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Sociedade Brasileira de Cardiologia • ISSN-0066-782X • Volume 108, Nº 1, January 2017

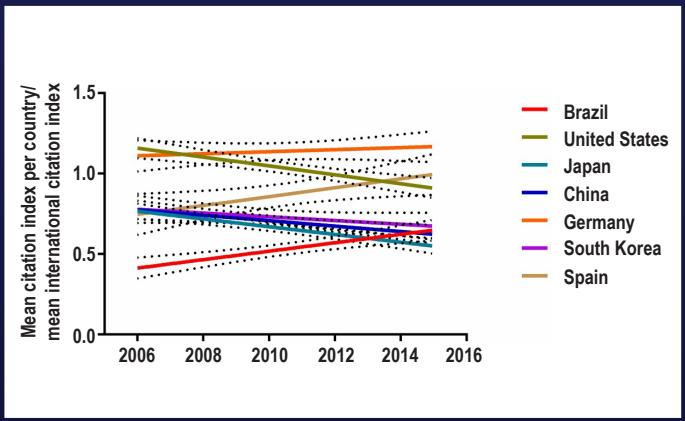

Figure 1 – *Relationship between the mean citation indexes of papers published in the fields of cardiology and cardiovascular sciences in each country and the mean international citation index in the period from 2006 to 2016. Page 2*

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Arquivos Brasileiros de Cardiologia

Volume 108, Nº 1, January 2017

Indexing: ISI (Thomson Scientific), Cumulated Index Medicus (NLM), SCOPUS, MEDLINE, EMBASE, LILACS, SciELO, PubMed

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Impact of Brazilian Papers in Cardiology and Cardiovascular Sciences in the Last Decade

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During the last decade, there has been a significant increase in the number of Brazilian publications in cardiology and cardiovascular sciences in important international citation indexing platforms. This occurred in Brazil and in most Latin American countries at approximately 13% per year between 1999 and 2008 according to the study by Colantionio et al.[1] This represents nearly 3% of all articles published in international journals indexed in the Web of Science platform, maintained by Thompson-Reuters, and Scopus-Scimago, provided by Elsevier.

Although most of our indexed articles have been published in international journals, the citation indexes achieved by Brazilian and Latin American authors are usually lower than those from countries with higher income or higher Human Development Index.[1] This is even more evident for studies conducted in national institutions in comparison with those developed with some degree of international cooperation.

As compared with international citations, using the data from Scimago country ranking,[2] the mean citation index of Brazilian papers in cardiology has progressively increased from less than 0.5 to nearly 0.65 in the last ten years (Figure 1). Today, this index is similar to that of countries like Japan, South Korea and China.

Advances in the quality of Brazilian papers in cardiology has occurred along with the improvement of Brazilian researchers' qualification thanks to Master and Doctoral degree programs and incentive programs for scientific research supported by agencies and national medical societies. On the other hand, approximately 35% of Brazilian papers in cardiology or cardiovascular sciences have been published in *Arquivos Brasileiros de Cardiology* or other Brazilian journals, and less than 50% of papers in journals with an impact factor greater than 1.6.

Previously, we have reported that the citation indexes of studies on major subjects in cardiovascular sciences, such as 'myocardial revascularization' and 'atrial fibrillation' published in Brazilian journals are not different from those of articles published in other countries.[3] Aiming to expand this analysis, we assessed 968 articles in cardiology indexed in the Web of Science, written exclusively by Brazilian authors and published between 2010 and 2014.

We considered the mean citation index of the 30 journals with the highest number of articles published, and assessed correlations between the number of times these articles were cited and the impact factor of the journals in which these articles were published. There was a weak correlation between the mean number of times these papers were cited within two years of publication and the impact factor of the journals, especially in higher impact journals (Figure 2).

These findings confirm that, despite recent advances, the citation indexes obtained from Brazilian authors are still lower than the mean international index. Besides, they highlight the importance to analyze the citation indexes of each study. These are currently available in the main international citation indexing systems that, in turn, provide an online, daily update of these parameters. For this reason, the 'value' of a publication may not be related to the journal impact. In fact, the citation index of scientific publications has been used by most of Brazilian fostering agencies and the Lattes Platform, a database of Brazilian researchers' resumes.

On the other hand, one of the main ranking criteria of graduate programs in Brazil, used by the Coordination for the Improvement of Higher Education Personnel (CAPES) of the Ministry of Health, is based on Qualis system. Qualis classifies scientific production of graduate programs according to the impact factors of journals in which the papers are published, without taking into consideration the indexes of each publication. Thus, a revision of this method is required to promote adequate fostering of research and incentive for the publication of Brazilian papers in internationally indexed journals.

A positive attitude towards the progress of scientific research in Brazil also depends on the maintenance of government and private funding to scientific research, on the expansion of training programs on clinical and laboratory studies, and improvement of Master's and Doctoral programs. Besides, the development of clinical trials and multicenter or multinational studies on major cardiovascular diseases by our centers and medical societies[4,5] represent important initiatives of great impact and an adaptation of scientific knowledge to national conditions.

Keywords

Cardiovascular Diseases; Cardiology; Periodicals as Topic; Bibliometrics.

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DOI: 10.5935/abc.20170005

Editorial

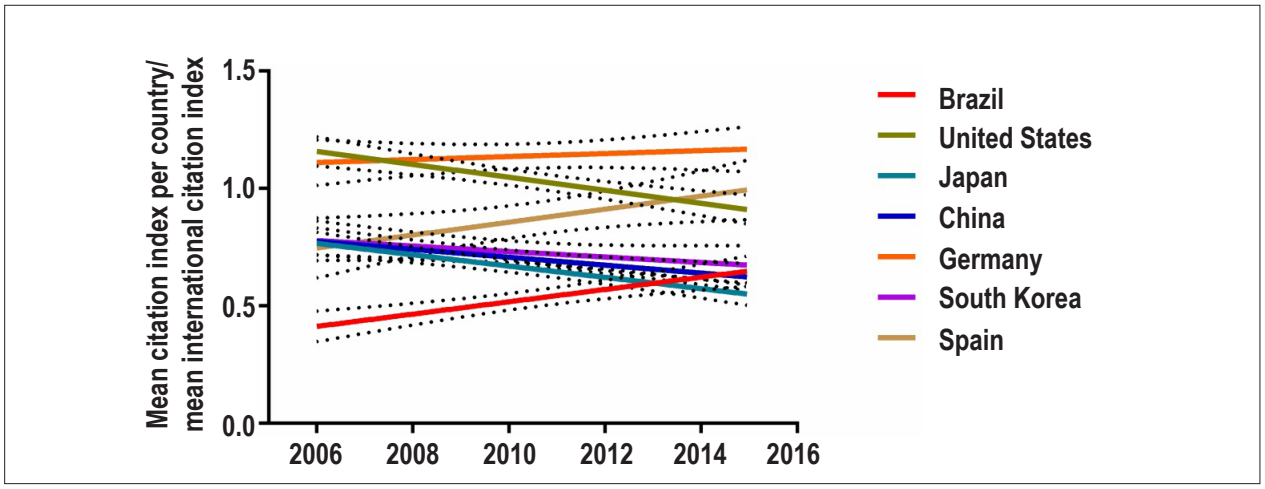

Figure 1 – *Relationship between the mean citation indexes of papers published in the fields of cardiology and cardiovascular sciences in each country and the mean international citation index in the period from 2006 to 2016.*

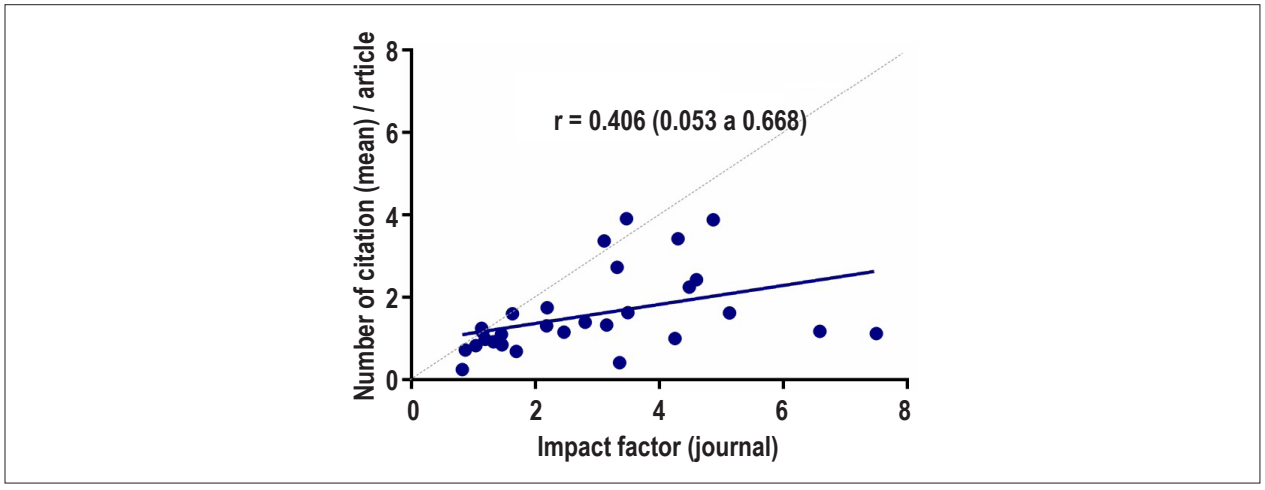

Figure 2 – *Correlation between mean number of citations obtained from 968 articles of Brazilian authors published in 30 journals in cardiology and cardiovascular sciences within 2 years of their publication and the impact factor in which these articles were published.*

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Intima-Media Thickness in the Carotid and Femoral Arteries for Detection of Arteriosclerosis in Human Immunodeficiency Virus-Positive Individuals

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Abstract

Background: The prevalence of atherosclerosis is higher in HIV-positive people, who also experience it earlier than the general population.

Objectives: To assess and compare the prevalence of atherosclerosis evaluated by the intima-media thickness of carotid and femoral arteries, and by the ankle-brachial pressure index (ABPI) in HIV patients treated or not treated with protease inhibitors (PIs) and controls.

Methods: Eighty HIV+ subjects (40 using PIs and 40 not using PIs) and 65 controls were included in the study. Atherosclerosis was diagnosed by (carotid and femoral) ITM measurement and ABPI. Classical risk factors for atherosclerosis and HIV were compared between the groups by statistical tests. A $p \leq 0.05$ was considered significant.

Results: An IMT > P_{75} or the presence of plaque was higher in the HIV+ than in the control group (37.5% vs 19%, p = 0.04). Comparative analysis showed a significant difference (p=0.014) in carotid IMT between HIV+ with PIs (0.71 ± 0.28 mm), without PIs 0.63 ± 0.11 mm and, and controls (0.59 ± 0.11 mm). There was no significant difference in femoral IMT between the groups or in ABPI between HIV+ subjects and controls. However, a significant difference (p=0.015) was found between HIV+ patients not treated with PIs (1.17 [1.08 – 1.23]), and controls 1.08 [1.07 – 1.17]).

Conclusion: In HIV patients, atherosclerosis is more prevalent and seems to occur earlier with particular characteristics compared with HIV-negative subjects. (Arq Bras Cardiol. 2017; 108(1):3-11)

Keywords: Carotid Artery Disease; Atherosclerosis; Carotid Intima-Media Thickness; HIV; Ankle Brachial Index.

Introduction

HIV-positive individuals (HIV+) experience different conditions in terms of morbidity and mortality of atherosclerosis and related cardiovascular events, as compared with subjects free of infection.[1,2] Cardiovascular disease (CVD), particularly atherosclerotic disease, is more prevalent and occurs earlier in HIV+ individuals than in those without the infection.[3-5]

The traditional risk factors for CVD are age, male sex, smoking, diabetes mellitus (DM), dyslipidemia, and systemic arterial hypertension (SAH). Studies have shown that these factors may be more prevalent in HIV+ people.[6,7]

The highly active antiretroviral therapy (HAART) is associated with a variety of adverse effects, which have proatherogenic effects and are also associated with CVD.[8,9] While some authors have suggested that protease inhibitors (PIs) may be associated with early atherosclerosis and CVD,[10] others have shown that the lipid profile is less affected by newer medications, which hence mitigates the increase in cardiovascular risk by endothelial dysfunction.[11] In a study comparing HIV+ subjects with dyslipidemia treated with PIs with healthy controls, no difference was found in endothelial function between the groups.[12]

Intima-media thickness (IMT) is a non-invasive, early marker of atherosclerosis; an increase in this measure may reflect an increase in cardiovascular risk.[13] This is an independent predictor of CVD, and may be considered as a marker for the assessment of subclinical atherosclerosis, including in HIV+ individuals.[14] In addition, common femoral and right subclavian arteries have been used for IMT measurements, and suggested as early markers of atherosclerosis.[15-17]

The ankle-brachial pressure index (ABPI) is a simple, non-invasive method, with high predictive value of peripheral artery disease (PAD) and cardiac disease. Values lower than 0.9 are associated with a significant increase in cardiovascular risk, independent of other risk factors.[18]

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Manuscript received April 28, 2016; revised manuscript July 29, 2016; accepted September 28, 2016.

DOI: 10.5935/abc.20160197

The detection of subclinical atherosclerosis allows a more adequate approach of HIV+ patients at risk for cardiovascular disease.

In the present study, the primary objective was to assess the prevalence of atherosclerosis by IMT of the common carotid and femoral arteries, and by the ABPI. The secondary objective was to compare classical risk factors of atherosclerosis and HIV-specific risk factors between patients treated and not treated with PIs.

Methods

Study design and population

This is a cross-sectional, prospective, analytical study on HIV+ patients in HAART including or not PIs during the period from June 2015 to February 2016. The sample was empirically determined by the authors based on the literature on the theme,[19,20] and included 40 HIV+ patients in HAART with PIs, 40 HIV+ patients in HAART without PIs, and 65 controls.

Phases of the study

Patients were selected at the outpatient service of infectious and parasitic diseases of this institution according to inclusion and exclusion criteria. For reference of outcome measures, we included 65 healthy subjects, who were sex- and age-matched with HIV+ patients – 40 patients in use of PIs were matched with 40 controls, whereas 40 patients not using PIs were matched with 25 controls. The HIV+ patients were enrolled in the program of prevention, control and treatment of AIDS, and control group was composed of individuals accompanying patients.

Inclusion criterion of HIV+ patients was time on HAART of five years or more, and the exclusion criteria were history of cardiovascular diseases – angina pectoris, acute myocardial infarction (AMI), stroke, PAD, hospitalization in the last two months, very low CD4 levels and/or very high viral load (VL). Exclusion criteria of healthy volunteers were history of cardiovascular diseases, smoking, DM and/or SAH.

Clinical data and complementary tests were collected by questionnaires, and detailed data about the treatment were obtained from patients' medical records. Participants had a Framingham risk score lower than 10% (low risk).

The atherosclerosis risk factors assessed were SAH, smoking, DM, hypercholesterolemia, hypertriglyceridemia, and history of any cardiovascular event – AMI, angina, stroke or PAD. Obesity was assessed by body mass index (BMI). A BMI between 18.5 and 24.9 kg/m^2 was considered healthy weight, and a BMI between 25.0 and 29.9 kg/m^2 was considered overweight. HIV-related factors assessed were current CD4, current VL, time of disease, time of treatment and type of HAART.

The study was approved by the local Ethics Committee, and all participants signed the informed consent form.

Protocol of intima-media thickness measurement

Ultrasonographic assessment of common carotid IMT was performed by B-mode ultrasonography (LOGIQe, DICOM with a 12-RS linear transducer, General Electric®), by a blinded observer. The common carotid IMT measurement was used as reference. Common carotid was analyzed by cross sections and longitudinal sections from the proximal segment of the common carotid to the bifurcation of the internal and external carotid arteries. The IMT measurement was performed in the posterior wall of the common carotid, in an area free of plaque, defined as the distance between two echogenic lines represented by the lumen-intima interface and media-adventitia interface of the arterial wall. The mean value and the maximum value are commonly used as references for IMT measured in the common and internal carotid, respectively.[15] Carotid atherosclerotic plaque was defined as a focal structure that extends at least 0.5 mm into the arterial lumen and/or measures 50% or more of the adjacent IMT value and/or has an IMT value greater than 1.5 mm.[15] The automated measurement of IMT was determined, using a software, in the right and left common carotid arteries in mean, medium and minimum value. When atheromatous plaques were identified, IMT was determined both automatically and manually. The mean automated measurement of the thickest common carotid artery was used as reference, be it the right or the left artery. As our study population was composed of patients aged less than 65 years, thickened intima-media was defined as an IMT equal to or higher than 0.8 mm.[17,21,22] The 75 percentile of the study group was also calculated,[15] and the presence of atheromatous plaque was defined as an IMT higher than 1.5 mm.[15] The IMT was also measured in the right common femoral artery (RCFA) and left common femoral artery (LCFA), using the same criteria for IMT and atheromatous plaque definition.

Protocol of measurement of the ankle-brachial pressure index

ABPI was calculated after measurement of right and left ankle pressure, which was measured at the dorsalis pedis artery and the posterior tibial artery. Pressures in the upper and lower extremities were measured using a sphygmomanometer (Becton Dickinson®) and a Doppler ultrasound device as above described. The ABPI was calculated by dividing the highest systolic ankle pressure by the highest systolic pressure of the arms. An ABPI of 0.9-1.3 was considered normal; ABPI values above 1.3 indicated incompressible arteries, and values below 0.9 were an indication of PAD.[21]

Statistical analysis

The Kolmogorov-Smirnov test was used to test the normality of data. Normally distributed data were presented as mean and standard deviation, and data without a normal distribution were expressed as median and minimum and maximum values. A descriptive and analytical analysis of the data was performed. A $p \leq 0.05$ was considered significant.

The prevalence and 95% confidence interval of atherosclerosis were calculated in the group of HIV+ patients and controls, and the compared by the Pearson's chi-square test.

The odds ratios (ORs) were calculated taking the control group as reference, and the OR of each HIV+ group (with PIs and without PIs) was estimated.

In between-groups comparison of classical risk factors for atherosclerosis, age and BMI were compared by ANOVA and Bonferroni's post-test. Median values were compared by the

nonparametric Kruskal Wallis test, and categorical variables by Pearson's chi-square test.

The chance of atherosclerosis according to HIV and use of PIs was assessed by multivariate logistic regression analysis, adjusted by skin color, hypercholesterolemia, hypertriglyceridemia, DM and BMI. These variables were significantly different between the groups.

For IMT validation analysis, the common carotid IMT was used as reference, and the ROC curve was applied to determine the femoral IMT cutoff. The analysis was performed by c-statistic (area under the ROC curve), measurement of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), using the using the Stata software, version 12.0.

Results

Forty subjects with HIV/AIDS in HAART with PIs (26 men, mean age of 42.7 ± 8.8 years), 40 subjects with HIV/AIDS in HAART without PIs (21 men, mean age of 42.2 ± 9.1 years), and 65 controls (37 men, mean age of 39.7 ± 9.7 years) were recruited.

The 75 percentile calculated for the total sample was 0.66. When the IMT was increased (> 0.66 mm), comparative analysis of the IMT of carotid arteries between control, HIV+ with PIs and HIV+ without PIs groups revealed an IMT of 0.59 ± 0.11 mm *vs*. 0.63 ± 0.11 mm *vs*. 0.71 ± 0.28 mm, respectively (p=0.014) (Table 1). The presence of IMT > P75 or plaque was detected in 19.0% (9.1-29.0) in controls, and in 37.5% (21.8 – 53.2) of HIV+ without PIs patients (p = 0.041) (Table 1).

The prevalence of IMT > 0.8 mm or atherosclerotic plaque in carotid arteries was 3.2% (0.0-7.6) in the control group and 15% (7.0-23.0) in HIV+, p=0.032. Between-groups comparisons of some of the IMT in carotid and femoral arteries and ABPI data are presented in Table 1.

No significant difference in ABPI was found between the control and HIV+ groups. The ABPI in the control group and HIV+ without PIs group was 1.08 (1.07 – 1.17) *vs* 1.17 (1.08 – 1.23), respectively (p = 0.015). The IMT in femoral artery was 0.74 mm ± 0.30 *vs*. 0.79 mm ± 0.33 in the control and HIV+ with PIs, respectively (p = 0.373) (Table 1).

The following risk factors for atherosclerosis were identified in the 80 HIV+ patients: smoking in 6 patients (7.5%), SAH in 7 (8.75%), hypercholesterolemia in 15 (18.75%), hypertriglyceridemia in 24 (30%) and DM in 6 (7.5%). Mean BMI was within the normal range in the HIV+ with PIs, at the lower limit for overweight in the group without PIs and classified as overweight in the control group. The BMI in the control, HIV+ without and with PIs was 26.2 ± 5.4 *vs*. 25 ± 3.6 *vs*. 24.7 ± 3.7 kg/m^2, respectively (p = 0.193) (Table 2).

The time of disease was significantly different between the HIV+ with PIs (13.6 ± 6.2 years) as compared with the HIV + without PIs group (7.3 ± 6.8 years) (p<0.001). The time of HAART was 12.1 ± 6.7 years in the group with PIs *vs*. 6.6 ± 6.7 years in the group without PIs, p < 0.001. Only 4 (5%) HIV+ patients had CD4 levels lower than 200, and only 7 (8.75%) of studied population had a detectable VL sample, with a maximum of 3,231 copies (Table 3).

There was a positive Pearson correlation between common carotid IMT and femoral carotid IMT ([ρ = 0.354 (p<0.001)] (Figure 1).

Figure 2 shows the ROC curve of IMT in femoral artery, considering atherosclerosis as the carotid artery IMT >0.66 mm. Using a cutoff of 0.7mm in the femoral artery, we observed a 72.5% sensitivity, 46.6% specificity, area under the ROC curve of 0.661 and kappa of 14.3% (Table 4).

Discussion

The present study demonstrated that the IMT of carotid arteries was greater in HIV+ patients as compared with controls, regardless of the use of PIs.

Eira et al.[20] studied 118 patients divided into 4 groups (HIV patients in HAART, HIV patients without treatment – naïve group, noninsulin-dependent diabetes and control). Right carotid IMT was greater in the naïve group than in HAART and control groups (0.55 ± 0.02 mm *vs*. 0.52 ± 0.02 mm *vs* 0.52 ± 0.02 mm, respectively; p<0.0011), whereas the IMT in the left carotid artery was greater in HAART group than in naïve and DM groups (0.64 ± 0.04 mm *vs*. 0.53 ± 0.04 mm *vs*. 0.52 ± 0.04 mm, respectively; p<0.0001). Therefore, these results are in agreement with ours by showing greater IMT in HIV+ patients than in other groups.[20] Other authors have also reported higher prevalence of atherosclerosis in HIV+ patients treated with HAART.[23]

Our findings differ from those of Godoi et al.[21,22] on 70 HIV patients and 70 controls, showing no difference between these groups. However, the study involved younger subjects, and included smoking, hypertensive and DM controls, which may have contributed to greater IMT values.

In our study, HIV+ patients had a mean IMT higher than the 75 percentile of the study group and the control group.

The 75 percentile depends on the studied population, as it varies with sex, race and age. In the Elsa-Brasil study, this parameter was estimated in pardo, male subjects with similar age as our study group; the 75 percentile was 0.58 – 0.63 mm.[15]

In HIV+ patients, chronic immune activation and chronic inflammation are associated with increased risk for atherosclerosis. Ultrasonography was one of the first diagnostic methods to identify high incidence of subclinical atherosclerosis in HIV-infected individuals as compared with healthy controls.[24,25]

It has been hypothesized that the HAART activates endothelial function and promotes atherosclerosis. Thus, HIV, immune reconstitution response and HAART may promote early endothelial activation, and hence represent proatherogenic factors and/or accelerators of atherosclerosis.[26,27] In our study, there was no significant difference in IMT between the HIV+ patients treated with PIs and not treated with PIs. Despite the hypothesis of HAART-related endothelial dysfunction, many issues need further clarification.

Nolani et al.[12] compared HIV-infected subjects with dyslipidemia treated with PIs and healthy controls and did not find any difference between the groups.

Table 1 – Prevalence of atherosclerosis assessed by common carotid and femoral intima-media thickness (IMT) and ankle-brachial pressure index (ABPI) in HIV-negative subjects, and HIV-positive subjects in antiretroviral therapy treated or not treated with protease inhibitor (PI)

Atherosclerosis	HIV-negative subjects % (95%CI)	HIV-positive subjects % (95%CI)	HIV carriers		OR control *x* HIV (95%CI)[b] P value	OR (95%CI)[b] p value (control *x* without PI)	OR (95%CI)[b] valor de p (control *x* with PI)	p value (3 groups)
			Without PI % (95%CI)	With PI % (95%CI)				
Intima-media thickness								
Carotid								
IMT	0.59 ± 0.11	0.70 ± 0.27	0.63 ± 0.11	0.71 ± 0.28	0.004	0.007	0.006	0.014
IMT >75P or presence of plaque	19.0% (9.1 - 29.0)	35.0% (24.3 - 45.7)	37.5% (21.8 - 53.2)	32.5% (17.3 47.7)	2.28 (1.05 - 4.98) p = 0.037	2.55 (1.04 - 6.25) p = 0.041	2.05 (0.82 -6.25) p = 0.124	0.095
IMT >0.8mm or presence of plaque	3.2% (0.0 - 7.6)	15.0% (7.0 - 23.0)	15.0% (3.4 - 26.6)	15.0% (3.4 - 26.6)	5.38 (1.16 - 25.1) p = 0.032	5.38 (1.03 - 28.1) p = 0.046	5.38 (1.03 - 28.1) p = 0.046	0.061
Femoral								
IMT[d]	0.74 ± 0.30	0.79 ± 0.33	0.75 ± 0.27	0.82 ± 0.38	0.373	0.661	0.155	0.351
IMT >75P or presence of plaque	50.8% (38.1 -63.5)	65.0% (54.3 - 75.7)	60.0% (44.1 - 75.9)	70.0% (55.1 - 84.8)	1.80 (0.92 - 3.53) p = 0.088	1.45 (0.65 -3.24) p = 0.361	2.26 (0.98 -5.22) p = 0.056	0.153
IMT >0.8mm or presence of plaque	11.1% (3.1 - 19.1)	20.0% (11.0 - 29.0)	20.0% (7.0 32.9)	20.0% (7.0 - 32.9)	2.00 (0.77 - 5.21) p = 0.156	2.00 (0.66 - 6.03) p = 0.218	2.00 (0.66 - 6.03) p = 0.218	0.356
Changes in ABPI[c]								
ABPI[e]	1.08 (1.07; 1.17)	1.15 (1.08; 1.2)	1.17 (1.08; 1.23)	1.08 (1.07; 1.17)	0.190	0.015	0.797	0.019[a]
Normal (0.9 – 1.3)	95.2% (92.0 - 100)	96.3% (92.0 - 100)	90.0% (80.3 - 99.9)	100% (-)	Reference	Reference	Reference	-
Incompressible (> 1,3)	4.8% (0.0 - 10.2)	5.0% (0.1 - 9.9)	10.0% (0.03 - 19.7)	0% (-)	1.05 (0.23 - 4.88) p = 0.948	2.22 (0.47 - 10.5) p = 0.314	Not calculated	0.116

ABPI: ankle-brachial blood pressure index; OR: Odds Ratios; HIV: human immunodeficiency virus. a 75 Percentile of IMT of 0.66 mm in the study population; b Reference group: HIV-negative subjects; c IMT < 0.9 was not detected in the study group; d Data with logarithmic transformation for normalization of distribution; e Median (P25; P75) - Kruskal-Wallis test for between-group comparisons.

Although our HIV+ patients using PIs had a significantly longer time of treatment compared with the HIV+ patients without IPs, apparently, the use of these medications did not affect the IMT in HIV+ patients.

In some situations, the treatment of HIV+ patients may be started without PIs. In the present study, the possibility that patients treated with PIs had been previously treated without these drugs cannot be ruled out, which may explain the longer time of disease and treatment in this group.

Newer PIs are relatively less related with metabolic disturbances, and hence, have a smaller effect on the increase in cardiovascular risk induced by endothelial dysfunction.[28,29] This may explain the absence of a significant difference in IMT between the patients treated and not treated with PIs in the present study.

The principles of the HAART are viral control and stabilization of the immune system, resulting in increased life expectancy and reduction of opportunistic infections.[1] HIV patients have cardiovascular changes caused by exposure to classical CVD risk factors, virus infection and cardiotoxicity to antiretroviral drugs.[8] The HIV infected patients in our study were immunologically stable. Most of them had CD4 levels above 200 cells/ mm^3, and only seven patients had a detectable VL. There was no significant difference in CD4 and VL between patients with PIs and without PIs.

Previous studies[3-5] have demonstrated that atherosclerosis is more prevalent and occurs earlier in patients with HIV infection as compared with non-infected individuals, which are in agreement with our findings.

