampsia was similar when compared to pregnant women without dyslipidemia by any of the criteria. p = 0.005) and HDLc (39 vs 51 mg/dl, p = < 0.0001) were lower in patients with dyslipidemia by the criterion of percentiles, while the TG concentration was significantly higher (224 vs. 191 mg/dl, p = 0.002). There were no correlations between cholesterol and fractions with gestational age at birth and neonatal weight. Total and LDL cholesterol were inversely related to maternal weight gain (r = –0.173, p 0.003 and r = –0.177, p 0.003, respectively).

The agreement between the two criteria defining dyslipidemia was 0.09 (Kappa). All pregnant women with dyslipidemia according to percentile criteria were included in the guideline criteria. However, 80.2% of the women without dyslipidemia according to percentile criteria were dyslipidemic under Brazilian Guideline. The area under the curve (AUC) ROC for birth weight and gestational age revealed a lack of accuracy of both dyslipidemia criteria to predictthe risk of macrosomia and prematurity: dyslipidemia according to the National Guideline criteria, AUC 0.49 (95% CI 0%, 39 to 0.58) for birth weight and 0.51 (95% CI 0.43 to 0.59) for gestational age at birth; Dyslipidemia by percentile criterion, AUC 0.49 (95% CI 0.4 to 0.59) for birth weight and 0.47 (95% CI 0.44 to 0.60) for gestational age at birth, according to figure 3.

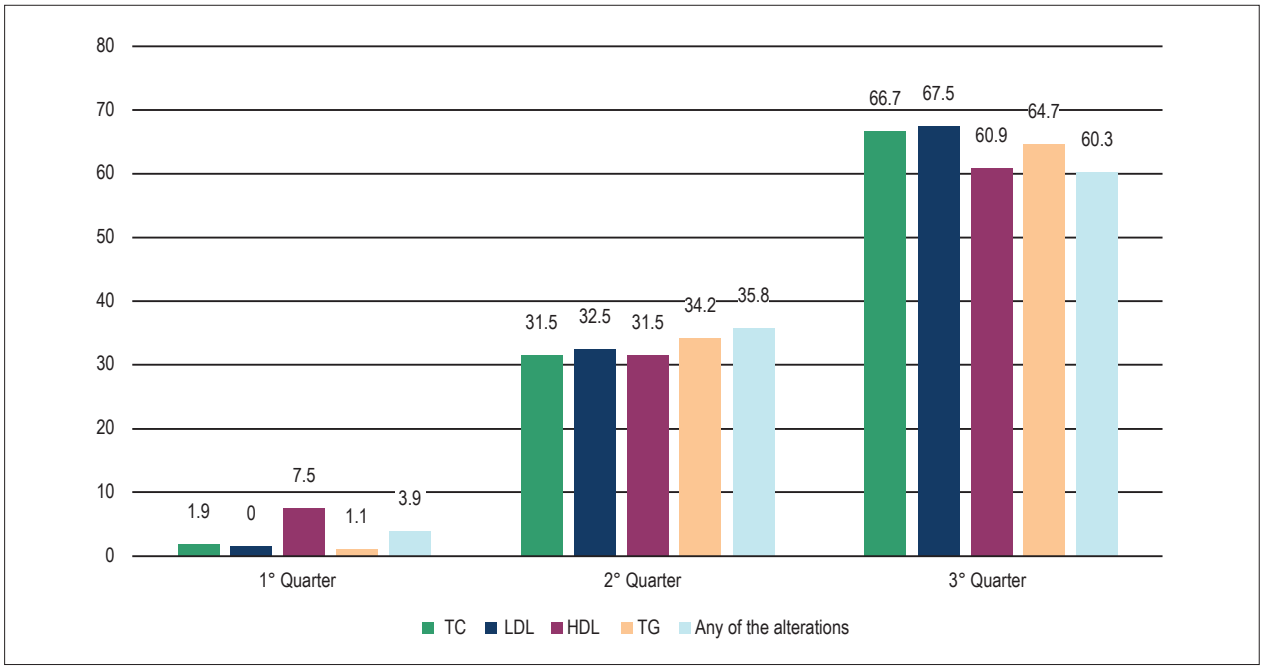

Figure 2 – *Prevalence of dyslipidemia according to guideline criteria. TC: total cholesterol; LDL: low density lipoproteins; HDL: high density lipoproteins; TG: triglycerides.*

Discussion

The present study revealed that, in a population of pregnant women with high gestational risk, the frequency of diagnosis of dyslipidemia according to the V guideline criterion was higher than that identified by the percentile criterion and that none of the criteria was able to predict risk of macrosomia and prematurity in the offspring of affected pregnant women. These findings show the impact of using different criteria to determine the prevalence of the same disease. However, the lack of association between the presence of dyslipidemia and fetal outcomes raises questions about the clinical importance of the detection of dyslipidemia during pregnancy by the criteria evaluated in this study.

The motivation to compare two diagnostic criteria for the definition of dyslipidemia during gestation was the lack of common understanding on the best way to diagnose dyslipidemia during pregnancy. The agreement between the two criteria was poor and this explained the significant difference in prevalence.

The adoption of a diagnostic strategy with the institution of cut-off points and defining criteria of a disease is not a simple task. In pathologies in which continuous variables such as lipids, blood pressure and blood glucose are used for diagnosis, choosing the best cut-off point to determine the disease is often difficult.[31] One of the strategies used to establish cut-off points is the frequency-based statistical definition and distribution of the variable in the population. However, the determination of the optimal cut-off point for a diagnosis in the case of measurement of continuous variables depends on the finding of a value that maintains the balance between the medical, social and economic costs of making the diagnosis in people without substantial risk of adverse effects of one disease and not to diagnose those at real risk of disease damage.[32] Establishing the diagnosis of a disease by statistical definition does not always reveal the association with risk and thus the value of the diagnosis. Glucose cutoff points for the diagnosis of diabetes, for example, were justified by the association with the dramatic increase in the prevalence of microvascular complications considered specific for diabetes[33] which was not determined for lipids and poor fetal maternal outcomes.

The use of the criterion for the definition of dyslipidemia in adults resulted in a prevalence 4.3 times higher than that found by the criterion of specific percentiles for pregnant women. However, the frequency of comorbidities SH, DM and previous preeclampsia was similar in pregnant women with dyslipidemia by any of the criteria, when compared to those without dyslipidemia, and in the studied population there was no superiority of one of the criteria to identify pregnant women at greater risk . The areas under the ROC curve revealed a lack of accuracy in any of the criteria to establish the highest risk of macrosomia and prematurity. It is known that hypertriglyceridemia is associated with gestational diabetes and preeclampsia[15] but it is unknown whether the strength of association with morbidities differs according to the criterion for dyslipidemia, however the present study revealed no association with both criteria. Using different criteria to diagnose the same disease, the result was the meeting of different prevalences that could have generated additional investigations, costs and unnecessary therapies. The similar proportion of comorbidities can be explained by the homogeneity of the population of the present study, consisting of pregnant women with high risk pathologies in which almost 90% were carriers of diabetes.

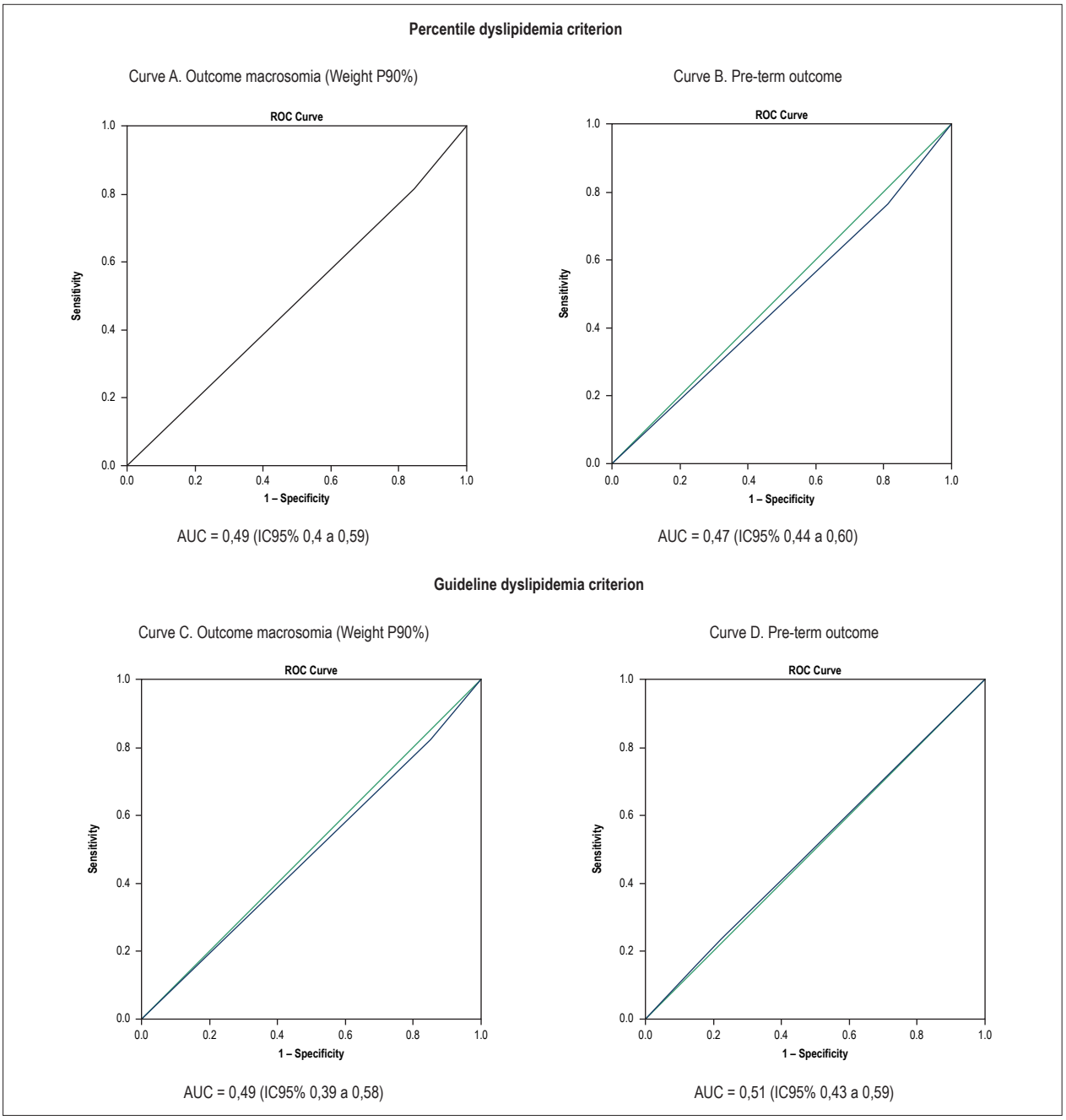

Figure 3 – *ROC curves of fetal outcomes and dyslipidemia by Percentile and Guideline criteria.*

The present study revealed that the prevalence of dyslipidemia increased during the quarters when the criterion of V guideline was used. This finding is compatible with the physiological behavior of gestational hyperlipidemia and has already been demonstrated in several studies.[34;35] However, the frequency of lipid changes was not progressive with the progression from gestation to cholesterol and TG when the criterion of percentiles was used. It is possible that the limited number of patients with alterations in these lipid fractions favored chance and not demonstrated the physiological behavior of the lipids for the percentiles criterion. However, when analyzing dyslipidemia for any lipid alteration, increasing the sample size for each lipid fraction, we found that, for both criteria, dyslipidemia was more frequent with the advancement of the quarters.

Controversies and questions about different definitions for the same disease are international. Regarding dyslipidemia in pregnant women, the scientific community claims for attention and research on lipid metabolism during gestation.[3] The fact that there is no standardization of the ideal criterion may result in the doctor who assists the pregnant woman choosing any of the criteria without the benefit of the use being demonstrated. In the absence of evidence of cut-off points that identify the possible risk(s), it seems reasonable to use the percentiles definition strategy based on the frequency of lipid distribution during pregnancy. However, there are problems that limit the use of percentile criteria: it is necessary to have a reference table for pregnancy lipids categorized by quarter to establish cut points for each lipid and there is no established and internationally accepted standard. There are few studies that report reference values per quarter.[2;34;36] and none in Brazil, to our knowledge. There are several Brazilian publications that demonstrate associations between lipids and BMI,[37] depressive symptoms,[38] changes in pressure,[39] risk of gestational diabetes,[40] without, however, using cut-off points to determine the increase in morbidities or specific risks for dyslipidemia during pregnancy. The present study demonstrated that pregnant women with dyslipidemia defined by guideline criteria had higher TC and HDL and lower TG concentrations than pregnant women with dyslipidemia according to percentile criteria, suggesting that the guideline criterion selects cases of greater severity in relation to dyslipidemia . However, the study did not show superiority of any of the criteria in relation to the association with other maternal or fetal morbidities raising the question of why to diagnose a disease that does not modify maternal-fetal clinical outcomes. These findings, however, should be confirmed with a higher number of pregnant women and in low-risk pregnancies to define the importance of dyslipidemia during pregnancy and which diagnostic criteria to use.

This task presents, however, some limitations. It is a unicentric study with pregnant women from the state of Bahia. The sample is presumed to be mixed, however, it is known that important population differences occur according to the region of the country and Bahia is the state with the highest percentage of African contribution to ancestry.[41] While the impact of ethnic/racial differences in the relation between dyslipidemia and rates of cardiovascular disease lack determination, non-hispanic blacks and whites are less likely to have dyslipidemia than Asian and Mexican Americans,[42] and we do not know if the same is true for the Brazilian population. The results of the present study therefore do not allow national or international generalization and the absence of association with clinical outcomes may have been a result of racial influence and/or limited sample size.

Most of the pregnant women had only one determination of the lipidogram and we know that the lipid fractions, especially the concentration of triglycerides, undergo significant changes depending on diet, exercise and intra and inter-laboratory variations. Despite recognizing the possibility of the influence of an isolated determination on the reduction of the robustness of the findings, a significant portion of the observational studies investigating associations between dyslipidemia and outcomes, do it with only determination, so that our study does not differ from the method commonly used in research in this area. All measurements were made in a 12-hour fasting period, which is also the most used method to determine the lipidogram and to investigate the outcomes related to dyslipidemia.

The cross-sectional nature of the study with data collection in medical records is a limitation, as far as confounding factors could have been neglected. Lack of recognition and appropriate assessment of the influence of confounding factors possibly interferes with results. Most of the pregnant women investigated were diabetic and obese, morbidities associated with dyslipidemia, and the independent contribution of each morbidity in the findings was not established. While the limitation in establishing the causal relationship due to lack of evidence of the temporal sequence between exposure to the risk factor and disease development is a recognized disadvantage of cross-sectional studies, this type of study is important for calculating disease prevalence, the main focus of the present study.

Conclusion

The prevalence of dyslipidemia assessed by the V Brazilian guideline for adult dyslipidemia was higher than the prevalence identified by the criterion of the specific percentiles of pregnancy without, however, showing superiority in the association with morbidities.

Author contributions

Conception and design of the research: Feitosa ACR; Acquisition of data: Feitosa ACR, Barreto LT, Silva IM, Silva FF; Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content: Feitosa ACR, Feitosa Filho GS; Statistical analysis: Feitosa ACR, Barreto LT; Writing of the manuscript: Feitosa ACR, Barreto LT, Feitosa Filho GS.

Potential Conflict of Interest

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

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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Analysis of the Economic Impact of Cardiovascular Diseases in the Last Five Years in Brazil

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Abstract

Background: **There is growing concern about the economic impact of cardiovascular diseases (CVD) in Brazil and worldwide.**

Objective: **To estimate the economic impact of CVD in Brazil in the last five years.**

Methods: **The information to estimate CVD costs was taken from national databases, adding the direct costs with hospitalizations, outpatient visits and benefits granted by social security. Indirect costs were added to the calculation, such as loss of income caused by CVD morbidity or mortality.**

Results: **CVD mortality accounts for 28% of all deaths in Brazil in the last five years and for 38% of deaths in the productive age range (18 to 65 years). The estimated costs of CVD were R$ 37.1 billion in 2015, a 17% increase in the period from 2010 to 2015. The estimated costs of premature death due to CVD represent 61% of the total cost of CVD, Direct costs with hospitalizations and consultations were 22%, and costs related to the loss of productivity related to the disease were 15% of the total. Health expenditures in Brazil are estimated at 9.5% of GDP and the average cost of CVD was estimated at 0.7% of GDP.**

Conclusion: **CVD costs have increased significantly in the last five years. It is estimated that CVD costs increase as the Brazilian population ages and the prevalence of CVD increases. (Arq Bras Cardiol. 2017; 109(1):39-46)**

Keywords: **Cardiovascular Diseases / economy; Cadiovascular Diseases / mortality; Costs and Cost Analysis; Hospitalization.**

Introduction

Non-communicable chronic diseases (CDNs) - mainly cardiovascular disease (CVD), cancer, chronic respiratory diseases - are the leading cause of death, causing approximately 38 million deaths annually worldwide.[1] Approximately 82% of premature deaths from non-communicable diseases occur in low- and middle-income countries, which can be largely avoided. Statistics show that approximately half of these deaths occur during the productive life of individuals[2] and CVD accounts for most of them, accounting for 37%.[1]

The socioeconomic impact of chronic diseases is increasing and is considered a problem for the world public health. In addition to premature deaths, NCDs are responsible for incapacity for work, reduction of family incomes and reduction of productivity.[3,4]

Current health spending in Brazil is approximately 9.5% of GDP per capita. Data from the World Health Organization indicate health expenditures of $ 1078 per capita in 2012 in Brazil. Of these 47.5% are financed by the government, which corresponds to 7.9% of the total expenditure of the Brazilian government.[5] In this same period, developed countries spend an average of 4632 dollars per capita, that correspond to the average of 16,8% of the governmental expenses with health.

The cost of hospitalizations for cardiovascular diseases is considered the largest cause of hospital admissions in Brazil[6] and recent IBGE data show that Brazil is changing its age structure very rapidly, increasing the proportion of elderly people and life expectancy of the Brazilian.[7] Aging tends to increase the incidence of CVD and, consequently, its costs exponentially.[8]

This study was designed to estimate the economic impact of CVD based on Brazilian data. Our estimate of socioeconomic impact is based on government expenditures in Brazil, once the information from the public database of the health system was used. The methodology proposed in the present study includes the direct and indirect costs related to CVD.

Methods

Health costs can be divided into: 1. Direct costs: costs of direct **medical care** to the patient, such as medical services rendered and treatments performed, and **non-medical costs** (non-medical visits);[9] 2. Indirect costs: costs of morbidity and mortality. **Morbidity costs** are defined as expenses for the temporary or permanent loss of work activities due to the disease studied. **Mortality costs** are the costs estimated for premature death as a consequence of illness.[1;9]

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Data Sources

Data sources are publicly available information on the Hospital Mortality System (SIM),[10] the hospital morbidity was obtained at the approved hospital admission events of the DATASUS Hospital Information System (SIH) and outpatient information system (SIA),[11] in addition to information on social security expenses for temporary and / or permanent removal – DATAPREV.[12]

In order to estimate the total cost of diseases in Brazil, information from previous observational studies[13] was collected, as well as from the access to the World Health Organization (WHO) database.[14] To estimate the costs of private care, the sources of information in the National Health Agency (ANS) were used. In order to evaluate the impact of the cost of mortality, estimates from the Brazilian Institute of Geography and Statistics (IBGE) were used, such as: population estimates, life expectancy by sex and age group, average salary of the Brazilian population, and rate of unemployment.[7] A productive range was considered from 18 to 65 years.

Hospital Admissions

The number of hospital admissions for CVD is available in the billing data approved in the SIH-SUS,[11] and corresponds to all events registered in Chapter IDC-10: IX Circulatory diseases in the SUS.[15] To estimate the number of hospital admissions in private care, public data banks (ANS)[16] were used, which show the coverage rate of the beneficiaries of the private health plans, that is, the percentage of beneficiaries who use private plans each year. The formula used to estimate the number of admissions to supplementary care was:

$$\text{Number of hospitalizations of the supplementary care} = \frac{\text{number of hospitalizations SUS x coverage rate ANS}}{(100 - \text{ANS coverage rate})}$$

The number of medical consultations performed by CVD was estimated through the Outpatient Production of the SUS - Brazil - by location of care, available in the SIA / SUS.[11] We extracted the information of quantities approved in the Subgroup procedure: 0301 Consultations / Attendance / Monitoring. As the number of consultations performed by ICD of illness is not available in the health information system, in the present study the percentage of hospitalizations for CVD was calculated on the total hospitalizations. This percentage value was applied in the total number of consultations performed in the SUS (10% of the total), since it may be underestimated. To estimate the number of outpatient visits performed in the private sector, the coverage rate of the ANS was applied.

SUS referrals to private sector beneficiaries are accounted by the ANS[16] and reimbursement amounts for hospitalizations and/or outpatient visits were accounted for the direct costs calculations.

Direct costs

The direct costs were calculated in Brazilian currency (reais) for the year 2016.

The hospital costs related to CVD events were separated in didactic form in: 1. Clinical treatments of CVD - subgroup 0303, titled "Clinical treatments (other specialties)"; 2. Surgical treatments of CVD - subgroup 0406, entitled "Circulatory System Surgery" - which include: cardiological surgical procedures, arrhythmia procedures (pacemakers, cardiac defibrillators and electrophysiological studies - FES), coronary angioplasty, and vascular (surgical and / or percutaneous).[11]

The costs of surgical hospitalizations were increased by estimates of costs of orthotics, prostheses or special materials (OPME). In order to estimate costs incurred with OPME, is used the price base available at ANVISA, with the lowest price practiced in Brazil for each type of OPME.

Indirect costs

Indirect costs are calculated through the costs of morbidity (loss of productivity caused by CVD) together with the costs of mortality, cost of CVD premature death.[17] The costs of morbidity can be defined as time and economic production lost by the patient's absence from their usual activities and work as a direct result of CVD or their treatment.[18] The calculations were grouped into two components: 1. Costs for temporary removals from the work of the patients employed (absenteeism): in this component are included the removals for hospitalizations and medical consultations, and added the values spent in the displacement, that is, the transportation for each consultation carried out; 2. Costs of patients who are no longer in working condition as a consequence of CVD (permanent or temporary removals paid by the government). Costs of pensions and sickness-help caused by CVD.

Social security benefits

The information available on the Social Security website was used to estimate the impact of temporary (sickness) and permanent (retired) removal. An analysis was made of the number of benefits granted because of CVD excluding benefits from other diseases outside the ICD-10 Chapter IX. Diseases of the circulatory system.[12]

The benefits granted by Social Security were analyzed by the frequency of events in the sample in the CVD disease group in the period from 2008 to 2013. For the estimation of removals occurred in the years 2014 and 2015, a projection was made based on the time series of the period from 2008 to 2013 from a model (ARIMA). The ARIMA model is a type of moving average model. A methodology widely used for the elaboration of forecasting studies, applied in the spss software forecasting module.[19] The benefits granted because of CVD correspond to 25% of the total expenses granted by the Social Security.Mortality costs are estimated by estimating the years of productive life lost due to premature death as a consequence of CVD. The calculation is done by multiplying the number of deaths because of CVD according to the age group by the number of days lost (difference between the life expectancy of the Brazilian population and the age of premature death).[17] This data is expressed in economic value multiplying the days lost by the estimated income of the Brazilian until the age of 65 years. Figure 1. In the present study, the salary used for the calculation was the average salary of the Brazilian population, corrected by the rate of unemployment in the same period.[7]

$$\text{Cost of morbidity} = \left[\left(\text{number of days of hospitalization for CVD} \times \text{Average salary by age group} - \frac{\text{number of outpatient visits}}{2}\right)\right]$$

$$\text{Cost of morbidity} = \left(\text{Number of CVD deaths by age group} \times \text{Lost days} \times \text{income on the same period}\right)$$

Figure 1 – *Formulas used to estimate cost.*

Results

Table 1 shows that there was an increase of 3% of the Brazilian population in the last five years, there were 195,497,797 Brazilians in 2010 and 204,450,649 Brazilians in 2015, with a percentage of 49% male and 51% female. There was an aging of the Brazilian population in this period, with an increase of 22% of the population over 65 years. Deaths from CVD represented 28% of total deaths in Brazil.

There was a decrease in the number of clinical admissions due to CVD from 874,949 in 2010 to 807,304 in 2015. However, there was an increase in CVD surgical hospitalizations from 246,038 to 279,010, with a 55% increase in hospitalizations for procedures related to vascular surgeries, 35% for coronary angioplasty, and 34% for electrophysiological studies (FES).

Direct costs

The estimated direct costs of public sector with CVD in Brazil from 2010 to 2015 are shown in Table 2. The estimated expenditures with cardiac consultations increased from approximately R$ 1.2 to R$ 1.5 billion. Expenditures on hospital admissions for CVD increased 28%, with higher expenses paid for surgical hospitalizations. The total direct expenditure on hospitalizations and consultations for CVD in 2015 was R$ 5,103,930,001.38.

The expenses related to the OPMEs were included in the amounts spent on the surgical hospitalizations in CVD in the SUS. The average values in Brazil spent on OPME were multiplied by the number of procedures with codes in the procedure table (TABNET)[20] that use each type of material. The estimated values are shown in Table 3. The average price practiced in Brazil was taken from the table of market values published by ANVISA. The estimated expenditures in OPME increased from R$ 557,624,803.82 in 2010 to R$ 715,347,170.25 in 2015 (a 28% increase in the percentage).