The prevalence of hypercholesterolemia and hypertriglyceridemia was higher in HIV+ patients using PIs than in other groups, suggesting that the use of PIs may affect these parameters.

The high prevalence of ABPI > 1.3 in HIV+ subjects may be influenced and mediated by vascular elasticity and atheroma

Table 2 - Comparison of classical risk factors for atherosclerosis between HIV-negative subjects and HIV-positive subjects in antiretroviral therapy treated or not treated with protease inhibitor (PI)

Atherosclerosis risk factors	Groups			p value (control *x* without PI)	p value (control *x* with PI)	p value (3 groups)
	HIV-negative subjects (n = 63)	HIV-positive subjects				
		Without PI (n = 40)	With PI (n = 40)			
Age, years (mean ± SD)[b]	39.7 ± 9.7	42.2 ± 9.1	42.7 ± 8.8	0.550	0.351	0.215
Male sex (%)	37 (58.7%)	21 (52.5%)	26 (65.0%)	0.998	0.276	0.525
Years of school (%)						
0 - 4 years	55 (87.3%)	33 (82.5%)	33 (82.5%)	0.501	0.501	0.732
5 - 7 years	8 (12.7%)	7 (17.5%)	7 (17.5%)			
Skin color (%)						
White	27 (42.9%)	8 (20.0%)	10 (25.0%)	0.017[a]	0.066	0.030[a]
Not white	36 (57.1%)	32 (80.0%)	30 (75.0%)			
Smoking (%)	0 (0%)	4 (10.0%)	2 (5.0%)	Not calculated	Not calculated	0.046[a]
SAH (%)	0 (0%)	2 (5.0%)	5 (12.5%)	Not calculated	Not calculated	0.062
Hypercholesterolemia (%)	3 (4.8%)	3 (7.5%)	12 (30.0%)	0.563	<0.001[a]	<0.001[a]
Hypertriglyceridemia (%)	1 (1.6%)	2 (5.0%)	22 (55.0%)	0.315	<0.001[a]	<0.001[a]
Diabetes (%)	0 (0%)	1 (2.5%)	5 (12.5%)	Not calculated	Not calculated	0.007[a]
BMI, kg/m^2 (mean ± SD)[b]	26.2 ± 5.4	25.0 ± 3.6	24.7 ± 3.7	0.585	0.285	0.193
Overweight/obese (%)	36 (57.1%)	19 (47.5%)	19 (47.5%)	0.339	0.339	0.519

[a] ANOVA with Bonferroni post-test; [b] Despite the significant difference between the groups, the analyses were not adjusted for hypercholesterolemia, hypertriglyceridemia and race conditions, due to the low frequency of the variables. HIV: human immunodeficiency virus; HAS: systemic arterial hypertension; BMI: body mass index; SD: standard deviation.

Table 3 – Comparison of risk factors, laboratory parameters, and HIV-related data between HIV-positive subjects in antiretroviral therapy treated with protease inhibitor and not treated with protease inhibitor (PI)

Factors	Without PI	With PI	p value
Laboratory[a]			
Cholesterol (mg/dL)[b]	182.3 ± 32.9	188.0 ± 57.9	0.642
HDL cholesterol (mg/dl)[b]	52.5 ± 13.6	45.4 ± 10.6	0.071
LDL cholesterol (mg/dl)[b]	103.2 ± 31.0	81.8 ± 25.2	0.044
Triglycerides (mg/dL)[c]	95.7 (73.6; 143.5)	238.1 (140; 375.9)	<0.001
Related to HIV			
Time of HIV infection (in years)[b]	7.27 ± 6.78	13.62 ± 6.20	<0.001
Time of HAART (in years)[b]	6.62 ± 6.65	12.1 ± 6.69	<0.001
CD4 count (%)			
< 200 cells/mm^3	2 (5.0%)	2 (5.0%)	0.599
200 - 500 cells/mm^3	9 (22.5%)	13 (32.5%)	
> 500 cells/mm^3	29 (72.5%)	25 (62.5%)	

a Thirty-six of 40 patients in the group without PI, and 20 of 40 patients without PI had available laboratory data; b Mean ± standard deviation; Student's t-test; c Median (P25; P75) - nonparametric Mann-Whitney test. HIV: human immunodeficiency virus; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; HAART: highly active antiretroviral therapy.

plaque formation.[10] In the present study, no significant difference in ABPI was detected between HIV groups and controls, which may be explained by impairment of vessel wall elasticity. The ABPI in HIV+ patients without PIs was within normal range.

A metanalysis investigated the ABPI in HIV-infected patients. Variable selection criteria of the study groups were used in the studies, and there was no consensus on the risk factors for an abnormal ABPI. The prevalence of increased ABPI was higher in HIV+ patients than in the general population. In addition, it has not been established whether a high prevalence of altered ABPI is associated with high incidence of cardiovascular events.[30]

The common carotid IMT is still the reference for other artery measures, and has been shown the best accuracy in studies correlating increased cardiovascular risk and HIV infection. The IMT of common femoral artery and right subclavian origin may also be considered and suggested by some authors as an early marker of atheromatosis. However, these parameters do not combine both high specificity and high sensitivity, which was corroborated by our study.[16,17,22]

When the IMT in the common carotid artery and femoral arteries were compared, we found a positive but weak correlation between the groups. We believe that further studies involving a higher number of patients would improve the understanding of this correlation.

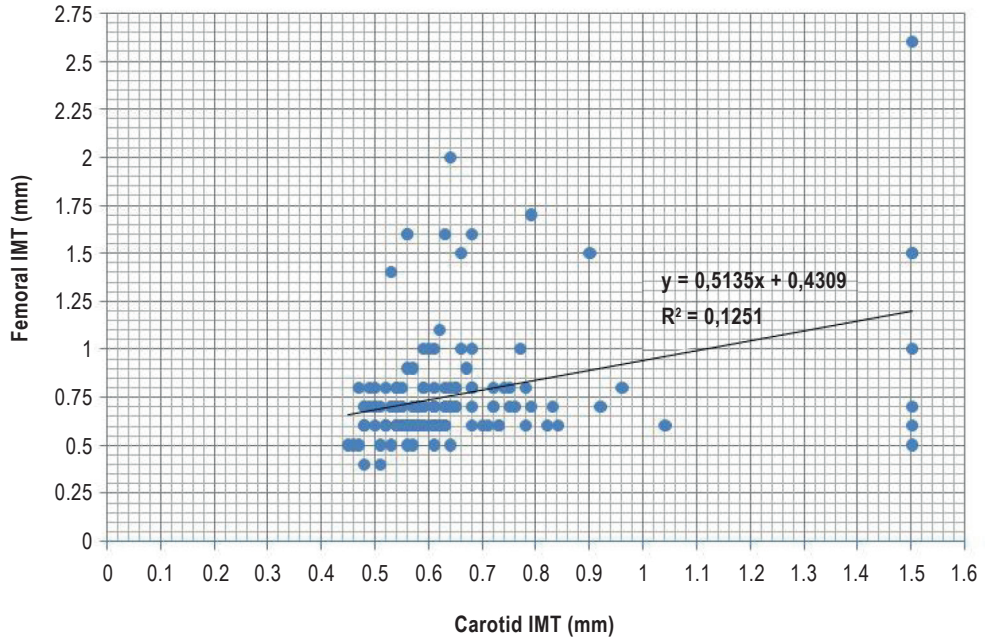

Figure 1 – *Correlation between intima-media thickness (IMT) in the common carotid and in the common femoral artery; Pearson correlation = 0.354 (p < 0.001)*

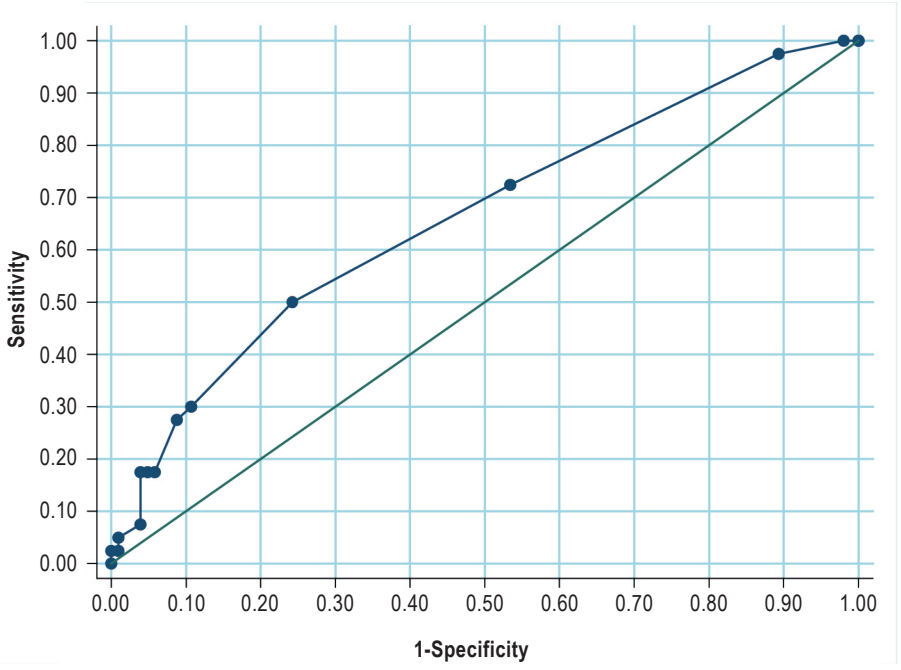

Figure 2 – *ROC curve of femoral artery intima-media thickness (IMT), considering 'atherosclerosis' as an IMT in the common carotid above 0.66 mm; *Area under the curve = 0.6614 (95%CI: 0.563 – 0.760)*

Table 4 – Accuracy of intima-media thickness (IMT) in the femoral and carotid arteries in the studied patients

	Carotid IMT [a]		Statistics (95%CI)
	With atherosclerosis	Without atherosclerosis	
Femoral IMT[b]			
With atherosclerosis	29	55	Sensitivity: 72.5% (58.0 - 86.9)
Without atherosclerosis	11	48	Specificity: 46.6% (36.8 - 56.4)
Total	40	103	PPV: 34.5% (24.1 - 44.9)
			VPN: 18.6% (8.4 - 28.9)
			Area under the curve: 0.661 (0.563 - 0.760)
			Kappa: 14.3% (0.8 - 27.8)

[a] Atherosclerosis by the 75 percentile of IMT in the study population equal to or greater than 0.66 mm; [b] Atherosclerosis by the femoral IMT cutoff equal to or greater than 0.7 mm ,estimated by the ROC curve. PPV: positive predictive value; NPV: negative predictive value.

Another study[17] has demonstrated a correlation (Pearson correlation), also not strong, between common carotid artery and right subclavian artery.

In this study, the ROC curve was used to compare two tests, the common carotid IMT (used as reference) and the femoral artery IMT. This analysis allows the comparison of two or more diagnostic tests, which is one of the greatest advantages of the method.[31]

By using a cutoff > 0.7mm for IMT, we tested the accuracy of IMT in the femoral and carotid arteries in HIV+ patients. Based on sensitivity, specificity, PPV and NPV, the IMT in the femoral artery could not be used as a surrogate for the measurement in the carotid artery.

The main limitations of this study were the empirical definition of the population sample size, and the study design that does not allow the establishment of a cause-effect relationship. It is worth mentioning, however, that other studies on the theme, available in the literature, have been performed on smaller sample sizes. In addition, whether the fact that we did not evaluate pulse wave velocity or flow mediated dilation of the brachial artery, which are also non-invasive methods for the diagnosis of subclinical atherosclerosis, may represent a limitation of the study, is a matter of discussion. Nevertheless, there are reliable data on the role of IMT as a cardiovascular risk predictor.[19,20]

Conclusions

Higher values of IMT and higher prevalence of IMT above the 75 percentile and IMT > 0.8 mm or presence of atherosclerotic plaque in HIV+ patients suggest an earlier occurrence of atherosclerosis in this population as compared with healthy controls. However, no difference was found in the occurrence of abnormal ABPI between the groups.

The prevalence of smoking was higher in HIV+ patients without PIs, whereas cholesterolemia, hypertriglyceridemia, and DM were more prevalent in HIV group without PIs. Time of disease and time of HAART were higher in HIV+ patients using PIs.

Common carotid IMT measurement is still the reference method for detection of atherosclerosis, since the femoral artery IMT showed a moderate sensitivity and low specificity to the former.

Author contributions

Conception and design of the research and Analysis and interpretation of the data: Godoi ETAM, Brandt CT, Lacerda HR, Godoi JTAM, Oliveira DC, Santos Junior GG, Godoi JTAM, Vasconcelos AF; Acquisition of data: Godoi ETAM, Costa GFAS, Santos Junior GG, Leite KME, Vasconcelos AF; Statistical analysis: Godoi ETAM, Brandt CT, Oliveira DC; Obtaining financing: Godoi ETAM, Santos Junior GG; Writing of the manuscript: Godoi ETAM, Godoi JTAM, Oliveira DC; Critical revision of the manuscript for intellectual content: Godoi ETAM, Brandt CT, Lacerda HR, Godoi JTAM, Oliveira DC, Godoi JTAM.

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 article is part of the thesis of Habilitation (professorship) by Emmanuelle Tenório Albuquerque Madruga Godoi, from Universidade Federal de Pernambuco.

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Comparison between Radionuclide Ventriculography and Echocardiography for Quantification of Left Ventricular Systolic Function in Rats Exposed to Doxorubicin

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Abstract

Background: **Radionuclide ventriculography (RV) is a validated method to evaluate the left ventricular systolic function (LVSF) in small rodents. However, no prior study has compared the results of RV with those obtained by other imaging methods in this context.**

Objectives: **To compare the results of LVSF obtained by RV and echocardiography (ECHO) in an experimental model of cardiotoxicity due to doxorubicin (DXR) in rats.**

Methods: **Adult male Wistar rats serving as controls (n = 7) or receiving DXR (n = 22) in accumulated doses of 8, 12, and 16 mg/kg were evaluated with ECHO performed with a Sonos 5500 Philips equipment (12-MHz transducer) and RV obtained with an Orbiter-Siemens gamma camera using a pinhole collimator with a 4-mm aperture. Histopathological quantification of myocardial fibrosis was performed after euthanasia.**

Results: **The control animals showed comparable results in the LVSF analysis obtained with ECHO and RV (83.5 ± 5% and 82.8 ± 2.8%, respectively, p > 0.05). The animals that received DXR presented lower LVSF values when compared with controls (p < 0.05); however, the LVSF values obtained by RV (60.6 ± 12.5%) were lower than those obtained by ECHO (71.8 ± 10.1%, p = 0.0004) in this group. An analysis of the correlation between the LVSF and myocardial fibrosis showed a moderate correlation when the LVSF was assessed by ECHO (r = -0.69, p = 0.0002) and a stronger correlation when it was assessed by RV (r = -0.79, p < 0.0001). On multiple regression analysis, only RV correlated independently with myocardial fibrosis.**

Conclusion: **RV is an alternative method to assess the left ventricular function in small rodents in vivo. When compared with ECHO, RV showed a better correlation with the degree of myocardial injury in a model of DXR-induced cardiotoxicity. (Arq Bras Cardiol. 2017; 108(1):12-20)**

Keywords: **Radionuclide Ventriculography / methods; Echocardiography / methods; Ventricular Function, Left; Comparative Study; Rats; Doxorubicin.**

Introduction

In recent decades, imaging methods assessing functional and structural cardiac parameters in small animals *in vivo* have been widely used to study the pathophysiological mechanisms of ventricular dysfunction in different models of cardiac disease, and to develop new therapies for heart failure (HF).[1-7] These methods allow a longitudinal study of the animals, increasing the power of observation at lower costs.

Among measurable parameters, the left ventricular systolic function (LVSF) is a key variable to evaluate myocardial remodeling, degree of ventricular dysfunction, and prognosis of myocardial disease. Echocardiography (ECHO) has been widely used to assess the ventricular function in humans and models of cardiac disease, as it is a low-cost tool for rapid acquisition of images without requirement of radioactive isotopes.[1,2,8] However, the echocardiographic evaluation, especially in small rodents, depends largely on the observer and has restricted interobserver reproducibility, limiting the detection of subtle changes.[9]

Radionuclide ventriculography (RV) is a technique often used in clinical practice with good accuracy and high reproducibility levels in serial evaluations for LVSF quantification.[10,11] In addition, RV is considered by many as the gold-standard method to assess ventricular function, as it faithfully represents the volumes of the ventricular chambers at each moment of the cardiac cycle without assumptions

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Article received on March 06, 2016; revised manuscript March 18, 2016; accepted June 28, 2016.

DOI: 10.5935/abc.20160194

of myocardial shape and/or geometry.[12-14] However, few studies have demonstrated its application in models of cardiac diseases in small animals.[15,16]

Although the use of RV in models of experimental cardiac disease in small rodents has been described for quite some time,[15,16] there have been no studies comparing results obtained with RV with those obtained by other imaging methods *in vivo*. The objective of this study was to conduct a comparative analysis of the ability of ECHO and RV in evaluating the global systolic performance of the left ventricle and correlate these results with the severity of cardiac structural changes detected by histopathological analysis in a model of anthracycline-induced cardiotoxicity.

Methods

Experimental animals

After approval by the Ethics Committee on Animal Experimentation of our institution, the study was performed in 29 male Wistar rats weighing approximately 250 g, obtained from the institution's animal room. The animals were kept in an air-conditioned room, with free access to water and standard chow, subjected to a rhythm of 12 hours of light/dark and controlled temperature. During all procedures, maximum care was taken to avoid inflicting unnecessary suffering to the animals.

General Study Design

To achieve a broader observation of the accuracy of both imaging methods in variable ranges of LVSF impairment, we used different doses of intravenous infusion of doxorubicin (DXR). This approach also allowed us to obtain a wide dispersion of the investigated variables to correlate better the changes in cardiac function measured *in vivo* and the degree of *in vitro* histological lesions that served as the gold-standard method to assess myocardial injury.

Based on that, the animals received three different cumulative doses of DXR over 8 weeks: D-8 mg: total infusion of DXR 8 mg/kg administered as four weekly injections of 2 mg/kg (n = 8); D-12 mg: 12 mg/kg accumulated over six weekly injections of 2 mg/kg (n = 7); D-16 mg: 16 mg/kg administered as eight weekly injections of 2 mg/kg (n = 7). Seven control animals received injections of saline solution over 8 weeks.

All animals underwent noninvasive LVSF evaluation with *in vivo* imaging methods, ECHO, and RV at baseline and 2 weeks after the end of the period of infusion of the respective doses of DXR or saline.

Medications administered

Adriablastina® RD (doxorubicin hydrochloride, Pharmacia, Milan, Italy) was dissolved in saline solution (10 mg/5 mL) and infused intravenously. A solution containing ketamine hydrochloride (Vetbrands, Jacareí, São Paulo, Brazil; 20 mg/kg) and xylazine (Bayer, São Paulo, Brazil; 8 mg/kg) was administered by intramuscular injection for anesthetic induction before each intravenous injection of DXR and imaging evaluations. To euthanize the animals, we used an overdose of these anesthetics (40 and 16 mg/kg, respectively).

Echocardiography

After sedation and trichotomy of the anterior chest region, the animals, while breathing spontaneously, were placed in the left lateral decubitus position and evaluated with ECHO Doppler with a two-dimensional, high-resolution ECHO system (Sonos 5500 Philips, Andover, MA, USA) and a sectorial transducer with a frequency of 12 MHz. The ventricular function was estimated by the calculation of the left ventricular fractional shortening, measured from the short-axis view of the left ventricle. The area shortening was determined by the formula: Delta Areas = (AD - AS)/AD x 100,[17] in which AD and AS are the areas in the diastole and systole, respectively. The fractional area shortening has been shown to be effective in detecting left ventricular systolic dysfunction in experimental models of myocardial infarction in rats[18-20] and has the advantage of considering the two-dimensional image of the short axis of the left ventricle, compared with the ventricular shortening fraction (ΔD%), which takes into account only a linear ventricular dimension in the diastole and systole.

The images obtained were recorded and archived for later off-line analysis by an observer blinded to the group to which the animals were allocated. All measurements were obtained by the same investigator and revised by another; both were experienced in obtaining and analyzing ECHOs in small animals.

Radionuclide ventriculography

After anesthesia, 75 μg of stannous agent was injected into the tail vein. After an interval of 15 min, the animals received a new tail vein injection of 15 mCi of technetium pertechnetate-99m. Immediately after the administration of Tc-99m, the animals were taken to the gamma camera and positioned in a dorsal decubitus position under the detector. Three electrodes were implanted in the animals' hypodermis for electrocardiographic monitoring, positioned in the two anterior limbs and on the upper portion of the abdomen, as shown in Figure 1.

RV was performed with the gamma camera Orbiter-Siemens (Siemens, Erlangen, Germany), equipped with a pinhole collimator with a 4-mm aperture. Images were obtained in the left anterior oblique projection in a word format, 64 x 64 pixels matrices, synchronized with the electrocardiogram (ECG) with an acceptance window of 20% around the mean QRS duration value and with 32 frames per cardiac cycle. We acquired 200,000 counts per frame. The symmetric energy window of 20% focused on the Tc-99m energy photopeak was 140 keV.

To process the images, we used a commercially available software (planar gated blood pool, SMV America) in a dedicated workstation (NXT-P, Sopha Medical Vision). After semiautomatic detection of the edges of the left ventricle and with the help of parametric images of phase and count amplitude variation, a time *versus* activity curve was generated. From this curve, we calculated the LVSF, expressed as percentage (%), defined as the difference between the values corrected for the background radiation of the end diastolic and systolic counts divided by the value of the end diastolic count (Figure 2).

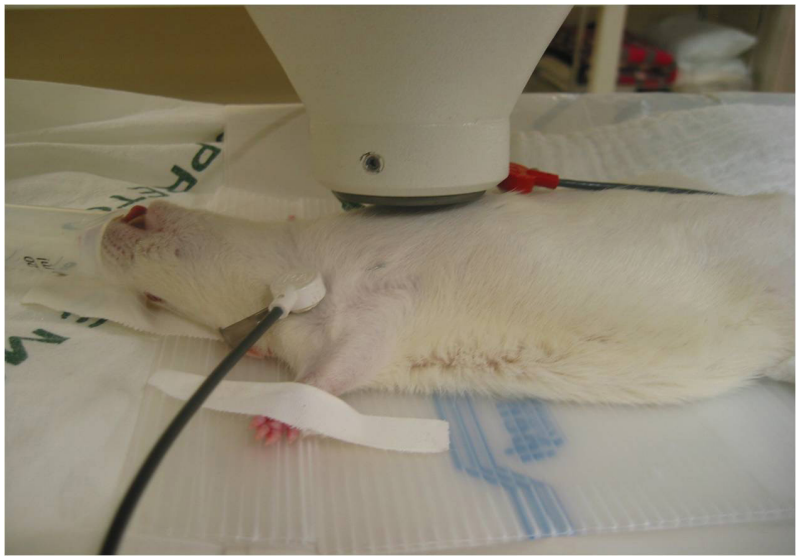

Figure 1 – *Positioning of the animal and the electrodes for radionuclide ventriculography.*

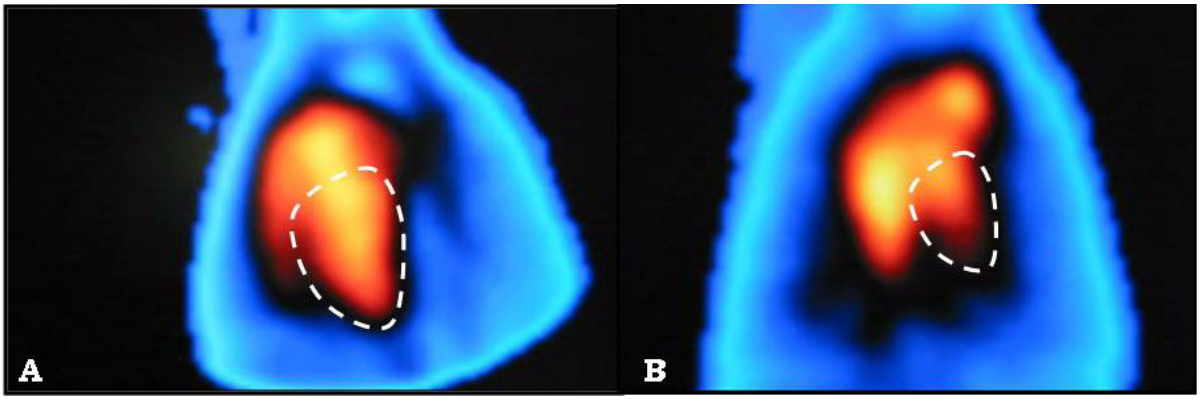

Figure 2 - *Images of the cardiac blood compartment labeled with ^{99m}Tc in the left anterior oblique projection, in diastolic (A) and systolic (B) frames, allowing quantification of the LVSF after regions of interest were traced. LVSF = 76%.*

Histology

After the animals had been euthanized, we quantified the extent of the myocardial fibrosis by measuring the collagen area in the myocardium. The hearts were sliced transversely, embedded in paraffin, and stained with picrosirius red. To quantify the collagen, we used the Leica QWin Software V 3.2.0 (Leica Imaging Systems Ltd., Cambridge, England) along with an optical microscope Leica DMR (Leica Microsystems Wetzlar GmbH, Wetzlar, Germany), a video camera (Leica DC300F, Leica Microsystems AG Heerbrugg, Switzerland), and an online computer. The values corresponding to the areas of fibrosis were obtained in relation to the total area of the left and right ventricles and septum, expressed as percentages. We evaluated 10 fields in the free left ventricular wall.

Statistical analysis

To calculate the sample size, we established a (two-tailed) confidence interval of 95% and test power of 90%, assuming a standard deviation of 5 and 3 percentage units of LVSF for ECHO and RV, respectively; these values were obtained from a pilot study in control animals. As a result, a sample size of 22 animals was deemed appropriate to detect a difference of 5 ejective units between the methods. The sample size was calculated with a tool available online at www.openepi.com.

The results are expressed as mean and standard deviation. The Gaussian distribution of the variables was assessed with the Kolmogorov-Smirnov test. For comparison between mean LVSF values evaluated by the methods, we used paired Student's *t* test. To compare the mean values

of the extension of the myocardial fibrosis between the control animals and the animals that received DXR, we used non-paired Student's *t* test. To analyze the correlation of the LVSF obtained by the imaging methods, we used the linear regression test and the least squares correlation. The Bland-Altman plot method was used for further analysis of the agreement between the LVSF measurements obtained by the two evaluated methods. Linear regression was also used to evaluate the correlation between the LVSF and the area of fibrosis.

All analyses were performed using the software GraphPad InStat, version 3.05, with a significance level of 5% ($p < 0.05$, two-tailed) for differences.

Results

Assessment of the left ventricular systolic function

Table 1 and Figure 3 summarize the results obtained.

The analysis of the LVSF in control animals showed values comparable to those obtained with RV and two-dimensional ECHO (82.8 ± 2.8% *versus* 83.5 ± 5%, $p > 0.05$).

A comparison between the control animals and the animals that received DXR indicated that both imaging methods showed lower LVSF values in the group receiving DXR ($p < 0.005$). These animals also showed a greater area of fibrosis when compared with the control animals (8.7 ± 3.2% *versus* 2.3 ± 1%, respectively, $p < 0.05$).

Additionally, the animals that received DXR exhibited significantly lower LVSF values assessed by RV (60.6 ± 12.5%) when compared with those obtained with ECHO (71.8 ± 10.1%, $p < 0.05$).

Analysis of correlation and agreement

Considering the entire study sample (including controls and animals exposed to DXR), individual LVSF measurements obtained by RV showed a significant and moderate positive correlation with those obtained by ECHO ($r = 0.72$, $p < 0.0001$) (Figure 4).

A Bland-Altman plot analysis of the agreement between measurements (Figure 5) showed a mean difference (RV - ECHO) of -7.6 ± 10.3%, with limits of agreement of -28.1% to 12.9%. Analyzing the dispersion plot, we observed a significant positive correlation ($r = 0.47$, $p = 0.01$) between the mean values of the measurements obtained by RV and ECHO plotted against the difference of these same measurements, indicating that the methods do not have a good agreement for different ranges of LVSF values. This result suggests that RV estimates

Table 1 – Summary of the mean and standard deviation results obtained from the evaluation of the left ventricular systolic function by echocardiography and radionuclide ventriculography in animals in the control group and animals that received doxorubicin

	ECHO (%)	RV (%)	Fibrosis (%)
DXR (n=22)	71.8 ± 10.1	60.6 ± 12.5	8.7 ± 3.2
Control (n=7)	83.5 ± 5	82.8 ± 2.8	2.3 ± 1
p	0.002	<0.0001	<0.0001

ECHO: echocardiography; RV: radionuclide ventriculography, DXR: doxorubicin.