The amounts reimbursed by the ANS to the public sector for Hospital Admissions Authorizations (AIH) and/or High Complexity Procedure Authorization (APAC) for visits to the SUS are added to the direct cost calculation. Table 4 shows the coverage rate of the ANS year by year and the expenses with reimbursements to the SUS for hospitalizations and outpatient visits of its beneficiaries. The value of the year 2015 was estimated through the calculation of linear progression, since the value is not available until the present date.

The costs of the drugs used for CVD were estimated through the information collected in the transparency portal of Brazil. All direct expenditures of the federal government with pharmaceutical medicines were added.[21] For the calculation of the percentage of total expenditure with CVD, the same percentage of 10% was used year by year.

Indirect costs

Social Security spending on pensions and sickness-help because of CVD is available with data open on the DATAPREV portal.[12] The data available refer to the years 2008 to 2013, without updates until the completion of the present study. The values spent in the years 2010 to 2013 in Brazil by CVD are detailed in Table 5. In order to estimate the amounts spent in the years 2014 and 2015, a statistical analysis of the previous years was made and the amount spent was estimated by linear progression.

Table 5 shows that in 2010 R$ 318,131,078.08 were spent due to temporary or permanent removals because of CVD in Brazil. The estimated cost for 2015 is R$ 380,402,308.87. Disability pension expenses related to CVD grew exponentially. This increase cannot be justified by the Brazilian per capita GDP. The GDP per capita in 2010 of the total benefits for CVD was 1.63 and in 2015 was 1.2. The amount of benefits granted by Social Security because of CVD corresponds to 8% of total benefits granted. The number of benefits granted in CVD disability pensions has been declining in Brazil in recent years (a 10% drop), with an increase in the number of sickness-help (6% increase).

The costs of morbidity (absenteeism) are shown in Table 6. The number of admissions and medical consultations in the period from 2010 to 2015, by sex, was used to calculate the number of days lost by CVD. The number of days lost with medical appointments was halved, considering that the worker loses half of his day worked to undergo a medical consultation. The minimum cost for transportation is added to the value, considering the minimum value of public transportation for each year in the same period. The cost of morbidity in 2010 was R$ 4,264,270,533.06, while the cost of morbidity in 2015 was R$ 5,657,186,269.96

Table 1 – Population, deaths, consultations, hospitalizations for CVD per year. Brazil, 2010-2015

Data/Year	2010	2011	2012	2013	2014	2015
Brazilian population	195.497.797	197.397.018	199.242.462	201.032.714	202.768.562	204.450.649
Population aged > 65 years	13.253.407	13.749.501	14.289.040	14.870.086	15.489.166	16.143.835
Total deaths	1.136.947	1.170.498	1.181.166	1.210.474	1.199.937*	1.217.673*
DCV Deaths	326.371	335.213	333.295	339.672	328.367*	334.076*
DCV SUS hospitalizations	1.153.213	1.159.210	1.137.024	1.133.235	1.140.792	1.124.156
Medical consultations DCV SUS	121.060.024	129.182.174	131.666.891	138.680.162	147.495.929	144.879.479
Surgical admissions for CVD SUS	246.038	259.888	267.323	275.838	285.109	279.010
Hospitalizations for coronary angioplasty SUS	55.980	62.221	67.113	70.744	73.939	75.410
Hospitalizations for SUS pacemaker implantation	19.937	20.857	21.959	22.448	23.426	23.300
Hospitalizations for the realization of FES SUS	5.532	6.052	6.392	6.962	6.751	7.417
Hospitalization for vascular surgeries SUS	10.238	10.719	11.586	13.403	14.931	15.907
Clinical hospitalizations for CVD SUS	874.949	870.306	844.018	831.130	819.789	807.304

*Source: IBGE, SIA/DATASUS e SIH/DATASUS. * The total number of deaths and the number of CVD deaths in the years 2014 and 2015 was estimated by calculating linear progression*

Table 2 – Values spent on consultations, hospitalizations in the SUS per CVD per year. Brazil, 2010-2015

Data/Year	2010	2011	2012	2013	2014	2015
Estimated cardiological medical consultations SUS	R$ 1.210.600.244,00	R$ 1.291.821.736,00	R$ 1.316.668.909,00	R$ 1.386.801.616,00	R$ 1.474.959.285,00	R$ 1.448.794.790,00
DCV SUS hospitalizations	R$ 2.094.586.170,18	R$ 2.280.690.735,84	R$ 2.381.639.909,14	R$ 2.490.327.299,45	R$ 2.616.411.987,59	R$ 2.672.683.530,36
Surgical admissions for CVD SUS	R$ 1.220.173.241,66	R$ 1.408.938.230,39	R$ 1.476.651.259,61	R$ 1.523.040.930,90	R$ 1.591.102.088,65	R$ 1.595.198.657,90
Coronary angioplasty SUS	R$ 334.006.069,71	R$ 374.975.648,22	R$ 409.312.529,41	R$ 431.199.989,60	R$ 459.208.716,25	R$ 470.525.283,05
Pacemaker / CDI SUS	R$ 239.463.794,84	R$ 255.854.307,54	R$ 271.049.370,77	R$ 283.030.018,64	R$ 305.711.764,52	R$ 314.135.570,00
Hospitalizations to carry out FES	R$ 19.133.666,93	R$ 21.114.282,38	R$ 23.329.967,02	R$ 25.291.313,54	R$ 24.932.895,30	R$ 27.324.342,93
Vascular Surgery	R$ 158.714.446,97	R$ 164.802.452,92	R$ 172.418.564,05	R$ 195.286.271,67	R$ 212.540.472,21	R$ 205.203.059,86
Clinical admissions for CVD	R$ 850.627.032,61	R$ 867.021.396,62	R$ 889.182.386,96	R$ 914.259.698,41	R$ 951.621.476,71	R$ 982.451.681,02
SUS direct expenses	R$ 4.155.813.446,79	R$ 4.439.533.868,46	R$ 4.587.491.205,10	R$ 4.791.388.613,86	R$ 5.042.992.749,30	R$ 5.103.930.001,38

Sourcee: SIA/DATASUS and SIH/DATASUS.

Mortality costs are shown in Table 7 and were performed using the formula given in Figure 1. The years of life lost by premature death by sex are multiplied by the average salary each year, corrected for the unemployment rate in the same period. The cost of mortality reached R$ 22,275,402,229.74 reais in 2014 and was estimated at almost 22 billion reais in the year 2015.

The total costs estimated with CVD in Brazil in the period from 2010 to 2015 is shown in Table 8. There was a 17% increase in CVD costs between 2010 and 2015, with an increase in the minimum salary of 55% in the same period. This means that there has been an increase in the per capita costs of the Brazilian with CVD in the last five years. The per capita expenditure in 2010 was R$ 154.41 and was estimated at R$ 172.62 in 2015. Despite this increase, the amount represented 30% of the minimum salary in 2010 and now represents 22 % in 2015.

The costs estimated for premature death with CVD represent 61% of total cost for CVD, direct costs were 22% and costs for morbidity were 15% of total CVD costs.

The percentage of GDP with CVD by the study estimate was 0.8% in 2010, 0.7% in the years 2011 to 2014 and 0.6% of GDP in 2015, with an average of 0.7% of GDP over the last five years.

Table 3 – Amounts spent on special materials in SUS by CVD per year. Brazil, 2010-2015

Data/Year	2010	2011	2012	2013	2014	2015
Expenditures on coronary stents	R$ 213.729.307,50	R$ 237.557.185,46	R$ 256.234.637,63	R$ 270.097.644,33	R$ 282.296.021,21	R$ 287.912.237,92
Prosthetic expenses and vascular stents	R$ 101.859.798,52	R$ 101.813.096,10	R$ 109.033.160,55	R$ 131.874.682,40	R$ 138.307.046,37	R$ 139.058.871,31
Pacemaker costs	R$ 172.383.272,80	R$ 181.073.385,60	R$ 190.190.120,80	R$ 193.936.978,40	R$ 201.300.852,00	R$ 200.252.844,80
CDI Expenses	R$ 69.652.425,00	R$ 68.775.320,39	R$ 74.915.052,67	R$ 79.300.575,72	R$ 88.793.943,28	R$ 88.123.216,22
Total expenses with OPME	R$ 557.624.803,82	R$ 589.218.987,54	R$ 630.372.971,64	R$ 675.209.880,86	R$ 710.697.862,85	R$ 715.347.170,25

Source: Table of SIH / DATASUS procedures and material values of ANVISA.

Table 4 – ANS coverage rate and amounts spent reimbursed to SUS due ANS beneficiaries. Brazil. 2010 - 2015

Data/year	2010	2011	2012	2013	2014	2015
Coverage rate (%)	23,6	24	24,6	25,5	26	25,6
Amount reimbursed to SUS	R$ 78.850.898,00	R$ 74.994.805,00	R$ 88.213.668,00	R$ 84.807.361,00	R$ 96.928.835,33	R$ 103.459.587,90*

*Source: ANS. * Estimated by linear progression calculation.*

Table 5 – Social Security expenditures with pensions and sickness-help per CVD per year. Brazil, 2010-2015

Data/Year	2010	2011	2012	2013	2014*	2015*
32-Ap Disability	R$ 37.615.271,29	R$ 40.444.323,27	R$ 42.972.030,79	R$ 47.495.480,16	R$ 46.720.481,83	R$ 50.581.850,60
Ap Disability Det. Ignored	R$ 3.044.567,55	R$ 3.070.944,17	R$ 3.212.029,12	R$ 3.419.950,57	R$ 3.572.476,76	R$ 3.742.351,63
Retired due accident	R$ 760.432,36	R$ 841.083,89	R$ 847.858,46	R$ 840.913,39	R$ 1.011.029,71	R$ 1.046.865,58
Sickness-help Accident	R$ 5.243.965,12	R$ 5.298.365,35	R$ 4.958.255,43	R$ 5.447.369,98	R$ 5.034.996,01	R$ 4.860.224,76
Sickness-help	R$ 271.466.841,76	R$ 305.155.132,76	R$ 353.206.016,30	R$ 397.833.261,78	R$ 423.022.209,26	R$ 467.767.157,75
Total Social Security Expenses	R$ 318.131.078,08	R$ 354.809.849,44	R$ 405.196.190,10	R$ 455.036.975,88	R$ 479.361.193,58	R$ 527.998.450,32

Source: DATAPREV.

Discussion

It is essential today, with scarce resources, to discuss health costs based on sources of secure and real-time information.[22] The present study has a methodology that proposes to use the data sources with greater reliability and with greater speed in obtaining the information. The sources of billing expenses in Brazil occur with a maximum delay of about 2 months after the current month, for which reason they were prioritized. It is important to point out that the present study was based on the use of the largest possible amount of information available in public databases. As the nationally based information system is still settling in Brazil, billing data on SIH and SIA may be underestimated, and this is a limitation of the study.

Another limitation of the study is information related to mortality, pensions and sickness-help, as well as the amounts of direct reimbursements from ANS to SUS. These data were not available for the years 2014 and 2015 in their fullness, in this way, it was chosen to estimate these values through linear progression. Expenses with drugs for treatment of cardiovascular disease are not available in Brazilian public information systems. The present study estimated expenditures for CVD drugs, considering that 10% of the pharmaceutical care expenses in Brazil are for CVD treatment. This strategy can be considered conservative. The criteria adopted for the selection of sources was to use as much information as possible in public databases, and to strengthen this information as a basis for the future of the country's public policies.[13,23]

The direct costs of supplementary health should be underestimated, since using the coverage rate available in the ANS assumes that the costs of supplementary medicine are at least similar to those spent in the SUS, which is known not to be a reality.

The number of CVD pensions has been decreasing in the country in the analyzed period, and the number of sickness-help has increased. This can be considered an indirect indication that the Brazilian population is living with CVD, without interruption of labor activities. This may have been due to improved health, or changes in pension legislation.

Table 6 – Costs of CVD morbidity per year. Brazil, 2010-2015

Data/year	2010	2011	2012	2103	2014	2015
Hospitalizations, male	569.537	574.593	567.461	565.431	569.142	569.604
Hospitalizations, female	583.676	584.617	569.563	567.804	571.650	556.175
TMP	6,5	6,6	6,6	6,6	6,7	6,5
Days of hospitalizations, female	3.793.894	3.858.472	3.759.116	3.747.506	3.830.055	3.615.138
Days of hospitalizations, male	3.701.991	3.792.314	3.745.243	3.731.845	3.813.251	3.702.426
Consultations	121.060.024	129.182.174	131.666.891	138.680.162	147.495.929	144.879.479
Average salary (R$), female	R$ 983,37	R$ 761,00	R$ 824,00	R$ 902,00	R$ 1.000,00	R$ 895,20
Average salary (R$), male	R$ 1.390,99	R$ 1.340,00	R$ 1.430,00	R$ 1.540,00	R$ 1.664,00	R$ 1.611,36
Cost of absenteeism for hospitalizations, female	R$ 124.360.051,43	R$ 97.876.578,14	R$ 103.250.380,64	R$ 112.675.025,76	R$ 127.668.500,00	R$ 107.875.703,00
Cost of absenteeism for hospitalizations, male	R$ 171.647.725,52	R$ 169.390.016,40	R$ 178.523.230,60	R$ 191.568.022,80	R$ 211.508.344,32	R$ 198.864.705,31
Cost of absenteeism per consultation	R$ 2.395.333.996,12	R$ 2.261.764.556,11	R$ 2.473.143.100,74	R$ 2.822.141.288,56	R$ 3.274.409.612,70	R$ 3.026.242.557,35
Cost days lost due to morbidity	R$ 2.691.34.773,07	R$ 2.529.031.150,65	R$ 2.754.916.711,98	R$ 3.126.384.337,12	R$ 3.613.586.457,02	R$ 3.332.982.965,66
Minimum ticket price (R$)	R$ 2,34	R$ 2,34	R$ 2,44	R$ 2,66	R$ 2,66	R$ 3,02
Cost of Transportation (R$)	R$ 566.560.914,19	R$ 604.572.572,45	R$ 642.534.427,59	R$ 737.458.428,57	R$ 784.337.964,40	R$ 875.963.619,18
Cost Morbidity SUS	R$ 3.257.902.687,26	R$ 3.133.603.723,10	R$ 3.397.451.139,57	R$ 3.863.842.765,69	R$ 4.397.924.421,42	R$ 4.208.946.584,85
Coverage Rate	23,6	24	24,6	25,5	26	25,6
SUS + private morbidity cost	R$ 4.264.270.533,06	R$ 4.123.162.793,55	R$ 4.505.903.368,13	R$ 5.186.366.128,44	R$ 5.943.141.110,03	R$ 5.657.186.269,96

Source: SIA/DATASUS, SIH-DATASUS, PME/IBGE. TMP: mean length of stay.

Table 7 – Costs of CVD mortality per year. Brazil, 2010-2015

Data/year	2010	2011	2012	2013	2014	2015
Women's Lost Years	520.810	527.350	511.302	512.366	517.223	516.891
Men's Lost Years	791.003	805.339	799.250	799.692	804.724	807.857
Cost of mortality (R$)	R$ 19.349.117.782,66	R$ 17.765.622.096,07	R$ 18.770.886.111,48	R$ 20.324.158.368,20	R$ 22.275.402.229,74	R$ 21.173.626.058,79

Source: SIM, PME/IBGE.

Table 8 – Estimated total CVD costs per year. Brazil, 2010-2015

Data/Year	2010	2011	2012	2013	2014	2015
Direct costs	R$ 6.169.421.794,00	R$ 6.616.780.073,69	R$ 6.920.244.266,23	R$ 7.337.716.100,29	R$ 7.775.257.583,99	R$ 7.821.609.101,66
ANS reimbursement	R$ 78.850.898,00	R$ 74.994.805,00	R$ 88.213.668,00	R$ 84.807.361,00	R$ 96.928.835,33	R$ 103.459.587,90
Cost of Morbidity	R$ 4.264.270.533,06	R$ 4.123.162.793,55	R$ 4.505.903.368,13	R$ 5.186.366.128,44	R$ 5.943.141.110,03	R$ 5.657.186.269,96
Mortality cost	R$ 19.349.117.782,66	R$ 17.765.622.096,07	R$ 18.770.886.111,48	R$ 20.324.158.368,20	R$ 22.275.402.229,74	R$ 21.173.626.058,79
Cost of pensions and helps	R$ 318.131.078,08	R$ 354.809.849,44	R$ 405.196.190,10	R$ 455.036.975,88	R$ 479.361.193,58	R$ 527.998.450,32
Cost of medication	R$ 968.489.393,60	R$ 1.286.742.089,14	R$ 2.277.654.330,14	R$ 1.418.869.356,06	R$ 2.162.470.925,74	R$ 1.819.345.140,75
Total cost DCV	R$ 31.148.281.479,40	R$ 30.222.111.706,89	R$ 32.968.097.934,08	R$ 34.806.954.289,87	R$ 38.732.561.878,41	R$ 37.103.224.609,38

With the epidemiological transition in Brazil, associated with population aging, it is vehement that several studies estimate health costs, especially CVD, as the main cause of death due to illness in the country. As shown in the last five years, CVD costs are growing, and it is essential that health promotion measures occur to reduce premature deaths.[18,24]

Conclusions

The direct and indirect costs of CVD in Brazil have increased in the last five years in Brazil. This increase was more significant in drug costs (88%), followed by social security costs (66%) and morbidity costs (33%). These data are indirect indications that there is an increase in the population living with CVD. The number of social security benefits has increased in the last five years in proportion to the sickness-help, since it is possible to visualize the fall of the pensions occurred by CVD. When analyzed as a percentage of GDP, CVD costs are stable, probably due to the lower number of years of life lost. The goal of the WHO was to reduce 25% of CVDNCs by 2025,[25] and in line with the global targets for the reduction of CVD, the *Sociedade Brasileira de Cardiologia* (SBC) launched in 2013 an important publication aimed at increasing CVD prevention in Brazil.[24] In order to achieve a reduction in the impact of CVD in Brazil, it is essential to involve everyone in society. The fight to prevent and improve the quality of life of the population is urgent, especially in a developing country such as Brazil where resources are scarce.

Author contributions

Conception and design of the research, Analysis and interpretation of the data and Statistical analysis: Siqueira ASE, Land MGP; Acquisition of data: Siqueira ASE; Writing of the manuscript: Siqueira ASE, Siqueira-Filho AG; Critical revision of the manuscript for intellectual content: Siqueira-Filho AG, Land MGP.

Potential Conflict of Interest

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

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Hypertriglyceridemic Waist Phenotype and Changes in the Fasting Glycemia and Blood Pressure in Children and Adolescents Over One-Year Follow-Up Period

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Abstract

Background: The hypertriglyceridemic waist (HTW) phenotype is defined as the simultaneous presence of increased waist circumference (WC) and serum triglycerides (TG) levels and it has been associated with cardiometabolic risk in children and adolescents.

Objective: The objective was to evaluate the influence of HTW phenotype in the fasting glycemia and blood pressure in children and adolescents over one-year follow-up period.

Methods: It is a cohort study involving 492 children and adolescents from 7 to 15 years old, both genders, who were submitted to anthropometric, biochemical and clinical evaluation at the baseline, and also after 6 and 12 months of follow-up. Generalized Estimating Equation (GEE) models were calculated to evaluate the longitudinal influence of the HTW phenotype in the glycemia and blood pressure over one-year.

Results: It was observed a prevalence of 10.6% (n = 52) of HTW phenotype in the students. The GEE models identified that students with HTW phenotype had an increase of 3.87 mg/dl in the fasting glycemia mean (CI: 1.68-6.05) and of 3.67mmHg in the systolic blood pressure (SBP) mean (CI: 1.55-6.08) over one-year follow-up, after adjusting for confounding variables.

Conclusions: The results of this study suggest that HTW phenotype is a risk factor for longitudinal changes in glycemia and SBP in children and adolescents over one-year follow-up period. (Arq Bras Cardiol. 2017; 109(1):47-53)

Keywords: Hypertriglyceridemic Waist, Phenotype; Glycemic Index; Fasting; Blood Pressure; Child; Adolescent; Cohort Studies.

Introduction

The hypertriglyceridemic waist phenotype (HTW) is defined as the simultaneous presence of increased waist circumference (WC) and serum triglycerides (TG) levels.[1] This phenotype has been associated with the cardiometabolic risk, with elevated insulin, apolipoprotein B, C-reactive protein and LDL cholesterol levels, increasing the risk of coronary artery disease.[2]

The prevalence of HTW phenotype has been widely investigated. A meta-analysis performed by Ren et al. (2016)[3] demonstrated variation in the prevalence of the HTW phenotype from 4% to 47%, with combined prevalence of 19% (95% CI 14-24%). Esmaillzadeh et al.[4] verified prevalence of 6.5% for HTW in Iranian adolescents (7.3% in boys and 5.6% in girls). In Brazil, transversal studies with adolescents from 10 to 19 years old identified HTW prevalence fluctuating from 6.4% to 20.7%.[5-7] However, no longitudinal studies involving children and adolescents were identified in the literature.

Evidences suggest that individuals with HTW phenotype are more likely to develop metabolic syndrome and risk factors for cardiovascular diseases[4,8-10]. Among these factors, there are increased glycemia and systemic hypertension, characterized by increased and sustained blood pressure, with multifactorial determination[8]. Therefore, HTW is a simple and reliable indicator to detect these diseases and metabolic risks associated to visceral obesity[9], and may become a practical, feasible, and low-cost approach, especially in Primary Care.

Given the above, and considering the absence of cohort studies involving the subject, this study aimed to assess the influence of the hypertriglyceridemic waist phenotype on the fasting glycemia and blood pressure of students after one-year follow-up.

Methods

Sample and study design

This is a cohort study including 492 children and adolescents from 7 to 15 years old, both male and female, from 10 public,

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urban and part-time schools. It was conducted a simple random sampling, selecting students of each school from a list of elementary school children registered with the Municipal Education Department of a municipality of Bahia, Brazil, in 2006. This sample has power of 97.6% to detect a 10% change in the mean fasting glycemia of the participants, during a 12 months period, considering the mean of 90.2 mg/dL ± 0.3SD.[7] For systolic blood pressure (SBP), the sample has power of 95% to detect alteration of 10% in the average values, considering the mean of 111.1 mmHg ± 12.9 SD; and power of 94% to detect a change of 10% in the mean of diastolic blood pressure (DBP), considering an average of 70.3 ± 9.3SD.[11] Calculations of sample power (1-β) were based on the significance level of 5% and two tailed-tests, indicating that this sample size is sufficient to carry out unbiased estimates of the parameters of the studied population.

All the measurements were collected in the beginning and after 6 and 12 months follow-up.

Exclusion criteria

State of pregnancy, lactation or physical disabilities that prevented anthropometric evaluation were adopted as exclusion criteria. However, these conditions were not identified among the selected students.

Anthropometric evaluation

In order to measure the weight, it was used a Filizola® portable weighing machine with capacity for 150kg and 100 g precision, allowing the variation of 100g. Height was measured by a stadiometer brand Leicester Height Measure, with the measure performed in the closest millimeter. Both measures were performed in duplicate and the mean of the two measures was adopted as final measure.[12] The anthropometric state was assessed by Body Mass Index (BMI) by age, using as reference, the recommendations of World Health Organization[13] for individuals from 5 to 19 years old.

The waist circumference (WC) was measured at the midpoint between the iliac crest and the outer face of the last rib. The measures were performed in duplicate and the average of these two measures was adopted as definitive. The cut off adopted to classify abdominal fat excess was the 90th percentile of the own sample, as proposed by Freedman et al. (1999).[14]

Sociodemographic and lifestyle data

The person responsible for the child/adolescent referred this information. The demographic variables included the student gender and age. The socioeconomic status included number of rooms in the house and people who lived there, the main source of illumination and the occupation of the head of the family. These variables originated the socioeconomic index. The status of household water supply, the source of drinking water and the destination of the garbage and waste were used to compose the environmental index. These variables had responses varying from 0 (worst condition) to 4 (best classification). Thus, the socioeconomic and environmental indexes were ranging from 0 to 16 and were categorized into terciles. Despite knowing that the maternal education is associated to socioeconomic conditions, this variable was not included in the socioeconomic index and it was assessed individually, once this also represents the cultural and dietary aspects of the society where the individual is inserted.

The physical activity level was assessed by a questionnaire structured with questions referring to the frequency of physical activities that were not included in the pedagogical content of the school, which is performed once a week. Therefore, the practice of two or more days of physical activity outside the school classifies the student as active; and less than two days of physical activity outside classifies the student as less active / sedentary.

Blood pressure

For blood pressure (BP) measurement it was adopted the technique recommended by the 4th Brazilian Guidelines on Hypertension. The BP value was classified according to the *National High Blood Pressure Education Program Working Group on High Blood Pressure in Children* (2004),[15] considering high pressure levels for children and adolescents BP > p90, according to age, gender and height percentile.

Biochemical exams

The blood collection was performed in the morning observing at least 12-hours fasting, 10 mL of blood were collected by venepuncture and deposited in sterile and disposable vacutainer tubes (BD®), without anticoagulant. The blood was centrifuged at 3000 rpm for 5 minutes for posterior serum separation, which was used for biochemical determinations. The biochemical parameters were performed at the Reference City Laboratory, provided by the Health Department. The triglyceride and glycemia values were determined by the enzymatic method. For the glycemia classification, the recommendation of the Brazilian Diabetes Society (2009)[16] was adopted, which characterizes the adequate conditions (≤ 100mg/dL) and increased conditions (>100 mg/dL). Triglyceride values < 100 mg/dL were considered adequate.[17]

Hypertriglyceridemic waist phenotype

The HTW phenotype was defined by the presence of increased waist circumference (> 90 percentile by age and sex of the sample itself) and increased serum triglycerides (> 100 mg/dL), simultaneously.[4]

Statistical Analysis

Prevalence of categorical data and measure of mean and standard deviation for continuous variables were calculated. The comparison of outcome variables and other variables of interest according to exposition variable was performed using Pearson^2- square test. The p-value lower than 0.05 was adopted as significant level.

The mean and standard deviation of the glycemia, the systolic and the diastolic BP were calculated. The Shapiro-Wilk normality test was applied, and the statistic indicates normality[1] when close to one.[8] Distributions of the mean values of glycemia, the systolic and the diastolic BP according to HTW phenotype at baseline and after the follow-up were calculated using the statistical test of mean comparison for continuous variables (Student t test for equal or unequal variances), adopting the p-value lower than 0.05 as significant level.[18]

Aiming to assess the influence of the HTW phenotype on fasting glycemia and BP of the students during 12 months, models of Generalized Estimating Equations (GEE) were built, which is adequate for continuous answers and repeated measures, reflecting the relationship between the variable and independent responses, considering the correlation between the measures in each moment of time. Furthermore, GEE model does not require normality assumption. For the present study, the correlation matrix chosen was the autoregressive, considering that the measures have an autoregressive relationship in function of time.[19]

Initially, the univariate analysis was conducted, aiming to select the variables candidate to the multivariate model, selecting those with p value lower than 20%. These variables were included in the model as covariables. In the final model, the remaining variables were those that presented significance level lower than 5%.

To assess the data adjust of the model, the criterion of quasi-likelihood under the corrected independence model (QIC_c) was used, which is a modification of the *Akaike's information criterion* (AIC) for GEE analysis. The QIC is calculated from the comparison of the quasi-likelihood of the independence model with that of the complete model. The lower the QIC, the better the model adjustment.[20]

All the statistical analyses were performed in the statistical package *Stata/IC for Mac* (*StataCorp, College Station*), version 12.0.

Ethical Aspects

The protocol of the present study was approved by the Nutrition Ethics Committee of the School of Nutrition by the number 03/06.

The participation of the student in the study depended on a written authorization of the parents and/or responsible person. After receiving the letter-invitation, knowing the study objectives and agreeing with the insertion of the minor in the investigation, the parents and/or responsible person signed the informed consent form (ICF).

Results

In Table 1 are presented the sociodemographic, clinical and anthropometrical characteristics of the students, according to the HTW phenotype. There was higher prevalence of the HTW phenotype among students with lower socioeconomic status (14,3%), with fasting glycemia above 100 mg/dL (22,9%) and with excess weight, according to the BMI (31,1%).

Considering the initial sample of this study, the loss of 37 (7.5%) individuals was registered after one year of follow-up. The analysis of the sociodemographic and clinical data indicated that there were no significant statistical differences among these factors for the students lost and those that continued in the study (data not shown).

The outcome variables (fasting glycemia, systolic BP and diastolic BP) presented normal distribution, according to the Shapiro-Wilk test; being applied at the mean comparison t-test. Thus, the Table 2 shows the mean values of the variables of interest at the beginning and at the end of the follow-up, according to the HTW phenotype. It was observed that in the beginning of the study, the students with HTW phenotype presented higher mean values of glycemia and systolic BP ($p = 0,003$ and $p = 0,03$, respectively). After one year of follow-up, it was identified that individuals with HTW phenotype had higher mean values of glycemia than those without this phenotype ($p = 0,04$).

The GEE models, constructed to evaluate the influence of HTW phenotype in the glycemia and blood pressure of the students after one-year follow-up, are presented in Table 3. For fasting glycemia, it was observed that students with the presence of HTW phenotype had the increase of 3.11 mg/dl in the mean of fasting glycemia after one-year follow-up, when compared to individuals without the HTW phenotype. Adjusting by gender, age, maternal education, socioeconomic status, BMI and physical activity level, the increase in the mean of fasting glycemia after one-year follow-up was 3.87 mg/dl ($p = 0,001$).

For the SBP, students with HTW phenotype had increase of 2.97 mmHg in the average of this measure after one-year follow-up, when compared to individuals without this phenotype. This raise in the SBP mean increased for 3.67 mmHg in individuals with HTW phenotype after one-year follow-up when adjusting by sociodemographic variables, BMI and physical activity level ($p = 0,02$).

The presented models were well adjusted to the data, according to the QICc criterion, considering that there was a reduction of this indicator in the final models when compared to the crude models (Table 3).

Significant statistical changes were not identified in the DBP mean in children and adolescents with HTW phenotype after the 12-months follow-up.

Discussion

The results of this investigation indicate higher prevalence of HTW phenotype among students with lower socioeconomic status, with altered fasting glycemia and weight excess, indicating important environmental component in this phenomenon. Furthermore, the presence of HTW phenotype favored the increase of mean values of glycemia and systolic BP after one year, especially after the adjustment by sociodemographic variables, BMI and physical activity level.

The prevalence of HTW phenotype identified in this study was higher than that found in children and adolescents in Iran (3.3% and 8.5%, respectively)[4,21] and in the United Kingdom (varying from 6.3% to 8.2%).[22] Investigations involving Brazilian adolescents found the occurrence of HTW phenotype from 2.6% to 20,7%.[5,6] These differences among prevalence of HTW phenotype can reflect the lack of world standardization for measuring the waist circumference, and the serum concentrations of triglycerides for the age, moreover the variations of the cutoff used to classify the HTW phenotype, which is an obstacle to the comparison of the investigations. Besides, the differences of lifestyle, genetic background and ethnicity interfere with the accumulation of abdominal fat and can explain the divergent results of the HTW phenotype prevalence.[21,23,24]

Table 1 – Sociodemographic, anthropometric and clinical characteristics at baseline of students from a city of Bahia, Brazil, 2006

	HTW Phenotype N (%)			
	Total N	Absent	Present	p value
Gender				
– Female	492	255 (88.8)	32 (11.1)	0.620
– Male		185 (90.2)	20 (9.8)	
Age				
– <10 years	492	124 (90.5)	13 (9.5)	0.620
– ≥ 10 years		316 (89.0)	39 (11.0)	
Environmental index				
– 3rd tercile	492	177 (88.9)	22 (11.1)	0.770
– 1st e 2nd terciles		263 (89.8)	30 (10.2)	
Socioeconomic index				
– 3º Tercile	492	206 (94.1)	13 (5.9)	0.003*
– 1º e 2º Terciles		234 (85.7)	39 (14.3)	
Maternal education				
– ≥ 6 years	439	171 (89.5)	20 (10.5)	0.090
– < 6 years		233 (93.9)	15 (6.1)	
Physical activity				
– Active	492	133 (86.9)	20 (13.1)	0.113
– Low active/sedentary		284 (91.6)	26 (8.4)	
Blood pressure				
– < P90	492	324 (91.0)	32 (9.0)	0.467
– ≥ P90		117 (86.0)	19 (14.0)	
Glycemia				
– <100mg/dL	492	413 (90.4)	44 (9.6)	0.01*
– ≥ 100 mg/dL		27 (77.1)	8 (22.9)	
BMI/age				
– < P85	492	369 (94.8)	20 (5.2)	0.000*
– ≥ P85		71 (68.9)	32 (31.1)	

** Significant p value for Pearson chi-square; HTW: hypertriglyceridemic waist.*

Table 2 – Mean comparison test for the variables of interesting according to the hypertriglyceridemic waist phenotype at the baseline after one-year follow-up in students from a city of Bahia, Brazil, 2006

	Baseline			After one-year follow-up		
	HTW(-)	HTW(+)	p value	HTW (-)	HTW (+)	p value
	Mean (SD)	Mean (SD)		Mean (SD) final	Mean (SD) final	
Glycemia (mg/dL)	81.8 (10.2)	86.0 (11.7)	0.003	83.5 (10.3)	86.1 (11.6)	0.04
Systolic BP (mmHg)	101.3 (12.0)	105.1 (12.1)	0.03	101.3 (11.8)	104.1 (11.0)	0.10
Diastolic BP (mmHg)	64.3 (10.1)	66.9 (10.3)	0.09	64.5 (10.6)	66.1 (10.6)	0.09

HTW: hypertriglyceridemic waist.

Table 3 – Models of Generalized Estimating Equation for the relationship between HTW phenotype and fasting glycemia, systolic and diastolic blood pressure after one-year follow-up in students from a city of Bahia, Brazil, 2006

	Fasting glycemia	
	Coefficient (95% CI); p value*	
- HTW phenotype	**Crude**	**Final model†**
Absent	Reference	Reference
Present	3.11 (1.35-4.86); 0.001	3.87 (1.68-6.05); 0.001
QIC_c ‡‡	128.540	112.716
	Systolic Blood Pressure	
	Coefficient (95% CI); p value*	
- HTW phenotype	**Crude**	**Final model†**
Absent	Reference	Reference
Present	2.97 (-0.11- 6.06); 0.06	3.67 (1.55-6.08); 0.02
QIC_c ‡	125.375	118.265
	Diastolic Blood Pressure	
	Coefficient (95% CI); p value*	
- HTW phenotype	**Crude**	**Final model†**
Absent	Reference	Reference
Present	1.43 (-1.20- 4.05); 0.28	1.45 (-1.20- 4.10); 0.29
QIC_c ‡	90.724	83.872

*Sample size- 492; HTW: hypertriglyceridemic waist. *Generalized Estimating Equation – GEE; † Adjusted by gender, age, maternal education; socioeconomic status and level of physical activity; ‡ QIC_c: quasi-likelihood corrected under the criterion of Independence model for.*

Data from this study indicate that the HTW phenotype increases the fasting glycemia mean after one-year follow-up when compared to the individuals without the HTW phenotype. This is an important clinical situation among the studied population, because the glycemia is related to visceral obesity, favoring the higher risk of developing others chronic and non-communicable diseases with expression in adult life.[25,26] However, different studies did not find the relationship between HTW phenotype and fasting glycemia in children and adolescents[4,7,21] but it worth noting the transversal design of these investigations.

The increase of HTW phenotype is associated with metabolic modifications. It is suggested that with increased values of the waist circumference, there are greater concentrations of free fatty acids, especially in the liver, muscle and pancreas, resulting from lipolysis of triglycerides. The excess of free fatty acids provides negative feedback of the glycogen synthase, which can induce the peripheral resistance to insulin and glucose intolerance, in both muscle and liver[27,28] which can explain the longitudinal relationship between HTW phenotype and glycemia identified in this study.

Even though some evidences identified the association between HTW phenotype and lipid alterations and in the hyperglycemia in children and adolescents, the relationship of the HTW phenotype and blood pressure levels is still not established for this age, because of the reduced number of publications that test this association and the limitations of the transversal study designs. In the study performed by Kelishadi et al.[23] SBP greater than 90th percentile for the age, gender and height was observed in 8.8% of the boys and in 17.6% of the girls with HTW phenotype, while diastolic blood pressure was 4.4% and 9.6%, respectively. Bailey et al.[22] observed higher means of DBP in children and adolescents with HTW phenotype.

The prevalence of high BP in people with HTW phenotype is 2 to 3 times greater when compared to those who do not present this phenotype.[4,20] This association is usually weakened or becomes statistically insignificant when BMI adjusts are conducted, suggesting that obesity can influence intensely the blood pressure and the distribution of isolate fat.

Although it is a cohort study, it is known that it is not possible to completely establish a causal relationship, being necessary to undertake confirmatory studies about these relationships identified in the present study. Moreover, maybe the follow-up period was not enough to identify the outcome variables in this population. However, the study was methodologically well designed, robust statistical techniques were adopted, and, in addition, the results are biologically plausible and consistent with the scientific evidence on this theme.

Therefore, in the present study, the longitudinal relationship between HTW phenotype and SBP was stronger after one-year follow-up, even when adjusted by BMI. This can be the reflection of the strength of a cohort study and it suggests that the presence of the phenotype is characterized as risk factor for progressive increase of SBP in this group. This relationship can be related to the presence of higher serum level of insulin in people with abdominal obesity, regardlessly the body weight,[29] since the insulin hormone induces various signals that promote increase of BP, which includes induction of vasoconstriction and proliferation of smooth muscle cells in blood vessels; promotion of pro-inflammatory activity; stimulus of renal absorption of sodium and sympathetic response.[30-32]

Conclusions

The results of this study suggest that HTW phenotype is a risk factor for longitudinal changes in glycemia and SBP in

children and adolescents. Considering that the components of this phenotype are already present early in life, the monitoring of HTW phenotype should be adopted in the pediatric group, since it is a simple and low-cost screening tool to identify cardiometabolic risk, and the diagnosis of the risk provided prematurely can favor the nutritional and lifestyle interventions, promoting health and preventing chronic non-communicable diseases in the later age.

Author contributions

Conception and design of the research and Acquisition of data: Costa PRF, Assis AMO; Analysis and interpretation of the data, Statistical analysis, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Costa PRF, Assis AMO, Cunha CM, Pereira EM, Jesus GS, Silva LEM, Alves WPO; Obtaining funding: Assis AMO.

Potential Conflict of Interest

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

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Cardioprotective Effect of Crocin Combined with Voluntary Exercise in Rat: Role of Mir-126 and Mir-210 in Heart Angiogenesis

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Abstract

Background: Crocin is reported to have a wide range of biological activities such as cardiovascular protection. Recent epidemiologic studies have shown that exercise reduces cardiovascular morbidity and mortality in the general population.

Objective: The aim of this study was to evaluate the effect of crocin and voluntary exercise on miR-126 and miR-210 expression levels and angiogenesis in the heart tissue.

Methods: Animals were divided into 4 groups: control, exercise, crocin, and exercise-crocin. Animals received oral administration of crocin (50 mg/kg) or performed voluntary exercise alone or together for 8 weeks. Akt, ERK1/2 protein levels, miR-126 and miR-210 expression were measured in the heart tissue. Immunohistochemical method was used to detect CD31 in the heart tissue.

Results: Akt and ERK1/2 levels of the heart tissue were higher in crocin treated group and voluntary exercise trained group after 8 weeks. Combination of crocin and exercise also significantly enhanced Akt and ERK1/2 levels in the heart tissue. MiR-126, miR-210 expression and CD31 in the heart increased in both crocin and voluntary exercise groups compared with control group. In addition, combination of exercise and crocin amplified their effect on miR-126 and miR-210 expression, and angiogenesis.

Conclusion: Crocin and voluntary exercise improve heart angiogenesis possibly through enhancement of miR-126 and miR-210 expression. Voluntary exercise and diet supplementation with crocin could have beneficial effects in prevention of cardiovascular disease. (Arq Bras Cardiol. 2017; 109(1):54-62)

Keywords: Rats; Angiogenesis Modulating Agents; Exercise; Crocus Sativus; Antioxidants; miR-126; miR-210.

Introduction

Crocin is a bioactive constituent found in the fruits of gardenia and in the stigmas of saffron.[1] Crocin has long been used in traditional medicine and has been reported to have various pharmacological activities, such as antioxidant, anti-cancer, anti-inflammation, anti-atherosclerotic effects, and protection against cardiovascular diseases.[2] The cardioprotective effects of crocin has been reported in some studies that are related to modulation of endogenous antioxidant enzymatic activities and cardiac biomarkers.[3,4] Recent evidence has indicated the protective effect of crocin on hypoxic damage of myocardial cells by elevation of vascular endothelial growth factor (VEGF), as a proangiogenic factor.[5]

Physical activity plays a critical role in metabolism, cardiovascular function, and immune function. In the last years it became evident that exercise training is a very powerful therapeutic strategy for prevention of development and progression of cardiovascular disease.[6] Nevertheless, exhaustive exercise may be problematic, as they are stressful through production of reactive oxygen species and can cause damage to muscle tissue and other organs.[7,8] It has been suggested that voluntary exercise may be a better model with more beneficial effects.[9] In the animal model of voluntary exercise, the animal has free access to a running wheel and uses the wheel according to its physiological threshold for physical activity. It has been discovered that physical activity triggers extension of the capillary network or angiogenesis. This process is known to be VEGF dependent.[10] However, the underlying mechanisms of exercise have yet to be fully elucidated.

Micro-RNAs (miRs) are small non-coding 18–25 nucleotide RNAs that play a key role in regulating gene expression by inhibiting protein translation or enhancing messenger RNA degradation.[11,12] Their participation in cardiovascular disease has been recognized during recent years.[11-13] MiR-126 is one of the few miRNAs that is an endothelial cell-specific miRNA and plays an essential role in neoangiogenesis. MiRNA-126 is strongly expressed in the heart endothelium and targets Sprouty-related protein-1 (Spred-1), PIK3R2, a regulatory subunit of PI3K.[14,15] Downregulation of these targets activates survival kinases ERK and Akt and enhances the actions of

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vascular endothelial growth factor (VEGF).[16,17] VEGF exerts many of its effects on angiogenesis via the Akt and ERK1/2 pathways. During developmental vasculogenesis, the Akt pathway regulates venous specification, whereas the ERK pathway regulates arterial specification.[18,19] MiR-210 overexpression in normoxic endothelial cells stimulated the formation of capillary like structures as well as VEGF-driven cell migration.[20] MiR-210 induction is a virtually constant feature of the hypoxic response that is important in the pathogenesis of several human diseases, such as heart disease.[21]

According to the advantage effects of crocin and voluntary exercise on diabetes that mentioned above, we hypothesized that, compared with crocin or voluntary exercise alone, 8 weeks of crocin combined with voluntary exercise in diabetic rats would produce a larger improvement in cardiovascular complications of type 2 diabetes. The present study was undertaken to clarify the effect of crocin and voluntary exercise on miR-126 and miR-210 expression in cardiac myocytes of diabetic rats.

Methods

Animals

Male Wistar rats (200-250g) were obtained from Tabriz medical faculty (Iran-Tabriz). Animals were housed in a room with a constant temperature of 24°C, a relative humidity of 50%, and a 12h dark/light cycle with access to food and water ad libitum. Animals in the *exercise group* were placed in individual wheel-*cage* units while the sedentary *group*s were *housed* in normal plastic *cages*. This study was approved by the Animal Ethics Committee (document number 92197) in accordance with the instruction for the care and use of laboratory animals prepared by Tabriz University of Medical Sciences.

Experimental design

Forty animals were randomly divided into four groups. *Ten animals were allocated* to each experimental *group a*t the beginning of the study. Group 1: Rats received NaCl 0.9 % Solution as a control group (Con). Group 2: Rats received a single dose of crocin (50 mg/kg) for eight weeks (Cro). Group 3: Rats performed voluntary exercise for eight weeks (Exe). Group 4: Animals received crocin and simultaneously performed voluntary exercise for eight weeks (Cro-Exe).

Crocin powder (Sigma, Germany) was diluted by normal saline (0.9%). Crocin was gavaged (50 mg/kg) 6 days a week for 8 weeks.[22] In addition, NaCl 0.9 % solution was gavaged in groups 1 and 3 during experiment.

For the assessment of voluntary exercise, rats were housed individually in a cage containing a wheel (1.00 m circumference, TajhizGostar). Each exercising rat had a separate running wheel in its cage that allowed it to run voluntarily during 8 weeks of the study. This stainless-steel running wheel was equipped with a digital magnetic counter that was activated by wheel rotation and wheel revolutions were recorded daily. Then the running distance per day was calculated as the number of wheel revolutions each day. Rats with running distance lower than ~2000 m per day were eliminated before statistical analysis.[23] Considering the excluding criteria (exercise below standard protocols) statistical analysis was performed for 7 animals in each group.

Quantification of Akt and ERK1/2 in heart by ELISA

On the final day of experiment, rats were sacrificed under deep anesthesia with ketamine/xylazine (88/10 mg/kg, i.p.). Heart tissue immediately removed and washed with saline 0.9%. Tissue samples were weighted, homogenized in PBS (pH 7.2-7.4) and centrifuged for 20 min at the speed of 3000 rpm and 4°C. Then supernatants were collected in new tube and Akt and ERK1/2 levels were measured using sandwich rat ELISA kits. The ELISA assay was performed according to the manufacturer's instructions. Akt protein activation by phosphorylation at serine residue 473 (P-Akt) and ERK1/2 phosphorylation (PT202/Y204) was assayed with ELISA (Akt: Cat. No. CK-E91385; Hangzhou Eastbiopharm Co., Ltd., Hangzhou, China. ERK1/2:Abcam Cambridge, UK) and normalized to the total protein concentration for each sample as determined by the Bradford assay.[24]

Total RNA extraction, cDNA synthesis and real time PCR

Expression of miR-126 and miR-210 was assessed by qRT–PCR. Triplicate assays were performed for each RNA sample. MicroRNA was extracted from the heart tissue using the miRCURYTM RNA Isolation Kit (Exiqon, Denmark) according to the manufacturer's protocol. The procedure was performed based on spin column using a proprietary resin as a separation matrix for RNA from other cell components. RNA content and purity were measured at a wavelength of 260–280 nm using Nanodrop 1000 spectrophotometer (Thermo scientific, Wilmington DE 19810 *U*SA).

cDNA synthesis was done according to LNA universal RT miRNA PCR kit (Exiqon, Denmark). Briefly, total RNA containing microRNA was polyadenylated and cDNA was synthesized using a poly (T) primer with a 3′ degenerate anchor and a 5′ universal tag.

Syber Green qPCR Mix purchased from Exiqon (denmark) and used for real time PCR. Real time PCR was done using Rotor-Gene 6000 Corbett. The $2^{-(\Delta\Delta Ct)}$ method was used to determine relative quantitative levels of miR-126 and miR-210. The results were expressed as the fold-difference to the control group. Mir-1 was used as the endogenous control miRNA.

Immunohistochemical assessments

For the investigation of angiogenesis in the heart tissue, samples from left ventricle were immersed into 10% formalin after excision, embedded in paraffin, and cut into 4 μm-thick slices. Sections were deparaffinized in xylene and dehydrated in a graded series of ethanol. Slides were incubated sequentially in proteinase K and treated by 0.3% hydrogen peroxide to block endogenous peroxidase activity. Sections were overlaid by primary antibody CD31 (Santa Cruz, USA) a marker of angiogenesis and incubated at +4°C overnight. Sections were then washed and incubated with standard avidin–biotin complex (ABC; Santa Cruz) according to the manufacturer's instructions. Then, the slides were incubated in DAB (di-amino-benzidine, Santa Cruz), as the

chromagen, and counterstained with Mayer's hematoxylin. Finally, sections were cleared in xylene, mounted with Entellan and assessed by light microscope (Olympus BX 40, Japan). Capillaries were visualized in the myocardium as a brown precipitate. Vascular structures positive for CD31 were counted for 5 to 6 slides per animal and 10 fields per slide.

Statistical analysis

Results are presented as mean ± SD. Statistical analysis was performed using SPSS version 21.0 statistical software for Windows. All parameters were tested for normality using the one-sample Kolmogorov-Smirnov test. Average daily running distances for rats in each exercise group were averaged for each week and were compared using repeated measures ANOVA. Physical activity between Exe and Cro-Exe groups was analyzed using independent t-test. For Akt, ERK, CD31, miR-126, and miR-210 parameters, data were analyzed using two-way ANOVA followed by Tukey's post hoc test. A *p*-value less than 0.05 was considered statistically significant.

Results

Voluntary exercise

The Figure 1 illustrates the average running distance per week over the 8-week period of experiment. Animals ran voluntarily an average of 2.949 ± 178 m/week in the exercise group and an average of 3.090 ± 140 m/week in the crocin-exercise group. There was no significant difference in physical activity between Exe and Cro-Exe groups based on an independent t-test analysis.

Average running distance increased gradually in both Exe and Cro-Exe groups from first to eighth week. This increase in Exe group was significantly different from the prior week in fourth ($p < 0.05$) and sixth ($p < 0.05$) weeks. In Cro-Exe group, animals ran significantly more distance in second ($p < 0.01$), third ($p < 0.01$), fifth ($p < 0.05$), and sixth ($p < 0.05$) weeks than the prior week.

Effects of crocin combined with voluntary exercise on Akt levels in the heart tissue

After 8 weeks of administration of crocin or performing voluntary exercise, the level of p-Akt increased significantly in Exe ($p < 0.01$), Cro ($p < 0.01$) and Cro-Exe ($p < 0.001$) groups in comparison with Con group (Figure 2). A comparison between the Cro-Exe group with Exe and Cro groups exhibited significant difference among these groups ($p < 0.01$ and $p < 0.001$, respectively).

Effects of crocin combined with voluntary exercise on ERK1/2 levels in the heart tissue

Two-way ANOVA showed that the p-ERK1/2 levels were significantly higher in rats treated with crocin or voluntary exercise than in control rats (Exe: $p < 0.01$, Cro: $p < 0.05$, and Cro-Exe: $p < 0.001$). Administration of crocin combined with exercise significantly increased p-ERK1/2 levels of the heart tissue compared to Exe ($p < 0.01$) and Cro ($p < 0.001$) groups (Figure 3). Figure 2 also indicates that crocin combined with voluntary exercise has a synergistic effect in p-ERK1/2 protein levels in heart tissue.