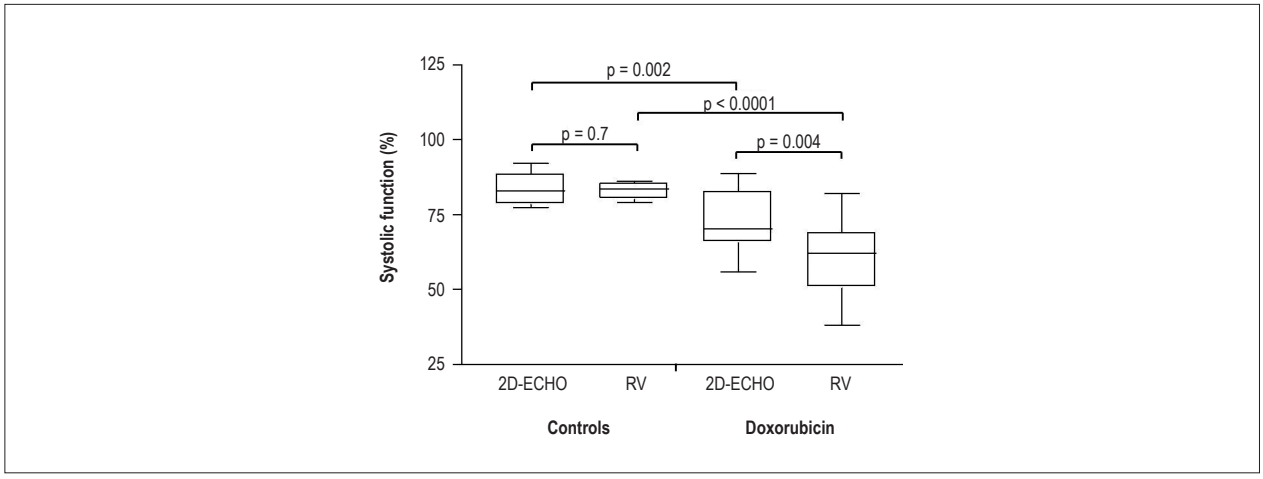

Figure 3 – *Graph showing the left ventricular systolic function obtained by two-dimensional echocardiography (2D-ECHO) and radionuclide ventriculography (RV) in animals in the control group and animals exposed to DXR.*

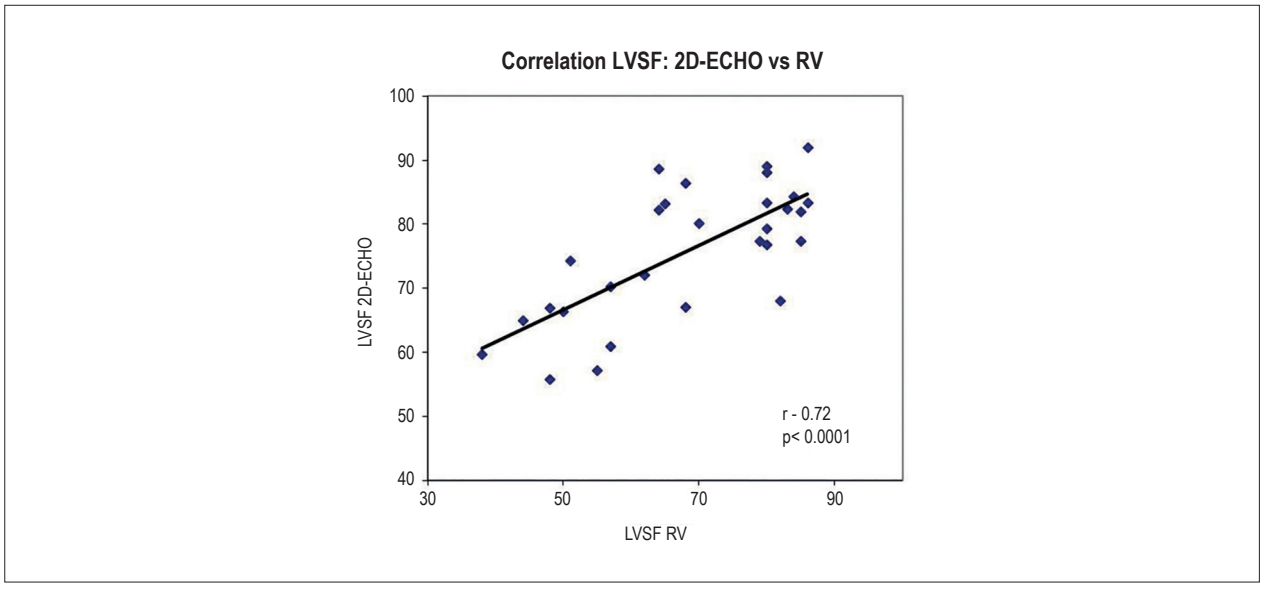

Figure 4 – *Graph correlating the left ventricular systolic function (LVSF) assessed by two-dimensional echocardiography (2D-ECHO) and radionuclide ventriculography (RV) in animals receiving different doses of doxorubicin (r = 0.72, p < 0.0001). (*Statistical test performed: linear regression analysis and Pearson's correlation coefficient).*

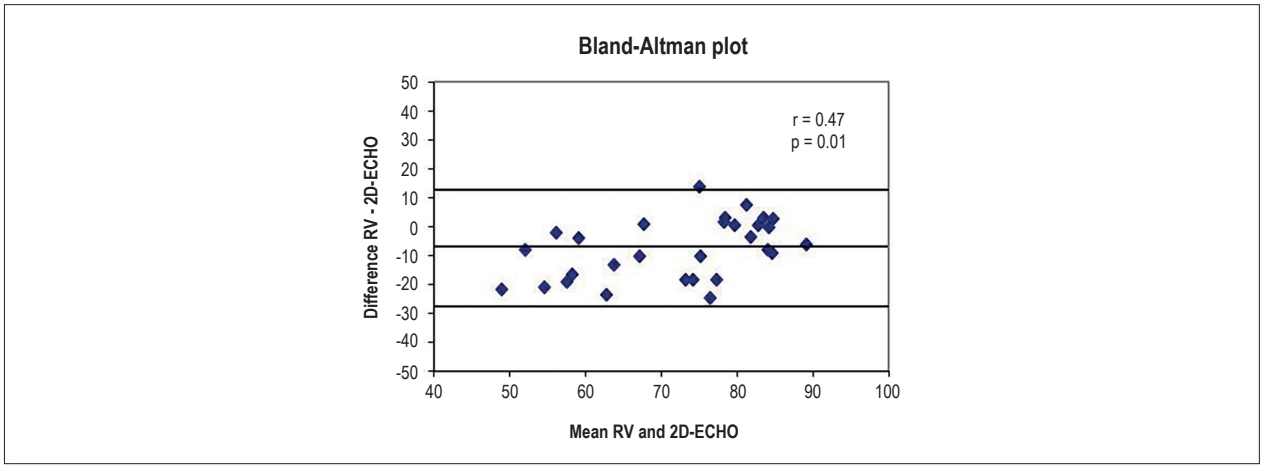

Figure 5 – *Bland-Altman plot indicating poor agreement between the imaging methods, showing lower values observed by radionuclide ventriculography (RV) in animals with decreased left ventricular systolic function.*

lower LVSF values than those estimated by ECHO in animals with a more compromised global systolic function.

Correlation between the methods of in vivo functional evaluation and histological analysis

After applying linear regression, we observed a moderate and significant negative correlation between the LVSF values measured by ECHO and the area of accumulated collagen on histology (r = -0.69, p = 0.0002). We found a stronger significant negative correlation between the LVSF assessed by RV and the area of accumulated collagen (r = -0.79, p < 0.0001) (Figure 6). Using multiple regression analysis, in which both imaging methods were included in the regression model, only the ventricular function measured by RV correlated independently with the percentage of accumulated collagen in the myocardium.

Discussion

In many experimental models of cardiac disease in small animals, the serial assessment of the LVSF is the most widely used parameter to follow the changes in myocardial function due to disease progression or the impact of pharmacological intervention.[4]

In the present study using *in vivo* imaging methods, we evaluated the LVSF in control animals and in animals

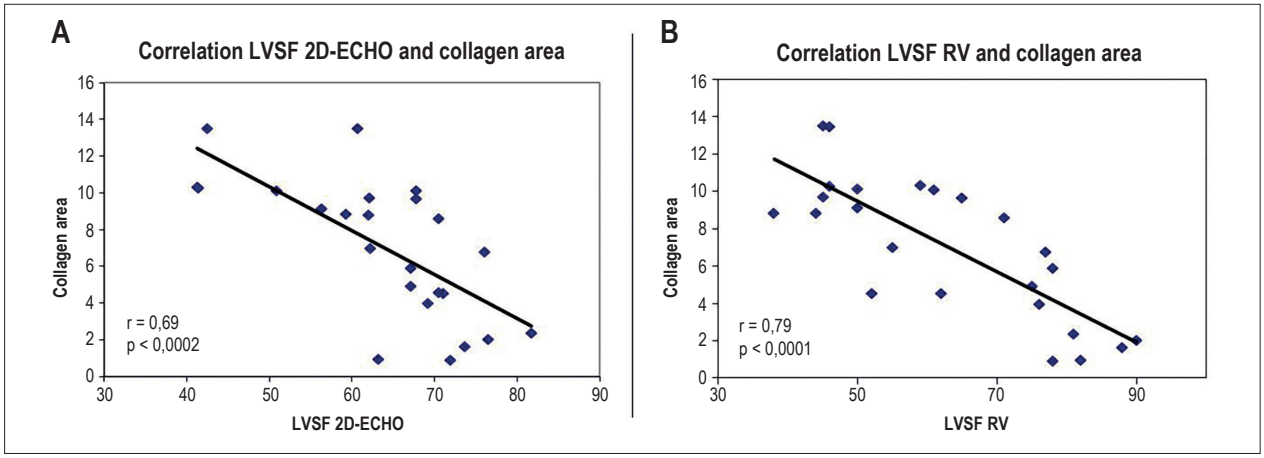

Figure 6 – *Graph correlating the mean left ventricular systolic function (LVSF) values evaluated by (A) two-dimensional echocardiography (2D-ECHO) and (B) radionuclide ventriculography (RV) and the percentage of accumulated collagen on histological analysis in animals receiving an infusion of 8, 12, and 16 mg/kg of doxorubicin.*

subjected to an experimental model of cardiotoxicity induced by DXR. This allowed us to compare two-dimensional ECHO and RV in the quantification of the LVSF in animals with different degrees of LVSF impairment, as well as to correlate these data with the quantification of the area of collagen tissue on histopathological analysis, which is considered a gold-standard method to assess the degree of myocardial injury in this cardiotoxicity model.

Our results showed that the LVSF values obtained by RV and ECHO were similar in control animals. However, in animals with severe ventricular dysfunction due to the cumulative infusion of DXR, the LVSF values obtained by RV were lower when compared with those obtained by ECHO.

Additionally, we observed a significant moderate Correlation between the LVSF results obtained by RV and ECHO. However, this type of analysis does not necessarily demonstrate the agreement between both methods.[21-23] The Bland-Altman plot analysis indicated that despite showing a significant correlation in the regression analysis, the measurements were not in agreement in regards to different ranges of LVSF values. This analysis shows that, in addition to very wide agreement limits, ECHO evaluates relatively higher LVSF levels than does RV in animals with a more depressed systolic function.

Overall, these results indicate a greater sensitivity of RV when compared with ECHO in detecting left ventricular systolic dysfunction in this experimental model.

This interpretation of the data is reinforced by the results of the agreement analysis between the LVSF values obtained by each method and the extension of the collagen accumulation, which is an index of the degree of myocardial injury in this model. For this analysis, the RV results reached a stronger correlation than that obtained with ECHO, with the RV emerging as the only method displaying an independent correlation in the multiple regression model. This set of data reinforces the impression that RV provides a more accurate assessment of the LVSF.

Although no experimental studies have compared both imaging methods, prior clinical studies are consistent with our results when they showed that although ECHO and RV have a good general Correlation for LVSF measurement, both methods only have a moderate degree of agreement.[24-26]

In a study in patients after acute myocardial infarction (AMI), Ray et al. (1995) demonstrated a mean difference of -8 ± 10% in the LVSF evaluation between RV and ECHO, with limits of agreement of -28% to +12%,[27] results that mirror to some degree those described in this study. Another study in patients after AMI has also shown that the LVSF was overestimated when evaluated by ECHO in comparison with RV, with wide limits of agreement.[28] Bellenger et al.,[29] while studying patients with stable HF, have shown a significant difference in LVSF evaluated by both methods. They also showed a moderate correlation (r = 0.44), but with wide limits of agreement between them, from -45% to 13%.

A low agreement between both methods of LVSF assessment has also been observed in patients with permanent pacemaker[30] and after cardiac transplantation,[31] studies in which ECHO overestimated the LVSF when compared with RV and cardiac magnetic resonance.

These differences between techniques, which have already been observed in the clinical setting, can be enhanced in the experimental scenario given the mechanical and geometrical differences of the myocardium in small animals. It should be noted that the contribution of the apical shortening in rodents can be different from that observed in humans, which would further compromise the geometric assumptions adopted in the ECHO for volume estimates.[32]

ECHO is the imaging method most widely used in large clinical trials and experimental studies, but it is dependent on geometric myocardial assumptions to estimate the LVSF. This becomes disadvantageous in several situations, as in the case of progressive dilation and consequent

geometric changes of the left ventricle in HF.[33] In this sense, biplane methods are considered more accurate than the M-mode method; however, they still extrapolate ventricular volumes measurements through geometric assumptions of the left ventricular cavity.[29] In experimental animal models, especially rodents, another limiting factor of two-dimensional ECHO in quantifying the ventricular function is the loss of image quality in apical windows, generating poor endocardium definition and impairing the measurement of the left ventricular cavity shortening.[34-36]

It is plausible to assume that this limitation in accurately estimating the LVSF in dilated ventricular cavities with a more spherical conformation, concomitant to a more severe systolic dysfunction, is the main explanation for the results obtained in the present study. It is worth mentioning that to estimate the left ventricular ejection fraction, the RV assessment is based on the variation of counts directly proportional to the volume of blood in the ventricular cavity, and is a method, therefore, that is not significantly influenced by changes in the left ventricular cavity shape and geometry. In contrast, values obtained with two-dimensional ECHO are based on planar measurements, which are highly dependent on the geometric shape of the left ventricular cavity. Due to that, their accuracy changes in situations with more severe ventricular dysfunction in which the left ventricle takes a more spherical shape, in addition to presenting other deformations in cavity shape. The advent of three-dimensional ECHO and its availability in new equipment dedicated to small animals will probably bring substantial accuracy improvement in estimating the LVSF by ECHO, as it allows a direct measurement of the volumes of the ventricular cavities.[34-36]

It is important to remember that RV has several technical limitations, such as low spatial resolution, need for background correction, structure overlap, attenuation errors, and need for manipulation of ionizing radiation.[37] In small rodents, limited spatial resolution is the greatest limitation of acquiring scintigraphic images with a gamma camera dedicated for clinical use. In fact, as the structures to be imaged in small rodents are 10 times smaller than the human organs, the spatial resolution of a conventional scintigraphic study (around 1.0 cm) should be proportionately increased to 1 mm. In our study, the use of a pinhole collimator with a 4-mm aperture was sufficient to considerably increase the spatial resolution of the image, with due visualization of the separation between the right and left ventricles (Figure 2) and maintenance of the 15-minute image acquisition, which is still suitable for an experimental study.

On the other hand, it is essential to emphasize that despite the high performance of RV in measuring the LVSF, this imaging method is more expensive and lacks information on other parameters of left ventricular remodeling such as diameter, wall thickness, and changes in the valvar apparatus. Therefore, we believe that RV, combined with ECHO, may become an additional tool for a thorough evaluation of models of cardiac diseases in small animals.

Study limitations

Some limitations of this study should be highlighted. For example, we were unable to quantify the left ventricular ejection fraction by ECHO using the biplane Simpson's method due to a limitation in the resolution of the endocardium in the animals' apical images. It is possible that the use of a high-resolution ECHO equipment and a 30-MHz transducer dedicated to obtaining and analyzing images in small rodents could have yielded more reliable results. Similarly, a three-dimensional ECHO might have allowed the measurement of ventricular volumes and provided a far more accurate LVSF assessment. It is also noteworthy that our study did not include a gold-standard *in vivo* imaging method to measure the LVSF, such as cardiac magnetic resonance imaging.

Conclusion

RV is an alternative method to evaluate the degree of left ventricular dysfunction *in vivo* in small rodents. When compared with ECHO, RV showed a better correlation with the degree of myocardial injury assessed by histopathology in a model of cardiotoxicity by DXR.

Our results suggest that although ECHO is a more available option, easy to use, and of low cost, RV may have in comparison a better performance, especially in sequential LVSF measurements in models of cardiac disease with changes in the left ventricular geometry.

Author contributions

Conception and design of the research: O'Connell JL, Pulici ECC, Romano MMD, Maciel BC, Simões MV; Acquisition of data: Oliveira LFL, O'Connell JL, Carvalho EEV, Pulici ECC, Romano MMD; Analysis and interpretation of the data: Oliveira LFL, O'Connell JL, Carvalho EEV, Pulici ECC, Simões MV; Statistical analysis: Oliveira LFL, Simões MV; Obtaining financing: Simões MV; Writing of the manuscript: Oliveira LFL, Carvalho EEV, Simões MV; Critical revision of the manuscript for intellectual content: Oliveira LFL, Romano MMD, Maciel BC, Simões MV.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by FAEPA.

Study Association

This article is part of the thesis of Doctoral submitted by João Lucas O'Connell, from Faculdade de Medicina de Ribeirão Preto.

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Speckle Tracking and Transthyretin Amyloid Cardiomyopathy

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Abstract

Background: Amyloidosis is a disease caused by deposits of insoluble fibrils in extracellular spaces. The most common type of familial amyloidosis is mediated by mutation of transthyretin, especially Val30Met. Symptoms and ejection fraction decrease may occur in cardiac amyloidosis only in case of poor prognosis. Myocardial strain detected by two-dimensional speckle tracking echocardiography can indicate changes in myocardial function at early stages of the disease.

Objective: To determine the accuracy of left ventricular longitudinal strain by two-dimensional speckle tracking echocardiography in patients with familial amyloidosis caused by Val30Met transthyretin mutation.

Methods: Eighteen consecutive patients, carriers of transthyretin mutation, were evaluated by two-dimensional speckle tracking echocardiography, by which myocardial strain curves were obtained, following the American Society of Echocardiography recommendations.

Results: Patients were divided into three groups: 1- Val30Met with cardiac amyloidosis; 2-Val30Met with extracardiac amyloidosis; 3 - Val30Met without evidence of disease. As the three groups were compared by the Mann-Whitney test, we found a statistically significant difference between groups 1 and 2 in the mean longitudinal tension (p=0.01), mean basal longitudinal strain (p=0.014); in mean longitudinal tension and mean longitudinal strain between groups 1 and 3 (p=0.005); and in the ratio of longitudinal strain of apical septum segment to longitudinal strain of basal septum (p=0.041) between groups 2 and 3.

Conclusion: Left ventricular longitudinal strain detected by two-dimensional speckle tracking echocardiography is able to diagnose left ventricular dysfunction in early stages of familial amyloidosis caused by transthyretin Val30Met mutation. (Arq Bras Cardiol. 2017; 108(1):21-30)

Keywords: Amyloidosis, Familial / complications; Cardiomyopathy, Restrictive / complications; Diagnostic Imaging, Echocardiography / methods; Prealbumin / analysis.

Introduction

Amyloidosis is a rare disease caused by deposits of proteins in the extracellular space of organs and tissues. The familial forms of the disease are commonly associated with mutations of genes related to proteins. The most frequent is transthyretin (TTR), a protein that is synthesized in the liver, choroid plexus and retina, and that acts in the transport of thyroxine (T4) and retinol-binding protein in the blood.[1] The TTR gene is located on chromosome 18q12.1.[2] The best described, most prevalent mutation is Val30Met (a methionine substitution for valine at position 30), which predominantly affects patients from Japan, Portugal, Sweden and Brazil.[3] Amyloidosis symptoms appear in the third to fifth decades of life, including progressive polyneuropathy, postural hypotension, and mild myocardial infiltration. The main clinical manifestations of cardiac amyloidosis (CA) are: restrictive heart disease, systolic dysfunction, postural hypotension and conduction disturbances. Rapezzi et al. reported a 98% survival after two years of TTR CA.[4]

Echocardiography is the cornerstone for evaluation of CA due to ease of image acquisition and interpretation, relative low cost, and capacity for unparalleled assessment of diastolic function and serial studies. The echocardiogram may show symmetrical thickening of left ventricular (LV) wall, hypokinesia, right ventricular free wall thickening, atrial septal thickening, valve thickening or valve failure, atrial dilatation and pericardial effusion[5] (Figure 1). Two dimensional (2D) speckle tracking echocardiography (STE) consists in the capture and tracking of speckles along the cardiac cycle, generating motion vectors and deformation curves. The method has been used to measure myocardial deformation and the percentage of deformation. LV global systolic function remains normal until the final stages of CA. However, in contrast to LV ejection fraction (LVEF) and shortening fraction, the global longitudinal strain may be altered in the first stages of the disease. Thus, new imaging techniques, as the STE, have been suggested for the evaluation of patients with CA.[6-14] The aim of our study was to determine the accuracy of LV longitudinal strain obtained

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Manuscript received March 15, 2016; revised manuscript September 08, 2016; accepted September 13, 2016.

DOI: 10.5935/abc.20160191

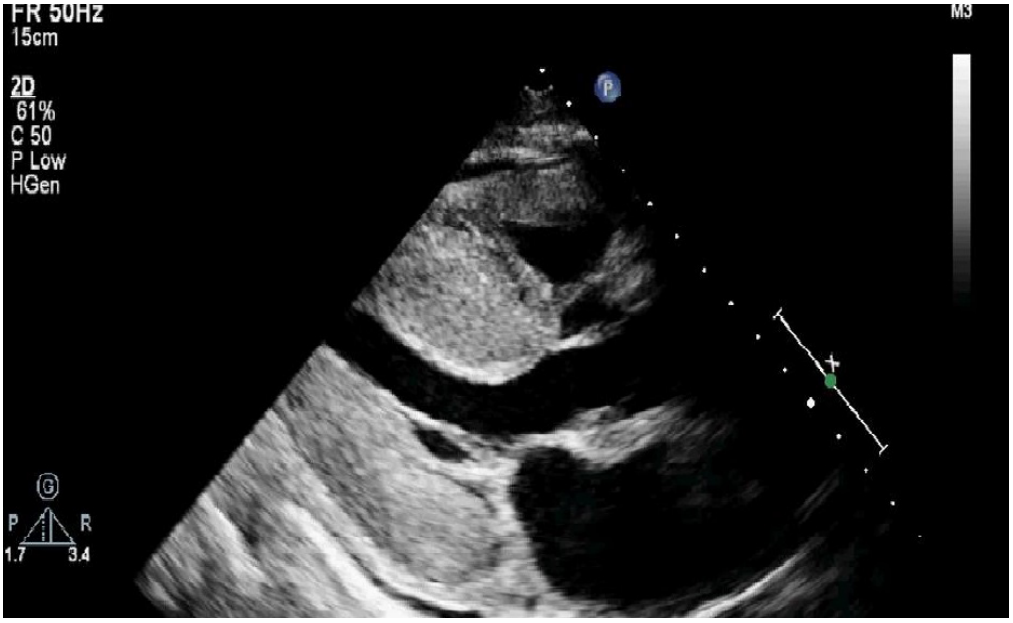


Figure 1 – *Echocardiography of cardiac amyloidosis with important left ventricular and right ventricular hypertrophy, increased thickness of mitral and aortic valves, and mild pericardial effusion.*

by two-dimensional STE in a group of patient with familial amyloidosis caused by the Val30Met mutation of TTR.

Methods

This is a cross-sectional, descriptive, comparative study on CA patients and genetic carriers of Val30Met mutation, and individuals without the mutation and without cardiovascular diseases as controls. Twenty-eight patients were selected and assessed from February 2014 to March 2015. Patients were divided into four groups – patients with familial CA with Val30Met mutation (n=6); patients with Val30Met mutation with diagnosis of familial extracardiac amyloidosis confirmed by biopsy (n=4); patients with Val30Met without amyloidosis (n=4); and a control group (n=14).

Patients were enrolled in the outpatient neurology service of Antonio Pedro University Hospital and in private offices of neurology run by professors of this hospital, and by contact to the Brazilian Association of Paramyloidosis by internet.

Sample calculation was based on the following assumptions:

In a similar study, Phelan et al.[15] selected 26 patients with TTR. We took this number as a reference for our sample calculation.

The expected margin of error is 5%, with 95% confidence error. The distribution of CA in this population is unknown; however, we extrapolated the value of 50% of primary amyloidosis (AL) distribution, and used it as reference for our sample calculation.

We used the online sample calculator available at http://www.raosoft.com/samplesize.html, and the sample size calculated was 26.

Inclusion criteria

Age > 18 years.

Agreement to participate and signature of the informed consent form

Patients carriers of TTR genetic mutation and/or with diagnosis of TTR amyloidosis (ATTR).

Exclusion criteria

1 - Poor quality of two-dimensional echocardiogram, established as the presence of artifacts or poor visualization of more than two cardiac segments.

2 - Tachycardia (heart rate over 100 bpm).

3 - Atrial fibrillation or other arrhythmias with variation in the R-R interval.

4 - Other causes of ventricular hypertrophy – systemic arterial hypertension (SAH), hypertrophic cardiomyopathy (HCM), aortic stenosis, Fabry disease.

Patients attended a medical visit, during which their demographic data were collected, and anamnesis and physical exam were conducted. Electrocardiography (ECG), conventional echocardiography (echo) and two-dimensional STE were performed.

The echocardiographic images were obtained using a Philips IE33 equipment (Philips Medical Systems, Bothel, Washington, USA), with a 5-1 MHz Sector S5-1 transducer. The quantification of cardiac chambers, hemodynamic measurements, tissue Doppler study and two-dimensional RTE were performed offline, using Q-Lab 5.0 (Advanced

Quantification Software – Philips Medical Systems, Bothell, WA, USA), a specific software for analysis of digital images, according to the protocols of the American Society of Echocardiography.[13,14,16,17]

Echocardiographic measurements were taken three times, and the mean of these values was recorded.

Numerical data were described by descriptive analysis in tables, and expressed as mean, standard deviation and median.

The Mann-Whitney test was used to assess differences in clinical variables of ECG, echo and two-dimensional STE between the paired groups. The normality of variable distribution was tested by the Shapiro-Wilk test. Significance level was set at 5%. Statistical analysis was processed with the SAS 6.11(SAS Institute, Inc., Cary, NC) software.

Results

Tables 1, 2, and 3 present numerical clinical variables in mean, standard deviation and median of numerical clinical variables of ECG, echo and two-dimensional STE according to the pairs of groups G1 x G2, G1 x G3, and G1 x G4, and corresponding descriptive level (p-value) of Mann-Whitney test.

Group 1 corresponds to patients with familial CA, according to the international criterion for the disease - mean LV wall thickness ≥ 12 mm, diastolic dysfunction ≥ stage 2, or global longitudinal strain lower than -18%. Group 2 corresponds to patients with familial extracardiac amyloidosis confirmed by biopsy. Group 3 corresponds to patients with TTR mutation with no evidence of CA. Group 4 is the control group (see Tables and Graphs 1, 2 and 3).

Discussion

As we compared the mean values of longitudinal strain per LV segments between G1 and G3, we found a statistically significant decrease in these values in the basal, anterolateral (p=0.019) and in the medial inferolateral segments in G1 (p=0.042). The same was observed in the basal inferoseptal (p=0.009), anteromedial (p=0.010), inferior medial (p=0.054), inferolateral medial (p=0.010) and apical septum (p=0.032) in G2, and in the basal inferoseptal (p=0.006), basal anterior (p=0.017), basal inferior (p=0.031), basal anterolateral (p=0.001), basal inferolateral (p=0.010), medial inferior (p=0.025) and apical anterior (p=0.013) in G4. It is of note that mean values of longitudinal strain were decreased in G1 not only as compared with G3, but also in absolute values (<-18%). On the other hand, longitudinal strain of apical segments, even when decreased in relation to other groups, was not decreased in absolute values.

The two-dimensional STE showed that apical segments were not affected by amyloidosis, ATTR or AL, which differs from the pattern of both HCM and aortic stenosis that do not spare the apex. Only apical strain was different between ATTR and AL, and significantly lower in ATTR patients. However, no difference was detected in global longitudinal strain or in the mean basal or medial longitudinal strain between the two types of amyloidosis[15] (see Figure 2).