Effects of crocin combined with voluntary exercise on miR-126 expression in the heart tissue

Two-way ANOVA showed that the miR-126 expression were significantly higher in rats treated with crocin ($p < 0.001$), voluntary exercise ($p < 0.01$) and crocin

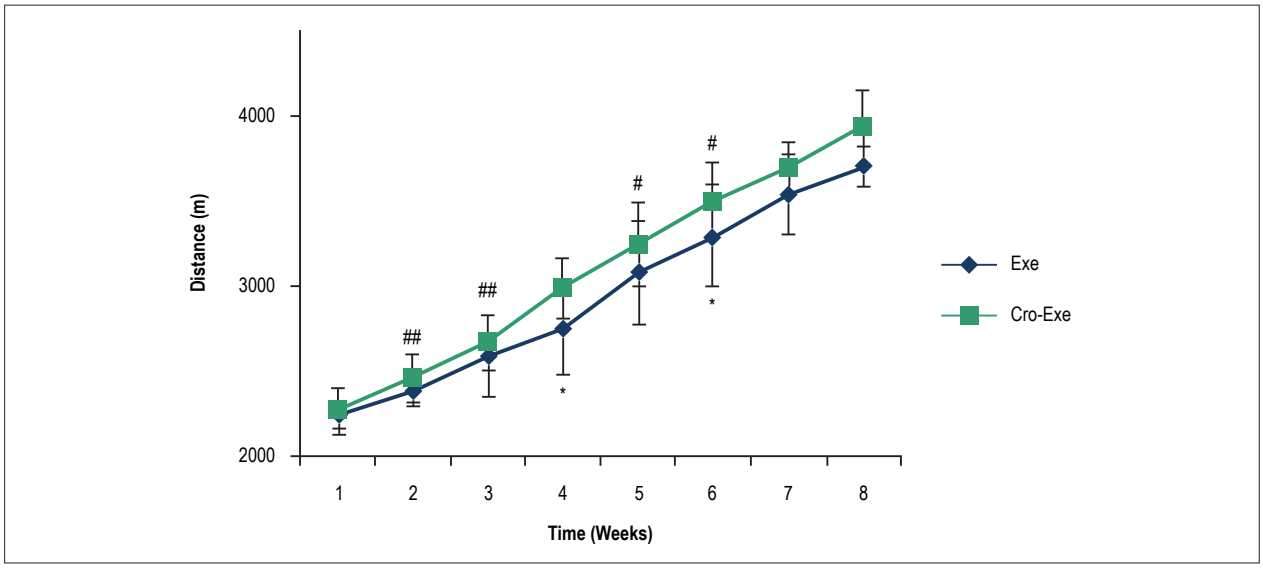

Figure 1 – *Running distance was averaged over each week of running wheel access (mean ± SD) for the 8-week duration of experiment, for both Exe and Cro-Exe groups (n = 7/group). There was no significant difference between groups. Animals in both Exe and Cro-Exe groups increased their average weekly running distance over the subsequent weeks. *p < 0.05 indicates a significant difference between consecutive weeks in Exe group. #p < 0.05 and ##p < 0.01 indicate significant differences between consecutive weeks in Cro-Exe group.*

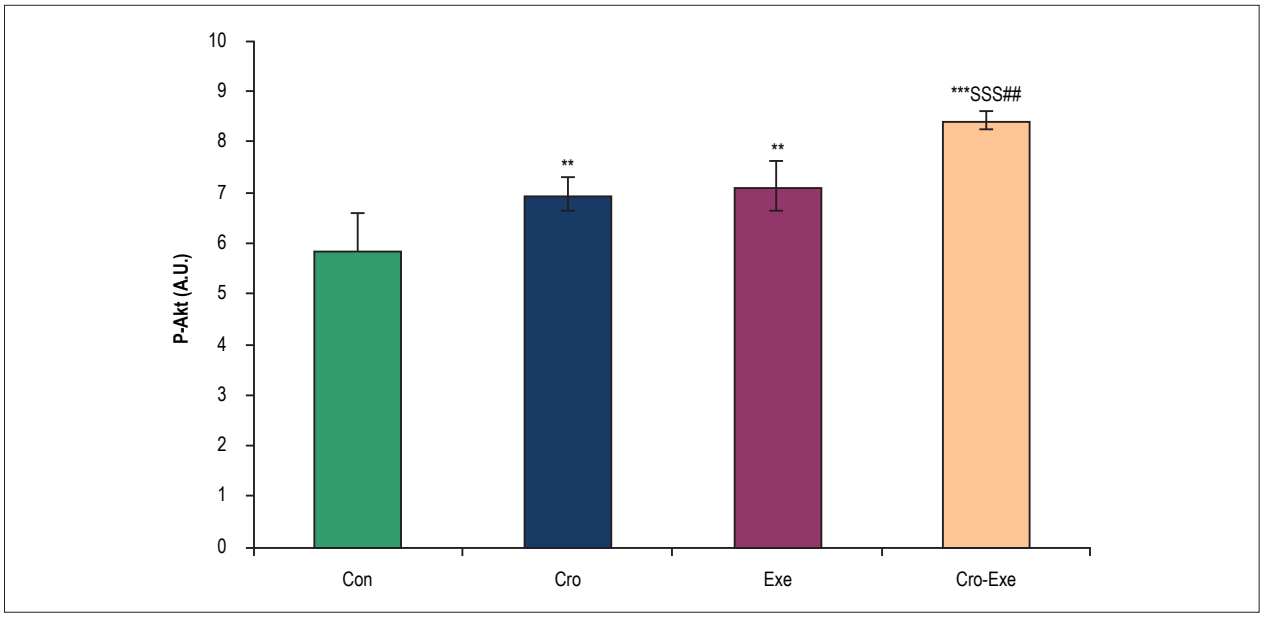

Figure 2 – *Effect of crocin and voluntary exercise on p-Akt levels. Data are shown as mean ± SD for n = 7 animals. $^{***}p < 0.001$ and $^{**}p < 0.01$ indicate significant differences with control group, $^{\$\$\$}p < 0.001$ indicates a significant difference with Cro group, and $^{\#\#}p < 0.01$ indicates a significant difference with Exe group.*

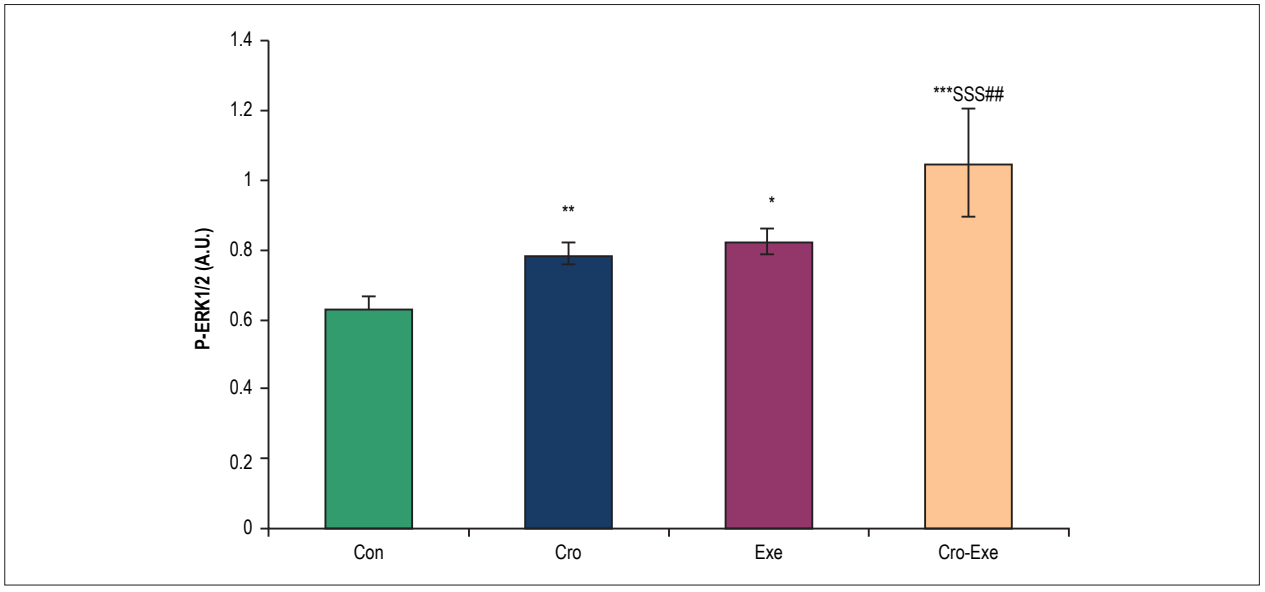

Figure 3 – *Effect of crocin and voluntary exercise on p-ERK1/2 levels. Data are shown as mean ± SD for n = 7 animals. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ indicate significant differences with control group. $^{\$\$\$}p < 0.001$ indicates a significant difference with Cro group and $^{\#\#}p < 0.01$ indicates a significant difference with Exe group.*

combination with exercise ($p < 0.001$) than in control rats. In the rats that underwent voluntary exercise and simultaneously received crocin for 8 weeks, expression of heart miR-126 significantly increased compared with Exe ($p < 0.01$), and Cro ($p < 0.001$) groups (Figure 4).

Effects of crocin combined with voluntary exercise on miR-210 expression in the heart tissue

As shown in Figure 5, following crocin administration and exercise performing, the heart expression level of miR-210 was significantly upregulated in Cro and Exe groups when compared to control group ($p < 0.01$ and $p < 0.001$, respectively). On the other hand, the expression of miR-210 increased significantly in Cro-Exe group compared with control group ($p < 0.001$). In addition, there is a significant difference between Cro-Exe and Cro groups ($p < 0.01$).

Effects of crocin combined with voluntary exercise on $CD31^+$ cells in myocardial capillary network

As demonstrated in figure 6, number of $CD31^+$ cells were higher in animals that received crocin ($p < 0.05$) or performed exercise ($p < 0.05$) compared with control group

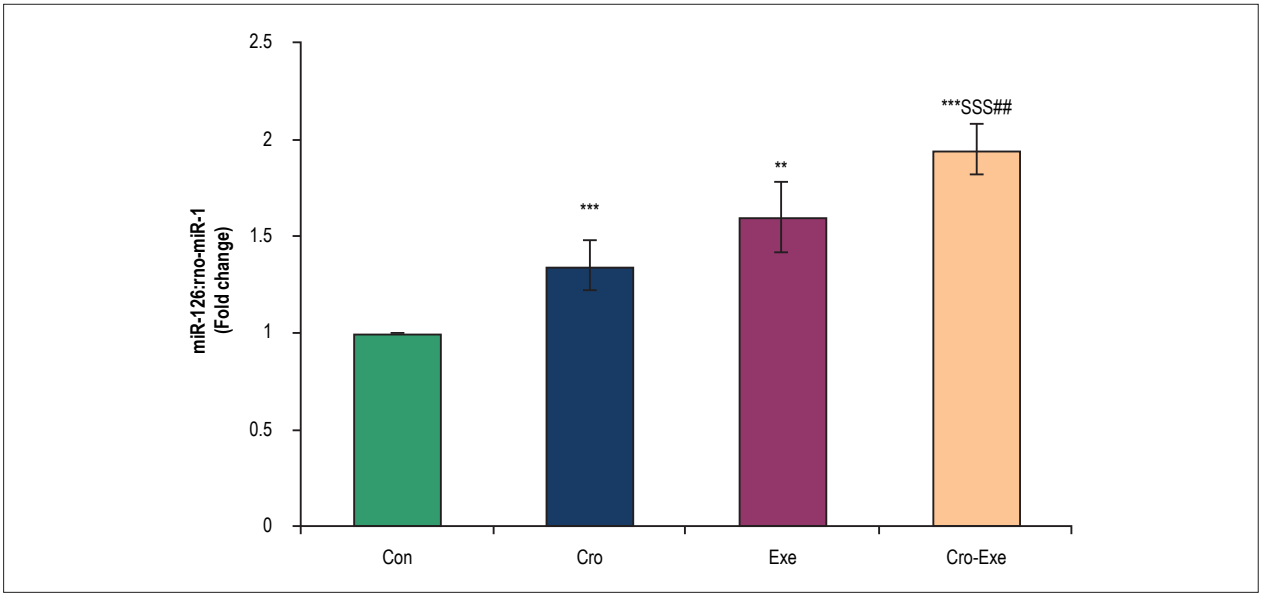

Figure 4 – *Effect of crocin and voluntary exercise on miR-126 expression levels. Data are shown as mean ± SD for n = 7 animals. $^{**}p < 0.01$ and $^{***}p < 0.001$ indicate significant differences with control group. $^{\$\$\$}p < 0.001$ indicates a significant difference with Cro group and $^{\#\#}p < 0.01$ indicates a significant difference with Exe group.*

(Figures 6A, B, C). In addition, $CD31^+$ cells were significantly higher in sections from the heart of Cro-Exe group than Exe ($p < 0.01$) and Cro ($p < 0.01$) groups (Figure 6D). Thus, crocin combination with voluntary exercise appears to enhance vasculogenic response.

Discussion

In the present study, we demonstrated that miR-126 and miR-210 expression of rat cardiac tissue increased in crocin, voluntary exercise, and exercise-crocin groups. In addition, crocin and voluntary exercise stimulated Akt and ERK1/2 proteins and angiogenesis in the heart tissue. For the first time, our study demonstrated that heart miR-126 and its related pathways including Akt and ERK1/2 upregulated in response to crocin combined with voluntary exercise in rats. Furthermore, our findings showed that crocin administration and voluntary exercise performing increased the expression of heart miR-210.

MiR-210, a hypoxia-specific miRNA, depends on HIF activation and upregulated after hypoxia.[25] When miR-210 is overexpressed in endothelial cells, the ability of these cells to form blood vessels becomes pronounced, more than that of cells with normal levels of expression. Confirming miR-210 proangiogenic role, up-regulation of miR-210 in CD34+ cells increased tissue perfusion and capillary density in a mouse model of hind limb ischemia.[26] Previous research has indicated that miR-210 may improve angiogenesis through the negative regulation of its target gene, ephrin A3, which is an important member of the ephrin angiogenesis regulatory gene family.[27] Heart tissue expresses a variety of miRNAs, but little is known about the cardiac angiogenic response to crocin and exercise.[28] Preclinical work demonstrated that intracardiac injections with a minicircle vector carrying miR-210 in a mouse model of myocardial infarction promoted significant improvement of left ventricular fractional shortening, decreased cellular apoptosis, and increased neovascularization.[29] In the current study, we observed that crocin and voluntary exercise increased miR-210 expression levels in heart tissue using quantitative real-time PCR analysis. The miR-210 upregulation seems to be dependent on the exercise performing because the group that performed voluntary exercise showed a stronger miR-210 expression than crocin group. A probable mechanism is that during exercise, local hypoxic conditions in the cardiac muscle can occur and hypoxic situation trigger a number of physiological responses such as angiogenesis through HIF-1α-induced miR-210 expression.[30,31] These data are in line with the observations made by Anja Bye et al.[32] regarding significant increase in miR-210 expression in subjects with low Vo2max following the exercise activity.

MiR-126 is a pro-angiogenic miR, which is strongly expressed in the heart endothelium and directly targets SPRED1 and PIK3R2 for repression and functions to promote VEGF signaling.[14,15] In fact, miR-126 activates survival kinases including ERK and Akt by downregulation of its targets and enhances the actions of VEGF.[16,17] In endothelial cells, VEGF promotes angiogenesis through the phosphorylation of ERK1 and Akt. ERK and Akt are well known kinases that activate and promote cell proliferation by stimulating growth factors.[18]

In the present study, we showed that miR-126 regulates heart angiogenesis via Akt and ERK1/2 pathways in response to crocin and voluntary exercise. However, a few studies available say that exercise can increase miR expression in cardiac tissue. In line with our results, Uhlemann et al.[33] reported that miR-126 expression increased after acute endurance exercise. Major findings also emerge from Fernandes et al.[34] study indicating that exercise training restored the levels of peripheral miR-126 associated with

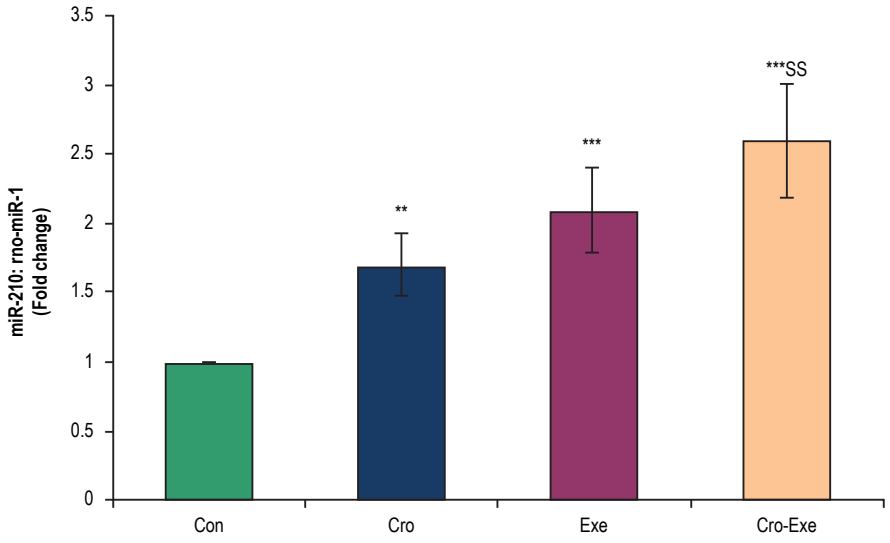

Figure 5 – *Effect of crocin and voluntary exercise on miR-210 expression levels. Data are shown as mean ± SD for n = 7 animals. $^{**}p < 0.01$ and $^{***}p < 0.001$ indicate significant differences with control group and $^{\$\$\$}p < 0.001$ indicates a significant difference with Cro group.*

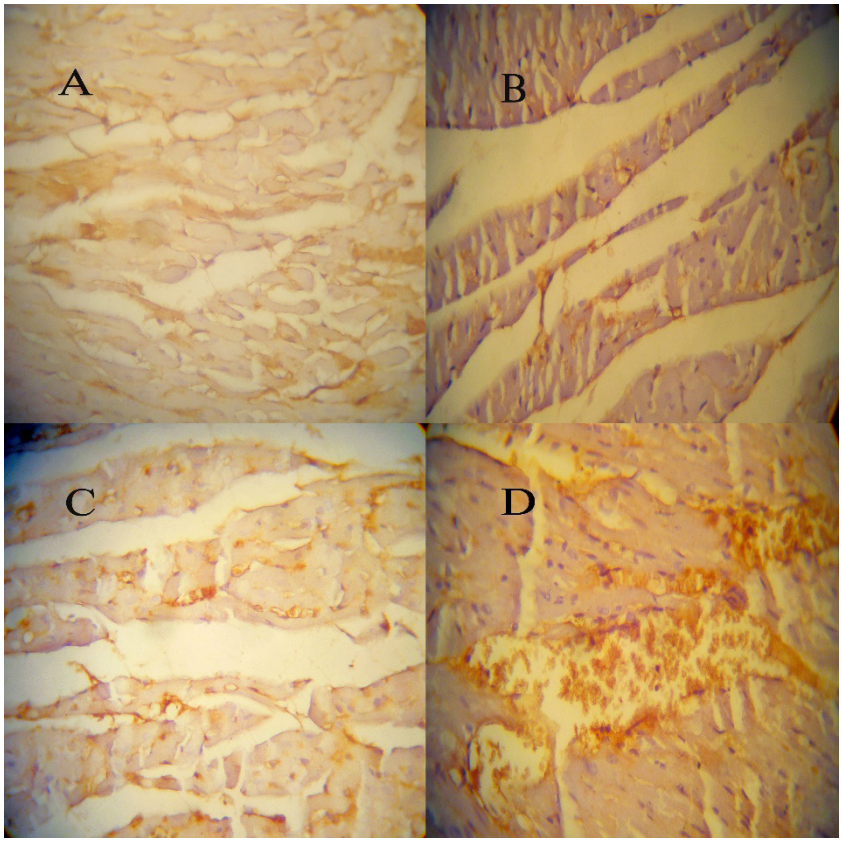

Figure 6 – *A) Representative images of CD31 staining (brown) in cardiac vessels of control, exercise, crocin and exercise-crocin groups (Magnification ×400). A: Con, B: Exe, C: Cro, D: Cro-Exe. B) Microvessel density was analyzed by immunohistochemistry for CD31. Microvessel density was quantified using 6 slides per animal and 10 fields per slide. Data are shown as mean ± SD for n = 7 animals. ***p < 0.001 indicates a significant difference with control group. ###p < 0.001 and $$$p < 0.001 indicate significant differences with exercise group and crocin group, respectively.*

revascularization in hypertension. Despite the observation that exercise affects endothelial function, the exact mechanism remains speculative. It is well established that exercise can increase VEGF levels, which is one of the major regulators of angiogenesis and cell survival.[35] VEGF binds to VEGFR2 and promotes endothelial survival and angiogenesis signals which are intermediated by PI3K and its downstream target of the Akt and ERK1/2. In addition, we showed that ERK1/2 and Akt levels increased under high expression of miR-126. Therefore, it seems that voluntary exercise relieves the repressive influence of Spred-1/PI3K on the Akt and ERK1/2 by miR-126 overexpression, which finally improves cardiac angiogenesis. This finding is in agreement with a previous study that showed that PI3KR2 mRNA expression in the heart decreased in the exercise groups and it was associated with increase in protein expression of PI3K and phosphorylated Akt.[36]

In this study, we also showed that crocin regulates heart angiogenesis through miR-126 and its related Akt and ERK1/2 pathways. Crocin, a carotenoid pigment of saffron, has different pharmacological functions on the nervous,[37] cardiac,[38] and renal[39] systems. Cardioprotective effects of crocin have been reported in some studies that are related to improvement of antioxidant activities and cardiac biomarkers.[3,40] Although many researchers have explored the roles of crocin on different tissues, only a few studies have investigated the effects of crocin on angiogenesis in the heart tissue. Bie et al[41] demonstrated that saffron increased expression of VEGF-R2 and promoted angiogenesis following brain injury in rats. Furthermore, it has been reported that the PI3K/Akt pathways are activated by crocin in the ganglion cell layer after retinal IR injury.[42] Kang et al[43] also showed that saffron increased the phosphorylation of mitogen-activated protein kinases (MAPKs), as one member of ERK family, in the muscle cells. Also our previous study confirms that crocin increases VEGF-A levels in the heart tissue of diabetic and non-diabetic rats.[40]

Based on the present results it could be concluded that crocin pretreatment improved cardiac angiogenesis, the effect which can be attributed to its ability of increasing Akt and ERK1/2 levels via enhancement of miR-126. Preservation of histoarchitecture of heart tissue by crocin pretreatment confirms these effects. Regarding the limitations of this study, we did not measure other factors involved in angiogenesis and we referred to previous studies. Further studies are needed to explore other possible mechanisms and pathways that might be directly or indirectly involved in its cardioprotective effects. Therefore, we suggest that crocin by increasing of miR-126 and enhancement of VEGF signaling pathways through Akt and ERK1/2 can induce cardiac capillary formation. In addition, we showed that crocin combination with voluntary exercise has synergistic effects on miR-126 expression and Akt, ERK1/2 levels in heart tissue, which was the first study in rat cardiac angiogenesis.

Conclusion

This study shows that crocin in combination with voluntary exercise promotes cardiac angiogenesis and this may be related to expression of miRNA-126 and miR-210. Further studies about the mechanism of crocin and voluntary exercise on cardiac angiogenesis may provide a basis for the development of new therapeutic or preventive approaches to some overcome cardiovascular diseases.

Author contributions

Conception and design of the research, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Ghorbanzadeh V, Mohammadi M, Mohaddes G; Acquisition of data: Ghorbanzadeh V, Dariushnejad H, Abhari A, Chodari L; Analysis and interpretation of the data: Ghorbanzadeh V, Dariushnejad H, Abhari A; Statistical analysis:Ghorbanzadeh V, Dariushnejad H, Mohaddes G.

Potential Conflict of Interest

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

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Gestational Protein Restriction Increases Cardiac Connexin 43 mRNA levels in male adult rat offspring

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Abstract

Background: The dietary limitation during pregnancy influences the growth and development of the fetus and offspring and their health into adult life. The mechanisms underlying the adverse effects of gestational protein restriction (GPR) in the development of the offspring hearts are not well understood.

Objectives: The aim of this study was to evaluate the effects of GPR on cardiac structure in male rat offspring at day 60 after birth (d60).

Methods: Pregnant Wistar rats were fed a normal-protein (NP, 17% casein) or low-protein (LP, 6% casein) diet. Blood pressure (BP) values from 60-day-old male offspring were measured by an indirect tail-cuff method using an electro sphygmomanometer. Hearts (d60) were collected for assessment of connexin 43 (Cx43) mRNA expression and morphological and morphometric analysis.

Results: LP offspring showed no difference in body weight, although they were born lighter than NP offspring. BP levels were significantly higher in the LP group. We observed a significant increase in the area occupied by collagen fibers, a decrease in the number of cardiomyocytes by 10^4 μm^2, and an increase in cardiomyocyte area associated with an increased Cx43 expression.

Conclusion: GPR changes myocardial levels of Cx43 mRNA in male young adult rats, suggesting that this mechanism aims to compensate the fibrotic process by the accumulation of collagen fibers in the heart interstitium. (Arq Bras Cardiol. 2017; 109(1):63-70)

Keywords: Pregnancy; Fetal Development; Connexin 43; Metabolism.

Introduction

Maternal dietary restriction is a recognized cause of mortality at birth[1] and low-birth weight.[2] The concept of "programming" is used to associate prenatal events to changes in fetal growth that may become pathological in adulthood.[3,4] Although molecular and physiological changes resulting from nutritional imbalance during pregnancy allow the offspring to survive, the long-term cardiovascular effects imposed by these changes promote structural modifications and changes in components of the renal, respiratory, endocrine, and central nervous systems.[5,6] Recent data have shown bioenergetic changes in liver mitochondria of 30-day-old pups born from mothers undergoing protein restriction during gestation.[7] In addition, gestational protein restriction (GPR) has been shown to be an important risk factor for cardiovascular disorders later in life.[8]

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During electrical activation of the heart, all myocytes are individually activated by currents flowing through intercellular junctions. In the cardiovascular system, these gap junctions include one or more of four connexins – namely, Cx37, Cx40, Cx43, and Cx45 – that work together during the initial cardiovascular development.[9] Gap junctions also ensure the mechanical and electrical communication between different types of muscle cells.[10] This role is crucial in the heart since proper ejection of blood to the circulation depends necessarily on a coordinated contraction of both atrial and ventricular cardiomyocytes.[11,12] Pathological conditions such as diabetes and hypertension are associated with deletions and changes in the regulation of connexin expression[13] while connexin genes may have deleterious effects on cardiac function.[14]

The aim of this study was to evaluate the effects of GPR on cardiac structure in male rat offspring at day 60 (d60) after birth. We specifically investigated their blood pressure (BP) values during the 8th week of life, morphological and morphometric parameters of left ventricular cardiomyocytes, and Cx43 mRNA levels. Our choice to study the molecular profile of Cx43 was based on the fact that this is the most abundant and expressed connexin in the heart. This is the first study describing the cardiac expression of this gene in rats subjected to GPR.

Methods

Animal care

All experiments were conducted in strict agreement with the Guide for the Care and Use of Laboratory Animals and approved by the local Animal Care and Use Committee (Permit N°. 056/2014). Ten-week-old virgin female Wistar rats weighing 180 to 250 g were mated with males. After confirming the pregnancy with observation of sperm in a vaginal smear (day 1 of pregnancy), we randomly allocated the pregnant rats (n = 12) on individual cages to receive an isocaloric and normal sodium semisynthetic diet (AIN 93G, Pragsoluções, Jau, SP, Brazil) with a normal protein content (17% casein, normal-protein [NP] group, n = 6, numbered from 1 to 6: 1NP to 6NP) or a low protein content (6% casein, low-protein [LP] group, n = 6, numbered from 1 to 6: 1LP to 6LP) (Table 1), as previously described.[7,15] The animals were maintained at a controlled temperature (21 ± 1°C) on a 12-h light/dark cycle, with free access to water until they delivered pups at 22 days of gestation. The anogenital distance was measured in all pups[16] and litters were culled to a maximum of 8 males pups to minimize variation in nutrition during the suckling period. All liveborn male offspring of each mother were used in the experiments. When the number of male pups was less than 8, the number was increased by female pups until this value was reached. After weaning, the pups were housed in cages for a maximum of 4 animals, numbered and identified according to their affiliation. The number of cages followed the identification number of the mothers (from 1 to 6, NP or LP). When the number of male pups of each mother exceeded four, the cages were identified by the number of the mother plus the letters A or B. The identification of the pups in the cages was done by the marking on the tail: 1st (without tail marking), 2nd (one tail trace), 3rd (two tail traces) and 4th (three tail traces). All the animals were identified by this method and the total number of male rats was 21 for the NP group and 37 for the LP group. They received *ad libitum* water and a standard laboratory diet (21.6% protein and 4.0% lipid, Nuvilab CR-1, Nuvital, Colombo, PR, Brazil). On the 8th week of life, their BP levels were measured and, after anesthesia with ketamine (75 mg·kg^{-1} body weight, i.p.) and xylazine (10 mg·kg^{-1} body weight, i.p.), their hearts were removed for analysis. The hearts were weighed, and fragments from the middle third of the left ventricles were processed for morphological and molecular analyses. Twelve animals (NP, n = 6; LP, n = 6; 1 male rat for each of the mothers, randomly) were perfused for measurement of the cross-sectional area of the cardiomyocytes.

Blood pressure measurement

Systemic arterial pressure was measured in conscious 7- and 8-week-old rats (LP, n = 12; NP, n = 12; 2 rats for each of the mothers, randomly) by an indirect tail-cuff method using an electrosphygmomanometer combined with a pneumatic pulse transducer/amplifier (IITC Life Science Inc., CA, USA). This indirect approach allowed repeated measurements with a close correlation (correlation coefficient = 0.975) to direct intra-arterial recording. The mean of three consecutive readings represented the BP level of the animal.

Tissue collection: histology and morphometric analysis

Hearts were removed and longitudinally sectioned in the middle region in two halves. For histological analysis, the upper and lower halves of the six hearts from each experimental group animal were fixed using Millonig's solution and treated for paraffin embedding. Six-micrometer-thick sections were obtained from each blocked tissue from the middle region and stained with toluidine blue (TB) and picrosirius-hematoxylin (PH). Three of the sections stained with TB were used for cardiomyocyte counting (number per $10^4 \mu m^2$) and three other sections stained with PH were used for quantification of collagen fibers using polarization microscopy (% of birefringence area in $10^4 \mu m^2$). Five representative fields obtained from each longitudinal section of the left ventricle of each rat (150,000 μm^2 of total area by animal heart) were analyzed by light microscopy (Leica DM 2000 Photomicroscope) and captured with a digital Leica DFC 425 digital camera (Leica Microsystems, Wetzlar, Germany). Each digital image was photographed with the × 40 objective and formatted at fixed pixel density (8 × 10 inches at 150 dpi) using Sigma Scan Pro (v.6.0). At each digital image,

Table 1 – Composition of the diets fed to the pregnant rats: normal protein (NP, 17%) and low protein (LP, 6%)[7,15]

g/kg	NP (17%)	LP (6%)
Cornstarch	397	480
Casein (84%)	202	71.5
Dextrin (90-94%)	130.5	159
Sucrose	100	121
Soybean oil	70	70
Fiber	50	50
Salt mixture (AIN 93 GMX)	35	35
Vitamin mix (AIN 93 VX)	10	10
L-cystine	3	1
Choline bitartrate	2.5	2.5

the cardiomyocytes were counted following recommendation by Olivetti et al.[17] and the area of birefringent collagen fibers was calculated as described by Mendes et al.[18] For the analyses, the investigators were blinded to the group allocation.

Measurement of cardiomyocyte cross-sectional area

The animals were anesthetized and perfused by the left carotid artery with saline containing heparin (5%) for 15 min and subsequently with 0.1 M phosphate buffer (pH 7.4) containing 4% (w/v) paraformaldehyde for 25 min. After perfusion, myocardial tissue samples were obtained from the septum and free wall of the middle part of the left ventricle and fixed in 4% phosphate-buffered formalin during 24 h for paraffin embedding. Five-micrometer-thick sections were cut from the blocked tissue and stained with hematoxylin-eosin (HE). The cross-sectional area of the cardiomyocytes was determined in at least 100 myocytes per slide stained with HE. The measurements were performed under a Leica DM 2000 microscope (x40 magnification lens) attached to a digital camera (Leica DFC 425, Leica Microsystems, Wetzlar, Germany) and connected to a personal computer equipped with the image analyser software Image J (National Institutes of Health, Bethesda, MD, USA). The cardiomyocyte area was measured with a digitizing pad, and the selected cells were transversely cut with the nucleus clearly identified in the center of the myocyte.[19]

RNA isolation and semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was isolated from ~100-mg samples of left ventricular tissue with the TRIzol® reagent (Invitrogen, CA, USA) and digested with DNAse I, Amplification Grade (Invitrogen) according to the manufacturer's instructions. RNA concentration was determined by measuring UV absorbance at 260 nm using a spectrophotometer, and integrity was confirmed by formaldehyde gel electrophoresis. Samples of total RNA were stored at -80°C until further use for analysis. cDNA was synthesized from 2 μg of RNA in the presence of dithiothreitol, dNTP, random primers, RNAseOUT, and SuperScript™ II Reverse Transcriptase (Invitrogen) in a final volume of 20 μL. Semiquantitative analysis of Cx43 mRNA expression was performed by RT-PCR in a final volume of 25 μL containing 1 μL of cDNA, 1.6 mM of $MgCl_2$, 200 μM of each dNTP, 0.2 pmol of each primer, and 0.04 U of Taq DNA polymerase (Invitrogen, Itapevi, SP, Brazil). Cx43 was amplified using gene-specific forward (5´-GATTGAAGAGCACGGCAAGG-3') and reverse (5´-GTGTAGACCGCGCTCAAG-3´) primers with an expected amplicon of 144 bp (Tm 58°C). ACTB (β-actin) was used as a housekeeping gene (Tm 57°C; forward primer 5´-AGAGGGAAATCGTGCGTGACA-3' and reverse primer 5´-CGATAGTGATGACCTGACCGTCA-3´) yielding an amplification product of 178 bp that was used to normalize the Cx43 mRNA levels.

The amplified products were separated on 1.5% agarose gel stained with ethidium bromide, visualized, and photographed with the gel documentation system Syngene G: Box®. The signal intensity of the bands was measured densitometrically using the Scion Image software. Each value was determined as the mean of three densitometric readings. The results are expressed as average ratios of the relative optical densities of Cx43 PCR products in relation to the β-actin gene.

Data analysis

The results were analyzed using the GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) and are reported as the mean ± standard deviation (SD) of the measurements from six different animals. In cases in which two groups were compared, we used unpaired Student's t-test with a significance level of 5% ($p < 0.05$). When appropriate, we used analysis of variance (ANOVA) followed by Tukey's post-hoc test.

Results

Characteristics of the animals

A previous analysis of the weight of pups on day 1 after birth (published by our research group and shown in Figure 1A[7,20] for validation of the GPR model) showed that male offspring of mothers fed a low-protein diet (LP, n = 37, □ symbols, weight 6.40 ± 0.21 g) were significantly lighter than offspring of mothers fed a normal-protein diet (NP, n = 21, ■ symbols, weight 7.805 ± 0.51 g, * p = 0.0048, Figure 1A).[7,20] Figure 1B shows the body weight gain of the animals over a period of 60 days after birth. At d60, there was no significant difference in weight between the NP and LP male offspring (LP, n = 37, □ symbols, final weight 271.8 ± 66.66 g; NP, n = 21, ■ symbols, final weight 298.3 ± 68.68 g).

Effect of GPR on systemic arterial pressure and cardiac mass at d60

The mean systemic BP values of the offspring at the 8th week of life are shown in Figure 2A. Values in the LP group (131.8 ± 2.7 mmHg) were significantly higher than those in the NP group (120.3 ± 3.33 mmHg, p = 0.021). Hearts isolated at d60 were quickly weighed after sacrifice, and their weights showed no significant difference (NP, 1.71 ± 0.34 g; LP, 1.48 ± 0.22 g), as shown in Figure 2B. Similarly, the ratio of heart tissue weight (mg) and body weight (g) (Figure 2C) showed no significant difference between groups (NP, 3.89 ± 0.48 mg/g; LP, 3.86 ± 0.28 mg/g).

Effect of GPR on cardiac morphology

Figure 3A shows the quantification of the area of collagen fibers in the heart of rats at d60. We observed a significant increase in the collagen fiber area in the heart of LP animals compared with NP ones. Morphometric analysis by TB staining allowed quantification of the number of myocytes present in the heart of NP and LP male offspring. The results showed a significant decrease in the number of myocytes in the hearts of LP offspring when compared with NP ones (Figure 3B). After perfusion of some animals (n = 6), the left ventricles were collected, weighed and processed for quantitation of the cardiomyocyte area. The ratio of left ventricle weight (mg) and body weight (g) (Figure 3C) showed no significant difference in the NP (2.28 ± 0.25 mg/g) and LP (2.49 ± 0.27 mg/g) groups.

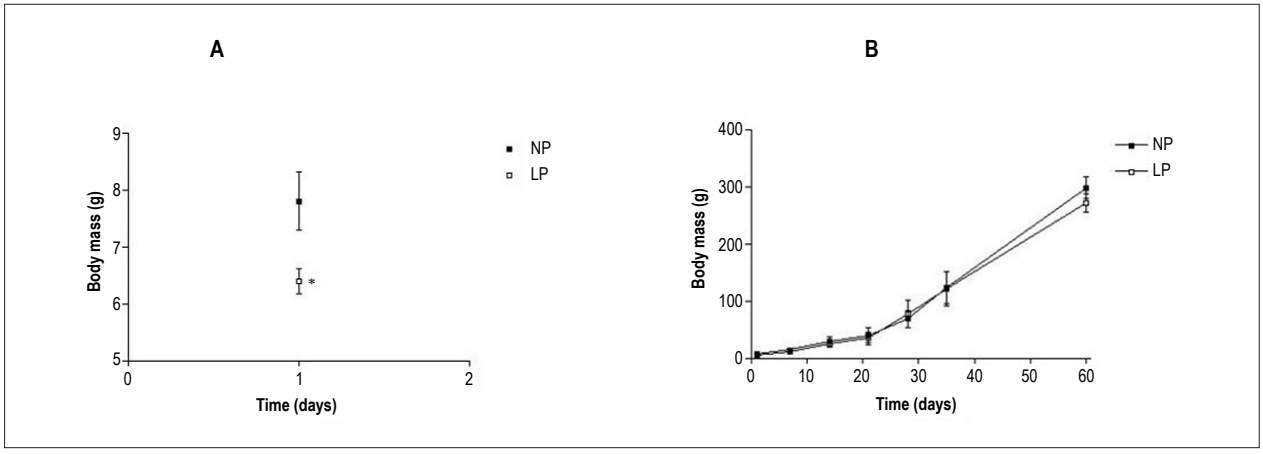

Figure 1 – *Effect of gestational protein restriction on the offspring weights. (A) weights on day 1 of male offspring of rats fed a normal-protein diet (NP, 17% protein, ■ symbol) or low-protein diet (LP, 6% protein, symbol □) during pregnancy (X ± SD, * p = 0.0048 versus NP); (B) growth curve of the offspring from the 1st to the 60th day after birth.*

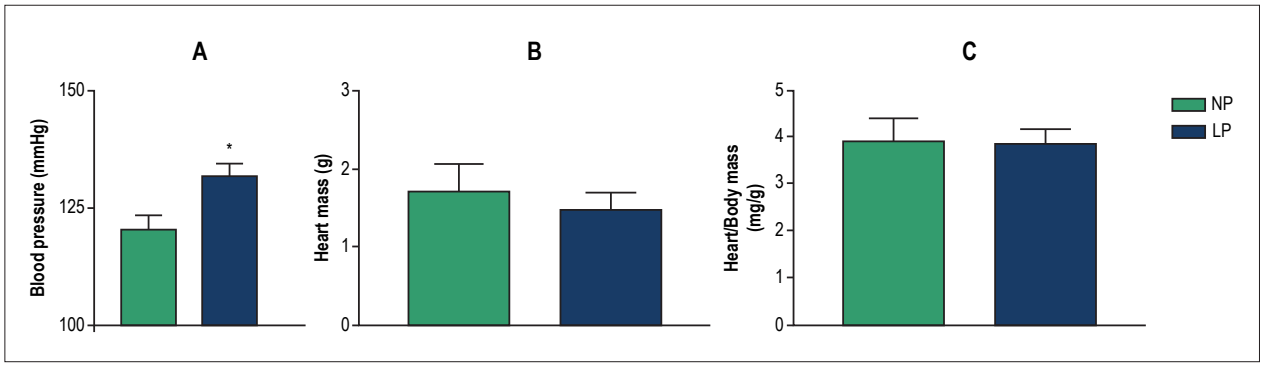

Figure 2 – *Effect of gestational protein restriction on the blood pressure levels and their cardiac mass of the offspring at d60. (A) blood pressure (mmHg); (B) cardiac mass (g); (C) ratio (mg/g) of the cardiac mass and body mass in young male offspring of rats fed a normal-protein diet (NP, full bar) or low-protein diet (LP, empty bars) (n = 12; X ± SD, * p = 0.021 versus NP).*

As seen in Figure 3D, the area of myocytes was significantly larger in the LP group (188.2 ± 4.14 μm^2) compared with the NP group (160.8 ± 2.57 μm^2).

Modulation of Cx43 in the heart

We collected left ventricular fragments for analysis of Cx43 expression. The values after densitometric analysis are shown in Figure 4. Compared with the NP group, the LP group showed significant increases in Cx43 mRNA levels (NP, 0.695 ± 0.058, n = 4, rats born to 4 different mothers; LP, 0.799 ± 0.032, n = 4; rats born to 4 different mothers).

Discussion

As expected and described in the literature,[7,20,21] offspring of rats that received a low-protein diet (LP group) were born lighter than offspring of rats fed a normal-protein diet (NP group). Fetal exposure to glucocorticoids (GC) has been proposed as one of the main risk factors for chronic diseases in adulthood.[22] Exogenous or endogenous (maternal stress) fetal exposure to excess GC reduces fetal growth.[23-25] During pregnancy, high levels of cortisol (in women)[26] and corticosterone (in rats)[27] are present in the maternal circulation.[24] Several studies in rats have shown that maternal malnutrition in response to maternal stress increases corticosterone levels in the plasma, decreases placental expression of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), and increases placental expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1).[24,28] A study in sheep fetuses has shown that mineralocorticoid (MR) and GC (GR) receptors, as well as 11β-HSD1 are abundantly expressed in myocytes and cardiac blood vessels.[29] The authors suggested that GC have access to both MR and GR in the fetal heart, and when GC plasma levels are elevated during a low-protein diet, the GC action in the cardiac MR and GR receptors also increases. GC could stimulate cardiac growth, either by hypertrophy or hyperplasia, or possibly even both. Cardiac hypertrophy could also result from high BP levels.[30] At d60 in our study, the LP offspring had increased systolic BP levels in parallel to an increased area of cardiac collagen fibers. However, these changes were not sufficient to increase the heart weight, which would then characterize

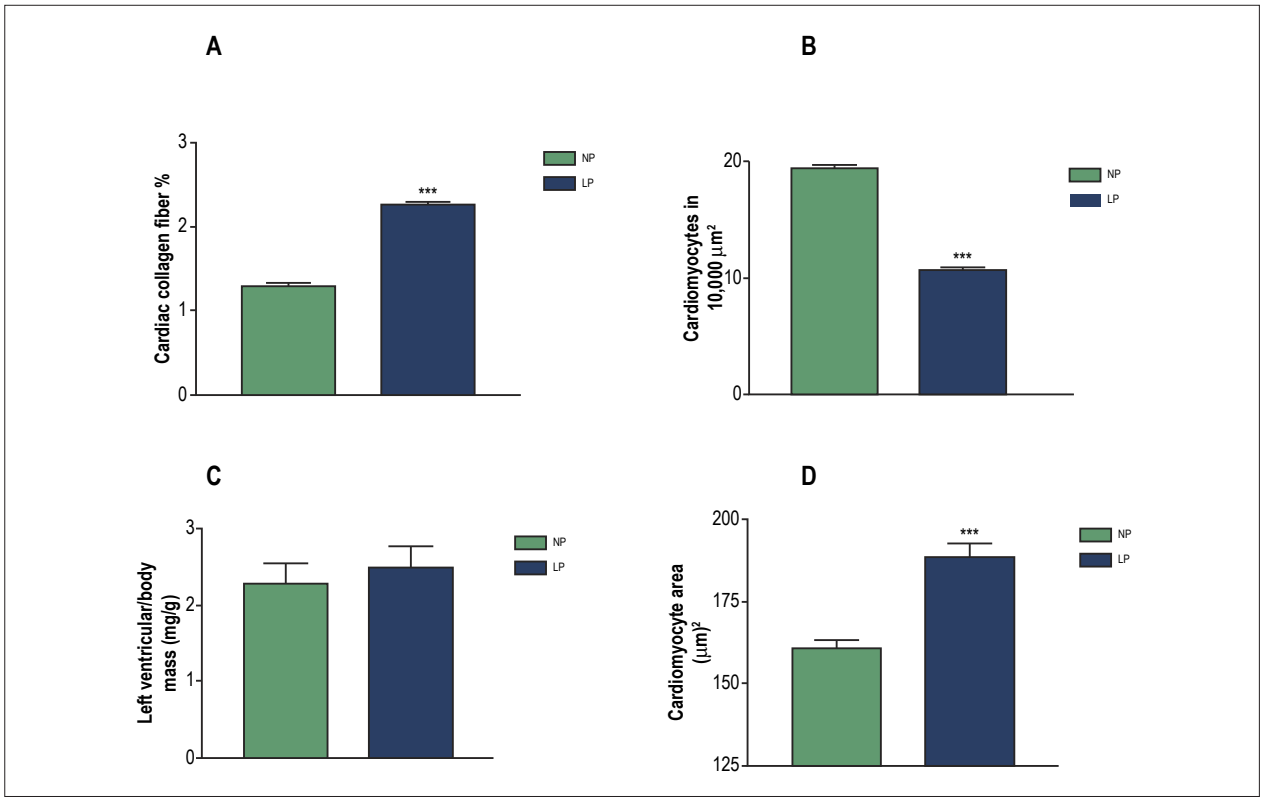

Figure 3 – *Effect of gestational protein restriction on cardiac morphometry at d60. (A) percentage of collagen fibers area/104 µm² in left ventricular sections stained with picrosirius-hematoxylin (n = 6; *** p < 0.0001 versus NP); (B) number of myocytes/104 µm² in left ventricular sections stained with toluidine blue (n = 6; * p < 0.0001 versus NP); (C) relationship between left ventricular weight and body mass (mg/g) at the age of 60 days in offspring of rats fed a normal-protein diet (NP, full bars) or low-protein diet (LP, empty bars); (D) cardiomyocyte cross-sectional area (µm²; X ± SD; n > 100 myocytes; *** p < 0.0001 versus NP).*

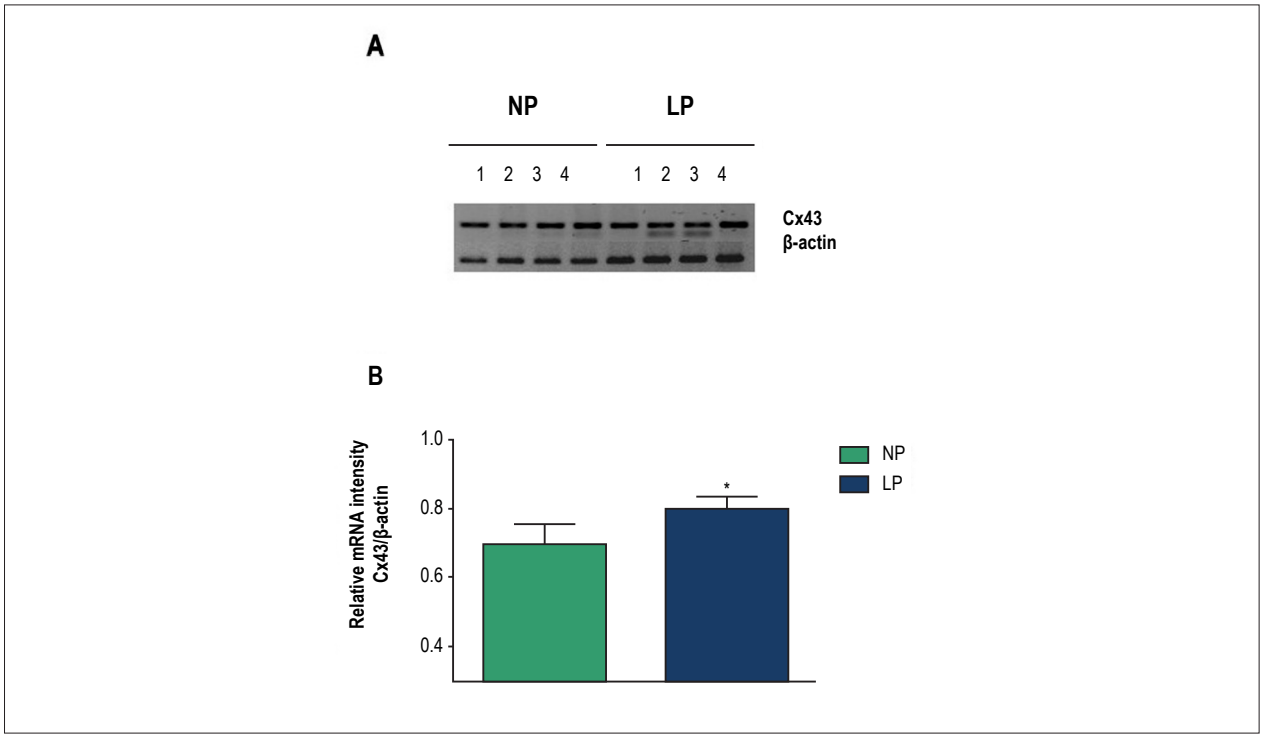

Figure 4 – *RT-PCR of Cx43 mRNA expression in the left ventricle (A) and densitometric analysis (B). Young male offspring of rats fed a normal-protein diet (NP, full bar) or low-protein diet (LP, empty bar) at d60 (n = 4; X ± SD of the optical density of Cx43 mRNA expression relative to β-actin; * p = 0.02 versus NP).*

the changes as cardiac hypertrophy. Although the area of the cardiomyocytes increased, the number of cardiomyocytes reduced. This finding, observed in young male offspring hearts in our study, support the evidence of interstitial collagen deposition, a symptom of cardiac hypertrophy in response to hypertension in adult human hearts.[30]

The renin-angiotensin system (RAS) plays an important role in primary and secondary forms of hypertension. Components of the RAS, such as angiotensin-converting enzyme (ACE) and angiotensin II, are locally produced in cardiac tissues[31] and are primary candidates for factors promoting remodeling, mainly cardiac myocyte hypertrophy and increased extracellular fibrosis, which lead to deterioration in cardiac function.[32] Various experimental animal models have been developed to investigate the associations between fetal undernutrition and cardiovascular disease later in life,[33,34] and a possible involvement of systemic RAS in the development of hypertension has been reported.[35,36]

The composition of the extracellular matrix in physiological and pathophysiological conditions can affect the degree of electrical coupling in cardiac myocytes.[37] The conduction of electrical impulses in the heart is determined mainly by three key parameters: electrical coupling between cardiomyocytes, excitability of individual cardiomyocytes, and connective tissue architecture.[37] These parameters of conduction are primarily mediated by Cx43, NaV1.5 sodium channels, and by the amount of collagen fibers, respectively. In cardiac arrhythmias,[38] abnormalities in any of these driving parameters have frequently been observed. Cx43 is generally down-regulated, less phosphorylated, and/or redistributed at the intercalated discs along the lateral aspects of the cardiomyocyte.[14,39,40] Our study provides the first evidence of increased Cx43 expression in rat hearts induced by GPR. Although our results are limited, we hypothesize that the increased deposition of collagen fibers in the heart associated with increased systolic BP lead to changes in the cardiac conduction of electrical impulses. In response to this injury and associated with the observed increased cardiomyocyte area, the preservation of cell-to-cell communication via upregulation of myocardial Cx43 may be attributed to a protective effect.