Mean basal longitudinal strain was lower in G1 as compared with G3 (p=0.010), G2 (p=0.014) and G4 (p=0.0005). According to Baccouche et al. this parameter has a differential diagnostic value between AC and HCM.[18]

When we evaluated global longitudinal strain in G1, the mean longitudinal strain in the apical two-chamber, four-chamber and three-chamber views (longitudinal apical), as well as the mean longitudinal tension were significantly lower than in G2, G3 and G4 groups.

Serial analysis showed that systolic strain rate and strain at the basal and mid ventricle were significantly reduced in asymptomatic patients with increased LV wall thickness.[13] In addition, the longitudinal systolic rate was reduced in all 16 segments of left ventricle in CA patients, who did not have abnormal echo findings.[19]

There are few studies showing functional changes in patients with CA using two-dimensional STE. Sun et al. showed that global longitudinal strain detected by two-dimensional STE was significantly lower (12%) in CA patients as compared with healthy controls, and also compared with patients with LV hypertrophy caused by HCM or hypertensive disease.[20]

Liu et al. showed that the ejection fraction was preserved whereas longitudinal strain was notably reduced in both compensated and decompensated patients (New York Heart Association functional class > 2).[21]

Disturbances in electrical conduction are among the main clinical manifestations of CA, and may be present in up to one third of patients.[5] Patients in G1 had increased PR interval (mean of 0.230s ± 0.060), which was significantly different from G2 (p=0.015), G3 (p=0.044) and G4 (p=0.005). Sayed et al.[22] also showed an association between disturbances in electrical conduction and decreased longitudinal strain, and a negative prognostic value for this association, although the study was conducted with cardiac AL amyloidosis.

Left atrial diameter was significantly greater in the G1 group (4.0 cm ± 0.18) than in G3 (p = 0.009), G2 (p = 0.009) and G4 (p= 0.0005). We also found increased left atrial volume in G1 in the apical four-chamber view (p=0.028), biplane (p=0.026) and indexed left atrial volume in the apical four-chamber view (p=0.010) as compared with G4. Such difference may be a sign of increased pressure in the left ventricle due to restrictive diastolic function, leading to pressure overload in the left atrium.[23]

Mean values of LV end-systolic diameter and end-systolic volume were significantly lower in G1 (p=0.009 and p=0.008, respectively) and G2 (p=0.025 and p=0.025, respectively) as compared with G4. LV end-systolic volume was also significantly lower (p=0.043) in G2 than G4. G1 showed mean LV end-diastolic diameter and end-diastolic volume significantly lower than G3 (p=0.033 and p=0.033, respectively), and G4 (p=0.029 and p=0.028, respectively). CA progresses with mild decrease in LV cavity.[24,25]

Table 1 – Comparison of echocardiographic findings between group 1 (G1) and group 2 (G2)

Variable	G1: Cardiac amyloidosis					G2: Extracardiac amyloidosis					p value *
	n	mean	±	SD	med	n	mean	±	SD	med	
Left atrial diameter (cm)	6	4.00	±	0.18	4.00	4	3.09	±	0.42	3.20	0.009
LV end-systolic diameter (cm)	6	1.97	±	0.47	2.03	4	2.14	±	0.19	2.06	0.67
LV posterior wall (cm)	6	1.07	±	0.39	1.01	4	0.660	±	0.098	0.680	0.033
LV end-diastolic diameter (cm)	6	3.86	±	0.66	4.11	4	4.53	±	0.51	4.49	0.20
Interventricular septum (cm)	6	1.17	±	0.60	1.08	4	0.725	±	0.033	0.735	0.086
Mean wall thickness (cm)	6	1.12	±	0.49	1.03	4	0.690	±	0.067	0.705	0.055
Relative wall thickness	6	0.600	±	0.346	0.500	4	1.200	±	1.867	0.300	0.38
End-systolic volume -Teicholz (mL)	6	12.7	±	7.7	11.7	4	15.1	±	3.7	13.6	0.52
% shortening -Teicholz (%)	6	50.0	±	6.8	48.8	4	52.9	±	2.6	52.5	0.29
Ejection fraction - Teicholz (%)	6	81.5	±	6.5	81.1	4	84.0	±	1.9	83.6	0.39
Ejection fraction - Simpson bp (%)	6	77.2	±	12.9	80.5	4	73.3	±	3.7	73.8	0.29
LAV - apical 4 chambers (mL)	6	39.3	±	12.6	37.0	4	32.8	±	15.5	35.0	0.52
Indexed LAV - apical 4chambers (mlLm^2)	6	23.9	±	7.1	25.0	4	18.6	±	8.1	19.8	0.20
LAV - apical 2 chambers (mL)	6	37.2	±	12.6	38.0	4	22.3	±	7.4	22.0	0.088
Indexed LAV - apical 2chambers (ml/m^2)	6	23.1	±	9.5	23.2	4	13.2	±	5.3	12.6	0.055
LAV - biplane (ml)	6	38.7	±	10.7	41.5	4	26.4	±	7.2	27.0	0.088
Indexed LAV - biplane (mL/m^2)	6	23.7	±	7.3	26.3	4	15.4	±	3.9	16.4	0.055
Medial E/E' ratio	5	13.4	±	5.5	12.5	3	8.2	±	1.8	9.0	0.10
Lateral E/E' ratio	5	10.7	±	7.9	7.5	4	6.4	±	0.1	6.4	0.14
Right ventricular lateral E'-wave velocity on tissue Doppler (cm/s)	5	11.1	±	1.1	11.6	1	11.7				0.55
Mean basal longitudinal strain(%)	6	-11.6	±	3.1	-12.0	4	-19.9	±	3.9	-20.5	0.014
Apical longitudinal strain- 2 chambers (%)	6	-16.0	±	3.9	-15.5	4	-23.0	±	1.4	-23.5	0.023
Apical longitudinal strain - 4 chambers (%)	6	-17.0	±	0.9	-17.0	4	-22.5	±	3.1	-22.5	0.009
Apical longitudinal strain.- apical longitudinal. (%)	6	-16.7	±	1.4	-16.5	4	-22.0	±	3.5	-23.0	0.030
Mean longitudinal tension - mean (%)	6	-16.8	±	1.8	-16.0	4	-22.3	±	1.3	-22.0	0.009

LV: left ventricular; LAV: left atrial volume; E/E' ratio: ratio between atrial flow E wave on Doppler and E' wave on tissue Doppler; (*) Mann-Whitney test; values in mean ± standard deviation (SD) and median (med).

Variables related to hypertrophy caused by amyloid deposit were statistically different between G1 and G3. Mean diastolic thickness of the interventricular septum in G1 was increased (1.17 ± 0.60 cm), and statistically greater than G3 (p=0.041) and G4 (p=0.010). The same was observed with LV posterior wall end-diastolic thickness, with mean of 1.07 ± 0.39 cm in G1, which was significantly different as compared with G3 (p=0.010), G2 (p=0.033) and G4 (0.008). Mean values of interventricular septum was greater in G1 (1.17±0.60 cm) than in G3 (p=0.041) and G4 (p=0.010). Mean wall thickness was significantly greater in G1 than in G3 (p=0.024) and G4 (p=0.011). Mean relative wall thickness was also increased in G1 (0.600 ± 0.346) compared with G3 (p=0.026) and G4 (p=0.005). Our study shows a relationship between LV wall hypertrophy and severity of disease. Mean wall hypertrophy greater than 15mm has been shown as an independent negative prognostic factor.[26]

Table 2 – Comparison of echocardiographic findings between group 1 (G1) and group 3 (G3)

Variable	G1: Cardiac amyloidosis					G3: TTR mutation without the disease					p value *
	n	mean	±	SD	med	n	mean	±	SD	med	
Left atrial diameter (cm)	6	4.00	±	0.18	4.00	4	3.15	±	0.35	3.15	0.009
LV end-systolic diameter (cm)	6	1.97	±	0.47	2.03	4	2.45	±	0.22	2.42	0.055
LV posterior wall (cm)	6	1.07	±	0.39	1.01	4	0.618	±	0.026	0.620	0.010
LV end-diastolic diameter (cm)	6	3.86	±	0.66	4.11	4	4.64	±	0.26	4.60	0.033
Interventricular septum (cm)	6	1.17	±	0.60	1.08	4	0.628	±	0.072	0.620	0.041
Mean wall thickness (cm)	6	1.12	±	0.49	1.03	4	0.623	±	0.048	0.620	0.024
Relative wall thickness	6	0.600	±	0.346	0.500	4	0.250	±	0.058	0.250	0.026
End-systolic volume -Teicholz (mL)	6	12.7	±	7.7	11.7	4	22.8	±	3.9	23.4	0.055
% shortening -Teicholz (%)	6	50.0	±	6.8	48.8	4	45.9	±	4.1	45.8	0.45
Ejection fraction - Teicholz (%)	6	81.5	±	6.5	81.1	4	77.1	±	4.3	77.0	0.39
Ejection fraction - Simpson bp (%)	6	77.2	±	12.9	80.5	4	74.7	±	7.9	75.2	0.45
LAV - apical 4 chambers (mL)	6	39.3	±	12.6	37.0	4	29.8	±	4.4	30.0	0.11
Indexed LAV - apical 4chambers (mlLm^2)	6	23.9	±	7.1	25.0	4	17.4	±	2.3	16.9	0.088
LAV - apical 2 chambers (mL)	6	37.2	±	12.6	38.0	4	26.0	±	3.5	27.0	0.20
Indexed LAV - apical 2chambers (ml/m^2)	6	23.1	±	9.5	23.2	4	15.0	±	1.1	15.4	0.087
LAV - biplane (ml)	6	38.7	±	10.7	41.5	4	27.1	±	4.2	26.7	0.14
Indexed LAV - biplane (mL/m^2)	6	23.7	±	7.3	26.3	4	15.9	±	2.6	15.6	0.088
Medial E/E' ratio	5	13.4	±	5.5	12.5	4	8.1	±	1.9	8.6	0.086
Lateral E/E' ratio	5	10.7	±	7.9	7.5	4	5.0	±	0.3	5.0	0.14
Right ventricular lateral E'-wave velocity on tissue Doppler (cm/s)	5	11.1	±	1.1	11.6	4	13.1	±	1.2	13.2	0.036
Mean basal longitudinal strain(%)	6	-11.6	±	3.1	-12.0	4	-20.6	±	2.6	-20.7	0.010
Apical longitudinal strain- 2 chambers (%)	6	-16.0	±	3.9	-15.5	4	-22.0	±	1.8	-22.0	0.041
Apical longitudinal strain - 4 chambers (%)	6	-17.0	±	0.9	-17.0	4	-20.5	±	2.5	-20.0	0.016
Apical longitudinal strain.- apical longitudinal. (%)	6	-16.7	±	1.4	-16.5	4	-19.5	±	2.9	-19.5	0.10
Mean longitudinal tension - mean (%)	6	-16.8	±	1.8	-16.0	4	-20.5	±	0.6	-20.5	0.016

TTR: transthyretin LV: left ventricular; LAV: left atrial volume; E/E' ratio: ratio between atrial flow E wave on Doppler and E' wave on tissue Doppler; (*)Mann-Whitney test; values in mean ± standard deviation (SD) and median (med).

Amyloid deposits cause restrictive heart disease. The pattern of echocardiographic parameters of diastolic dysfunction classification tend to worsen with the disease progression.[23] Patients of G2 also showed increased mean lateral E/E' ratio compared with G3 (p=0.020). Lateral E'-wave velocity on tissue Doppler was also decreased in G2 as compared with G3 (p=0.021). Medial and lateral E/E' ratio in G1 were increased (p=0.005 and p=0.033, respectively) compared with G4.

Tricuspid annular S'-wave velocity on tissue Doppler is an index of right ventricular systolic function, with normal values greater than 10 cm/s. Its mean values in G1 was significantly different from G3 (p=0.036) and G4 (p=0003). Capelli et al.[27] showed statistically significant difference in tricuspid annulus tissue Doppler analysis between patients with CA, patients with extracardiac amyloidosis and healthy controls, and reported a negative prognostic value for right ventricular systolic dysfunction.

Table 3 – Comparison of echocardiographic findings between group 1 (G1) and group 4 (G4)

Variable	G1: Cardiac amyloidosis					G4: control					p value *
	n	mean	±	SD	med	n	mean	±	SD	med	
Left atrial diameter (cm)	6	4.00	±	0.18	4.00	14	3.06	±	0.33	3.10	0.0005
LV end-systolic diameter (cm)	6	1.97	±	0.47	2.03	14	2.65	±	0.39	2.65	0.009
LV posterior wall (cm)	6	1.07	±	0.39	1.01	14	0.681	±	0.104	0.695	0.008
LV end-diastolic diameter (cm)	6	3.86	±	0.66	4.11	14	4.75	±	0.64	4.71	0.029
Interventricular septum (cm)	6	1.17	±	0.60	1.08	14	0.671	±	0.103	0.675	0.010
Mean wall thickness (cm)	6	1.12	±	0.49	1.03	14	0.674	±	0.080	0.653	0.011
Relative wall thickness	6	0.600	±	0.346	0.500	14	0.293	±	0.047	0.300	0.005
End-systolic volume -Teicholz (mL)	6	12.7	±	7.7	11.7	14	26.7		9.8	25.8	0.008
% shortening -Teicholz (%)	6	50.0	±	6.8	48.8	14	44.2		3.8	44.1	0.069
Ejection fraction - Teicholz (%)	6	81.5	±	6.5	81.1	14	75.2		4.2	75.4	0.032
Ejection fraction - Simpson bp (%)	6	77.2	±	12.9	80.5	14	71.7		6.2	70.5	0.14
LAV - apical 4 chambers (mL)	6	39.3	±	12.6	37.0	14	26.4		9.4	28.5	0.028
Indexed LAV - apical 4chambers ($mlLm^2$)	6	23.9	±	7.1	25.0	14	14.7		5.2	14.5	0.010
LAV - apical 2 chambers (mL)	6	37.2	±	12.6	38.0	14	29.1		12.9	26.0	0.11
Indexed LAV - apical 2chambers (ml/m^2)	6	23.1	±	9.5	23.2	14	15.6		5.2	13.3	0.083
LAV - biplane (ml)	6	38.7	±	10.7	41.5	14	28.3		9.4	28.0	0.063
Indexed LAV - biplane (mL/m^2)	6	23.7	±	7.3	26.3	14	15.3		4.1	16.1	0.026
Medial E/E' ratio	5	13.4	±	5.5	12.5	14	6.9		1.7	6.6	0.005
Lateral E/E' ratio	5	10.7	±	7.9	7.5	14	4.9		0.9	4.6	0.033
Right ventricular lateral E'-wave velocity on tissue Doppler (cm/s)	5	11.1	±	1.1	11.6	12	14.2		2.6	13.1	0.003
Mean basal longitudinal strain(%)	6	-11.6	±	3.1	-12.0	14	-21.2	±	3.2	-21.0	0.0005
Apical longitudinal strain- 2 chambers (%)	6	-16.0	±	3.9	-15.5	14	-19.2	±	3.3	-17.5	0.015
Apical longitudinal strain - 4 chambers (%)	6	-17.0	±	0.9	-17.0	14	-19.0	±	2.2	-19.5	0.054
Apical longitudinal strain.- apical longitudinal. (%)	6	-16.7	±	1.4	-16.5	14	-17.7	±	2.3	-18.0	0.26
Mean longitudinal tension - mean (%)	6	-16.8	±	1.8	-16.0	14	-18.6	±	2.3	-18.0	0.077

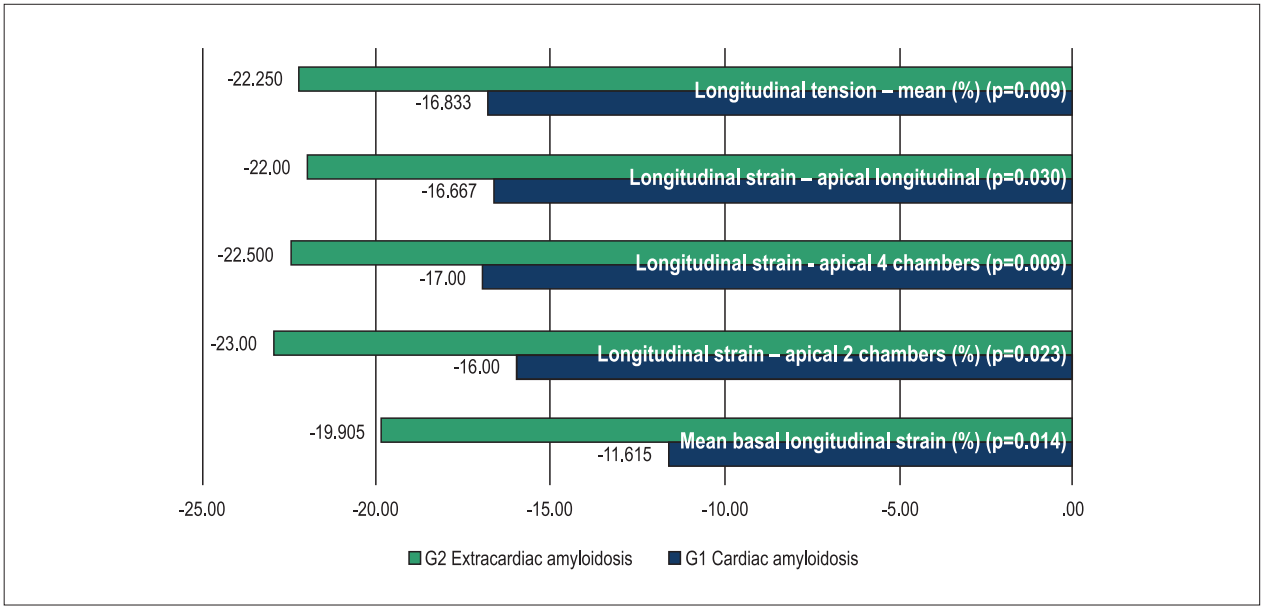
LV: left ventricular; LAV: left atrial volume; E/E' ratio: ratio between atrial flow E wave on Doppler and E' wave on tissue Doppler; (*)Mann-Whitney test; values in mean ± standard deviation (SD) and median (med).

Conclusions

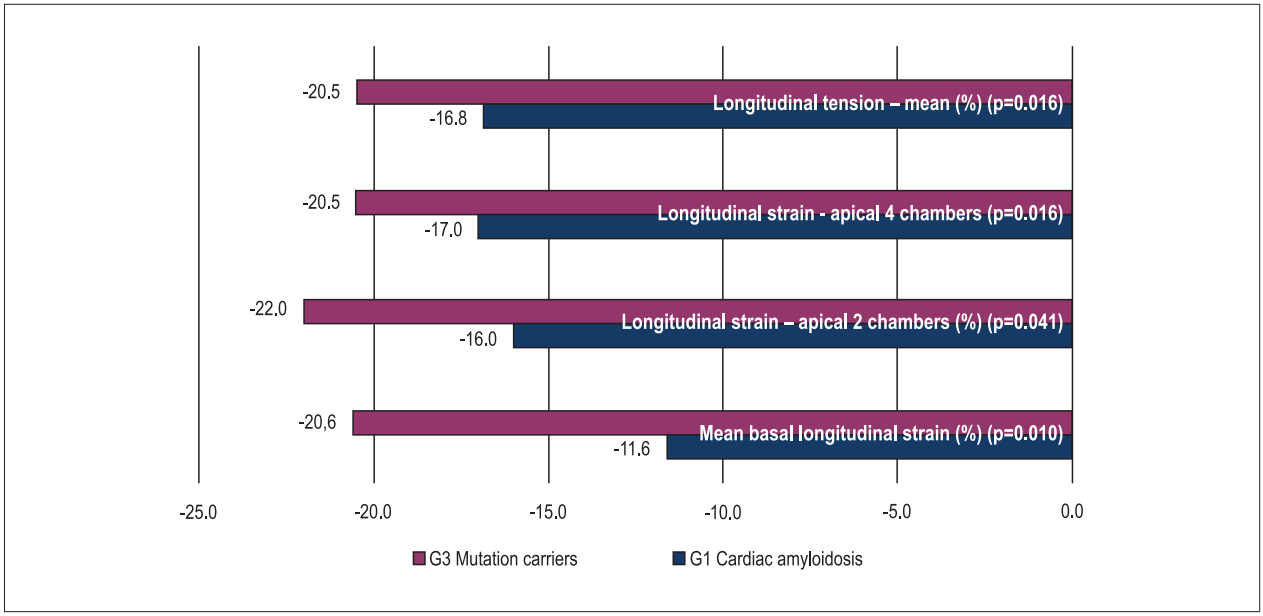
Two-dimensional STE increased the sensitivity of echo in diagnosing CA caused by TTR Val30Met mutation, since the adoption of a global longitudinal strain < -18% criterion increased the number of diagnosed patients from two (diagnosed by echo) to six patients.

Acknowledgements

We would like to thank Professor Claudio Tinoco Mesquita for his excellent guidance; our colleague Suzane Garcia Ferreira for the immeasurable collaboration; Professor Marcos Raimundo Gomes de Freitas for the trust placed on us in referring their patients to us and for



Graph 1 – *Comparison of longitudinal strain between group 1 (G1) and group 2 (G2)*



Graph 2 – Comparison of longitudinal strain between group 1 (G1) and group 3 (G3)

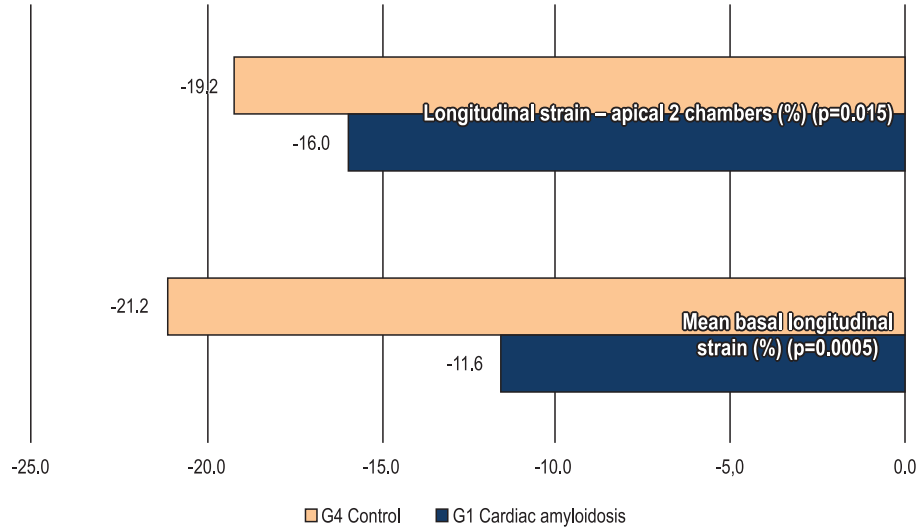
the careful review of our study; to Professor Mario Luiz Ribeiro for the guidance in our study and for teaching us echocardiography and to Professor Marcelo Souto Nacif for sharing with us his vast experience in research.

Author contributions

Conception and design of the research: Rocha AM, Nacif MS, Ribeiro ML, Mesquita CT; Acquisition of data: Rocha AM, Ferreira SG, Nacif MS, Ribeiro ML, Freitas MRG; Analysis and interpretation of the data: Rocha AM, Freitas MRG; Statistical analysis: Rocha AM, Mesquita CT; Obtaining financing: Mesquita CT; Writing of the manuscript: Rocha AM, Ribeiro ML, Mesquita CT; Critical revision of the manuscript for intellectual content: Ferreira SG, Freitas MRG, Mesquita CT.

Potential Conflict of Interest

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



Graph 3 – *Comparison of longitudinal strain between group 1 (G1) and group 4 (G4)*

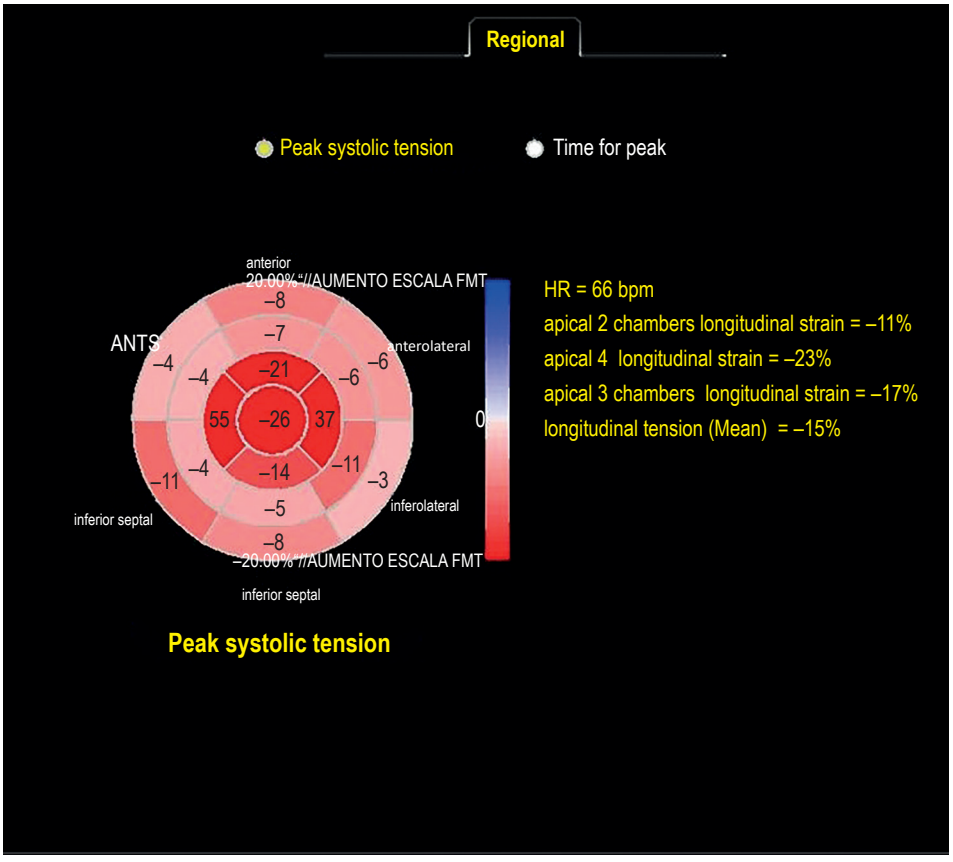


Figure 2 – *Bull's-Eye graph of cardiac amyloidosis patient. Apical longitudinal strain is preserved and there is important decrease in basal and medial segments.*

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of master submitted by Alexandre Marins Rocha, from Universidade Federal Fluminense.

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Original Article

Impact of High Risk for Obstructive Sleep Apnea on Survival after Acute Coronary Syndrome: Insights from the ERICO Registry

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Abstract

Background: Obstructive sleep apnea (OSA) is a very often clinical condition that can be associated with high mortality risk, particularly in coronary heart disease (CHD). The diagnosis of OSA is not always accessible via the gold-standard method polysomnography.

Objective: To evaluate long-term influence of the high risk for OSA on fatal and non-fatal outcomes after acute coronary syndrome (ACS) in the Acute Coronary Syndrome Registry Strategy (ERICO) Study using the Berlin questionnaire as a surrogate.

Methods: Berlin questionnaire, a screening questionnaire for OSA, was applied in 639 cases of ACS 30 days after the index event. Cox regression proportional-hazards model was used to calculate the hazard ratio (HR) of all-cause, cardiovascular and CHD (myocardial infarction) mortality, as well as, the combined endpoint of fatal or recurrent non-fatal CHD.

Results: The high-risk group for OSA had higher frequencies of previous personal/family history of CHD and diabetes, in addition to a poorer event-free survival, as compared to the low-risk group (p-log-rank=0.03). The HR for fatal or recurrent non-fatal CHD was 4.26 (95% confidence interval, 1.18 – 15.36) in patients at high risk for OSA compared to those at low risk for OSA after a 2.6-year mean follow-up.

Conclusions: Using Berlin questionnaire, we were able to identify high risk for OSA as an independent predictor of non-fatal reinfarction or CHD mortality in post-ACS individuals in a long-term follow-up. (Arq Bras Cardiol. 2017; 108(1):31-37)

Keywords: Acute Coronary Syndrome; Prognosis; Myocardial Infarction; Survivorship (Public Health); Risk Factors; Sleep Apnea, Obstructive.

Introduction

In addition to the classical cardiovascular risk factors, new risk factors associated with cardiovascular disease (CVD) have been detected in recent years. A promising candidate is obstructive sleep apnea (OSA), a common clinical condition characterized by partial or complete upper airway obstruction during sleep. These obstructive events elicit a series of mechanical, hemodynamic, chemical, neural, and inflammatory responses, with adverse consequences for the cardiovascular system. A recent meta-analysis of prospective cohort studies suggests that severe OSA significantly increases the risk of coronary heart disease (CHD), stroke, and all-cause mortality.[1] Moreover, subclinical atherosclerosis has been associated with OSA in many reports.[2-5] OSA may also affect the prognosis of patients with CHD. Some previous studies have shown an association of OSA with a poor long-term prognosis after percutaneous coronary intervention[6,7] and ST-elevation myocardial infarction (STEMI).[8] This was not confirmed in another study that evaluated acute coronary syndrome (ACS) in a short follow-up of 6 months.[9]

Polysomnography is the gold-standard test for diagnosis of OSA.[10] However, its use in large epidemiological studies is limited by its high cost. As a surrogate for polysomnography, several authors have attempted to develop screening questionnaires to detect individuals at high risk for OSA. The Berlin questionnaire, one of the available questionnaires for OSA diagnosis, has been used in other countries and in several studies in Brazil.[11-16] However, no previous study has evaluated OSA assessed by Berlin questionnaire in a sample of ACS with a long-term follow-up.

We aimed to evaluate the Berlin questionnaire, a screening tool of OSA, as a predictor of long-term survival measured in the Acute Coronary Syndrome Registry Strategy (ERICO Study).

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Manuscript received April 13,2016; reviewed August 17,2016; accepted September 13,2016.

DOI: 10.5935/abc.20160195

Methods

Design and population study

The ERICO Study is an ongoing prospective cohort study that enrolled all consecutive cases of ACS at the hospital affiliated to the University of São Paulo (HU-USP), an academic and teaching hospital situated in the district of Butantan, in the western region of the city. The design and baseline data of the ERICO Study are described in detail elsewhere.[17,18]

Individuals with ACS are treated in the emergency department, the internal medicine ward, or in a general intensive care unit. Patients requiring an interventional procedure are mostly referred to the Instituto do Coração of Hospital das Clínicas. The protocol of the study was approved by the local Institutional Review Board addressing research in human participants. All participants provided written informed consents.

All individuals with suspected ACS were invited to take part in the main study. The clinical interview included questions about education attainment (no formal education, elementary, high-school, or college), marital status (single, married, divorced or widowed), race (White, Mixed Race, Black or Asian), main cardiovascular risk factors, such as self-reported hypertension, diabetes, dyslipidemia, obesity, smoking (never, past or current), family and personal history of CHD, and physical inactivity. Further details about ACS definition are elsewhere.[17,18]

Additional data were obtained on cardiovascular risk stratification, such as urgent or scheduled percutaneous transluminal coronary angioplasty (PTCA) and/or coronary artery bypass graft surgery (CABG), echocardiogram findings and information about medications taken. Three physicians were responsible for reviewing all the medical charts, asking the participants for necessary information on hospital admission, and requesting electrocardiograms, laboratory tests (troponin I, MB-creatine kinase, serum glucose, total cholesterol, HDL/LDL-cholesterol, triglycerides and total blood cell count), and for the in-hospital medical treatment.

Six months and each year after the index event, all participants were contacted by telephone to update the information about their vital status, cardiovascular history, use of medications, depressive symptoms, and physical activity.

OSA definition

Berlin questionnaire was applied to all participants by trained interviewers, 30 days, 180 days, and one-year after ACS. The Berlin questionnaire includes 10 items divided into categories I (5 questions), II (3 questions) and III (2 questions). Two positive answers to questions 1 to 5 define category I as positive, and 2 positive answers to questions 6 to 8 define category II as positive. Category III is fulfilled if the subject presents hypertension or a body-mass index (BMI) ≥ 30 kg/m^2. The subject will be classified as having a high risk for OSA if at least two categories are positive.[19] The sensitivity and the specificity of the Berlin questionnaire for CHD were 70% and 48%, respectively.[19] Some studies in Brazil have similar results.[11,16]

Outcomes

We analyzed data from mortality [fatal endpoints: due to all causes, CVD and CHD] and a combined endpoint (fatal or recurrent non-fatal CHD). Each identified event was adjudicated using predefined international criteria.[20,21] Participants were defined as having death from a cardiovascular cause (CVD mortality) if we identified a cause of death classified in the 10th version of the International Classification of Diseases (ICD-10) chapter IX "Diseases of the circulatory system" or if we identified a cause of death classified as ICD-10 code R57.0 "Cardiogenic shock".[22]

Vital status was investigated periodically by a hot-pursuit strategy during the follow-up. Mortality information was confirmed by official death certificates with the collaboration of the municipal and State's health offices (*Programa de Aprimoramento das Informações de Mortalidade no Município de São Paulo, PRO-AIM,* and *Fundação Sistema Estadual de Análise de Dados-SEADE*, respectively).

Statistical analysis

Baseline characteristics were analyzed according to OSA risk (low and high). Categorical variables were expressed as proportions (%) and compared using the Chi-square or Fisher's exact tests, as indicated. We tested the probability of distribution of continuous variables by the normality test of Kolmogorov-Smirnov. Once continuous variables were parametric, all were expressed in mean (± standard deviation) and compared by OSA risk groups using Student *t*-test. We performed survival analyses (mean follow-up 2.6 years), considering the following endpoints: fatal (all-cause mortality, CVD mortality, CHD mortality), combined endpoint (recurrent non-fatal or fatal CHD) using Kaplan-Meier analysis with the log-rank test. Cox proportional hazard models for fatal and non-fatal outcomes were built and presented as crude, age-sex adjusted and after multivariate adjustment for age, sex, family history of CHD, previous history of ACS, diabetes (yes or no), dyslipidemia (yes or no), smoking (never, past or current), sedentary lifestyle (yes or no), type of ACS (angina, NSTEMI and STEMI myocardial infarction) and ejection fraction (%) on admission. We did not adjust for the presence of hypertension and obesity because the Berlin questionnaire includes these two risk factors as part of the classification criteria. All tests were two-sided, and $p<0.05$ was considered significant. The statistical analysis was carried out using the SPSS software, version 22.0.

Results

We included in the present analysis 639 (95.9%) participants with complete information on Berlin questionnaire 30 days after the index event. Individuals detected as having a high risk for OSA according to the Berlin questionnaire were mostly men (55.9%) compared to women (44.1%), $p=0.02$. In addition, individuals at high risk had higher BMI levels as compared to those at low risk (28.0 versus 25.9 kg/m^2, $p<0.001$). We detected

higher frequencies of previous history of CHD, obesity, hypertension, diabetes, and sedentary lifestyle in individuals at higher risk for OSA as compared to individuals at low risk. The Kaplan-Meier survival curves were not statistically different comparing individuals classified as at high and low risk for OSA regarding all-cause and CVD mortality or fatal CHD outcomes. However, when we analyzed the composite endpoint (recurrent non-fatal or fatal CHD), the high-risk group for OSA had lower event-free survival than the low-risk group after a mean follow-up of 2.6 years (p-log rank=0.03) (Figure 1). Cox regression analyses confirmed these findings (Table 1).

Multivariate adjusted hazard ratios (HR) for high-risk group compared to low-risk group of OSA were calculated for all-cause mortality [HR,1.29; 95% confidence interval (95% CI), 0.64-2.61]; CVD mortality (HR, 1.65; 95% CI, 0.63-4.38), CHD mortality (HR, 2.85; 95% CI, 0.54-15.12) and the composite endpoint (HR, 4.26; 95% CI, 1.18-15.36) (Table 2).

Discussion

Using the Berlin questionnaire as a surrogate, our results showed a positive association of high-risk for OSA with the composite endpoint including recurrent non-fatal or fatal CHD in patients with a mean follow-up of 2.6 years. The HR of death due to CHD or reinfarction was four times higher among individuals at high-risk as compared to low-risk for OSA. High-risk for OSA measured by Berlin questionnaire was not significantly associated with all-cause mortality, CVD mortality and death due to CHD. A recent meta-analysis by Wang et al.,[1] including 12 prospective cohort studies in which OSA was diagnosed by polysomnography, showed an association of severe OSA with significantly increased cardiovascular risk, stroke and all-cause mortality. Most of the studies that evaluate OSA as a prognostic factor for cardiovascular events studied specific subsamples of acute and chronic coronary syndrome,[5,9] STEMI,[8] unstable angina or CABG,[6,7,23] and used polysomnography to measure OSA with positive results. However, other studies using simple questions[24] or specific questionnaires to measure OSA[12,14] have also found positive associations.[25] Although some studies in Brazil used the Berlin questionnaire to evaluate the relationship between OSA and other endpoints,[11-13] only two evaluated the association of OSA defined by the Berlin questionnaire with cardiovascular events.[14,15] In a prospective cohort study of 200 individuals with ACS, Jesus et al.[14] evaluated the association of OSA with cardiovascular events using a composite endpoint of cardiovascular death, recurrent CHD events, acute pulmonary edema or stroke. In the multivariate logistic model, a positive association between high risk for OSA and the composite endpoint was reported (OR, 3.66; 95% CI, 1.22-11.0).[14] Our study has several similarities with that by Jesus et al. Both studies

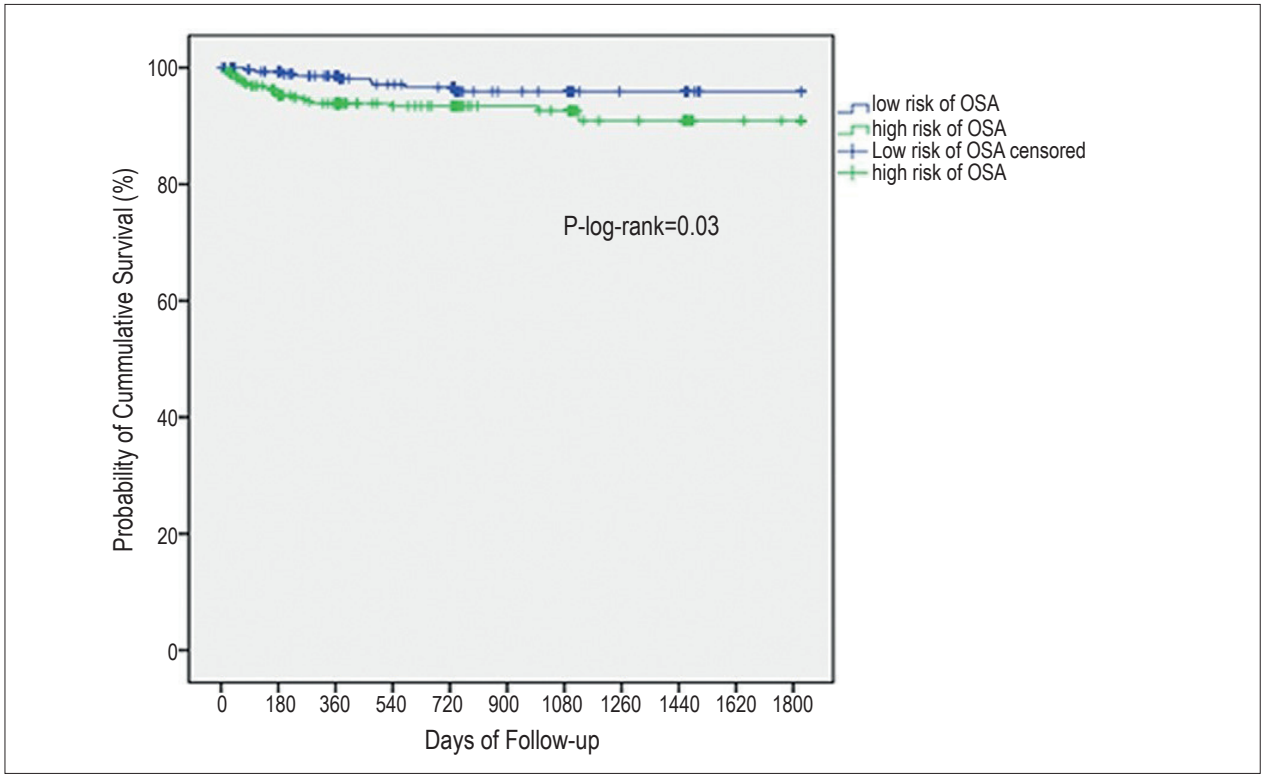

Figure 1 – *Obstructive sleep apnea as predictor of long-term survival measured by Berlin questionnaire in the ERICO study participants during a 2.6-year mean follow-up.*

Table 1 – General characteristics of the ERICO study participants according to the presence of low- and high-risk for obstructive sleep apnea (OSA) 30 days after acute coronary syndrome

Characteristics	Risk for OSA		p value
	Low n = 310	High n = 329	
Men (%)	201 (64.8)	184 (55.9)	0.02
Mean age (years) (± SD)	62.1 (13.1)	63.1 (12.2)	0.31
Body mass index (kg/m^2) (± SD)	25.9 (4.2)	28.0 (5.1)	<0.0001
Marital status (%)			0.50
Single	44 (14.2)	35 (10.7)	
Married	189 (61.2)	210 (64)	
Divorced	26 (8.4)	24 (7.3)	
Widowed	50 (16.2)	59 (18)	
Education (%)			0.23
No formal education	35 (11.3)	42 (12.8)	
Elementary	183 (59)	198 (60.2)	
High-school	56 (18.1)	66 (20.1)	
College	36 (11.6)	23 (7.0)	
Previous history of coronary heart disease (%)	61 (20.5)	101 (31.9)	0.001
Family history of coronary heart dis-ease (%)	71 (29.2)	102 (39.2)	0.02
Obesity (%)	41 (13.4)	113 (34.8)	<0.0001
Hypertension (%)	182 (59.9)	300 (92)	<0.0001
Diabetes (%)	100 (32.9)	131 (40.7)	0.04
Dyslipidemia (%)	135 (48.7)	168 (56)	0.08
Smoking (%)			0.29
Current	102 (33.2)	89 (27.5)	
Past	119 (38.8)	135 (41.7)	
Never	86 (28)	100 (30.9)	
Sedentary lifestyle (%)	201 (67.2)	240 (75.5)	0.02
Type of acute coronary syndrome (%)			<0.0001
Angina	74 (23.9)	112 (34.0)	
Non-ST myocardial infarction	127 (41.0)	148 (45.0)	
ST myocardial infarction	109 (35.2)	69 (21.)	
Mean ejection fraction (%) (± SD)	55.8 (13.1)	56.2 (13.2)	0.79

p-values were derived from Chi-Square for categorical variables or Student t-test for continuous variables. SD: standard deviation.

showed a positive association of OSA in a sample of ACS patients using composite endpoints of mortality and morbidity - though not exactly the same - and the same strategy for multivariate adjustment. However, one very important point is that in the study by Jesus et al.,[14] follow-up was restricted to the period of hospitalization in contrast to the 2.6-year mean follow-up of our analysis. In addition, there are differences in the structure of the two hospitals where the studies were conducted. The study performed by Jesus et al.[14] was conducted in a reference hospital with a proper hemodynamic facility, while ours was conducted in a general hospital that provides healthcare for people living in the Butantan borough, using the Instituto do Coração (INCor) as the reference center for cardiology. More recently, Correia et al.[15] tested the hypothesis that clinical suspicion of OSA is an independent predictor of worse in-hospital outcomes in patients with non-ST-elevation ACSs. Presence of a high risk for OSA was positively associated with risk for a cardiovascular event (OR, 3.4; 95% CI, 1.3-9.0), but the follow-up was also restricted to the period of hospitalization.[15] Our results have also found that OSA is associated with a poorer prognosis in ACS. However, we found that this association exists, including all types of ACS. We were probably underpowered to evaluate

Table 2 – Hazard ratio and 95% confidence interval of all-cause, CVD and CHD mortality, and combined endpoint including fatal and nonfatal CHD in the ERICO study participants at low and high risk for obstructive sleep apnea

	Crude	Age- and sex- adjusted	Multivariate adjusted
All-cause mortality			
Low risk for obstructive sleep apnea	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
High risk for obstructive sleep apnea	1.17 (0.632.17)	1.31 (0.83-2.07)	1.29 (0.64-2.61)
CVD* mortality			
Low risk for obstructive sleep apnea	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
High risk for obstructive sleep apnea	1.21 (0.453.24)	1.23 (0.66-2.29)	1.65 (0.63-4.38)
CHD† mortality			
Low risk for obstructive sleep apnea	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
High risk for obstructive sleep apnea	1.21 (0.453.24)	1.24 (0.46-3.34)	2.85 (0.54-15.12)
Combined endpoint (fatal and recurrent nonfatal CHD)			
Low risk for obstructive sleep apnea	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
High risk for obstructive sleep apnea	2.31 (1.06-5.02)	2.34 (1.07-5.08)	4.26 (1.18-15.36)

**CVD: cardiovascular disease; †CHD: coronary heart disease. Multivariate analysis adjusted for age, sex, diabetes, dyslipidemia, smoking, sedentary lifestyle, previous CHD, family history of CHD, acute coronary syndrome subtype and ejection fraction.*

prognosis according to the ACS subtype, but as follow-up of the study continues, we may address this objective in a future analysis.

The ERICO study has some diverse characteristics compared to other studies that evaluate ACS worldwide. The HU-USP is a general community-based hospital that cares for the community living in the Butantan borough. Even in that setting, we showed a positive association with the composite endpoint after a 2.6-year mean follow-up after the index event. However, we also have some important limitations that should be noted. The Berlin questionnaire performs more poorly than polysomnography in patients with CHD.[25] However, full polysomnography is a costly tool, and not readily available at all facilities. This important obstacle, together with the lack of an efficient and easy tool for OSA screening, may partially explain the under-diagnosis of OSA in the cardiology setting.[26] In our study, the Berlin questionnaire was applied 30 days after the ACS. Therefore, there is probably a survival bias in this analysis, with patients with more severe ACS, and probably also with a higher frequency of OSA, dying before they could enter the study. Even in this circumstance, we found a positive association that suggests real causality between high-risk OSA and CHD combined endpoint. This study also reports some interesting new data, as there have been few studies that exclusively evaluate the relationship of severe OSA with cardiovascular events only for ACS patients with a long-term follow-up. Another important point is the strict criteria used to define ACS, and the statistical analysis that used Cox proportional hazards, which are positive points of this analysis.

Conclusions

This prospective cohort of CHD demonstrates that a high risk for OSA, measured by the Berlin questionnaire, was an independent predictor of reinfarction or CHD mortality among individuals with ACS after a 2.6-year follow-up.

Acknowledgements

We are grateful to the people, physicians and hospital administrators in the study area for their help in data collection. In addition, we thank the municipal (PRO-AIM) and the state's health (SEADE) offices for their collaboration in this study.

Author contributions

Conception and design of the research, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Maia FC, Goulart AC, Drager LF, Staniak HL, Santos IS, Lotufo PA, Bensenor IM; Acquisition of data: Maia FC, Goulart AC, Staniak HL, Santos IS, Bensenor IM; Analysis and interpretation of the data: Maia FC, Goulart AC, Drager LF, Santos IS, Lotufo PA, Bensenor IM; Statistical analysis: Maia FC, Goulart AC, Bensenor IM; Obtaining financing: Lotufo PA, Bensenor IM.

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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Comparative Study between Perfusion Changes and Positive Findings on Coronary Flow Reserve

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Abstract

Background: Functional assessment of coronary artery obstruction is used in cardiology practice to correlate anatomic obstructions with flow decrease. Among such assessments, the study of the coronary fractional flow reserve (FFR) has become the most widely used.

Objective: To evaluate the correlation between FFR and findings of ischemia obtained by noninvasive methods including stress echocardiography and nuclear medicine and the presence of critical coronary artery obstruction.

Methods: Retrospective study of cases treated with systematized and standardized procedures for coronary disease between March 2011 and August 2014. We included 96 patients with 107 critical coronary obstructions (> 50% in the coronary trunk and/or ≥ 70% in other segments) estimated by quantitative coronary angiography (QCA) and intracoronary ultrasound (ICUS). All cases presented ischemia in one of the noninvasive studies.

Results: All 96 patients presented ischemia (100%) in one of the functional tests. On FFR study with adenosine 140 g/kg/min, 52% of the cases had values ≤ 0.80. On correlation analysis for FFR ≤ 0.80, the evaluation of sensitivity, specificity, positive and negative predictive values, accuracy, and ROC curve in relation to the stenosis degree and length, and presence of ischemia, no significant values or strong correlation were observed.

Conclusion: Coronary FFR using a cut-off value of 0.80 showed no correlation with noninvasive ischemia tests in patients with severe coronary artery obstructions on QCA and ICUS. (Arq Bras Cardiol. 2017; 108(1):38-46)

Keywords: Coronary Artery Disease / mortality; Percutaneous Coronary Intervention; Myocardial Ischemia; Fractional Flow Reserve, Myocardial / physiology.

Introduction

Coronary artery disease (CAD) is considered the most common cause of death due to cardiovascular diseases (CVD) in Brazil and worldwide. Nonetheless, the number of individuals aged more than 60 years who survive a first event increases at each year, a fact that is attributed to technological advancements in diagnostic methods and treatment techniques over the past 30 years.[1-3]

International guidelines recommend a combination of functional and anatomical assessments to define the ideal treatment strategy for CAD.[4,5] However, some studies[6-10] aiming at complete lesion revascularization, have proposed treatment of lesions with a ≤ 50% stenosis diameter with percutaneous coronary intervention (PCI), prioritizing the anatomical findings independent of their functional repercussions (assessed by noninvasive methods).

The DEFER study showed that it is safe to defer treatment of functionally nonsignificant coronary lesions.[11] More recently, the FAME study showed that in the presence of multivessel disease, treatment of epicardial lesions guided by fractional flow reserve (FFR) is associated with a reduction in ischemic complications when compared with treatment guided by angiography.[12]

Based on these findings, FFR measurement has become routine in guiding clinical decision making in CAD treatment. However, both the technique and its cut-off value of 0.80 have not been tested in some specific situations including severe coronary artery obstructions (the initial results involved minor and moderate lesions). Therefore, to evaluate the impact of FFR measurement on severe lesions with ischemia previously detected by noninvasive functional tests will be of great importance, as the decision to treat or not to treat these lesions may be substantiated by the results of the FFR study.

Thus, the objective of this study was to correlate the FFR results, using a cut-off value of 0.80, with the presence of ischemia, detected by noninvasive tests including stress

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Manuscript received March 03, 2016; revised manuscript July 15, 2016; accepted August 08, 2016

DOI: 10.5935/abc.20160184

echocardiography or nuclear medicine, in patients with severe coronary obstruction assessed by cineangiography and intracoronary ultrasound (ICUS).

Methods

Type of study

We conducted a retrospective study of cases treated with systematized and standardized procedures for coronary disease between March 2011 and August 2014 at the *Hospital Cardiológico Costantini (HCC)* in Curitiba.

Studied population

We screened 264 patients with suspected CAD who had undergone noninvasive functional tests, pharmacological stress echocardiography or nuclear medicine, and had an indication of cineangiography.

Inclusion criteria

The study's project was described in line with the Declaration of Helsinki and approved by the Research Ethics Committee of the *Hospital Erasto Gaertner* (2274/13). All patients read, understood, and signed an informed consent form prepared according to Resolution 466/2012 of the National Health Council. The study included patients who presented ischemia on perfusion studies with pharmacological stress echocardiography or nuclear medicine due to severe obstructive lesions with > 50% obstruction in the left coronary trunk (LCT) and/or ≥ 70% in other segments, leading to ischemia in the region supplied by the affected artery.

Exclusion criteria

We excluded from the study those cases with associated neoplasms, chronic obstructive pulmonary disease, renal insufficiency (creatinine > 2.0 mg/dL), hemorrhagic disease, acute myocardial infarction, stroke, or surgical treatment in the past 6 months, as well as coronary obstructions < 50% in the LCT territory and/or < 70% in other segments.

Noninvasive functional evaluation methods

All patients included in the study underwent noninvasive functional evaluation with myocardial perfusion scintigraphy (MPS) and/or pharmacological stress echocardiography.

Myocardial perfusion scintigraphy

MPS was performed according to a standard protocol recommended by the American Society of Nuclear Cardiology (ASNC),[13] both for the exercise and pharmacological stress (intravenous dipyridamole) protocols. The images were obtained with a tomographic gamma camera (Philips Cardio MD3), reconstructed with the program Cedars Quantitative Gated Spect, and interpreted by two independent investigators who concurred with the diagnosis of ischemia. The MPS images were qualitatively and quantitatively interpreted by more than one experienced investigator according to the ASNC recommendations. For the MPS quantification, we subjectively (visually) assigned a numerical value to each of the 17 segments in both phases, categorizing it as 0 (homogeneous uptake), 1 (slightly decreased uptake), 2 (moderately decreased uptake), 3 (markedly decreased uptake), or 4 (no uptake). The sum of the scores attributed to the 17 segments in the stress (SSS) and resting (SRS) phases allows a semiquantitative evaluation of the intensity and extent of the coronary disease.[13]

Exercise ECG was performed according to the Bruce protocol as per criteria established by the guideline of the Brazilian Society of Cardiology.[14] Pharmacological stress was induced by intravenous injection of dipyridamole 0.84 mg/kg for 3 minutes, followed 4 minutes later by injection of the radiotracer (sestamibi-^{99m}Tc) at a 555 to 740 MBq dose.[15]

The images were analyzed by two independent investigators and ischemia was considered to be present when both interpretations were in agreement.

Pharmacological stress echocardiography

The echocardiographic study with pharmacological stress was performed according to the criteria set by the guidelines of the Brazilian Society of Cardiology[13] with continuous infusion of dobutamine at increasing doses every 2 minutes, starting with 5 μg/kg/min; when the maximal heart rate was not reached, atropine bolus was used at an initial dose of 0.25 mg.[16]

Method of angiographic evaluation

All volunteers included in the study underwent coronary angiography. The coronary lesions diagnosed were initially classified according to their severity by quantitative coronary angiography (QCA). They were also assessed by ICUS for better quantification of the lesion areas. Additionally, the patients underwent FFR measurement and the results were compared with the ischemic areas suggested by noninvasive functional tests.

Quantitative coronary angiography

The angiographic images were evaluated by the main investigator (CRC) and the hemodynamic team of the *Hospital Cardiológico Costantini*. For that, we used a specific software to quantify obstructive coronary lesions (CASS version 5.7.4, Pie Medical Imaging B.V., The Netherlands).

In all cases, the images were obtained in different projections, always seeking a better visualization of the lesion and of the proximal and distal portions of the artery. Thus, it was possible to establish a mean reference diameter for the artery, the length of the lesion, the minimum luminal diameter, and the percentage of the diameter of the stenosis [(reference diameter - minimum luminal diameter)/(reference diameter x 100)] before and after the procedure. The calibration standard was established by the outer diameter of the catheter filled with contrast.[17]

Measurement of fractional flow reserve

To evaluate the impact of the lesion on the coronary flow, FFR was used according to established criteria,[18] in which the

distal pressure was measured with a 0.014-inche guide wire (Pressure Wire 4 Sensor, RADI Medical Systems, Uppsala, Sweden) or a Volcano Wave Wire (Volcano Inc., Rancho Cordova, California, USA) immediately distal to the stenosis, one at a time,[18] during the period of maximal hyperemia induced by intravenous injection of adenosine 140 μg/kg/min through a large venous access in the antecubital vein. The aortic pressure was measured with a 6 or 7 F guide catheter. Lesions with a FFR ≤ 0.80 were considered to be responsible for the ischemia, as determined by the guidelines.[19]

Intracoronary ultrasound

The ICUS images were obtained with a rotating single element transducer with a 40 MHz frequency within a 2.6 Fr sheath and an automated transducer pullback with a speed of 0.5 mm/s, connected to an iLAB 2 scanner (Boston Scientific Corporation, Natick, USA) and Eagle Eye Platinum Intravenous Ultrasound (IVUS) Catheter (Volcano Corporation, San Diego, California, USA).

The images were digitized and analyzed according to the criteria of the Clinical Expert Consensus Document on Standards for Acquisition, Measurement and Reporting of Intravascular Ultrasound Studies (American College of Cardiology)[20] and the program EchoPlaque 3.0.48 (INDEC Systems Inc., Mountain View, USA), respectively. Each millimeter of the arterial segments was analyzed with computerized planimetry to measure the lesion area and volume.[21]

Study design

See figure 1 below.

Statistical analysis

In the descriptive statistical analysis, the results of categorical variables are expressed as absolute frequencies and percentages. For continuous variables, we present mean ± standard deviation values. To verify homogeneity and normality, we applied the Levene and Shapiro-Wilk tests. To compare two groups in regard to quantitative variables, we used Student's *t* test for independent samples. When the comparison included more than two groups, we used one-way analysis of variance (ANOVA). Regarding categorical variables, the comparisons were performed using Fisher's exact test. To evaluate the cut-off values for quantitative variables associated with dichotomous outcomes of interest, we adjusted receiver operating characteristic (ROC) curves. Statistical significance was set at p values < 0.05. The data were analyzed with the programs IBM SPSS Statistics v.20 and GraphPad Prism v.6.05. We used logistic regression and ROC curve analysis to define the correlation coefficients between noninvasive and invasive functional evaluations with the FFR measurement.