Conclusion

Using a rat model of fetal protein restriction, we showed that GPR affects the organization and number of myocytes in the offspring heart and increases the amount of collagen fibers in the cardiac tissue, showing clearly a degenerative process compatible with fibrosis. This finding reinforces the association between maternal malnutrition with low birth weight and the risk of cardiovascular morbidity in adulthood. GPR increases the area of cardiomyocytes and expression of Cx43 in the myocardium of young adult male rats, suggesting that this mechanism aims to compensate the fibrotic process by the accumulation of collagen fibers in the heart interstitium.

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Author contributions

Conception and design of the research, Statistical analysis, Obtaining funding and Writing of the manuscript: Catisti R; Acquisition of data: Rossini KF, Rebelato HJ; Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content: Oliveira CA, Esquisatto MAM, Catisti R.

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 Amyloidosis and its New Clinical Phenotype: Heart Failure with Preserved Ejection Fraction

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Abstract

Heart failure with preserved ejection fraction (HFpEF) is now an emerging cardiovascular epidemic, being identified as the main phenotype observed in clinical practice. It is more associated with female gender, advanced age and comorbidities such as hypertension, diabetes, obesity and chronic kidney disease. Amyloidosis is a clinical disorder characterized by the deposition of aggregates of insoluble fibrils originating from proteins that exhibit anomalous folding. Recently, pictures of senile amyloidosis have been described in patients with HFpEF, demonstrating the need for clinical cardiologists to investigate this etiology in suspect cases. The clinical suspicion of amyloidosis should be increased in cases of HFPS where the cardio imaging methods are compatible with infiltrative cardiomyopathy. Advances in cardio imaging methods combined with the possibility of performing genetic tests and identification of the type of amyloid material allow the diagnosis to be made. The management of the diagnosed patients can be done in partnership with centers specialized in the study of amyloidosis, which, together with the new technologies, investigate the possibility of organ or bone marrow transplantation and also the involvement of patients in clinical studies that evaluate the action of the new emerging drugs.

Introduction

Heart failure (HF) with preserved ejectionfFraction (HFpEF) is now an emerging cardiovascular epidemic, being identified as the main phenotype observed in clinical practice in different countries, such as the United States, United Kingdom, Portugal and Brazil. It is more associated with female gender, advanced age, and comorbidities such as hypertension, diabetes, obesity, and chronic kidney disease.[1-6] Recently, a picture of senile amyloidosis has been described in patients with HFpEF, demonstrating the need for clinical cardiologists to investigate this etiology. Advances in cardio imaging methods combined with the possibility of performing genetic tests and identification of the type of amyloid material allow for greater ease in the diagnostic process in view of the clinical suspicion of the disease.[7-15]

Keywords

Amyloidosis; Heart Failure, Diastolic; Stroke Volume; Cardiomyopathy, Restrictive; Risk Factors.

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Amyloidosis is a clinical disorder resulting from the deposition of insoluble fibril aggregates originated from proteins that have anomalous folding. These proteins, mostly initially soluble and with alpha helix configuration, take the pleated beta form through the abnormal folding phenomenon, with tissue precipitation in the form of amyloid fibrillar aggregates. These aggregates have the characteristic of staining congo red, acquiring a shade described as "apple-green" in polarized light. Through the alteration of the affected organ, it determines numerous dysfunctions of irreversible, progressive and indolent course, as observed in cardiac amyloidosis.[7,10,16-23]

Cardiac involvement may lead to the development of a restrictive HF model. Deposits in the myocardium and blood vessels cause diastolic, systolic dysfunction, ischemia and arrhythmias, and late diagnosis is the main cause of the reduction of survival of these patients.[7,10,16-23]

The diagnosis of amyloidosis presents important non-invasive advances in characterizing its presence and its type. In the past, the diagnosis was centered on the endomyocardial biopsy stained by congo red. More recently, new techniques such as doppler echocardiography with analysis of myocardial strain, myocardial scintigraphy with radioisotopes such as Tc99m bound to pyrophosphate or 2,3-dicarboxypropane-1,1-diphosphonate (DPD) and magnetic resonance imaging of blood tests for genotyping evaluation have promoted important advances in this area.[11,12]

New treatments directed toward specific disease targets have already been incorporated into clinical practice and others are still being tested, gradually improving patients' survival and quality of life.[24,25]

According to data obtained in MedLine, publications on the cardiac amyloidosis framework date back to 1948, totaling over 1000 articles indexed in several languages. Over the last five years, there has been a continuous increase in the number of studies evaluating the various aspects of the disease, especially with regard to innovations in diagnostic methods and new therapies (Figure 1). This trend is confirmed by the fact that the material produced in the last five years represents a third of the total published so far.[26]

The increase in the number of studies on the disease provides sufficient evidence for physicians to increase their clinical suspicion of cardiac amyloidosis, especially of the senile type, in cases of HFpEF, and referral of these patients to specialized centers is recommended. At these sites, invasive and non-invasive diagnostic methods allow for a wide assessment, including genetic testing, multidisciplinary teaming, and access to new drugs.

In this present review we will discuss the recent advances in the etiology and pathophysiology of cardiac amyloidosis, especially the senile form, in a systematized way for the

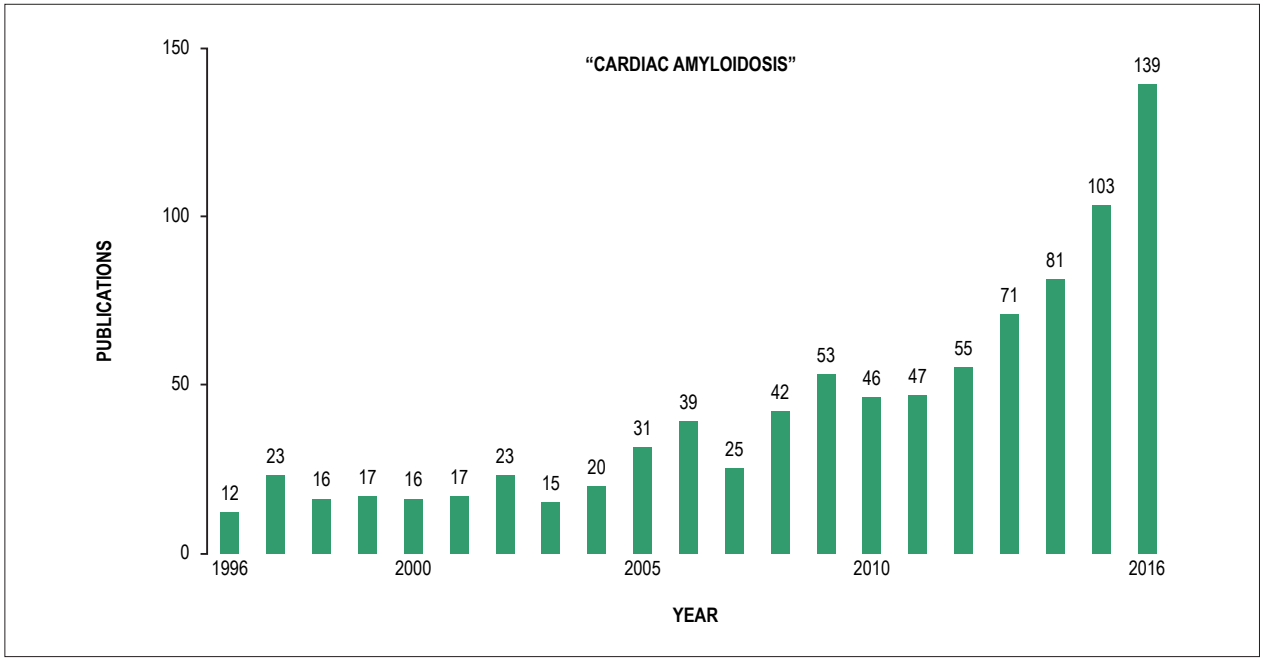

Figure 1 – *Search with the term Cardiac Amyloidosis from 1996 to 2016 showing the growth of articles related to the topic in the last ten years. (Medline)*

evaluation of individuals suspected of amyloidosis in the context of HFpEF and the emerging therapies currently available in clinical practice.

Classification and etiopathogenesis of amyloidosis

In the face of the complexity associated with the disease and its multiplicity of presentations, specific nomenclatures and classifications were established related to the predisposing condition and to the type of amyloid fibril deposited in the tissue. In general, amyloidosis can be classified as primary, secondary, related to dialysis and associated with transthyretin.[27-30]

It is classified as primary amyloidosis (AL) when it is defined by the production of amyloid protein composed of light chain immunoglobulins (kappa and lambda), synthesized under clinical conditions that present plasmacyclic dyscrasias, such as multiple myeloma and, less frequently, Waldenström's macroglobulinemia and non-Hodgkin's lymphoma. Classically, AL is a systemic disease that predominates in an older population and in male individuals.[27,31]

The clinical picture varies directly with the organ of predominance of amyloid deposition and its degree of functional impairment. The two most commonly affected organs are the kidney and heart, accounting for 60-80% of patients in most studies. Kidney involvement manifests as nephrotic syndrome or asymptomatic proteinuria. Cardiac involvement is related to the development of a HFpSC, in addition to the possible involvement of the heart conduction system and its corresponding complications. Autonomic neuropathy, or sensitivomotor peripheral neuropathy, may be present in up to 20% of patients.[27,32-34]

The accumulation of the amyloid material in the liver is frequent and comes along with isolated hepatomegaly or even hepatosplenomegaly, and may present a pattern of elevation of liver enzymes, compatible with cholestasis. Muscular infiltration may occur with pseudohypertrophy, as in classical macroglossia, as well as arthropathy due to deposits in the joints. Periorbital purpura (raccoon's eyes), despite being an uncommon finding, is strongly characteristic of the AL form. Hemorrhagic diathesis is an important condition that may be present and reports as possible causal links the connection between amyloid material's with coagulation factor X, the reduced synthesis of coagulation factors in the presence of a compromised liver and a possible acquired von Willebrand's disease.[27,32-34]

Secondary amyloidosis (AA) is identified in chronic inflammatory clinical conditions such as rheumatoid arthritis, psoriasis and, more recently, autoinflammatory diseases such as inflammatory bowel disease, Mediterranean family fever, and Muckle-Wells syndrome. Fibrils are composed of the amyloid A protein and are produced by the liver during the acute phase of inflammatory diseases. This protein originally has the function of increasing the affinity of high density lipoprotein (HDL) by macrophages and adipocytes, as well as mediating the chemoattraction and induction of the synthesis of proinflammatory cytokines. The chronic inflammatory picture increases its synthesis and, due to incorrect processing with cleavage and erroneous folding, results in its pathogenic form. The kidneys are involved in approximately 80% of patients, being the organ most affected by AA. However, there are also reports of cardiac involvement.[27,35-37]

Dialysis-related amyloidosis occurs as a function of the deposition of fibrils originating from beta-2 microglobulin proteins, which accumulate at increasing levels in patients with advanced renal disease and who undergo long-term dialysis. In this form, in particular, the predominant condition is osteoarticular involvement, such as carpal tunnel syndrome and rotator cuff involvement.[38,39]

Cardiac amyloidosis associated with transthyretin (TTR) is the second form of amyloidosis with a higher prevalence of cardiac involvement, and may be divided into hereditary and senile forms. The precursor protein is predominantly synthesized in the liver and plays a role as a carrier of retinol and thyroxine.[10,14-18]

In the senile form, we identifie tissue deposition of the TTR wild form, especially in the myocardium, and a clinical picture of HF is observed. The association with carpal tunnel syndrome has been described, whereas renal involvement is a rare finding. It has been observed in necropsy studies that deposition of this amyloid material in the heart is a frequent finding, especially in previously asymptomatic patients.[22] Data from the Mayo Clinic group indicate that the prevalence of this form among patients with amyloidosis is approximately 8,5%, with a mean age of 77 years and the male sex representing 82% of the affected individuals. It has been found that it rarely occurs in patients below 70 years of age. Observational study, observed that patients usually have a slow progression and a survival, after the diagnosis, of approximately 43 months, compared to the 26.6 months of the mutant form.[18,40-42]

The hereditary form, unlike senile, affects patients in different age groups, but predominates a mean age lower than that found in patients with the senile form. The coding of TTR occurs on chromosome 18 and more than 70 mutations associated with this protein have been identified. In view of the suspicion of an amyloidosis by TTR, the sequencing of this protein from tissue or blood sample should be performed for the diagnosis and identification of a possible specific mutation that allows us to define the prognostic course of the patient and guide the investigation of the relatives. The Val122Ile mutation is more associated with the elderly and is predominantly male, presenting in 90% of the cases a clinical manifestation of a cardiomyopathy.[7,10,14,15]

The most prevalent mutation in the world population is Val30Met, which presents marked neurological involvement, allied to a late cardiac involvement and is related to Corino de Andrade's disease, also known as familial amyloid polyneurophaty (FAP), which occurs along with sensory-motor peripheral polyneuropathy. Manifests, especially, at the age of 20 years, characterized by paresthesias, motor and autonomic disorders, besides studying with cardiac and renal impairment in the late phase of the disease. This condition has been identified as a genetic disease associated with the TTR mutation.[7,10,14,22]

Clinical presentations

Amyloidosis cardiomyopathy is classically described as a directt HF model, often occuring together with ascites, predating lower limb edema and allying with hepatomegaly on physical examination. Unlike cardiac dysfunction with increased filling pressures, pulmonary edema is an infrequent condition in amyloidosis cardiomyopathy.[7,8,10,14]

A rarer phenotype is the involvement of the interventricular septum with deposit of the amyloid material promoting the disproportionate thickening of the region, mimicking a hypertrophic cardiomyopathy. This presentation constitutes what is called a phenocopia, that is, a clinical condition that presents itself through manifestations typical of a disease of well-defined genetic origin.[21,24,41]

The report of syncope, due to autonomic nervous system involvement by amyloidosis, is a common finding in these patients and their presence in relation to physical exertion is associated with a worse prognosis, presenting a high mortality in three months, often due to sudden death.[21,41]

Ventricular arrhythmias are uncommon causes of syncope in this population. This is justified by the fact that the myocardium infiltrated by the amyloid material is more susceptible to episodes of hypoperfusion. Diseases of the conduction system may be present in the different forms of amyloidosis. However, they are more frequently found in the form associated with TTR, both senile and hereditary. Syncope in patients with cardiac amyloidosis is mainly associated with hypotension due to dysautonomia and bradyarrhythmias and less related to ventricular arrhythmias. Malignant ventricular arrhythmia, when present, is a common cause of death in patients with cardiac amyloidosis, and these patients are strong candidates for implantation of cardio-defibrillators.[24]

Involvement of the pericardium may occur in some cases, resulting in pericardial effusion which, most of the time, does not develop cardiac tamponade. Due to the alterations of cardiac amyloidosis itself, this condition may be masked and not have echocardiographic signs such as atrial and right ventricular compression.[24,41]

The accumulation of the material in the atrium promotes its electromechanical dysfunction and, consequently, considerably increases the risk of intracavitary thrombus formation. This process is evident, especially in patients with AL amyloidosis type and is an independent factor of atrial fibrillation, and when both factors are present, the risk of thromboembolism is extremely high. Therefore, the use of anticoagulants should be considered in these individuals.[24,41]

Cardiac amyloidosis and its new clinical phenotype

A new insight into HFpEF becomes critical, given its increasing relevance as the most prevalent clinical phenotype of HF in the world population. This data is present in our country, as evidenced by the DIGITALIS study, which investigated the prevalence of HF and its phenotypes in primary care in the city of Niterói. According to this study, among the population with HF, 59% had the HFpEF phenotype.[5]

Data from a specialized center in amyloidosis in Brazil point to a high prevalence of myocardial involvement in patients with amyloid polyneuropathy from abnormalities on the electrocardiogram (ECG).[43]

Despite numerous studies about this clinical condition, much is unknown about its etiophysiopathogeny, which has caused negative results in the studies of treatment of HFpEF. This is hampered in particular by the numerous phenocopies that mimic their presentation.[6,44]

In this scenario, it is worth highlighting the possibility that a portion of the patients with HFpEF actually present a cardiac amyloidosis. This is confirmed by recent studies with patients with a diagnosis of HFpEF, in the absence of arterial hypertension or diabetes, through the new cardio-imaging modalities, present accumulation of amyloid material in the myocardium. Next to this is the fact that amyloid material has been identified in necropsy studies of patients with HF.[7,8,15,45]

In order to facilitate the identification of a suspected cardiac amyloidosis, regardless of the type of fibril deposited, we must consider some clinical evidence and complementary tests as presented in Table 1.[7]

In this scenario where the most common etiologies applicable to the HFpEF are not confirmed, a cardiac amyloidosis scenario should be suspected. Therefore, we propose a guideline flow chart for the management of these patients (FIGURES 2 and 3).[18,22]

Diagnostic approach

Cardiac amyloidosis presents an indolent course and the diagnosis is made, often late, thus contributing to a worse prognosis. In several situations, cardiac involvement causes great morbidity to the patient, although the diagnosis is often not suspected, even in conditions where there is the characterization of restrictive cardiopathy.[7]

In clinical history, cardiac amyloidosis may be suspected in patients 50 years of age or older who have signs and symptoms of HF, with an ejection fraction of the left ventricle greater than or equal to 50% and that do not show improvement of the symptoms with the treatment. Extracardiac manifestations in the patient's history should be considered as guiding principles for the diagnosis of HF by amyloidosis, such as peripheral neuropathy and carpal tunnel syndrome, especially recurrent and bilateral. Signs and symptoms such as orthostatic hypotension, macroglossia, muscular consumption of the tenar and hypothenar region, haematomas of unknown origin and cardiac electrical conduction disturbances may also be present.[7,29]

The ECG is an easily accessible examination that may offer changes that raise the suspicion of amyloidosis in the presence of a CHF. The finding of a low voltage QRS complex, electrical axis deviations and branch block can be found. Arrhythmias are frequent, especially atrial fibrillation, which is related to amyloid infiltration of the atrium, and complex ventricular arrhythmias.[14,22]

ECHO is an important method of diagnostic investigation and the presence of significant left ventricular hypertrophy associated with ECG with low-voltage QRS may lead to

Table 1 – Clinical criteria and complementary tests in the investigation of cardiac amyloidosis

Categories	Criteria
History	Age of onset of HFpEF > 60 years
	Family history of unexplained HF at age 60
	Peripheral polyneuropathy
	Carpal tunnel syndrome
	Blood dyscrasia
	Orthostatic hypotension
Physical exam	Macroglossia
	Unexplained skin lesion
Medicines	Beta-blocker intolerance
	Vasodilator intolerance
ECG	Dissociation between low voltage ECG with ECHO hypertrophy
	Atrial Fibrillation / Flutter
	Bloqueio atrioventricular
	Pseudoinfarction pattern
ECHO	Unexplained ventricular hypertrophy
	Increased interatrial septum thickness
	Increased myocardial granulation
	Biatrial increase
	Restrictive filling pattern (Increased E/A and E/E ratio)
	Preservation of longitudinal strain
	Pericardial effusion

HfpEF: Heart failure with preserved ejection fraction; ECHO: doppler echocardiography; ECG: electrocardiogram.

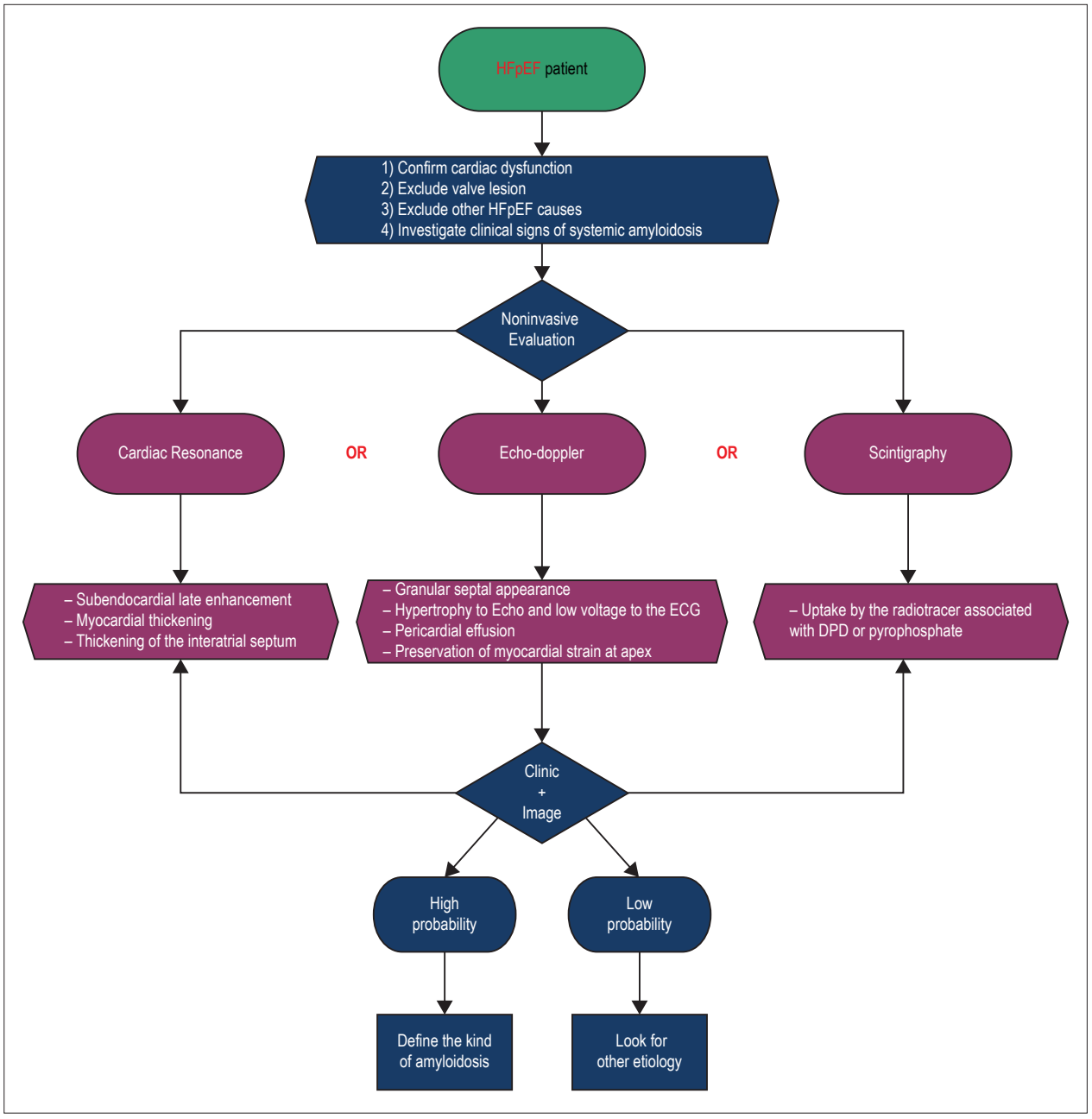

Figure 2 – *Flowchart for the evaluation of patients with HFpEF and suspicion of amyloidosis. HfpEF: heart failure with preserved ejection fraction; Echo: doppler echocardiography; ECG: electrocardiogram; DPD: 2,3-dicarboxypropane-1,1-diphosphonate.*

suspicion of cardiac amyloidosis. Another criterion for suspicion of cardiac amyloidosis is thickening of the left ventricular wall above 12 mm in the absence of a history of systemic arterial hypertension. Other findings that may be present in ECHO are the biatrial increase with normal-sized ventricles, pericardial effusion, and evidence of diastolic dysfunction due to the pattern of restive cardiomyopathy.[9,14,22]

Measurement of the thickness of the interventricular septum may suggest the type of amyloidosis present in the patient, and is often greater in cases of amyloidosis by TTR than the AL form, and may in many cases be greater than 20 mm. However, the separation between these two forms on a clinical basis is not always possible.[9,14,22]

In some patients with cardiac amyloidosis, we can observe the clinical phenotype similar to obstructive hypertrophic cardiomyopathy due to the presence of the dynamic pressure gradient that is related to an additional "narrowing" of the left ventricular outflow tract. More recently, longitudinal systolic strain has been used for the diagnosis of systolic dysfunction in patients with cardiac amyloidosis, and may