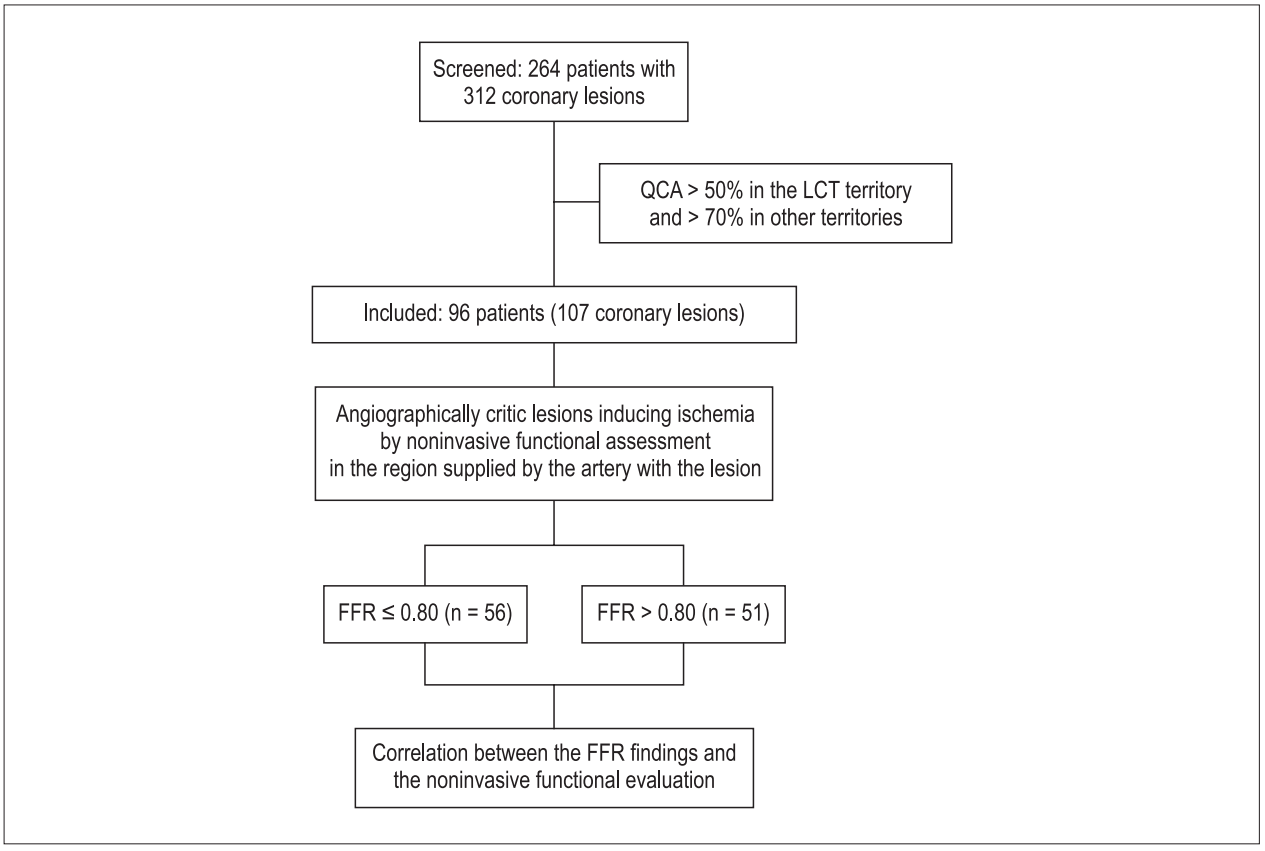

Figure 1 – *Study Design. QCA: quantitative coronary angiography; LCT: left coronary trunk; FFR: fractional flow reserve.*

Results

In total, 107 obstructive lesions were diagnosed by angiography in the 96 patients included in the study. In 34% of the cases, the obstructions affected multiple vessels and in 81 cases (87% of the sample), the obstructions were categorized as type B/C according to the classification of the American College of Cardiology/American Heart Association.[22] The anterior descending artery had the highest prevalence of lesions (52.34%).

Based on the assumption, grounded in the literature[19] that coronary lesions with a FFR ≤ 0.80 should be deemed responsible for the myocardial ischemia, the following variables were compared between the FFR > 0.80 and ≤ 0.80 groups in the sample with ischemia detected by functional tests: modifiable and non-modifiable risk factors, clinical characteristics of the patients prior to the initiation of the clinical investigation, findings of noninvasive functional tests, and angiographic findings (QCA, ICUS, and FFR).

Table 1 presents the characteristics of the sample with regard to risk factors and clinical characteristics in the FFR > 0.80 and ≤ 0.80 groups**.** We observed similar results between both groups.

Figure 2 presents the results of the noninvasive functional evaluations conducted in each group for the diagnosis of myocardial ischemia. In the FFR > 0.80 group, 41 patients (85%) underwent MPS, while seven (15%) underwent stress echocardiography. The corresponding numbers in the FFR ≤ 0.80 group were 42 (88%) and six (12%), respectively. Figure 2 also shows the results according to the classification of ischemia as mild, moderate, and important. We observed a higher frequency of mild ischemia in the FFR > 0.80 group and moderate ischemia in the FFR ≤ 0.80 group.

When we compared the groups in terms of angiographic characteristics, we observed a significant ($p < 0.03$) difference in regard to the anatomical location of the lesion, with a greater number of lesions in the anterior descending artery in the FFR ≤ 0.80 (Table 2).

Table 2 also shows that when the QCA was compared with respect to the diameter of the stenosis, there was no significant difference between lesions with FFR ≤ or > 0.80 (74.25 ± 7.2% *versus* 75.5 ± 6.84%, respectively). Also, no significant differences were observed when the length of the lesion was compared between the FFR ≤ 0.80 and > 0.80 groups: 12.12 ± 5.22 mm *versus* 10.53 ± 4.24 mm, respectively, on QCA evaluation and 20.92 ± 7.27 mm *versus* 18.76 ± 7.22 mm, respectively, on ICUS evaluation.

Table 3 shows the characteristics of the predictors of ischemia for a FFR ≤ 0.80. Considering the sensitivity, specificity, and positive and negative predictive values, we found a reference arterial diameter of < 2.62 mm, and minimal luminal diameters of < 0.36 mm on QCA and < 2.50 mm on ICUS.

Discussion

The main findings of this study were: 1) in the overall evaluation of the sample, the descending anterior artery showed the highest prevalence of lesions (52.34%), while 87% of the sample presented type B/C obstructions; 2) when patients with ischemia diagnosed by a noninvasive functional test were divided into FFR > 0.80 and ≤ 0.80 groups, there were no significant differences between both groups in regard to modifiable and non-modifiable risk factors, as well as clinical symptoms leading to the investigation. In the angiographic data evaluated, there was a significant difference with respect

Table 1 - Comparison of risk factors and clinical characteristics in the FFR ≤ 0.80 and FFR > 0.80 groups

Clinical Characteristics	Total 96 Patients	FFR ≤ 0.8 48 patients	FFR > 0.8 48 patients	p*
Age, mean ± SD	65.60 ± 10.34	65.8 ± 10.4	65.4 ± 10.4	0.90
Male gender, n (%)	66 (69)	31 (65)	35 (73)	0.46
Hypertension, n (%)	93 (97)	47 (98)	46 (96)	0.50
Obesity, n (%)	17 (18)	11 (23)	6 (12)	0.14
Diabetes mellitus, n (%)	48 (50)	23 (48)	25 (52)	0.41
Dyslipidemia, n (%)	93 (97)	46 (96)	47 (98)	0.50
Current smoking, n (%)	14 (15)	10 (21)	4 (8)	0.03
Clinical Symptoms	**Total 96 patients**	**FFR ≤ 0.8 48 patients**	**FFR > 0.8 48 patients**	**p***
Silent ischemia, n (%)	16 (17)	10 (21)	6 (13)	0.20
Stable angina, n (%)	40 (42)	20 (42)	20 (42)	0.09
Unstable angina, n (%)	33 (34)	13 (27)	20 (42)	0.09
Atypical angina, n (%)	6 (6)	4 (8)	2 (3)	0.33
Acute coronary syndrome, n (%)	1 (1)	1 (2)	0 (0)	0.50

(*) Fisher's exact test (categorical variables) or Student's t test for independent samples (quantitative variables); p < 0.05; n: number, SD: standard deviation.

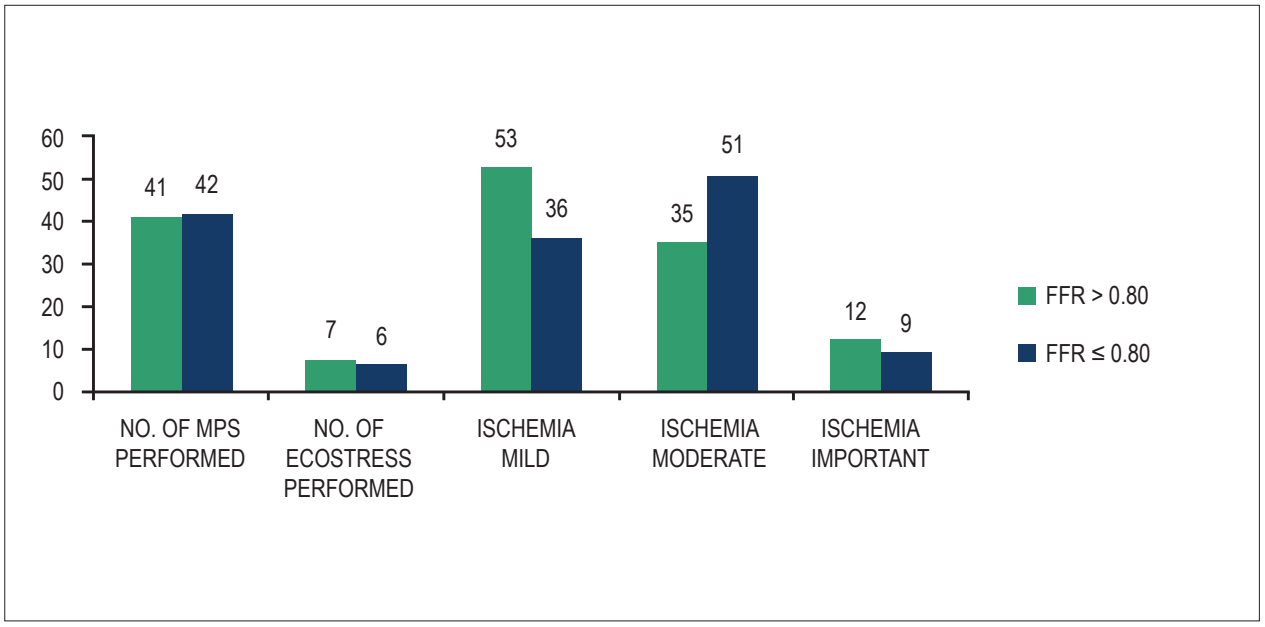

Figure 2 – *Percentage distribution of the functional tests performed. FFR: fractional flow reserve*

Table 2 – Comparison of angiographic characteristics in the general sample and in the FFR ≤ 0.80 and FFR > 0.80 groups

Angiographic characteristics	Total 107 lesions	FFR ≤ 0.8 56 lesions	FFR > 0.8 51 lesions	p*
Type B/C lesions, n (%)	87 (81)	42 (75)	39 (76.47)	0.07
Multivessel, n (%)	36 (34)	21 (37.5)	15 (29.41)	0.42
Bifurcation, n (%)	13 (12)	7 (12)	6 (11.76)	0.42
Left coronary trunk, n (%)	7 (6.54)	2 (3.57)	5 (9.80)	0.46
Left coronary trunk involving the proximal AD, n (%)	2 (1.87)	2 (3.57)	0 (0)	0.52
AD, n (%)	56 (52.34)	36 (64.29)	20 (39.21)	0.11
Diagonal, n (%)	5 (4.67)	3 (5.35)	2 (3.9)	0.65
Circumflex, n (%)	16 (14.95)	8 (14.28)	8 (15.68)	0.56
Circumflex marginal branch, n (%)	3 (2.8)	0 (0)	3 (5.88)	0.10
Right coronary, n (%)	15 (14.02)	4 (7.14)	11 (21.57)	0.05
Posterior descending - right coronary, n (%)	2 (1.87)	1 (1.78)	1 (1.97)	0.72
Saphenous vein graft, n (%)	1 (0.93)	0 (0)	1 (1.97)	0.47
QCA, RVD, mm (SD)	2.71 ± 0.63	2.70 ± 0.72	2.73 ± 0.53	0.31
QCA, stenosis diameter (%)	75.43 ± 6.68	75.5 ± 5.85	74.25 ± 8.5	0.39
QCA, length, mm (SD)	11.36 ± 5.19	12.12 ± 6.19	10.53 ± 3.71	0.11
Ultrasonographic Characteristics				
RVD, mm (SD)	2.99 ± 0.42	2.98 ± 0.40	3.15 ± 0.44	0.03
ICUS, stenosis diameter (%)	84.21 ± 8.46	84.25 ± 8.03	84.18 ± 9.00	0.96
ICUS, length, mm (SD)	19.89 ± 7.22	20.93 ± 8.02	18.76 ± 6.12	0.88
Fractional flow reserve (mean ± SD)	0.80 ± 0.10	0.72 ± 0.09	0.88 ± 0.04	0.00

*(*) Fisher's exact test (categorical variables) or Student's t test for independent samples (quantitative variables); p < 0.05. AD: anterior descending; SD: standard deviation; RVD: reference vessel diameter; QCA: quantitative coronary angiography; ICUS: intracoronary ultrasound. *Considered statistically significant at p < 0.05.*

Table 3 – Characteristics of the analysis of ischemia predictors for a FFR ≤ 0.80

Variable	AUC (%)	95% CI	Accuracy	Values associated with a FFR ≤ 0.80 (cut-off values)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
QCA diameter	0.5	0.39 - 0.62	53.3%	≥ 76%	48.2	58.8	56.3	50.8
ICUS diameter	0.49	0.38 - 0.60	52.3%	≥ 86%	57.1	47.1	54.2	50.0
QCA RVD (mm)	0.54	0.43 - 0.65	57.0%	< 2.62	57.1	56.9	59.3	54.7
QCA MLD (mm)	0.53	0.42 - 0.64	57.0%	< 0.36	48.2	66.7	61.4	54.0
ICUS MLD (mm)	0.54	0.43 - 0.65	57.9%	< 2.50	53.6	62.7	61.2	55.2
QCA LL (mm)	0.59	0.48 - 0.70	64.5%	≥ 9.68	66.1	62.7	66.1	62.7
ICUS LL (mm)	0.58	0.47 - 0.69	57.9%	≥ 20	51.8	64.7	61.7	55.0

QCA: quantitative coronary angiography; ICUS: intracoronary ultrasound; RVD: reference vessel diameter; MLD: minimal luminal diameter; LL: lesion length; PPV: positive predictive value; NPV: negative predictive value; AUC: area under the ROC curve; 95% CI: 95% confidence interval for the AUC. For these calculations, the prevalence of FFR ≤ 0.80 in this study population was estimated from the sample results (56/107 = 52.3%).

to the anatomical location of the lesion, with more common lesions in the left anterior descending artery in the FFR ≤ 0.80 group; 3) correlation analysis for FFR ≤ 0.80 considering the sensitivity, specificity, positive and negative predictive values, accuracy and ROC curve relative to the presence of ischemia and stenosis degree and length did not show values with significance or strong correlation.

For some authors, the cut-off value of 0.80 for the FFR may represent more than an anatomic evaluation. Pijls et al.[22] studied 45 patients with angiographically questionable stenoses according to their angiographic severity. In 24 and 21 patients with 44 ± 9% and 41 ± 8% percent stenoses, respectively, their results suggested that the FFR had a greater accuracy to distinguish stenoses with a potential hemodynamic impact (sensitivity of 88% and specificity of 100%) compared with exercise testing, MPS, and stress echocardiography.

Other studies have been published using the FFR as a measurement to recommend or not recommend PCI, including the DEFER study,[11] which evaluated 325 patients divided into three groups, none of whom had undergone functional evaluation to justify the procedure. The patients were randomized to group 1 (defer; immediate PCI or not, n = 91, no prior functional tests and FFR ≥ 0.75, undergoing optimized clinical treatment), group 2 (reference; n = 144, no prior functional tests and FFR < 0.75, undergoing immediate PCI), and group 3 (perform; n = 90, no prior functional tests, with FFR ≥ 0.75 and mean stenosis percentage of 48 ± 10%, undergoing, nonetheless, immediate PCI). The 5-year follow-up in the DEFER study[23] showed consistent results, with a risk of death or infarction of 1% per year in the population whose treatment was deferred based on the FFR. It is worth noting that the patients in the *perform* group who had no clinical or noninvasive functional criteria for PCI presented a 7.9% rate of death/acute myocardial infarction at 5 years. However, it is unclear whether these results would be similar had noninvasive diagnostic tests such as MPS been performed. In the present study, unlike the methodology of the DEFER study, patients undergoing coronary angiography had a positive functional assessment of myocardial ischemia and, as a result, we noted that there was no significant or strong correlation (sensitivity/specificity), positive/negative predictive values, and accuracy in relation to the degree or extension of the stenosis and presence of ischemia. Although the FAME study[19] showed that 60% of the patients had obstructive lesions > 70% and nearly 20% had lesions > 90%, these patients had not undergone noninvasive functional tests that could be confronted with the values obtained by FFR measurement.

It is clear that the decision of coronary intervention should be based on objective evidence of the functional and anatomical impact of the coronary narrowing;[24,25] this evidence helps to stratify the disease risk and future coronary events, providing better guidance in terms of therapeutic approach.[26,27] Patients with significant areas of ischemia have a worse prognosis when maintained on clinical treatment.[28] If the ischemia negatively affects the individual's daily life due to the occurrence of symptoms, revascularization may bring major benefits, as shown in the COURAGE study, which demonstrated better symptom control with revascularization;[29] even asymptomatic patients with moderate/important ischemia show better outcomes in terms of reduction of adverse events after revascularization of the lesion.[30]

A very important issue that should be addressed in this discussion is related to the numerous changes that the methodology used for FFR measurement has undergone during the evolution of interventional cardiology. These changes relate to:

A) The ideal dose of adenosine: Pijls et al.[22] have validated the method using an intravenous infusion of adenosine at a dose of 140 μg/kg/min to induce maximal hyperemia. The DEFER study[11] used two methods for adenosine administration: intravenous, at a dose of 140 μg/kg/min, and intracoronary, at a dose of 15 μg in the right coronary and 20 μg in left coronary. The ISCHEMIA study,[31] in turn, proposed that the dose of 140 μg/kg/min should be doubled when the FFR results are ≥ 0.81 or ≤ 0.82. In addition, De Luca et al.[32] showed that intracoronary adenosine at increasing doses of up to 720 μg progressively decreased the FFR values. We should also emphasize that the infusion of adenosine at a dose of 140

μg/kg/min may not produce absolute maximal vasodilation in the subepicardial infarction in all patients.[33]

B) Route of administration: different protocols suggest different administration routes, including intravenous, intracoronary, and central lines.

C) Time to maximal hyperemia: In 2013, Tarkin et al.[34] published a study showing that the measurements should only be obtained when steady-state hyperemia has been reached for ≥ 60 seconds during continuous intravenous infusion of adenosine, which is not consistent with protocols used in previous studies.[12]

D) Ideal cut-off value: The cut-off value to detect ischemia with a sensitivity of 90% and specificity of 100% is 0.75. Values below 0.75 are almost always associated with myocardial ischemia, while stenosis associated with FFR greater than 0.80 are almost never associated with ischemia, creating a gray area for FFR values between 0.75 and 0.80.[35] To increase to close to 100% the sensitivity to detect ischemia, a FFR cut-off value of 0.80 has been recently used.[12] In a recent study, Petraco et al.[36] suggested that the gray zone for the FFR measurement is between 0.75 and 0.85. In clinical practice, this means that each time a single FFR measurement falls between 0.75 and 0.85, there is a chance that a recommendation for revascularization guided by FFR may change if the measurement is repeated after 10 minutes; the chance becomes greater as the FFR result becomes closer to 0.80. Based on the classic flow dynamics equation, in which the resistance to the flow across the stenosis is dependent on both the length and diameter of the stenosis, Lopez-Lopez-Palop et al.[37,38] and Jaffe et al.,[39] recently showed that the length of the lesion is more important than its diameter when the functional impact of the lesion is estimated. It is important to emphasize that in our registry, the longer was the lesion, the greater was the correlation with the positive FFR, corroborating the theory defended by these authors.

It is questionable if the 0.80 cut-off value for the FFR measurement is ideal to quantify lesions and whether it is really possible to define a patient's therapy based on this method alone since this study was unable to show reproducibility in severe lesions with noninvasive functional tests to confirm its physiological meaning.

Based on the findings of this study and this sample, we believe that it is precocious to adopt the cut-off value of 0.80 for FFR measurement as a gold standard with a class of recommendation I and level of evidence A[40] in defining the treatment strategy for coronary artery disease. Some barriers still need to be overcome, such as the definition of the actual value of the ideal reference for the cut-off measurement, the time to hyperemia, and the dose and ideal administration route for FFR measurement.

Study limitations

The number of patients included in the study was low. A continuity of the study including a greater number of participants is suggested.

Conclusion

This study found no correlation between FFR values (cut-off value of 0.80) with the presence of myocardial ischemia obtained by noninvasive functional studies in angiographically severe coronary lesions assessed by QCA.

Author contributions

Conception and design of the research and Writing of the manuscript: Costantini CRF, Ramires JA, Costantini CO, Denk MA, Macedo RM; Acquisition of data: Costantini CRF, Costantini CO, Denk MA, Silveira CW, Macedo RM; Analysis and interpretation of the data and Statistical analysis: Costantini CRF, Costantini CO, Denk MA, Macedo RM; Critical revision of the manuscript for intellectual content: Costantini CRF, Ramires JA, Costantini CO, Denk MA, Tarbine SG, Santos MF, Zanuttini DA, Souza AM, Macedo RM.

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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Original Article

Electrocardiogram Performance in the Diagnosis of Left Ventricular Hypertrophy in Hypertensive Patients With Left Bundle Branch Block

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Abstract

Background: Left ventricular hypertrophy (LVH) is an important risk factor for cardiovascular events, and its detection usually begins with an electrocardiogram (ECG).

Objective: To evaluate the impact of complete left bundle branch block (CLBBB) in hypertensive patients in the diagnostic performance of LVH by ECG.

Methods: A total of 2,240 hypertensive patients were studied. All of them were submitted to an ECG and an echocardiogram (ECHO). We evaluated the most frequently used electrocardiographic criteria for LVH diagnosis: Cornell voltage, Cornell voltage product, Sokolow-Lyon voltage, Sokolow-Lyon product, RaVL, RaVL+SV_3, RV_6/RV_5 ratio, strain pattern, left atrial enlargement, and QT interval. LVH identification pattern was the left ventricular mass index (LVMI) obtained by ECHO in all participants.

Results: Mean age was 11.3 years ± 58.7 years, 684 (30.5%) were male and 1,556 (69.5%) were female. In patients without CLBBB, ECG sensitivity to the presence of LVH varied between 7.6 and 40.9%, and specificity varied between 70.2% and 99.2%. In participants with CLBBB, sensitivity to LVH varied between 11.9 and 95.2%, and specificity between 6.6 and 96.6%. Among the criteria with the best performance for LVH with CLBBB, Sokolow-Lyon, for a voltage of ≥ 3,0mV, stood out with a sensitivity of 22.2% (CI 95% 15.8 – 30.8) and specificity of 88.3% (CI 95% 77.8 – 94.2).

Conclusion: In hypertensive patients with CLBBB, the most often used criteria for the detection of LVH with ECG showed significant decrease in performance with regards to sensitivity and specificity. In this scenario, Sokolow-Lyon criteria with voltage ≥3,0mV presented the best performance. (Arq Bras Cardiol. 2017; 108(1):47-52)

Keywords: Hypertension; Electrocardiography / methods; Hypertrophy, Left Ventricular / diagnosis; Bundle-Branch Block.

Introduction

Left ventricular hypertrophy (LVH) diagnosis by electrocardiogram (ECG) in hypertensive patients involves clinical and prognostic decisions. Pioneering studies by Framingham have shown that alterations in QRS voltage and ventricular repolarization are important determining factors for cardiovascular events.[1,2]

Despite its relatively low sensitivity, ECG makes up for this limitation with high specificity in the identification of LVH. Moreover, it is a widely used method that is easily accessible and low cost. However, several situations may negatively alter ECG performance in LVH diagnosis, among which is the presence of complete left bundle branch block (CLBBB).[3] Because it interferes in the measurement of its criteria or parameters, alterations promoted by CLBBB in ECG tracings are described as restrictive for the electrocardiographic diagnosis of LVH.

The objective of this study was to evaluate CLBBB influence in the sensitivity and specificity of the main electrocardiographic criteria used in LVH diagnosis in patients with systemic arterial hypertension (SAH).

Methods

We analyzed ECG tracings in 12-lead of 2,240 hypertensive patients in outpatient care. Patients with valvular diseases, known coronary artery disease, previous myocardial infarction, Chagas disease, rhythm disturbances, right bundle branch block, use of digitalis compounds, ventricular pre-excitation, or inadequate technical quality of the echocardiogram were excluded from the present analysis.

Electrocardiogram

All participants were submitted to a 12-lead ECG at rest, recording at a speed of 25 mm/s and standardized calibration for 10 mm/cm (equipment - Dixtal® EP3, Brazil). For the precise analysis of the tracing, we used a magnifying glass that allowed a fivefold enlargement in its contact face. In all tracings

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Manuscript received July 04, 2016; revised manuscript August 24, 2016; accepted September 13, 2016.

DOI: 10.5935/abc.20160187

(analyzed by the same observer), a certified cardiologist with experience in ECG reading was brought in. We estimated the axis and duration of the QRS complex; R wave amplitude in aV_L, V_5 and V_6 leads; S wave amplitude in V_1, V_2 and V_3; and the strain pattern in V_5 e V_6. We separately analyzed 14 electrocardiographic criteria for LVH:

a) Cornell voltage criteria: $RaV_L + SV_3 \geq 20$ mm for women and ≥28 mm for men.[4]

b) Cornell criteria duration: ($RaV_L + SV_3$) x QRS duration – for women, add 8 mm, ≥2440 mm.ms.[5]

c) Sokolow-Lyon voltage criteria: $SV_1 + RV_5$ or $V_6 \geq 30$ mm and ≥35 mm.[6]

d) Sokolow-Lyon product criteria: ($SV_1 + RV_5$ or V_6) x QRS duration ≥3710 mm.ms.[7]

e) Gubner-Ungerleider score: RD1+SV3 >25 mm.[8]

f) R wave of $aV_L \geq 11$ mm.[9]

g) RaVL product: RaVL x duration QRS ≥1030 mm.ms.[7]

h) RaVL $+SV_3$ >16 mm in men and >14mm in women.[10]

i) RV_6/RV_5 ratio >1.[11]

j) (Biggest R wave + biggest S wave) x (QRS duration): >28 mm.ms.[12]

k) Presence of the strain pattern: defined as the convex depression of the ST segment with asymmetrical inversion of the T wave opposed to QRS complex in V_5 or V_6 leads.[13]

l) Left atrial enlargement: duration ≥120 ms; P wave alteration at D2 with slurrying in the apex or Morris signal in V1; terminal component with duration and amplitude ≥ 0,04 mm.s).[14]

Other analyzed electrocardiographic variables

a) QT interval: measured in ms, from the beginning of the Q wave to the end of the T wave (corrected through Bazett's formula: $QTc = QT/RR^{1/2}$; normal values from 350 to 440 ms).[15]

b) CLBBB was identified when: duration off the QRS ≥120ms; absence of "q" wave in D1, aVL, V5 and V6; widened R waves with slots and/or medium-terminal slurrying in D1, aVL, V5 and V6; "r" wave with slow growth of V1 to V3 with possible occurrence of QS; widened S waves with thickening and/or slots in V1 and V2; intrinsicoid deflection in V5 and V6 ≥0,05 s, electrical axis between -30º e + 60º; ST depression and asymmetrical T wave in opposition to medium-terminal delay.[16]

Transthoracic echocardiogram

The exams were performed with the device ATL® 1500, USA, with 2.0 and 3.5 MHz transducers. All measurements were obtained by the same observer who was unaware of participants' clinical characteristics, and according to the recommendations of the European Association of Echocardiography.[17] Images were obtained with the participant in left lateral decubitus from the left parasternal region between the fourth and fifth intercostal space, proceeding with the habitual sections for the complete study in M and two-dimensional modes, simultaneously with the recording of the ECG. According to the recommendations of the Penn Convention, the following measurements were performed: left ventricle size (LV) in systole and diastole; interventricular septum thickness in diastole (IVSD) and end diastolic left ventricular posterior wall thickness (LVPWd); LV end-diastolic diameter (LVDd); end systolic and diastolic volumes, and percentage of diastolic shortening and ejection fraction by the cube method. LV mass was calculated by the formula: LV mass = 0.8 X {1.04 [(IVSD + LVDd + LVPWd)^3 – (LVDd)^3]} + 0.6 g.[17] LV mass was indexed for body surface to adjust differences in heart size depending on the patient size. Body surface was calculated by the formula BS = (W – 60) X 0.01 + H, where: BS is the body surface in m^2, W is the weight in kg, and H is the height in meters.[18] Enlargement of the LV mass was considered when the mass index was ≥96 g/m^2 for women and ≥116 g/m^2 for men.

Statistical analysis

Continuous variables were expressed in mean and standard deviation. Categorical variables were expressed in percentages. We used Pearson's linear correlation coefficient to determine the association between LVMI and the numerous electrocardiographic criteria. To analyze the performance of LVH electrocardiographic criteria, we used the values obtained for sensitivity and specificity with the respective confidence intervals of 95%. In the evaluation of statistical differences between LVH electrographic criteria in patients with and without CLBBB, we used McNemar's paired test. A reproducibility study of ECG tracings was performed by three observers who interpreted 100 tracings randomly taken from the sample. To that end, we analyzed the amplitude of R and S waves and the duration of the QRS complex, and the Kappa test was used.[19] To verify statistical significance, in all comparisons, we considered confidence intervals of 95% and $p < 0.05$. All analyses were executed with the software SPSS (version 17.0, SPSS Inc., Chicago, IL, USA).

Results

Of the 2,240 studied participants, 684 were male (30.5%), and 1,556 were female (69.5%), with a mean age of 11.3 ± 58.7 years. Of these, 2,054 (91.7%) constituted the group of patients without CLBBB, and 186 (8.3%) formed the group with CLBBB. In the group without CLBBB, 46.8% had LVH whereas in the group with CLBBB, 67.7% had LVH, as shown in Table 1. In this series, we had 11.8% (22/186) of the patients with CLBBB with left anterior divisional block.

According to Pearson's correlation, in both groups there was a significant association between LVMI and the electrocardiographic variables for most LVH criteria (Table 2). However, the correlations between the several criteria and LVMI showed a moderate or weak correlation, suggesting that these criteria are not fully able to explain the presence of LVH, regardless of CLBBB in the electrocardiographic tracing. We did not perform correlations between LVMI with enlargement of the left atrium and the strain pattern considering these are qualitative variables.

In relation to the electrocardiographic criteria for LVH, patients with CLBBB presented significant alterations with expressive decrease in values. Sokolow-Lyon voltage criteria

Table 1 – Characteristics of the sample according to the presence or absence or CLBBB

No CLBBB (n=2054)		With CLBBB (n=186)	
Age	Male / Female	Age	Male / Female
11.4±58.3	610 (29.7%) / 1444 (70.3%)	8.5±63.4	74 (39.8%) / 112 (60.2%)

Data expressed as mean and standard deviation and n (%). CLBBB: complete left bundle branch block.

Table 2 – Pearson correlation between LVMI and the analyzed electrocardiographic criteria

Variable	Without CLBBB (n=2054)	With CLBBB (n=186)
Cornell voltage	0.400*	0.306*
Cornell duration	0.456*	0.392*
Sokolow-Lyon voltage	0.404*	0.124
R in aVL	0.300*	0.141
QTc	0.085*	0.210*
Gubner-Ungerleider	0.536*	0.305*
(Rmax+Smax) x dur QRS	0.546*	0.383*

**p< 0.05; LVMI: left ventricular mass index; CLBBB: complete left bundle branch block..*

(≥3.0 mV e ≥3.5 mV), R wave amplitude in aVL, and enlargement of the left atrium had the lowest reductions in specificity. Interestingly, this happened with an insignificant alteration in sensitivity (Tables 3 and 4). In the criteria in which there were substantial increases of sensitivity indices, such as Cornell voltage and Cornell voltage product, these increases were concomitant with the expressive loss of specificity, which hinders the application of these criteria in the scenario of ECG with the presence of LBBB.

With regards to the reproducibility study, the level of agreement among the three observers varied between 0.82 and 0.98, which are considered excellent numbers. The first figure corresponds to the duration of the QRS complex, and the last one to the amplitude of R and S waves, respectively.

Discussion

The presence of LVH is a consistent predictor of high cardiovascular risk, regardless of other comorbidities. In clinical and epidemiological studies, there is a clear relation between LVH and adverse cardiovascular events. Hence the importance of early detection, if possible, through low-cost, easily accessible diagnostic methods. Unquestionably, ECG is one of the most frequently used methods in the detection of LVH, be it for its low operational cost or wide availability. It is often an initial instrument in the identification of several cardiologic manifestations. In the scenario of LVH secondary to SAH, it is inarguably the most cost-effective exam. It is known, however, that several factors interfere in the diagnostic precision of LVH, more specifically the presence of conduction disturbances, especially CLBBB, which notoriously imposes limitations in LVH diagnosis.[20-22]

In the last few decades, ECHO has become the reference exam in the evaluation of LV mass and function. In this context, it is used not only to confirm LVH, but also other pathological manifestations. As opposed to ECG, ECHO found the limitation in LVH identification, and provided earlier diagnosis and more aggressive approaches to associated diseases, such as SAH. However, despite its relatively low sensitivity, ECG is still the most widely used exam to detect LVH in hipertensive patients. This is because it is an easily performed test that shows excellent inter/ intraobserver reproducibility. Conversely, besides having a much higher operational cost, ECHO is extremely dependent not only on the quality of the device, but also on the observer interpreting the images.

Since CLBBB interferes in several electrocardiographic parameter employed in LVH diagnosis, in this study we evaluated the main criteria used by the ECG in this situation.[23] Considering LV mass calculation presumes the heart to be in normal, ellipsoid shape, patients with dilated hearts were excluded. To increase homogeneity in the analysis of sample members, we used LVMI to compare individuals with different body compositions and, thus, obtain values that would better identify groups at high risk for cardiovascular events.[24-26]

LVMI association with LVH electrocardiographic criteria showed moderate or weak correlation in patients with and without CLBBB. However, in the group with CLBBB, even though Sokolow-Lyon voltage and RaVL criteria did not show statistically significant correlation with LVMI, they presented the best diagnostic performances.

In patients with CLBBB, sensitivity varied between 12.7% and 95.2%, and specificity between 6.6 and 96.6%. The electrocardiographic criteria that predominantly used

Table 3 – Sensitivity of electrocardiographic variables for LVH in patients with and without CLBBB

Criteria	Without CLBBB (n=2054)	With CLBBB (n=186)	p
	Sensitivity (CI95%)	Sensitivity (CI95%)	
Sokolow-Lyon voltage ≥35 mm	12.5 (10.6-14.8)	12.7 (7.90-19.6)	ns
Sokolow-Lyon voltage ≥30 mm	21.0 (18.5-23.6)	22.2 (15.8-30.8)	ns
Sokolow-Lyon duration ≥3710 mm.ms	7.6 (6.1-9.5)	46.8 (38.3-55.5)	*
Cornell Voltage ≥ 28 mm (m). ≥20 (f)	9.3 (7.6-11.3)	78.5 (67.6-86.5)	*
Cornell Voltage duration 2440 mm.ms	17.4 (15.2-19.9)	86.5 (79.4-91.4)	*
Gubner-Ungerleider ≥ 25 mm	33.2 (30.3-36.3)	59.5 (50.7-67.6)	*
RaVL ≥ 11 mm	10.0 (8.3-12.1)	11.9 (7.3-18.7)	ns
RaVL duration >103 mm.ms	8.9 (7.3-10.9)	46.0 (37.5-54.7)	*
RaVL+SV3 >16 mm (m). 4 mm (f)	40.9 (37.8-44.0)	88.1 (81.4-92.7)	*
QTc ≥ 440 ms	35.4 (32.4-38.5)	80.9 (73.2-86.8)	*
V6/V5 >1	12.4 (10.5-14.7)	72.3 (72.3-86.1)	*
(Rm+Sm) product ≥28 mm.ms	30.8 (28.0-33.8)	95.2 (90.0-97.8)	*
Enlarged left atrium	38.1 (35.1- 41.2)	32.5 (24.9-41.1)	ns
Strain pattern	16.6 (14.4-19.1)	51.5 (42.9-60.1)	*

**increase in sensitivity with p value < 0.05; CI 95%: confidence interval; ns: non-significant; m: male; f: female. LVH: Left ventricular hypertrophy;CLBBB: complete left bundle branch block.*

Table 4 – Specificity of electrocardiographic variables for LVH in patients with and without CLBBB

Criteria	Without CLBBB (n=2054)	With CLBBB (n=186)	p
	Specificity (CI95%)	Specificity (CI95%)	
Sokolow-Lyon voltage ≥35 mm	97.6 (96.5-98.3)	96.6 (88.6-99.0)	ns
Sokolow-Lyon voltage ≥30 mm	92.4 (90.7-93.9)	88.3 (77.8-94.2)	ns
Sokolow-Lyon product ≥3710 mm.ms	99.1 (98.4-99.5)	70.0 (57.4-80.1)	*
Cornell Voltage	99.2 (98.5-99.6)	38.2 (29.8-47.3)	*
Cornel Voltage product ≥28 mm (m). ≥20 mm (f)	96.7 (95.5-97.6)	20.3 (12.0-32.2)	*
Gubner-Ungerleider ≥ 25 mV	91.1 (89.2-92.6)	61.6 (49.0-72.9)	*
RaVL ≥ 11 mm	97.0 (95.8-97.2)	96.6 (88.6-99.0)	ns
RaVL.durQRS >103	98.5 (97.6-99.1)	71.6 (59.2-81.4)	*
RaVL+SV3 >16 mm (m). 14 mm (f)	84.2 (81.9-86.2)	18.3 (10.5-29.9)	*
QTc ≥ 440 ms	70.2 (67.4-72.8)	25.0 (15.7-37.2)	*
V6> V5	90.9 (89.0-92.5)	18.3 (10.5-29.9)	*
(Rm+Sm) product ≥28 mm.ms	90.4 (88.5-92.0)	6.6 (2.6 -15.9)	*
Enlarged left atrium	77.8 (75.2-80.2)	75.0 (62.7-84.2)	ns
Strain Pattern	97.7 (96.6-98.4)	50.0 (37.3-62.1)	*

**decrease in specificity with p value < 0.05; CI 95%: confidence interval; ns: non-significant; m: male; f: female. LVH: Left ventricular hypertrophy;CLBBB: complete left bundle branch block.*

QRS complex voltage presented an increase in sensitivity, but at the cost of a great reduction in specificity. We observed that the criteria that obtained the highest sensitivity increases, such as Cornell criteria, RaVL duration, RaVL+SV_3, also had the highest statistically significant reduction in specificity. Exceptions included only Sokolow-Lyon voltage and RaVL, which had discreet, non-significant reductions in specificity.

Generally speaking, there was a reduction in specificity, with mild or strong intensity, in all the criteria. However, among the criteria that showed the best performance in detecting LVH in the presence of CLBBB, Sokolow-Lyon for a voltage of ≥3,0mV with a sensitivity of 22.2% (CI 95% 15.8 – 30.8) and specificity of 88.3% (IC 95% 77.8 – 94.2) stood out. We would point out that these values have no statistical significance. It is known that sensitivity and specificity data are related to the prevalence of the phenomenon in the evaluated sample. It is also known that hypertensive patients with CLBBB are usually older and have had the disease for longer. This explains why, in the present study, the group of patients with CLBBB presented a prevalence of 67.7%. Conversely, the group without CLBBB have a lower prevalence (46.8%).

The reasons for the different performances of the several electrocardiographic criteria are not clear. However, they are related to the specificity of parameters that compose each criterion, with the limitations of each method, which essentially stem from the electrical activity of the cardiac muscle and are, deductively, correlated to the three-dimensional anatomic alteration. Moreover, besides the specific limitations of each criteria in particular, there are also individual characteristics of the studied sample.

Conclusion

CLBBB modifies ECG sensitivity and specificity in the detection of LVH. However, the best diagnostic performance of the ECG, in the presence of CLBBB, occurred with Sokolow-Lyon voltage and RaVL criteria. The other electrocardiographic criteria presented expressive losses in specificity, rendering them less indicated in the presence of this conduction disturbance. Considering this is a study performed in a relatively young, hypertensive population in outpatient care, caution is recommended when transferring these results onto a group of older patients with more advanced hypertensive diseases.

Author contributions

Conception and design of the research: Burgos PFM, Bianco HT, Póvoa R; Acquisition of data: Burgos PFM, Luna Filho B, Costa FA, Bombig MTN, Souza D, Bianco HT, Oliveira Filho JA, Póvoa R; Analysis and interpretation of the data: Burgos PFM; Writing of the manuscript: Burgos PFM, Bianco HT, Izar MCO, Fonseca FAH, Póvoa R; Critical revision of the manuscript for intellectual content: Póvoa R.

Potential Conflict of Interest

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

Sources of Funding

This study was partially funded by Capes.

Study Association

This article is part of the thesis of Doctoral submitted by Paula Freitas Martins Burgos, from Federal University of Sao Paulo.

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Multiprofessional Treatment of High Blood Pressure in Very Elderly Patients

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Abstract

Background: As the world population ages, patients older than 80 years, known as very elderly, are more frequently found. There are no studies in this age group aimed at analyzing the multidisciplinary intervention in the treatment of systemic arterial hypertension (SAH) and some comorbidities.

Objectives: To assess the effect of a multidisciplinary approach in very elderly hypertensives cared for at a specialized service.

Methods: Longitudinal retrospective cohort study in a multidisciplinary service specialized in the SAH treatment in the Brazilian West-Central region. Patients aged 80 years and older by June 2015 were included. Data from the first (V1) and last visit (Vf) were assessed. Anthropometric variables, blood pressure (BP), renal function, pharmacological treatment, lifestyle, comorbidities and cardiovascular events were studied, comparing data from V1 and Vf. Controlled BP was defined as systolic blood pressure (SBP) lower than 140 mm Hg and diastolic blood pressure (DBP) lower than 90 mm Hg. Statistical analyses were performed with SPSSR software, version 21.0. Values of p<0,05 were considered significant.

Results: Data of 71 patients were assessed with a mean follow-up time of 15,22 years. Their mean age at V1 was 69.2 years, and, at Vf, 84.53 years, and 26.8% of them were males. There was a significant reduction in mean SBP (157.3 x 142.1 mm Hg; p<0.001) and DBP (95.1 x 77.8 mm Hg; p<0.001), with an increase in BP control rates from V1 to Vf (36.6 x 83.1%; p<0.001). The number of antihypertensive drugs used increased (1.49 x 2.85; p<0.001), with an increase in the use of angiotensin-converting enzyme inhibitors (22.5 x 46.5%; p=0.004), angiotensin II receptor blockers (4.2 x 35.2%; p<0.001) and calcium-channel blockers (18.3 x 67.6%; p<0.001). There was a reduction in total cholesterol (217.9 x 191 mg/dL; p<0.001) and LDL-cholesterol (139.6 x 119.0 mg/dL; p<0.001), but worsening of the glomerular filtration rate (62.5 x 45.4 mL/min; p<0.001).

Conclusion: The multidisciplinary intervention in very elderly hypertensives increased BP control rate, with optimization of the pharmacological treatment. (Arq Bras Cardiol. 2017; 108(1):53-59)

Keywords: Hypertension; Aged, 80 and over; Patient Care. Team; Aging; Cohort Studies.

Introduction

From the chronological viewpoint, elderly are defined as individuals aged 65 years and older living in developed countries, or aged 60 years and older living in developing countries.[1] In that age group, those who have reached the eighth decade are called 'oldest old' or 'very elderly'.[2]

Aging, regardless of ethnical, social and cultural factors inherent in each population, is associated with a higher probability of chronic noncommunicable diseases (NCDs) secondary to morphophysiological and functional changes, as well as to lifestyle.[3]

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Manuscript received May 19, 2016; revised manuscript July 29, 2016; accepted September 13, 2016

DOI: 10.5935/abc.20160196

Systemic arterial hypertension (SAH) is the most common NCD among the elderly.[4] Its prevalence increases progressively as age advances, SAH being considered the major modifiable risk factor for cardiovascular disease in the geriatric population.[5] There is a direct and linear relationship of blood pressure (BP) and age, the prevalence of SAH being higher than 60% in those older than 65 years.[6]

Because SAH is a multifactorial clinical syndrome, a multiprofessional team to support hypertensives is desirable whenever possible.[7,8] That team should comprise all professionals who manage hypertensives,[9,10] an initiative recommended in national and international guidelines.[11,12]

To our knowledge, there is no study in very elderly hypertensives confirming the benefit of the multiprofessional management.

This study was aimed at assessing the result of the multiprofessional treatment of very elderly hypertensive patients undergoing regular follow-up in a reference service for the multidisciplinary treatment of SAH.

Methods

This study was assessed and approved by the Ethics Committee in Human and Animal Medical Research of the institution (protocol 700.942 of 06/26/2014).

This study assessed retrospectively data of very elderly patients undergoing regular follow-up in a multiprofessional service of reference for the treatment of SAH in the Brazilian West-Central region.

The service has been existing for more than 20 years, dedicated to the multiprofessional care of hypertensive patients, teaching and research. Its professional team consists of physicians (cardiologists, endocrinologists and nephrologists), nurses, nutritionists, physical therapists, physical education teachers, psychologists and musical therapists. Patients are followed up at maximum 3-month intervals between the appointments, regardless of the health specialty responsible for the appointment. In addition, educational and health promotion activities are routinely performed.

The medical team assesses symptoms, lifestyle habits and medications used, performs complete physical examination, interprets the complementary tests and establishes the management, which includes: prescription of drugs and nonpharmacological measures; request of complementary tests; and scheduling of return appointments, with definition of the time interval and designation of the assisting professional. In addition, if clinical decompensation is identified in the medical consultation, the patient is referred to emergency care or hospitalization.

The nurse team assesses symptoms, vital signs, lifestyle habits and medications used, in addition to instructing about treatment adherence in both pharmacological and nonpharmacological aspects. They define the interval of the nurse return appointment and refer patients for medical consultation, when necessary or when the time since the last medical consultation is longer than 6 months.

The group of nutritionists emphasizes nonpharmacological aspects of care, specifically the diet. They collect dietary data and assess anthropometric data and vital signs. The management is aimed at dietary guidance with emphasis on salt restriction and prescription of special diets, when necessary.

The other health specialties of the service do not conduct formal appointments, but rather a series of educational interventions to promote health with the hypertensive patients. The physical therapists and physical education teachers conduct periodical meetings previously scheduled or meet with patients at the waiting room to emphasize the importance of regular physical activity practice and preventive measures of injuries and falls. In addition, they promote assisted group physical activity for patients. Similarly, the psychology and musical therapy teams act mainly in the waiting room, providing instructions and interventions aimed at stress reduction and waiting time improvement.

Since the beginning of the multidisciplinary service more than 20 years ago, consultations have been registered in a standardized form, whose completion by all health professionals is mandatory, ensuring data reliability and reproducibility throughout the follow-up years.

This study included patients aged 80 years and older by June 2015, with at least three consultations attended in the service and reported in the medical record. We collected data of the first consultation, with the patient already diagnosed with SAH and on conventional treatment (nonmultiprofessional) at another health service. Those data were compared with the data of the last consultation at our service reported in the medical record after the institution of multiprofessional treatment, regardless of the time elapsed between both.

The treatment goals established for the very elderly followed the recommendations of the national guidelines at the time, which establish the management adopted at our service since the beginning of its activities. That management abides by the updates and changes of those guidelines.

Controlled BP was defined as systolic blood pressure (SBP) < 140 mmHg and diastolic blood pressure (DBP) < 90 mmHg, in accordance with the recommendations of national guidelines.[11]

The following data were collected from the medical records:

Anthropometric data:

- Consisted in weight, height and calculation of body mass index (BMI) with the Quetelet formula (BMI = weight in kg/height^2 in meter).

Blood pressure:

- The measures were taken with a mercury-column manometer after 5 minutes of rest, twice, at a 2-minute interval, on the upper limb, with the individual sitting with the arm supported. The mean of the last two measures was considered for data analysis.

Laboratory data:

- Renal function with creatinine measure;
- Creatinine clearance calculated with the MDRD formula;[13]
- Fasting glycemia and lipid panel: collected after a 12-hour fasting, and observing the recommendation of no alcoholic beverage consumption in the preceding 48 hours. The enzymatic colorimetric method was used to determine total cholesterol (TC), HDL-cholesterol (HDL), serum triglycerides (TG) and glycemia. The LDL-cholesterol (LDL) level was estimated with the Friedewald formula:[14] LDL = TC – (HDL + TG/5).

Medications being used:

- Anti-hypertensive drugs: analyzing the number and classes of drugs;
- Other drugs: statins and acetylsalicylic acid.

Lifestyle:

- Smoking: smoker or nonsmoker;

- Alcoholism: alcoholic beverage consumption or not;
- Sedentary lifestyle:
  - sedentary – no leisure physical activity
  - non-sedentary – any type of leisure physical activity.

Associated comorbidities:

- Dyslipidemia and diabetes mellitus.

Cardiovascular events:

- Acute myocardial infarction (AMI) – AMI reported in the medical record and confirmed by hospital discharge summary and/or altered levels of tissue necrosis markers;
- Stroke – reported in the medical record and confirmed by hospital discharge summary and/or imaging exam suggestive of cerebrovascular event;
- Need for surgical myocardial revascularization or angioplasty – reported in the medical record and confirmed by hospital discharge summary, surgeon's report and/or angioplasty report.

Data bank and statistical analysis

Data were stored in a data bank structured in Excel (Microsoft) and analyzed comparatively. Statistical analysis was performed using the SPSS software (*Statistical Package of Social Science*, version 21.0, Chicago, IL, USA). Kolmogorov-Smirnov test was used to check if the continuous variables had a normal distribution. Paired Student *t* test was used to compare the numerical variables, expressed as mean and standard deviation. Qualitative variables were compared using McNemar test. The significance level adopted was $p<0.05$.

Results

This study assessed 71 very elderly patients on regular follow-up at our service. The mean follow-up time was 15.22 years (ranging from 3 months to 23.5 years), 85.9% of the patients were followed up for more than 5 years, and only two patients for less than 1 year.

Male patients accounted for 26.8% of the sample. The patients' mean age at the first visit was 69.2 years (range, 57 to 91 years), and, at the final visit, 84.53 years (range, 80 to 94 years).

The BP control rate, which was initially 36.6% (n=26) with conventional treatment, passed to 83.1% (n=59) ($p<0.001$).

Mean BP levels decreased significantly during follow-up, with an increment in the number of anti-hypertensive drugs used and optimization of the drug classes prescribed. That optimization was characterized by an increased use of the first-line drug classes [angiotensin-converting-enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB) and calcium-channel blockers (CCB)] (Tables 1 and 2).

Analyzing the pharmacological treatment and comparing the initial and final prescriptions, a significant increase in the use of both statins (1.4% x 52.1%; $p<0.001$) and acetylsalicylic acid (11.3% x 39.4%; $p<0.001$) was found.

Analysis of laboratory variables evidenced an improvement in TC and LDL, after the institution of multiprofessional treatment, but worsening of the glomerular filtration rate during follow-up (Table 3).

Regarding lifestyle habits, no change was observed in the prevalence of smoking (5.6% x 1.4%; $p=0.250$) and of sedentary lifestyle (14.1% x 8.5%; $p=0.388$), but a significant reduction in the prevalence of alcoholism was observed with multiprofessional treatment (11.3% x 1.4%; $p=0.039$).

The patients' BMI decreased during follow-up, from 27.01 kg/m^2 to 25.6 kg/m^2 ($p=0.001$).

Regarding the comorbidities studied, the number of diabetic patients increased (8.5% x 28.2%; $p<0.001$), as increased the number of dyslipidemic patients (66.2% x 74.6%; $p=0.345$), but with no statistical significance for dyslipidemia. Only two cardiovascular events occurred in the population studied during follow-up.

Discussion

Several national and international studies[7-10,15] have shown the superiority of BP control with the multiprofessional treatment as compared to the conventional treatment. That evidence, however, is not available for very elderly hypertensives. This study showed a significant increase in the BP control rate, with 83.1% of the very elderly hypertensives on multiprofessional treatment showing BP control by the end of follow-up. That exceeds the BP control rates in very elderly hypertensives reported in different clinical contexts. In that age group, North American epidemiological data have shown BP control rates of 30.4%, between 1988 and 1994, and of 53.1%, between 2005 and 2010.[16]

The multiprofessional treatment of very elderly hypertensives reduced SBP levels by 15 mm Hg and DBP levels by 17 mm Hg. That more marked reduction in DBP as compared to SBP has been shown in other studies with the very elderly.[17,18] Similarly, the increase in the number of anti-hypertensive drugs used during follow-up in this study has also been reported in other follow-up analyses of elderly hypertensives.[19]

One marked feature of the pharmacological treatment observed in this study regards the therapeutic regimen optimization adopted during follow-up, characterized by the increased use of first-line drugs, such as ACEIs, ARBs and CCBs. This suggests the good quality of care provided, with alignment of the pharmacological treatment with the recommendations of current guidelines.[11,12]

Another relevant aspect of the pharmacological treatment was the increasing use of statins and acetylsalicylic acid to our patients during follow-up. This indicates the excellence of the treatment conducted by the multidisciplinary team, abiding by guidelines on cardiovascular disease prevention.[20,21]

Regarding the laboratory findings, there was a significant reduction in TC and LDL levels, despite the population's aging. This can be explained by the increase in the use

Table 1 – Mean levels of systolic blood pressure (SBP) and diastolic blood pressure (DBP), and mean number of anti-hypertensive drugs in the initial visit (V1) and final visit (Vf). Goiânia - GO

	V1 (n=71)	Vf (n=71)	p
SBP (mm Hg)	157.3 ± 21.5	142.1 ± 20.9	<0.001
DBP (mm Hg)	95.1 ± 13.9	77.8 ± 10.8	<0.001
Number of drugs	1.49 ± 0.9	2.85 ± 1.2	<0.001

Student t test of related samples; significant: p<0.05; values expressed as means ± standard deviations.

Table 2 – Distribution of the classes of anti-hypertensive drugs in the initial visit (V1) and final visit (Vf). Goiânia – GO

	V1	Vf	p
Diuretic	53.5% (38)	60.6% (43)	0.511
ACEI	22.5% (16)	46.5% (33)	0.004
ARB	4.2% (3)	35.2% (25)	<0.001
CCB	18.3% (13)	67.6% (48)	<0.001
BB	16.9% (12)	16.9% (12)	-
Spironolactone	0	5.63% (4)	0.125
Others	29.6% (21)	16.9% (12)	0.078

McNemar test; significant: p<0.05; values expressed as percentage and absolute numbers; ACEI: angiotensin-converting-enzyme inhibitor; ARB: angiotensin receptor blocker; CCB: calcium-channel blocker; BB: beta-blocker.

Table 3 – Mean levels of laboratory variables in the initial visit (V1) and final visit (Vf). Goiânia – GO

	V1	Vf	p
TC (mg/dL)	217.9 ± 40.5	191 ± 37.3	<0.001
HDL (mg/dL)	47.7 ± 9.8	47.3 ± 11.5	0.772
LDL (mg/dL)	139.6 ± 30.9	119.0 ± 33.2	<0.001
Triglycerides (mg/dL)	135.04 ± 66.85	122.48 ± 50.7	0.101
Glycemia (mg/dL)	102.5 ± 46.9	103.82 ± 29.7	0.819
GFR (mL/min)	62.5 ± 25.7	45.4 ± 15.2	<0.001

Student t test of related samples; significant: p<0.05; values expressed as means ± standard deviations; TC: total cholesterol; HDL: HDL-cholesterol; LDL: LDL-cholesterol; GFR: glomerular filtration rate.

of statins. However, despite the significant BP reduction, the glomerular filtration rate worsened during the 15-year follow-up. This can also be explained by the population's aging, because renal function loss is known to be progressive from the age of 40 years onward.[22]

The lifestyle change of the very elderly patients studied was small. This is expected for an octogenarian population, because age is one of the greatest limiting factors of lifestyle changes.[23,24] Nevertheless, there was a significant reduction in alcoholism in the group assessed.

Regarding nutrition, aging is associated with a decline in undernutrition and an expressive increase in obesity prevalence.[25,26] Obesity is not simply weight increase, but excessive body fat. Aging is associated with increased fatty mass and changes in its distribution pattern, with a 20% to 30% increase in total body fat (2% to 5%/decade, after the age of 40 years).[27,28] The BMI reduction observed in our sample has been reported in follow-up studies of elderly patients;[29] however, taking only BMI into consideration is a superficial way to assess the nutritional status of the elderly.