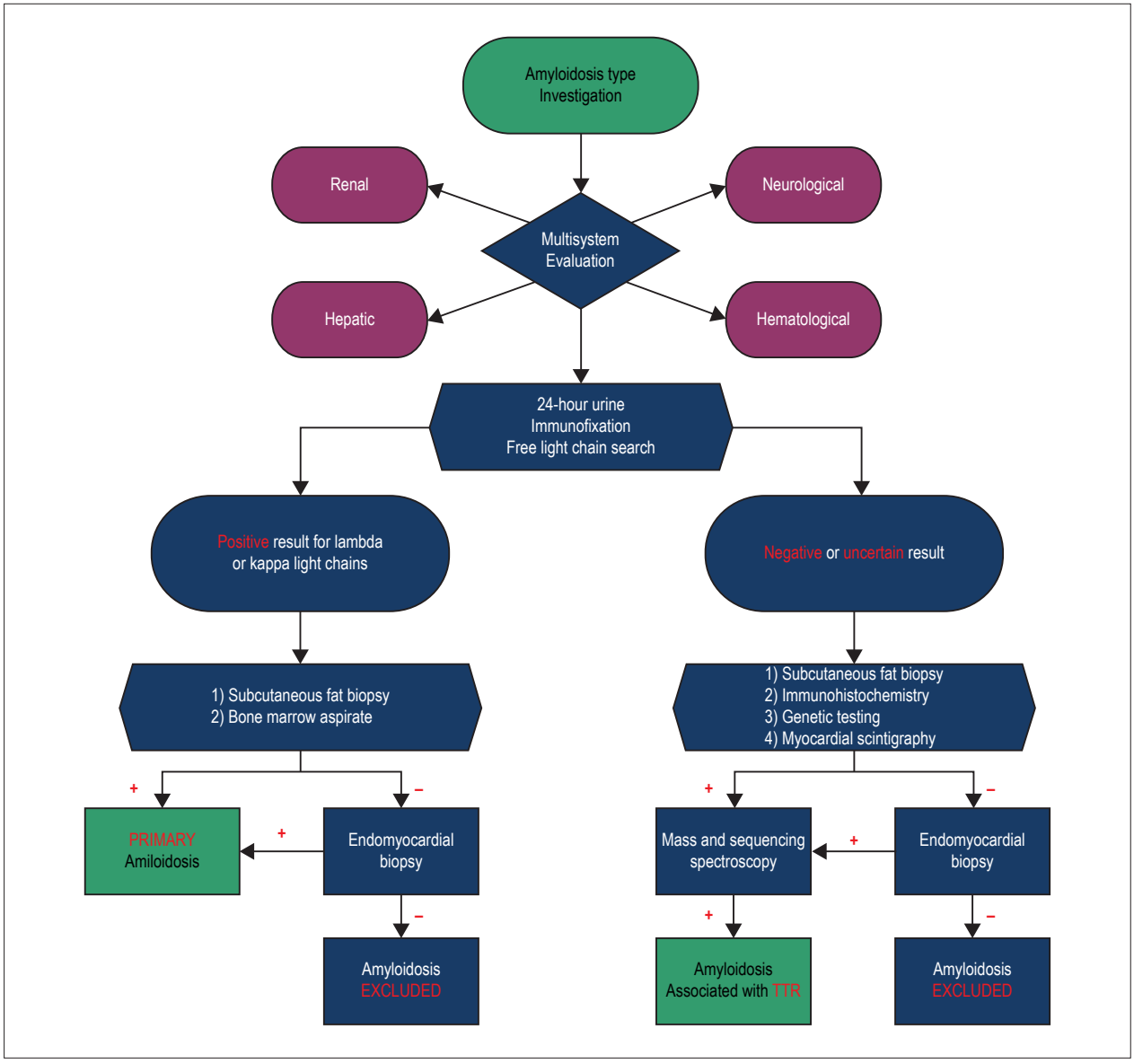

Figure 3 – *Flowchart for investigation of the type of amyloidosis. TTR: transthyretin.*

show the preservation of the heart tip in relation to other walls. In the presence of ECG and ECHO alterations we should resort to complementary tests. In a first step in the elucidation of a case of cardiac amyloidosis, we investigated the renal function status of this patient through the nitrogenous excoriations and, in particular, the quantification of protein loss and its type, made through the collection of 24h urine with dosage proteinuria and urinary protein electrophoresis. This stage in the investigation allows the identification of the primary form of amyloidosis and consists in the identification of the light chains that are in high titers in these patients. Immunofixation, when associated, allows an increase in the diagnostic accuracy of this type of presentation.[9,14,22,31,34]

The abdominal fat aspirate for histopathological study is a more accessible alternative, since it is a simple diagnostic procedure, easy to perform, safe and that presents good sensitivity, but having less accuracy in the form associated with TTR. However, when a negative result is obtained with abdominal fat aspirate, right ventricular endomyocardial biopsy may be essential for the diagnosis of cardiac amyloidosis. Through this method, the amyloid protein is stained by congo-red. Other tissues can also be evaluated as the rectum, gums, bone marrow, kidney, among others. The histochemical study of tissue samples is important in order to distinguish between the hereditary, senile, systemic and primary forms, due to differences in treatment and prognosis.[14,22,29]

Magnetic resonance imaging appears as another alternative for the diagnosis of cardiac amyloidosis, with a sensitivity of 87% and specificity of 96% for the form associated with TTR. Through this examination, it is possible

to identify the myocardial and atrial septal thickening, signs of diastolic dysfunction, and the typical pattern of late subendocardial enhancement in the left ventricle, which may also affect all cardiac chambers.[11,12,22,29]

Molecular imaging has also revolutionized diagnosis. The non-invasive method can be used from the use of the Tc99m radiotracer, which binds to TTR but not to the light chain derivatives, being an effective method of evaluating the mutant or wild forms of cardiac amyloidosis associated with TTR. Positron emission tomography in conjunction with the C-RiB plotter may be a new strategy to be used in the diagnosis of these patients.[14,22]

Bone marrow biopsy with immunohistochemical staining or flow cytometry analysis is critical in patients in whom the type of AL-amyloidosis has been identified. This will demonstrate a clonal population of plasma cells, which are producing defective light chains of the antibody. If these tests are negative, we should investigate the hereditary forms of the disease.[29,33]

There are new studies involving omic sciences that aim to increase the diagnostic accuracy of amyloidosis. Proteomics involves the study of all the protein expression of a cell in different conditions, being its study complementary to the genome, identifying any protein, with or without genetic mutations. The main technique employed is laser microdissection followed by mass spectrometry (LMD-MS), through which positive samples in the congo-red color are dissected and decomposed into smaller components called peptides.[25,28]

Treatment of cardiac amyloidosis

Treatment of cardiac amyloidosis is best performed in specialized centers of the disease. Treatment requires two approaches: control of heart-related complications due to amyloid deposition and treatment of the underlying disease to prevent new amyloid formations.

Treatment of cardiac amyloidosis aims to improve the signs and symptoms of HF. The use of low-dose diuretics improves symptoms related to congestion, while the use of the combination of beta-blockers and angiotensin-converting enzyme inhibitors has its undefined benefit in amyloidosis.[16,18,21,22,24]

The use of digitalis has no benefits in this group of patients, since the myocardium in dysfunction by the amyloid material is more susceptible to toxic effects, which predisposes to the occurrence of arrhythmias.[16,18,21,22,24]

The use of anticoagulants should be considered in case of atrial fibrillation and in the detection of intracavitary thrombi.[18,21,22,41]

Amyloidosis of AL form

Overall survival is approximately four years after diagnosis and has improved over the past three decades. AL-amyloidosis is often the result of a clonal increase of plasma cells in the bone marrow and thus therapy with cytotoxic chemotherapeutics may be effective. The performance of the hematologist in the staging process and definition of the therapeutic strategy in this scenario is fundamental.[21,31,33,34]

Patients who present a hematological response to treatment have symptomatic improvement and cardiac biomarkers, and can occur along with amyloid deposition, which is already evident in the first three months, confirming a better prognosis in these cases.[19,21,31,33,34]

Dexamethasone-associated melphalan therapy (MelDex) in patients ineligible for autologous stem cell transplantation had a response rate around 70%, which was worse in cases with advanced cardiac involvement. In a recent randomized clinical trial comparing MelDex with high doses of melphalan followed by stem cell transplantation showed a better survival rate in patients who took MelDex.[32,46-48]

Bortezomib has been shown to be an effective drug when combined with cyclophosphamide and dexamethasone, with a significant hematological response (71%) after two months of use. According to Mayo Clinic group, in the case of three patients initially ineligible for stem cell therapy, the use of bortezomib made the procedure possible. Stem cell transplantation is used in 25% of patients with cardiac amyloidosis. Following the procedure episodes of supraventricular tachycardia may occur, with mortality of 11% in these individuals. The positive hematological response in patients submitted to stem cell therapy is approximately 56%.[16,19,27,32,33]

A study evaluating the use of bortezomid, dexamethasone and alkylating agents (BDEX + AA) in 106 patients with symptomatic HF due to AL cardiac amyloidosis showed an improvement in survival after adjustment of clinical variables. (Hr: 0.209, 95% CI: 0.069 to 0.636, p = 0.006).[32,49]

Form associated with TTR

Tafamidis appears as an important option for the treatment of amyloidosis, acting as a kinetic stabilizer of the TTR tetramer. The interaction of molecules at certain TTR binding sites promotes stability to the protein in its tetrameric state, markedly decreasing its dissociation and, consequently, the amyloidogenesis. Tafamidis has the ability to selectively bind to one of the thyroxine sites in the TTR, promoting the kinetic stabilization of the tetramer.[7,22,24,32,50-54]

In a multicenter, randomized, double-blind, placebo-controlled study, the safety and efficacy of oral Tafamidis in patients with amyloidosis and involvement of the peripheral nervous system were demonstrated. Clinical trials show that this medication slows the progression of the disease, improves the function of small and large caliber nerve fibers and, consequently, reduces the functional loss of the affected systems, optimizing the quality of life of the patient. In another study, Tafamidis resulted in stabilization of transthyretin in 97% of patients with mild to moderate HF due to the wild type of cardiac amyloidosis.[7,22,24,32,50-54]

Another drug in the evaluation process for the cardiac amyloidosis associated with TTR is Diflunisal, a non-steroidal anti-inflammatory that can stabilize the tetramer, avoiding amyloidogenesis. One cohort evaluated the tolerance and effects promoted in 13 patients with cardiac amyloidosis by TTR, both mutant and wild type. No significant changes in cardiac structure and function were observed, as well as biomarkers.[32,55,56]

The use of doxycycline and tauroursodeoxycholic acid was carried out with a small number of patients, and a possible clinical improvement was identified. A new second-generation antisense therapy, ISIS-TTRrx, works by reducing the serum level of the TTR protein by suppressing the gene expression of its synthesis. In addition, a total of 28 studies are enrolled in the Clinical Trials database for interventions in patients with cardiac amyloidosis.[32,57,58]

An alternative treatment for some types of amyloidosis would be liver transplantation with the goal of replacing the mutated TTR gene that produces the majority of the circulating transthyretin by a gene found in a genetically normal donor organ. In this way, liver transplantation may be an alternative to slow the progression of the disease and prolong the patient's survival. However, chronic immunosuppression pertinent to transplantation leads to a high mortality rate in the first year, about 10%, and high morbidity. Transplantation does not prevent the extrahepatic synthesis of amyloid protein and thus does not delay the progression of the disease.[22,24,32]

Conclusion

Cardiac amyloidosis inaugurates a new era of personalized cardiology, where precise diagnosis through techniques involving molecular genetic analysis, biomarkers and cardioimaging methods make it possible to classify the form of amyloidosis and define its clinical course and prognosis and, in the future, guide the therapeutics of these frames.

The clinical suspicion of amyloidosis should be increased in cases of HFPSE in which the methods of cardiac imaging are compatible with the restrictive cardiomyopathy or signs of dissociation between ECHO and ECG findings. The partnership with centers specialized in amyloidosis combined with new technologies are fundamental in the management of these patients through specialized treatments, including organ transplantation, or even involving patients in clinical studies that evaluate the action of new emerging drugs.

Author contributions

Conception and design of the research: Mesquita ET, Jorge AJL; Writing of the manuscript and Critical revision of the manuscript for intellectual content: Mesquita ET, Jorge AJL, Andrade TR.

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Case 3/2017 – A 47-Year-Old Female with Refractory Heart Failure and Embolic Acute Myocardial Infarction

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The patient was a 47-year-old white single female referred for medical treatment to the Instituto do Coração, born and coming from the state of São Paulo, with four children, unemployed, but reporting having worked in coffee farming in inner Minas Gerais state.

At the time of her first medical consultation (September 25, 2015), she reported dyspnea on minimal exertion, orthopnea and anasarca, which had started 2 months earlier. She denied angina pectoris, previous myocardial infarction and syncope. She knew she had systemic arterial hypertension and diabetes mellitus, and was being treated at a basic health unit. She used to smoke (20 packs/year), but quit the habit 2 months before. She denied consuming alcoholic beverages and illicit drugs, as well as a family history of cardiovascular disease.

She was on metformin (2550 mg/day), furosemide (80 mg/day), carvedilol (12.5 mg/day), and losartan (50 mg/day).

On her first medical consultation, her physical exam showed regular general condition and dyspnea in the horizontal position. Her blood pressure was 100/70 mmHg, and heart rate, 102 bpm. Her pulmonary auscultation revealed no respiratory sound on the base of the right lung and no rales. Her cardiac auscultation showed regular gallop rhythm, due to the presence of the third heart sound, and no murmur. Her abdomen was globose, tense, painless, with signs of huge ascites. Her extremities were cold and edematous (++/4+), with symmetrical pulses.

The electrocardiogram on the medical consultation showed sinus rhythm, heart rate of 97 bpm, left atrial overload and indirect signs of right atrial overload (Peñaloza-Tranchesi sign), low voltage of the QRS complexes in the frontal plane and no progression of the R wave in V_1 to V_4 (probable electrically inactive area in the anterior wall), and diffuse changes of ventricular repolarization (Figure 1).

Her chest X-ray showed bilateral veiling of costophrenic sinus, with pleural effusion up to half of the right hemithorax, normal aorta and global heart enlargement (+++/4+) (Figure 2).

Keywords

Heart failure; Myocardial Infarction; Thromboembolism.

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Her laboratory tests were as follows: hemoglobin 14.8 g/dL; hematocrit 46%; leukocytes 9950/mm³; creatinine 0.87 mg/dL; sodium 141 mg/dL; potassium 4.3 mg/dL; and negative serology for Chagas disease.

On that medical consultation, spironolactone 25 mg was added, and, due to gastrointestinal intolerance, metformin was replaced by glicazide 30 mg/day.

Returning to medical consultation at the outpatient clinic (December 16th, 2015), she reported marked improvement of the dyspnea, then triggered only by large exertion. Her physical exam revealed no pathological jugular venous distention, blood pressure of 100/70 mmHg, heart rate of 100 bpm. Her pulmonary auscultation showed no respiratory sound on the base of the right lung. Her cardiac auscultation evidenced the presence of the third heart sound and no murmur. Her abdomen was globose, tense, painless, with liver palpable 3 cm from the right costal margin. Her lower limbs had mild edema (+/4+). She had not undergone the tests requested on her first medical consultation. Her prescription was then changed: carvedilol to metoprolol 50 mg/day.

After missing her subsequent medical consultation, the patient was hospitalized on July 25, 2016, due to heart failure decompensation. She had mixed shock, with decreased level of awareness and increased levels of myocardial injury markers. She required dobutamine for hemodynamic control. Empiric antibiotic therapy was initiated with ceftriaxone and clarithromycin, being the patient later submitted to orotracheal intubation for respiratory support.

Bedside chest X-ray on that day evidenced bilateral veiling of costophrenic sinus, with reduced transparency of the lower third of the right hemithorax (pleural effusion), increased pulmonary vascular bed with Kerley's B lines, and marked heart enlargement (Figure 3).

Her laboratory tests revealed: hemoglobin 14.1 g/dL; hematocrit 44%; leukocytes 15100/mm³ (band neutrophils 6%, segmented neutrophils 84%, lymphocytes 5%, and monocytes 5%); platelets 195000/mm³; CK-MB 9.7 ng/mL; troponin I 0.654 ng/mL; ALT 42 U/L; AST 80 U/L; urea 87 mg/dL; creatinine 1.18 mg/dL; sodium 132 mEq/L; potassium 3.5 mEq/L; International Normalized Ratio (INR) 2.3; ratio between activated thromboplastin times 1.06; magnesium 1.6 mEq/L; total bilirubin 3.14 mg/dL; direct bilirubin 2.19 mg/dL; C-reactive protein 156.50 mg/L; arterial lactate 22 mg/dL. Arterial blood gas analysis (with oxygen therapy) showed pH of 7.52, pCO_2 of 29.6 mmHg, pO_2 of 176 mmHg, oxygen saturation of 99.9%, bicarbonate of 24.1 mmol/L, and base excess of 2.2 mmol/L.

Bedside transthoracic echocardiography showed: left ventricular diffuse hypokinesia and ejection fraction of 20%; marked right ventricular hypokinesia; marked mitral and tricuspid regurgitation and poor leaflet coaptation;

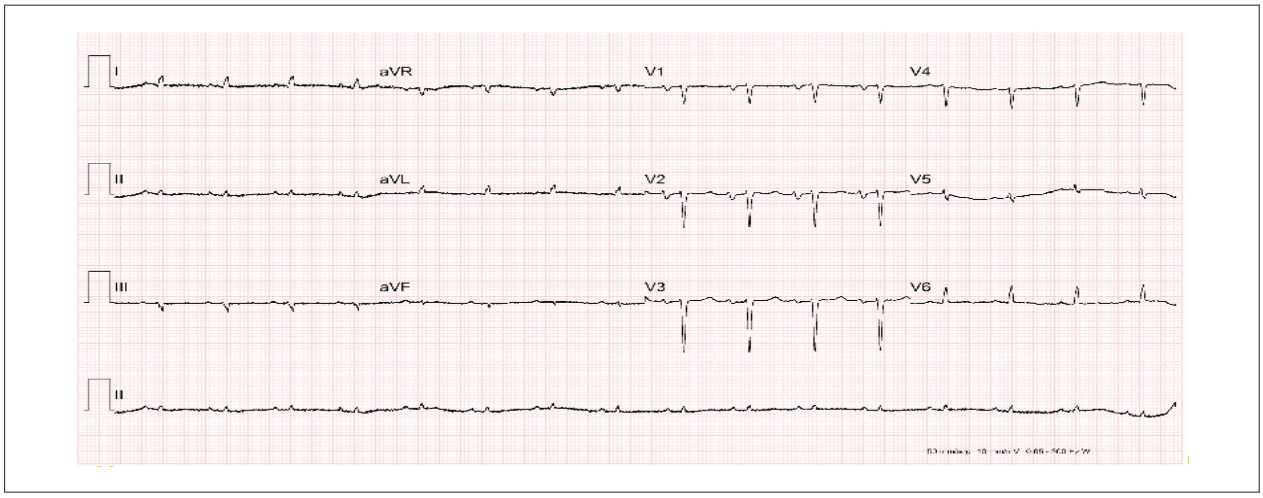

Figure 1 – *ECG: left atrial overload and indirect signs of right atrial overload Peñaloza-Tranchesi sign), low voltage of the QRS complexes in the frontal plane and no progression of the R wave in V_1 to V_4 (probable electrically inactive area in the anterior wall), and diffuse changes of ventricular repolarization.*

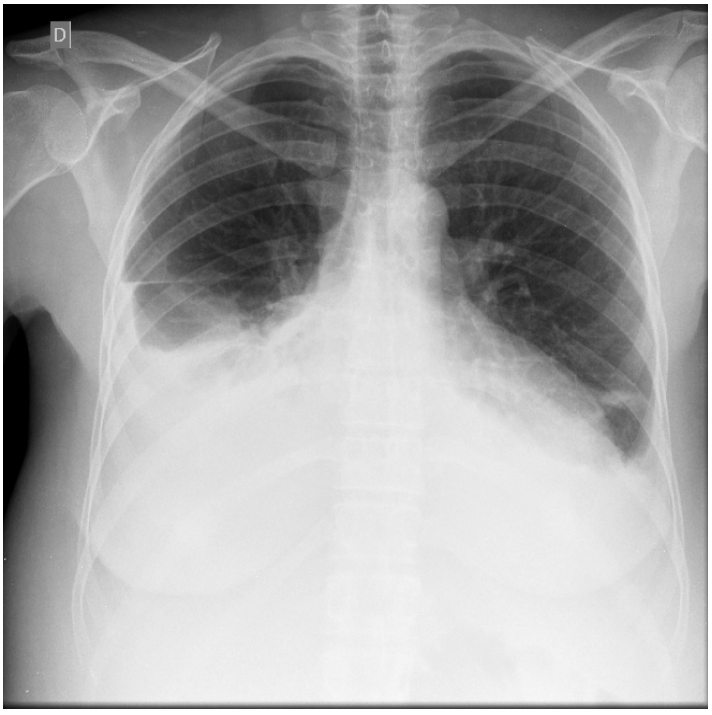

Figure 2 – *Chest X-ray showing bilateral veiling of costophrenic sinus, with pleural effusion up to half of the right hemithorax, normal aorta and global heart enlargement (++++/4+).*

pulmonary valve with signs of pulmonary hypertension; mild pericardial effusion and presence of large heterogeneous mass in the left ventricle, measuring 30x28 mm, compatible with intracavitary thrombus. Estimated systolic pulmonary artery pressure of 65 mmHg.

Coronary angiography revealed coronary arteries without proximal lesions, but the anterior interventricular branch showed a 95% distal lesion, and the diagonal branch showed distal occlusion. Neither the circumflex artery nor the right coronary artery showed any sign of obstruction (Figures 4A, 4B, 4C, 4D).

The hypothesis of infarction of embolic cause was raised. The patient was referred to the intensive care unit, with progressive increase of vasoactive drugs and later introduction of noradrenaline and widening of the antimicrobial spectrum to meropenem and vancomycin. The patient had refractory shock and died on July 27, 2016, with multiple organ dysfunction.

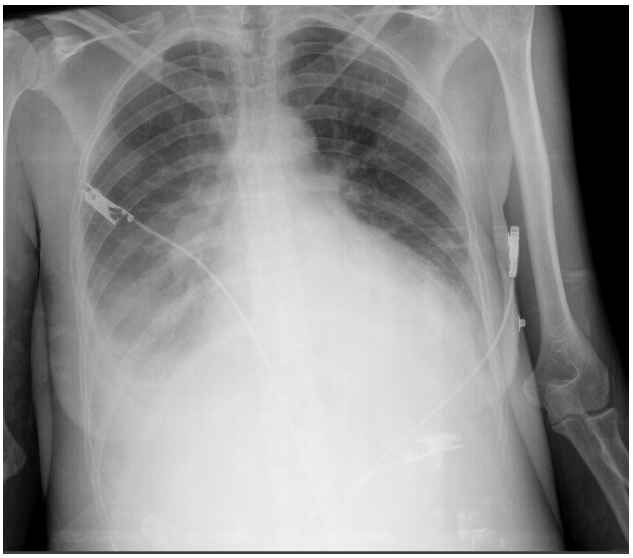

Figure 3 – *Bedside chest X-ray showing bilateral veiling of costophrenic sinus, with pleural effusion up to half of the right hemithorax, normal aorta and global heart enlargement (++++/4+).*

Clinical aspects

This case can be approached in two ways: chronic disease and acute decompensation. Taking the chronic disease way, some possible etiologies of heart failure can be considered. With the negative Chagas serology and her known comorbidities, the major hypotheses to be considered for this patient are hypertensive heart disease (dilated phase), microcirculation disease due to diabetes mellitus, and idiopathic dilated cardiomyopathy.[1-3] The patient attended to only two medical consultations, being her complementary investigation unfinished. Now, taking the acute decompensation way, it was relatively clear at the beginning that the infectious hypothesis was the most plausible, being the elevation in the levels of myocardial necrosis markers probably related to sepsis and hemodynamic instability (type 2 acute myocardial infarction). However, after the results of the other complementary tests (echocardiography and coronary angiography), the hypothesis of acute myocardial infarction of embolic cause gained strength, mainly due to the finding of an intracavitary thrombus on the first exam. Therefore, we hypothesize that the significant left ventricular dysfunction determined the formation of the thrombus, whose fragment embolized to the coronary circulation, causing an acute myocardial infarction, culminating with dysfunction worsening, thus triggering the cascade that led to the patient's death.

Some of the causes of coronary emboli are heart valvular disease, cardiomyopathy, coronary atherosclerosis and atrial fibrillation. In a postmortem study by Prizel et al.,[4] an intracavitary thrombus was present in 33% of the cases. Nevertheless, a superimposed infectious cause for decompensation cannot be ruled out. **(João Gabriel Batista Lage, MD)**

Diagnostic hypothesis: syndromic: heart failure due to heart disease with left ventricular ejection fraction reduction; etiological: dilated cardiomyopathy; final: acute myocardial infarction due to thromboembolism to the coronary arteries and cardiogenic shock. **(João Gabriel Batista Lage, MD)**

Postmortem examination

The heart showed global dilatation of the four chambers (Figure 5), and no significant changes in the valves and coronary arteries. The microscopic exam showed neither inflammatory infiltrate nor any type of deposit, and the muscle fibers were thin and had enlarged nuclei, denoting hypertrophy. Thrombi were present in the tips of both ventricles (Figure 5). The diagnosis of systemic arterial hypertension was based only on information provided by the patient, and there was no renal arteriolosclerosis. In the lack of genetic study, thus, neither decompensated hypertensive cardiomyopathy nor idiopathic dilated cardiomyopathy can be diagnosed for sure, the latter seeming more likely.

There was myocardial infarction of approximately 2 weeks, affecting the apical region of the left ventricular anterior and septal walls (Figures 5 and 6). On microscopic exam, the coronary arteries were normal or had minimal intimal lesions (Figures 7A and 7B). On the 6th centimeter of the anterior interventricular branch (anterior descending), there was lumen occlusion by a material with characteristics of thrombus-embolus (Figure 7C).

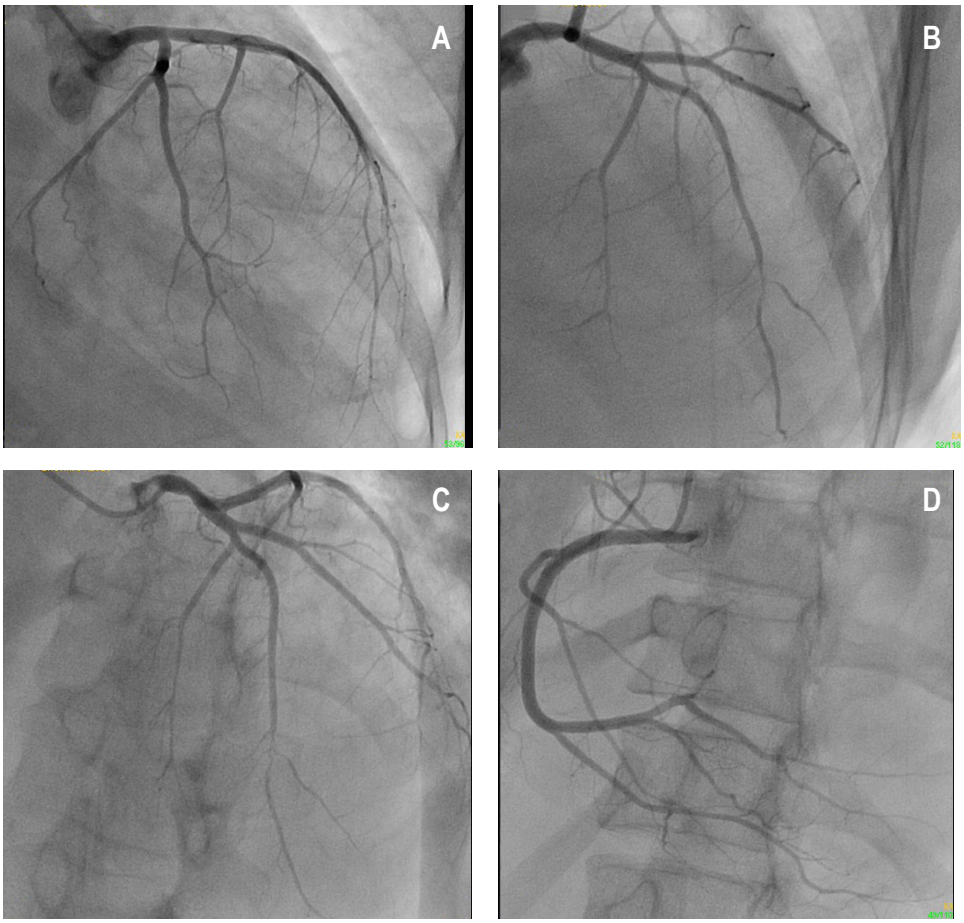

Figure 4 – *Coronary angiography: A) caudal RAO: 95% distal lesion in the anterior descending branch and occlusion of the diagonal branch; B) cranial RAO: 95% distal lesion and distal occlusion of the diagonal branch; C) cranial LAO: 95% distal lesion in the anterior descending branch; D) LAO: right coronary artery with no lesion.*

In the lower lobes of the lungs, there were infarctions, small to the left and large to the right (Figure 8), which were considered the final factor triggering death.

In the other organs, there were changes resulting from congestive heart failure, with chronic passive congestion, general visceral congestion, anasarca and cachexia. (**Paulo Sampaio Gutierrez, MD**)

Major disease: idiopathic dilated cardiomyopathy.

Cause of death: pulmonary thromboembolism (**Paulo Sampaio Gutierrez, MD**)

Comments

It is worth noting, in this patient with dilated cardiomyopathy, the presence of myocardial infarction, in whose region, there was mural thrombus in both the right and left ventricles. The major issue is the cause of the infarction. The coronary arteries, on both coronary angiography and morphological exam, had no significant atherosclerotic disease, except for distal embolization of the anterior interventricular branch (anterior descending). Adding the coronary angiographic finding with the presence of infarction, one might consider that the later resulted from embolization to a coronary artery. The myocardial infarction and later that of the lung might have been caused by embolism from the ventricular thrombi. It is worth noting that, coincidentally, the infarction happened in the same area of the thrombus originating it. Another possibility might be the infarction resulting from another process, such as vasospasm, generating thrombi, which caused the terminal embolism.

Although there are other similar cases in the literature,[5,6] the appearance of transmural infarction in patients with idiopathic dilated cardiomyopathy is uncommon. (**Paulo Sampaio Gutierrez, MD**).

Anatomopathological Session

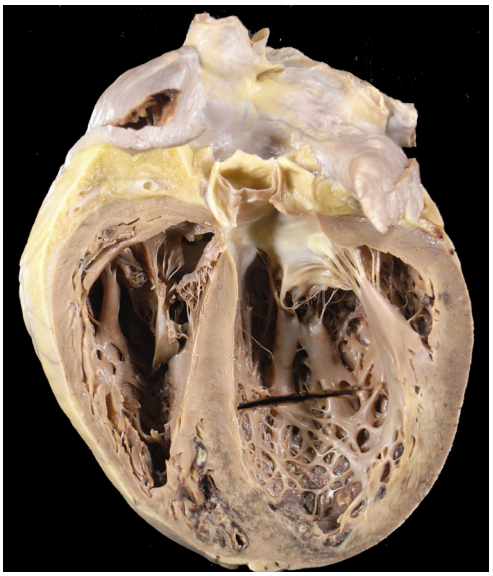

Figure 5 – *Longitudinal section of the heart showing dilatation of the cavities.*

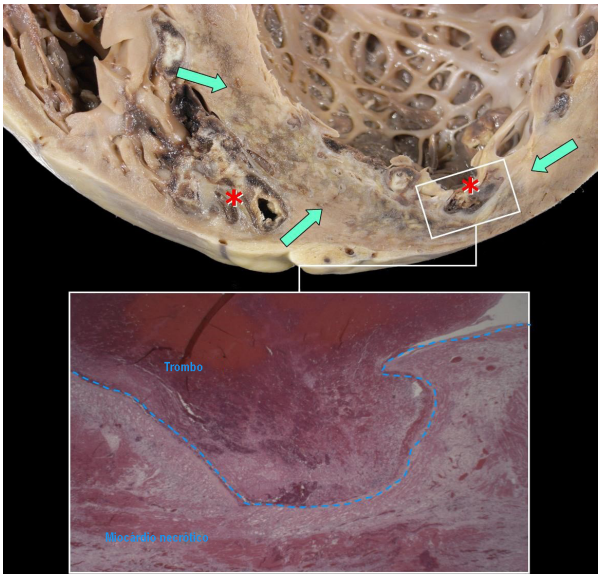

Figure 6 – *Longitudinal section of the apex of the heart showing thrombi in both ventricles (asterisks) and myocardial infarction (arrows).*

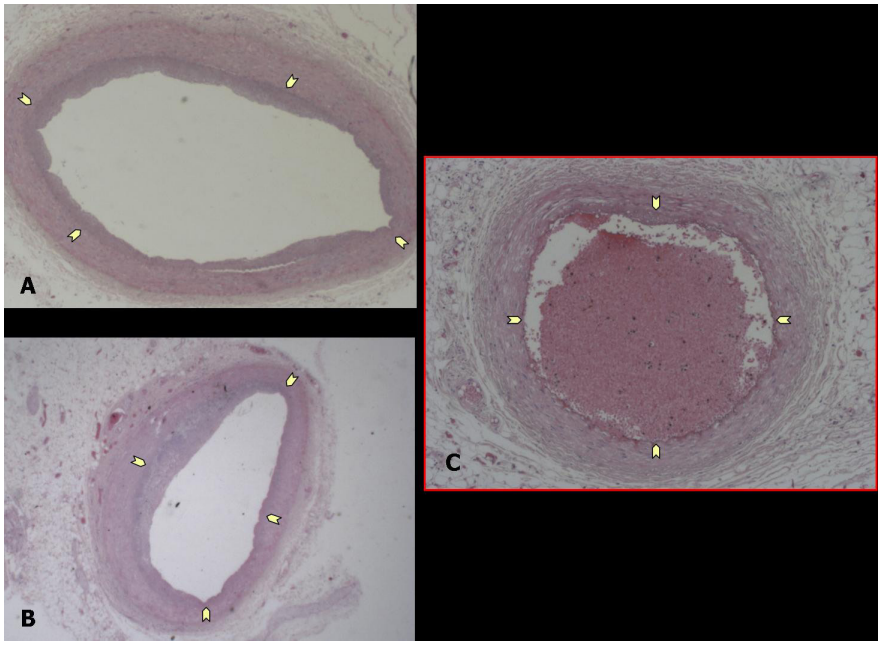

Figure 7 – *Cross-sectional histological sections of segments of the coronary arteries. A and B- right coronary artery and left main coronary artery, respectively, showing intima layer (delimited by the arrows) without significant obstructions; C- the 6th centimeter of the anterior interventricular branch (anterior descending) occluded with a thrombus-embolus.*

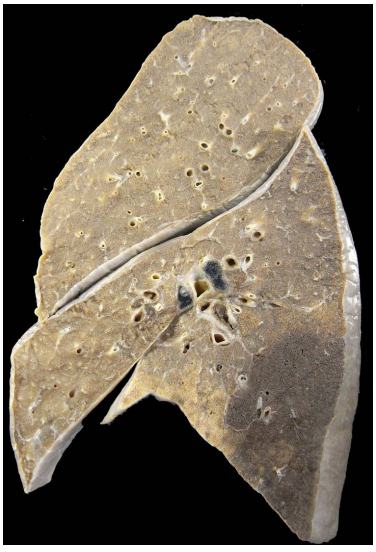

Figure 8 – *Gross section of the right lung showing hemorrhagic infarction in the lower lobe (darker triangular area).*

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Symptomatic Exercise-induced Intraventricular Gradient in Competitive Athlete

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Case Report

We describe the case of a 17-year-old caucasian male tennis player, training a mean of 20-24h/week, refereed for evaluation in Sport's Cardiology clinic due to symptoms of dizziness on strenuous exercise, relieving soon after decubitus. The athlete denied other concomitant complaints, namely thoracic pain, palpitations, syncope or decrease in physical performance. Although this is the most symptomatic episode, he revealed other prior episodes with similar presentation, but less intense and occurring in environments with high temperatures. Personal/family history was unremarkable and all pre-competitive evaluations were normal and without restrictions for competitive sport. Physical examination did not show significant findings – cardiac evaluation was normal, heart rate and blood pressure at rest were 52 bpm 121/64mmHg respectively.

The 12-lead electrocardiogram and transthoracic echocardiogram did not show pathological findings, only cardiac physiological adaptations to exercise (Figure 1). Subsequently the athlete underwent a treadmill exercise stress echocardiogram revealing an excellent functional capacity (19'09'' of Bruce protocol, 19.3METs), but with reproduction of symptoms (dizziness) in the peak of exercise with simultaneous decrease in systolic blood pressure (185 →90mmHg) and detection of intraventricular gradient (IVG) – at least 69mmHg (Figure 2). In the first minute of recovery the symptoms disappeared and blood pressure normalized.

The athlete was advertised to stop the sportive practice. An ambulatory 24h-Holter monitoring and cardiac magnetic resonance were subsequently performed, not showing pathological changes, namely arrhythmias or structural cardiac abnormalities.

After these investigations the case was discussed with involvement of the athlete, parents and coach. It was decided to reinitiate exercise with a gradual increase in intensity and volume of training, with the special advertising to increase hydration (apparently suboptimal according to the coach report) and to begin beta-blocker therapy if the symptoms persist. After 18 months of follow-up the athlete remain asymptomatic, with excellent performance and without need of pharmacologic therapy.

Keywords

Athletes; Echocardiography, Stress; Heart Ventricles/physiopathology; Exercise Test/adverse effects; Ventricular Dysfunction/etiology.

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Discussion

The development of significant exercise-induced IVG (>30mmHg at rest or >50mmHg with exercise) is uncommon, but can lead to several and unspecific symptoms such as dizziness, thoracic pain, or even ventricular repolarization changes and arrhythmias during exercise test.[1,2] This condition is usually associated to global or segmental left ventricular hypertrophy or an abnormal implantation of the papillary muscles, but the pathophysiological mechanisms are not well established. Three potential mechanisms are purposed for the development of IVG:

a) Increase of physiological non-obstructive IVGs;

b) End-systolic obstruction secondary to ventricular cavity obliteration;

c) Mid-systolic obstruction due to systolic anterior motion of the mitral valve with restriction of ejection flow.[3,4]

In a study performed by Zywca et al.[5] the independent predictors of dynamic left ventricular outflow tract obstruction in individuals without hypertrophic cardiomyopathy were: chordal systolic anterior motion, smaller left ventricle at end-systole, higher systolic blood pressure at peak, younger individuals and increased septal wall thickness.[5] However, as in the case reported, IVG can occur without structural cardiac changes, namely of the mitral valve apparatus, and eventually justified by extreme myocardial deformation in response to load conditions.[3] In this context, IVG is more frequently described in athletes or in situations with increased inotropic stimuli as during dobutamine stress echocardiogram.[6,7] Exercise stress echocardiogram plays a relevant role in the evaluation of symptomatic athletes, with reproduction of symptoms and the potential detection of significant IVGs.[1,8]

The clinical significance of IVG remains unknown – it could be one extreme physiological adaptation to exercise, one isolated pathological entity or in the other hand corresponds to a pre-phenotypic finding of cardiomyopathy.

Regarding the preventive/therapeutic measures to adopt in the presence of an athlete with IVG, maintenance adequate hydration during exercise is crucial, often sufficient for the remission of symptoms. Exercising under higher temperatures without adequate hydration can increase the gradient secondary to left ventricle cavity obliteration. Among the pharmacological therapy, the evidence indicates a significant effectiveness of beta-blocker therapy, both in the remission of symptoms and in the remission/disappearance of IVG.[1,9]

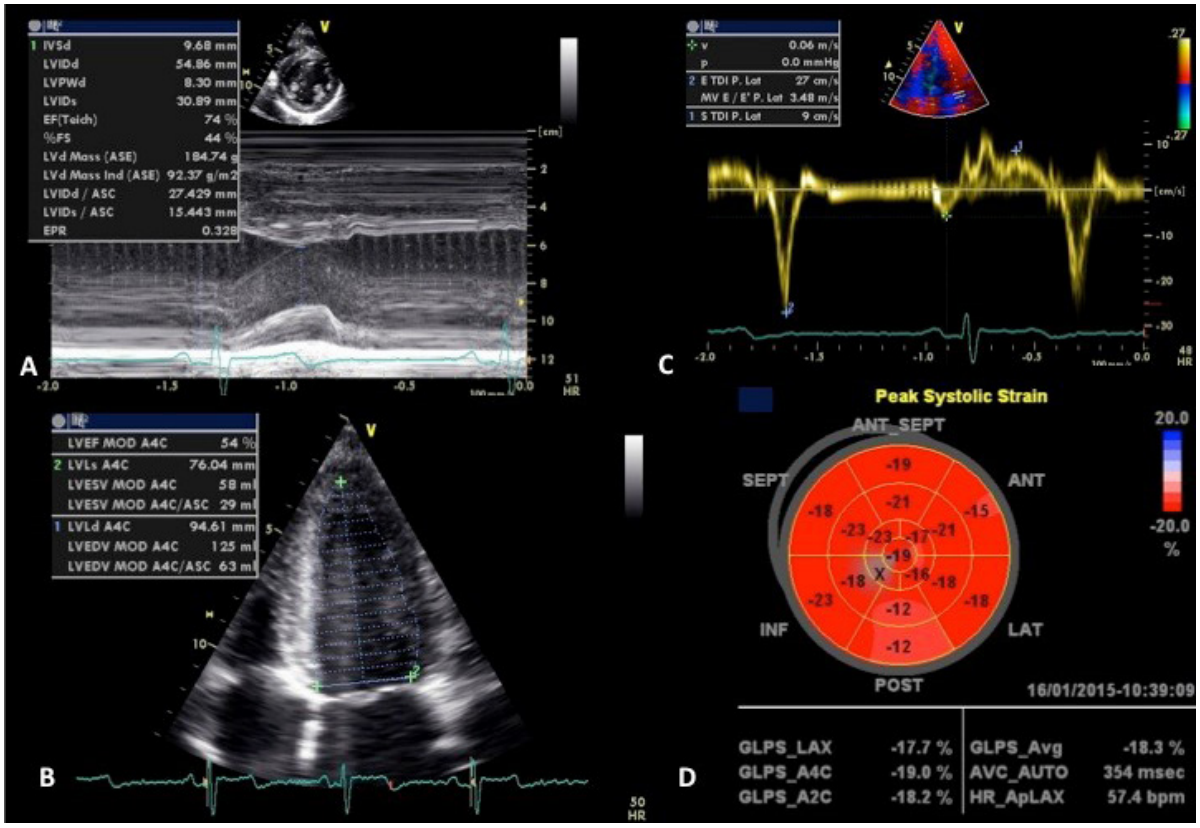

Figure 1 - *Transthoracic echocardiogram at rest without evidence of significant morfo-functional abnormalities – left ventricle dimensions (LV) by M Mode (A), volumes and LV ejection fraction (B), tissular Doppler at mitral ring (C) and global longitudinal strain (D).*

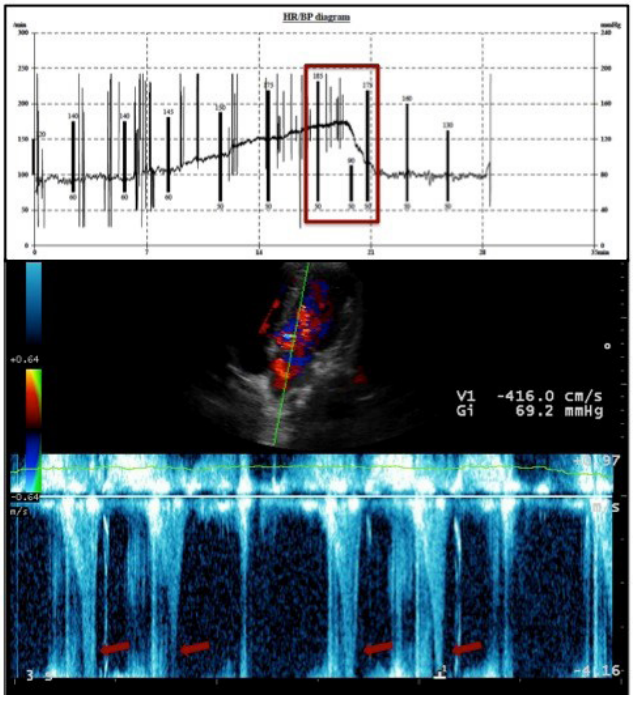

Figure 2 - *Exercise stress echocardiogram performed in treadmill with Bruce protocol, revealing a significant decrease in systolic blood pressure (185→90mmHg) in peak of exercise, with concomitant detection of IVG (bottom picture).*

The small published data and the short follow-up of athletes with IVG did not permit definite conclusions regarding the prognostic impact, but there are not described fatal clinical events in athletes with IVG without structural cardiac changes. In this setting there are not specific recommendations relatively to competitive sport in athletes with IVG.[10,11] In general, if an athlete is still symptomatic despite the stressed preventive/therapeutic measures, it is not advised to maintain sportive practice, especially with the intensity of exercise that precipitates the symptoms, and this should be regularly evaluated during follow-up.

Shortly, in the presence of an athlete with exercise-induced symptoms, IVG should be taken in consideration. The exclusion of potential pathologies associated to an increased risk for sudden cardiac death is fundamental in the reproduction of symptoms, in which exercise stress echocardiogram plays an important role. IVG remains poorly clarified and some questions unanswered:

- Which is the etiology/pathophysiology of IVG (physiologic versus pathologic)?

- Which is the clinical impact at long-term of IVG?

- Which should be the recommendations regarding the eligibility for competitive sport of athletes with IVG?

- Which should be the surveillance/follow-up of athletes with IVG?

Author contributions

Conception and design of the research: Dores H, Mendes L; Acquisition of data: Dores H, Mendes L, Ferreira A; Writing of the manuscript: Dores H; Critical revision of the manuscript for intellectual content: Dores H, Mendes L, Ferreira A, Santos JF.

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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Angiosarcoma Arising from the Main Pulmonary Artery Mimicking Pulmonary Thromboembolism

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A 79-year-old female with no relevant past medical history was admitted in our emergency department for dyspnea on minimal exertion and chest discomfort over 2 weeks. Blood gas analysis showed severe hypoxemia and hypocapnia. Troponin was slightly positive. Despite a negative D-dimer assay, contrast-enhanced chest CT was performed to exclude pulmonary embolism. It showed a large filling defect centered in the pulmonary valve plane (Panels A and B). Bedside transthoracic echocardiogram showed a large echodense mass, apparently mobile, extending across the right ventricle outflow tract, pulmonary valve, and the main pulmonary artery, with dilatation of the right sided chambers and transtricuspid peak gradient of 70 mmHg (Panels C and D). Lower-limb venous compression ultrasound was negative for deep vein thrombosis. The patient remained stable, but required high oxygen inspiration fraction to maintain saturation above 90%. As pulmonary embolism was deemed unlikely given the clinical findings, the patient underwent cardiac surgery. Surgery revealed a pearly mass in the main pulmonary artery obliterating almost the entire lumen and with upstream extension to the pulmonary valve and right ventricle outflow tract (Panel E). The tumor was excised as much as possible and the pulmonary valve was replaced by a homograft. Pathological examination was compatible with angiosarcoma.

Pulmonary artery angiosarcoma is exceedingly rare and carries a very poor prognosis. It can be clinical and radiologically indistinguishable from acute or chronic pulmonary artery thromboembolism. Our clinical suspicion was heightened by a negative D-dimer assay and venous ultrasound and the apparent infiltration of pulmonary arterial walls on CT.

Author contributions

Conception and design of the research: Ferreira JSSM; Writing of the manuscript: Ferreira JSSM, Moreira N; Supervision: Ferreira MJ, Antunes M.

Potential Conflict of Interest

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Sources of Funding

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Study Association

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Keywords

Echocardiography; Pulmonary Embolism; Thoracic Surgery.

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Image

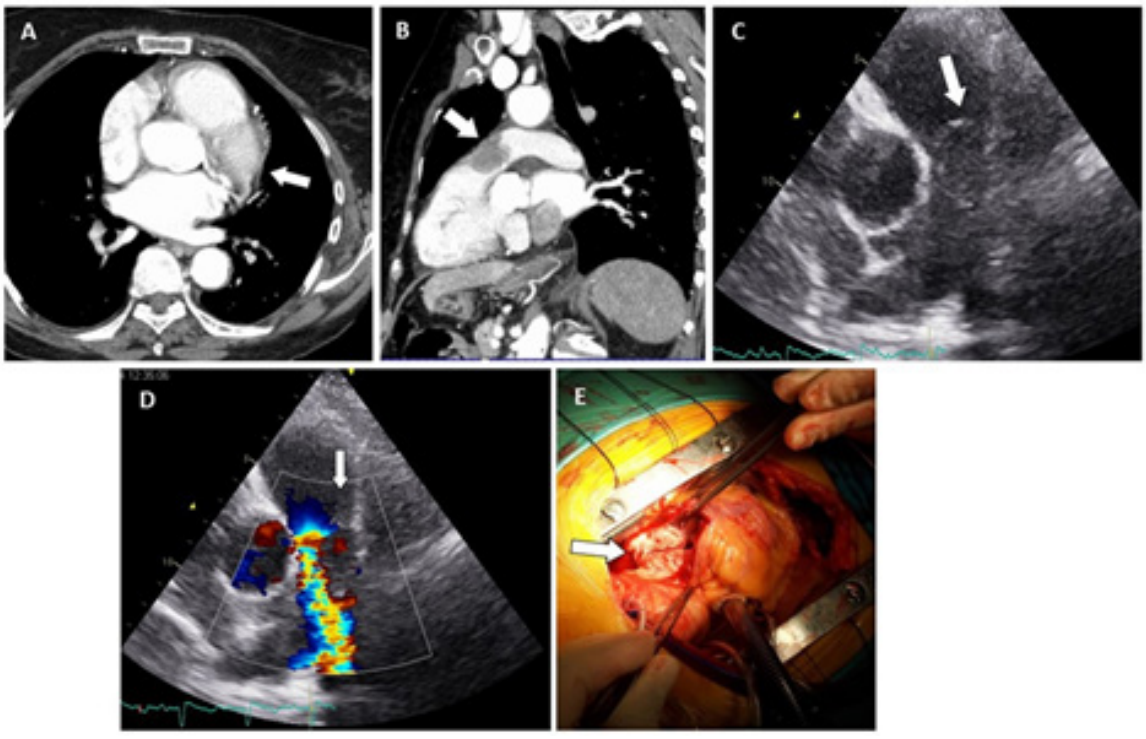

Figure 1 – *Angiosarcoma.*