Of the comorbidities considered in this study, a significant increase in new cases of diabetes was demonstrated and reproduces the findings of long-term follow-up studies of hypertensive patients.[29,30] This is in accordance with the degenerative character of diabetes, widely demonstrated in observational studies, even in non-elderly.[31,32]

It is worth noting the reduced number of events in the group studied, which should be further investigated. Even the

patients being very elderly and the follow-up time prolonged, only two cardiovascular events occurred. This might indicate that the multiprofessional treatment is capable of reducing cardiovascular outcomes in the very elderly.

One limitation of this study is its retrospective character. However, the fact that data collection was performed in a structured service since its conception for the generation of scientific knowledge reduces that limitation. The structure of the medical records is objective, and the completion of its mandatory fields is exhaustively trained. This ensures the ability of generating reliable data, although not prospectively.

In addition, the follow-up time was not homogeneous in this sample. Therefore, a minimum number of three consultations in our service was an inclusion criterion, ensuring not only the patient's minimum commitment to the service, but care provision by at least two professionals of different health areas within those three consultations. In addition, a more careful analysis of that follow-up time shows that most patients (85.9%) underwent the multiprofessional management for at least 5 years.

Another limitation was the lack of a control group. There was no comparison with a similar group, because all our patients undergo the same multidisciplinary treatment. This study compared the initial visit at the multidisciplinary service, when the patients were not on a multidisciplinary anti-hypertensive treatment, with the final visit, when they were already on the multiprofessional treatment. The use of a control group would be ideal for this study; however, the way we compared indirectly two treatment patterns in a little studied group of difficult follow-up should be highlighted. This study generates hypotheses, because the multiprofessional management of very elderly hypertensives has not yet been assessed with any methodology. In addition, the extremely positive results found will encourage further studies on that type of treatment, as well as its more comprehensive use.

The perspectives of investigating multiprofessional interventions in very elderly hypertensives are innumerous and extremely promising. Our data suggest that, similarly to other subgroups of hypertensive patients, the very elderly do benefit from a strategy of multifaceted treatment, which provides a more comprehensive and effective therapy.

Conclusions

The multiprofessional intervention in very elderly hypertensives reduced BP and increased its control rate, with optimization of the pharmacological treatment instituted.

Author contributions

Conception and design of the research, Acquisition of data, Analysis and interpretation of the data, Statistical analysis, Obtaining financing, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Jardim LMSSV, Jardim TSV, Souza WKSB, Pimenta CD, Sousa ALL, Jardim PCBV.

Potential Conflict of Interest

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

Sources of Funding

This study was partially funded by Fundação de Amparo à Pesquisa do Estado de Goiás.

Study Association

This article is part of the thesis of master submitted by Luciana Muniz Sanches Siqueira Veiga Jardim, from Universidade Federal de Goiás.

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Original Article

Lifestyle Intervention on Metabolic Syndrome and its Impact on Quality of Life: A Randomized Controlled Trial

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Abstract

Background: Lifestyle intervention programs can reduce the prevalence of metabolic syndrome (MetS) and, therefore, reduce the risk for cardiac disease, one of the main public health problems nowadays.

Objective: The aim of this study was to compare the effects of three types of approach for lifestyle change programs in the reduction of metabolic parameters, and to identify its impact on the quality of life (QOL) of individuals with MetS.

Methods: A randomized controlled trial included 72 individuals with MetS aged 30-59 years. Individuals were randomized into three groups of multidisciplinary intervention [Standard Intervention (SI) - control group; Group Intervention (GI); and Individual Intervention (II)] during 12 weeks. The primary outcome was change in the metabolic parameters, and secondarily, the improvement in QOL measures at three moments: baseline, 3 and 9 months.

Results: Group and individual interventions resulted in a significant reduction in body mass index, waist circumference, systolic blood pressure at 3 months and the improvement of QOL, although it was significantly associated with the physical functioning domain. However, these changes did not remain 6 months after the end of intervention. Depression and anxiety were significantly associated with worse QOL, although they showed no effect on the response to intervention.

Conclusion: Multidisciplinary intervention, especially in a group, might be an effective and economically feasible strategy in the control of metabolic parameters of MetS and improvement of QOL compared to SI, even in a dose-effect relationship. (Arq Bras Cardiol. 2017; 108(1):60-69)

Keywords: Metabolic Syndrome; Life Style; Quality of Life; Cardiovascular Diseases; Prevention; Risk Factors.

Introduction

Metabolic syndrome (MetS), considered a complex set of cardiovascular risk factors related to abdominal fat and resistance to insulin, has been increasing progressively and is strongly associated with high cardiovascular morbimortality,[1,2] with estimated prevalence around 23.7%, according to Adult Treatment Panel III criteria.[3] The main recommendations for MetS prevention and treatment are the change in lifestyle through a multifactor approach based on education, regular physical exercise and a healthy diet, as well as pharmacological strategies.[1]

Studies show that programs of lifestyle change that include nutritional education and supervised physical exercise were efficient to achieve the proposed goals for the treatment of MetS.[4,5] However, few studies use this multifactor approach in their interventions, including all main aspects in the intervention.[6-10]

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Manuscript received April 08, 2016; revised manuscript September 13, 2016; accepted September 13, 2016.

DOI: 10.5935/abc.20160186

Furthermore, an increasing number of studies support the idea that MetS is significantly associated with impaired quality of life (QOL),[11-13] and that this association can be predictive of mortality.[14] Otherwise, few intervention studies confirm the association between MetS and QOL, showing improvement in the MetS components, followed by better QOL scores after lifestyle change intervention,[7-10,15-17] in up to 24 months of follow-up.[7]

Moreover, studies also show association between depression, anxiety and MetS, although they are not conclusive. While some studies demonstrate the association between MetS and depression,[18-21] others reveal only association between MetS and anxiety.[22,23] For this reason, the analysis of the prevalence of these clinical situations was carried out in this study in order to identify whether there is some influence of these variables in the recovery or the improvement process of the metabolic condition.

The study of prevention and treatment strategies, as well as the relationship between MetS and QOL, due to its relevance, complexity and treatment possibility, have been receiving little attention in medical literature. Thus, the aim of this study is to test three different programs with a multidisciplinary approach for lifestyle change in the reduction of metabolic parameters and QOL improvement in the population of a rapidly developing country.

Methods

Participants

Randomized controlled trial was conducted at the *Centro de Reabilitação do Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul (HSL-PUCRS)*, a general university hospital in Southern Brazil. The trial was registered in clinical trial registry Brazil, ReBEC, number RBR9wz5fc.

Inclusion criteria: waist circumference (WC) measure > 88 cm for females and > 102 cm for males, followed by at least two criteria: a) systolic blood pressure (SBP) ≥ 130 mmHg, diastolic blood pressure (DBP) ≥ 85 mmHg; b) triglycerides (TGL): ≥ 150 mg/dL; c) high-density lipoprotein cholesterol (HDL-C): < 40 mg/dL for males and < 50 mg/dL for females;[1] and d) fasting glucose (FG): ≥ 100 mg/dL.[2]

Exclusion criteria: a) absolute contraindication for physical activity due to musculoskeletal, neurological, vascular, lung and cardiac problems; b) pregnancy; c) diagnosis of severe psychiatric disorders, significant cognitive impairment, assessed by the Mini Mental State Examination (scores under 24 as a cutoff point); d) unavailability to participate in the program.

Procedures

Individuals recruited by media advertising in newspapers, radio and websites participated in a screening meeting when they were told about the objectives, inclusion and exclusion criteria of the study. After identifying the participants who were able to join the study, they were consecutively randomized into the three kinds of intervention for lifestyle change, by simple randomization 1:1:1. This procedure occurred successively in four waves till the sample size was reached.

After randomization, each individual received the information regarding the procedures involved in the study, specific for each program, and signed the written informed consent previously approved by the Ethics Committee in Research of PUCRS, under number 10/05153. Initial interviews were scheduled, as well as the following appointments, according to the intervention program drawn. All interviews and interventions were previously confirmed by telephone and performed by the researchers, who were submitted to quality standard training for data collection and intervention procedures.

Standard intervention

The standard intervention (SI), considered in this study as the control group, was the non-pharmacological intervention recommended by the main guidelines for the clinical management of MetS. The volunteers in this group had two consultations: at baseline and 3 months. Consultations were carried out individually by the nursing staff: the first one for standard guidance on exercising, diet and self-care, according to the guidelines. The diet program is based on the healthy diet model of the Brazilian Ministry of Health[24] and the self-care program, focused on the administration of the medications in use and general health care. The second consultation approached the facility and difficulty to follow recommendations for changing eating habits and regular exercising.

Intervention group

The group intervention (GI) worked the change in lifestyle through the discussion of pre-defined themes of health education, focused on the main cardiovascular risk factors considered changeable which are associated with MetS, as well as motivation for changing behavior, based on the transtheoretical model of change.[25] The GI appointments occurred weekly during 1 hour and 45 minutes, coordinated by a psychologist, a nurse, a physical therapist and a nutritionist. During the first 45 minutes, volunteers discussed a health topic proposed by the team. Soon after that, they discussed and tested strategies for changing eating habits and regular exercising, which could be included in the volunteers' routine, according to the group's motivation. The groups were composed of 10 to 12 individuals.

Individual intervention

The volunteers in the individual intervention (II) group participated in weekly individual appointments with the psychology and nutrition teams, and exercised regularly with the physical therapy team.

Nutritional intervention: based on the needs of each participant according to the aspects that should be changed, respecting intrinsic and extrinsic conditions necessary for the changing process of eating habits. During the weekly appointments, body weight was measured and adhesion to the diet program was assessed through a brief 24-hour recall. In addition, possible difficulties in the adhesion to the strategies and goals agreed in the previous consultation were constantly recorded and monitored. MetS-related themes were developed based on a pre-defined program and addressed individually, aiming to improve the understanding and adhesion to the strategies for changing eating habits.

Psychological intervention: based on the transtheoretical model of change,[25] adapted for individual model, which worked on the different stages of change based on a structured program, with pre-defined objectives, as well as the specific change processes. Materials such as flyers were used and filled out by the volunteer.

Physical intervention: composed of 36 sessions on the treadmill for 60 continuous minutes each. They occurred three times a week, and the intensity was adjusted according to the recommended heart rate (HR) for each individual. The training range remained between 75% and 85% of the maximum HR, assessed by the graded exercise test (GXT). During physical exercise, BP, HR and symptoms of cardiovascular alterations were monitored. The speed and inclination were constantly adjusted to keep HR within the training range.

Measurements

All groups were assessed at baseline, end of interventions (3 months), and 6 months later (9 months). The assessment comprised physical, metabolic, behavioral and psychological aspects of the individuals studied.

Sociodemographic data

Data on personal identification, psychosocial and health aspects, such as diagnosis, medications in use and lifestyle (smoking habit, use of alcohol, physical activity),

were collected in individual interviews by use of a structured questionnaire.

Alcohol use: male intake - up to 1 oz (30 ml) of ethanol/day; female intake – up to 0.5 oz of ethanol/day.[26]

Physical activity: exercise at least once a week as opposed to no exercise, the latter characterizing a sedentary lifestyle.

Clinical parameters

The anthropometric profile assessment included measuring WC, with a millimeter non-extensible long tape at the abdomen's maximum extension,[27] body weight, and height, to calculate body mass index (BMI). Individuals were barefoot and lightly dressed having body weight measured, through the use of a properly calibrated 160-kg Cauduro® scale. The Sunny® vertical anthropometer was used for measuring height. Blood pressure values were assessed in three consecutive measurements, according to the American Hypertension Guidelines.[26]

Laboratory parameters

Blood samples were collected after fasting for the analysis of biochemical markers. Plasma and serum were separated and stored at -80°C for later analyses at HSL-PUCRS' laboratory. The tests analyzed were FG, total cholesterol, HDL-C and TGL, while low-density protein was determined indirectly.

Depression and anxiety

These variables were measured through the Adult Self Report (ASR),[28] self-administered scale of 126 items that aims to identify the aspects of adults' adaptive functioning between the ages of 19 and 59 years, identifying behavioral and emotional problems and higher incidence of psychopathological disorders, such as anxiety and depression. Scores range from 0 to 100, with higher scores indicating a greater number of behavioral problems. Individuals with scores above 60 within the internalization scale, who demonstrate borderline and clinical status or under drug treatment, were classified as depressed or anxious.

Quality of life

This variable was assessed using the Medical Outcomes Study Short Form, General Health Survey (SF-36)[29] that evaluates the QOL of individuals in relation to their disease. It consists of 36 questions, divided into the following 8 domains: physical functioning; limitations due to physical problem; bodily pain; general health perceptions; vitality; social functioning; role limitations due to emotional problems; and mental health. These domains were summarized into Physical and Mental Component Summary (PCS and MCS, respectively). The scores range from 0 to 100 for each domain, in which higher scores indicate better QOL.

Statistical analysis

For $\alpha = 0.05$, 90% power and estimating a difference between WC averages of 0.9 units of standard deviation, a sample number of 27 volunteers in each group was calculated. Considering maximum loss of 20%, the sample size became 34 per group.

Quantitative data were described as mean and standard deviation. Categorical variables were presented as counts and percentages. Comparisons of quantitative data used the one-way Anova for 3 groups and *t* test for 2 groups. For categorical data, we used the chi-square and Fisher's exact test, when necessary. To evaluate the outcomes, MetS components and QOL scores, considering adjustment for confounding factors, analysis of covariance and multiple linear regression were used. Additionally, analysis of covariance was used for comparisons at 3 and 9 months, adjusting for baseline measures and other confounding factors. The results were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) program, version 21, with an alpha level of significance at 5%.

Results

This study included 72 individuals who concluded the intervention, divided into three groups: SI, 19; GI, 25; and II, 28 (Figure 1). Individuals who did not complete the trial and the ones that remained in the study showed similar characteristics regarding race, marital status and BMI. However, there were more women with lower levels of education (data not shown).

According to Table 1, most of the population studied was female, white and had high levels of education. Groups showed similar distributions in terms of general characteristics, as well as MetS and QOL components, without statistically significant differences at baseline.

Table 2 presents results regarding MetS components in the three types of intervention. Although there was a reduction in TGL, FG and DBP, only BMI, WC and SBP showed significant reduction in their mean scores after 12 weeks. Compared to baseline, only II was associated with a significant reduction in SBP levels. On the other hand, regarding BMI and WC, both the GI and II showed a significant reduction in their mean scores, and GI was more effective in the reduction of BMI (Figure 2).

Regarding QOL scores, almost all domains in all types of intervention showed an increase in their mean scores after 12 weeks. However, only physical functioning showed significant association ($p=0.024$), although general health had borderline significance. Compared to baseline, in almost all SF-36 domains, QOL improvement was higher in the II, although no statistically significant difference among the groups was found. Considering the PCS and MCS scores, no significant difference was found after the intervention. Similarly, there was no significant difference among the groups, despite the fact that II encouraged a larger increase in the MCS (Table 3).

Nevertheless, these results concerning the improvement of metabolic parameters, as well as QOL, were not kept 6 months after the end of intervention.

The prevalence of anxiety and depression was 41.7% and 22.2%, respectively. Regarding metabolic parameters, there was no significant association between MetS components and depression and anxiety. Concerning QOL, the mean scores for individuals with anxiety were lower in all SF-36 domains compared to those who did not have anxiety, although they were significant only in 5 domains (Table 4).

Among the individuals who had depression, besides the lower QOL mean scores, all QOL domains, except for physical

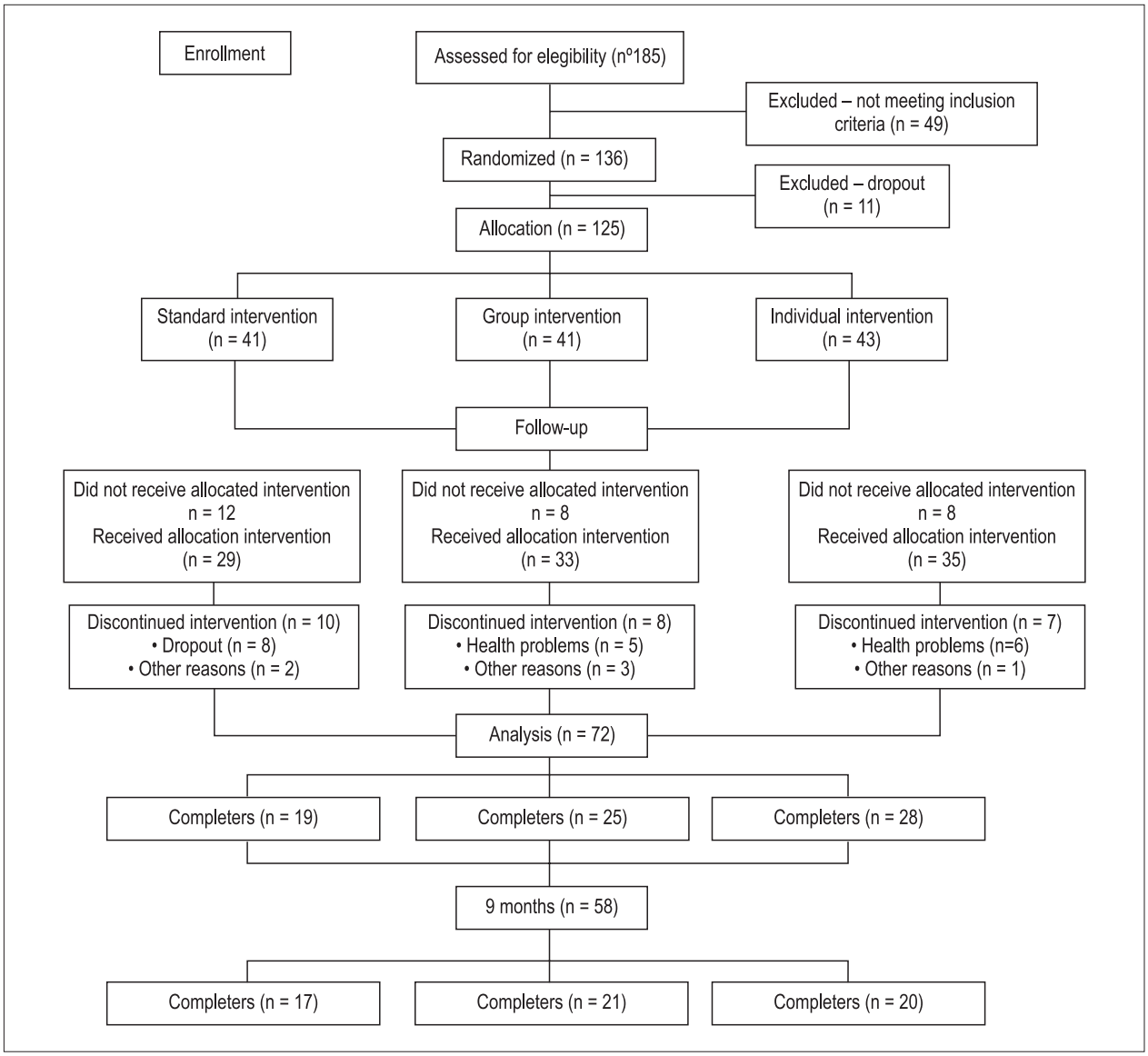

Figure 1 – *Flow chart of study participants.*

functioning and general health, showed significant difference when compared to those who did not have depression (Table 4). Regarding the influence of anxiety and depression in the intervention response, this study demonstrated that only depression had a negative significant effect on the scores of the SF-36 role emotional domain, although there was no statistical difference among the groups analyzed. MetS components, as well as the other QOL domains, showed no association with depression and anxiety.

Discussion

This study tested 3 types of multidisciplinary intervention for lifestyle change in individuals with MetS, followed during 9 months, to determine its effects in the reduction of metabolic parameters and improvement of QOL. Results suggest that GI, as well as II were associated with significant BMI and WC reduction, although only II had been significantly associated with lower SBP levels, which partially confirms the hypothesis previously established. It was surprising that GI reduced BMI levels more than II. According to a previous study,[30] which demonstrated that standard advice is not sufficient to obtain changes in lifestyle and cardiovascular risk factors, SI, even showing a slight reduction in WC, did not have positive results in the improvement of the other metabolic parameters or statistically significant improvements in QOL, and II and GI showed a better response to intervention. Despite the fact that GI showed a smaller WC reduction than II, considering the fact that a 3-cm reduction already results in significant improvement of cardiometabolic risk factors,[31] GI proved effective once it promoted a 4.4-cm reduction.

In accordance with previous reports,[7-9,15] this study demonstrated that lifestyle intervention produced beneficial

Table 1 – Baseline characteristics of the study participants

Characteristics	SI n=19	GI n=25	II n=28	p
Age, years	52.1±7.2	50.9±7.7	51.6±5.6	0.831 *
Female, n (%)	7 (36.8)	13 (52.0)	20 (71.4)	0.055 †
White, n (%)	17 (89.5)	23 (92.0)	24 (85.7)	0.763 †
Marital status, n (%)				0.768 †
With companion	12 (66.7)	15 (71.4)	17 (68.0)	
Single	3 (16.7)	5 (23.8)	6 (24.0)	
Widowed	3 (16.7)	1 (4.8)	3 (16.7)	
Level of education, n (%)				0.424 †
4 years of study	0 (0.0)	0 (0.0)	1 (3.6)	
5 to 8 years of study	0 (0.0)	0 (0.0)	1 (3.6)	
Over 9 years of study	19 (100.0)	25 (100.0)	26 (92.9)	
Sedentary lifestyle, n (%)	11 (57.9)	16 (64.0)	21 (75.0)	0.442 †
Smoking, n (%)	0 (0.0)	2 (8.0)	1 (3.6)	0.305 †
Use of alcohol, n (%)	0 (0.0)	2 (8.0)	2 (7.1)	0.280 †
BMI, kg/m^2	33.5±4.1	35.1±3.6	33.7±3.2	0.283 *
MetS Components				
WC (cm)	112.6±8.3	112.9±10.0	110.7±7.2	0.605 *
SBP (mmHg)	132.6±10.3	131.8±15.2	135.5±13.5	0.577 *
DBP (mmHg)	90.6±10.3	89.7±12.7	89.2±11.6	0.922 *
TGL (mg/dL)	174.6±60.2	266.5±227.0	200.4±84.9	0.101 *
HDL-C (mg/dL)	46.4±8.9	47.7±11.3	48.2±14.1	0.872 *
SF-36				
Physical functioning	76.8±20.6	74.8±18.1	77.0±17.2	0.898 *
Role-physical	75.0±35.4	77.1±26.5	86.6±30.0	0.365 *
Bodily pain	62.8±21.9	63.6±21.5	70.9±22.8	0.369 *
General health	73.2±14.8	72.8±18.3	72.0±18.6	0.973 *
Vitality	58.9±22.9	61.0±22.7	58.9±22.0	0.933 *
Social functioning	82.4±23.5	78.3±23.8	80.8±16.3	0.810 *
Role emotional	80.7±25.6	72.0±39.3	70.2±38.8	0.600 *
Mental health	71.6±18.8	71.7±22.8	68.9±16.8	0.842 *
Physical component summary	46.8±8.5	47.2±6.8	49.9±5.5	0.227 *
Mental component summary	50.2±10.2	48.9±14.1	47.1±9.7	0.664 *

ANOVA; †: Chi-square test; SI: standard intervention; GI: group intervention; II: individual intervention; BMI: body mass index; MetS: metabolic syndrome; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; TGL: triglycerides; HDL-C- High: density lipoprotein cholesterol.

effects on metabolic parameters, especially on weight loss and WC, and the average of WC reduction in the II group was similar to the one found in individuals who underwent an intensive lifestyle intervention program.[8] However, the present study, including all interventions, did not show statistically significant effects on FG, TGL,[32] DBP and HDL-C.[8,32] Although this study demonstrated improvement in QOL in both groups after intervention, in accordance with previous studies,[7-10,15-17,32] only the physical functioning domain, also shown in other studies,[7,10] showed a significant association. However, opposing data from most studies which demonstrated that QOL improvement is maintained after intervention for a period of 12,[9] 24[17] and up to 36 months,[7] this study showed this effect only after the end of intervention.[32] Due to the fact that there are no studies comparing the different approaches for individual and group lifestyle interventions in

Table 2 – Comparisons between the 3 groups at 3 and 9 months in metabolic parameters by ANCOVA

Variables	SI		GI		II		p	p *
	Month 3 (n=19)	Month 9 (n=17)	Month 3 (n=25)	Month 9 (n=21)	Month 3 (n=28)	Month 9 (n=20)		
MetS Components								
BMI (kg/m^2)	33.7±0.3	33.2±0.4	33.3±0.3	33.5±0.4	32.2±0.2	32.4±0.4	<0.001	0.144
WC (cm)	110.2±1.2	108.0±1.3	108.5±1.0	108.0±1.1	105.4±1.0	106.4±1.1	0.009	0.522
SBP (mmHg)	134.3±2.8	132.9±3.9	130.6±2.5	128.8±3.3	120.6±2.3	124.6±3.6	0.001	0.330
DBP (mmHg)	86.4±2.5	85.6±2.2	84.6±2.2	82.8±1.8	80.9±2.0	80.7±1.9	0.199	0.263
TGL (mg/dL)	215.0±14.5	182.1±19.7	203.6±12.8	210.7±16.1	176.2±12.3	203.4±15.9	0.103	0.539
HDL-C (mg/dL)	43.6±1.4	45.2±1.7	48.0±1.2	47.4±1.4	46.9±1.2	46.3±1.4	0.060	0.616
FG (mg/dL)	111.0±4.3	112.3±5.3	107.7±3.6	106.9±4.4	99.5±3.7	105.6±4.3	0.108	0.600

*p: statistical significance at 3 months; p *: statistical significance at 9 months; SI: standard intervention; GI: group intervention; II: individual intervention; MetS: metabolic syndrome; BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; TGL: triglycerides; HDL-C: high-density lipoprotein cholesterol; FG: Fasting glucose.*

individuals with MetS, the finding that II showed higher effect on most QOL domains suggests that this result might be attributed to the intensity of intervention. This occurs because, according to the results of previous studies comparing types of intervention related to their intensity (moderate x intensive), the individuals who took part in more intensive programs showed significantly better results in weight reduction[33] and in most QOL domains.[8] Similarly, it is inconclusive whether this improvement in QOL might be related to weight loss, due to the relationship between BMI increase and QOL impairment,[34] improvement in the physical condition[16] or both.[10]

Another important contribution of this study is the fact that it demonstrated the influence of depression and anxiety in the reduction of scores in most QOL domains for individuals with MetS. Previous studies have already shown the association between MetS and depression and anxiety,[18-23] but only a few analyzed its impact on QOL.[35] Despite the fact that there was no significant influence of these variables in the response to intervention, deserves attention, as these clinical situations lead to QOL impairment, which justifies the importance of screening individuals with MetS for depression and anxiety.

This study provides preliminary data that a group intervention program can present results similar to individual intervention and, for this reason, might be an important prevention strategy, although its effects were not kept after the intervention. Therefore, it seems important to carry out a regular follow-up, as well as measures that encourage individuals to continue the lifestyle changes to maintain these effects. Moreover, group programs for lifestyle change seem to be an alternative intervention strategy that presents the best cost-benefit ratio in the management of metabolic parameters, as well as QOL of individuals who suffer from this important clinical condition nowadays.

A limiting factor in this study was the dropout rate, which hindered the use of the intention to treat analysis. Although this rate was similar between GI and II interventions, SI presented a high figure. A possible explanation for this can relate to the fact that the SI did not meet the individuals' expectations, since they were looking for a new type of intervention. Although the dropout occurred during follow-up, individuals who did not complete the study showed no significant differences when compared to individuals who remained in the study, which might minimize the effect of these losses. Another limiting factor concerns the relatively small intervention period of 12 weeks. Although this is the period normally used in other trials, metabolic parameters and QOL improvement results might have been kept if the intervention had lasted longer.

Conclusion

Multidisciplinary intervention, especially in a group, might be an effective and economically feasible strategy to control the metabolic parameters of MetS and improvement of QOL compared to SI, even in a dose-effect relationship.

Acknowledgments

The authors thank their colleagues of the MERC research group, for their collaboration, especially academic students, for helping with data collection. We also thank Isabel Merlo Crespo for her important collaboration in our library searches and Dr. Mário Wagner for the statistical work developed throughout the project.

Author contributions

Conception and design of the research and Analysis and interpretation of the data: Saboya PP, Bodanese LC, Zimmermann PR, Gustavo AS; Acquisition of data: Saboya PP, Gustavo AS, Macagnan FE, Feoli AP, Oliveira MS; Statistical analysis and Writing of the manuscript: Saboya PP; Obtaining financing: Gustavo AS, Macagnan FE, Feoli AP, Oliveira MS; Critical revision of the manuscript for intellectual content: Saboya PP, Bodanese LC, Zimmermann PR, Gustavo AS, Macagnan FE, Feoli AP, Oliveira MS.

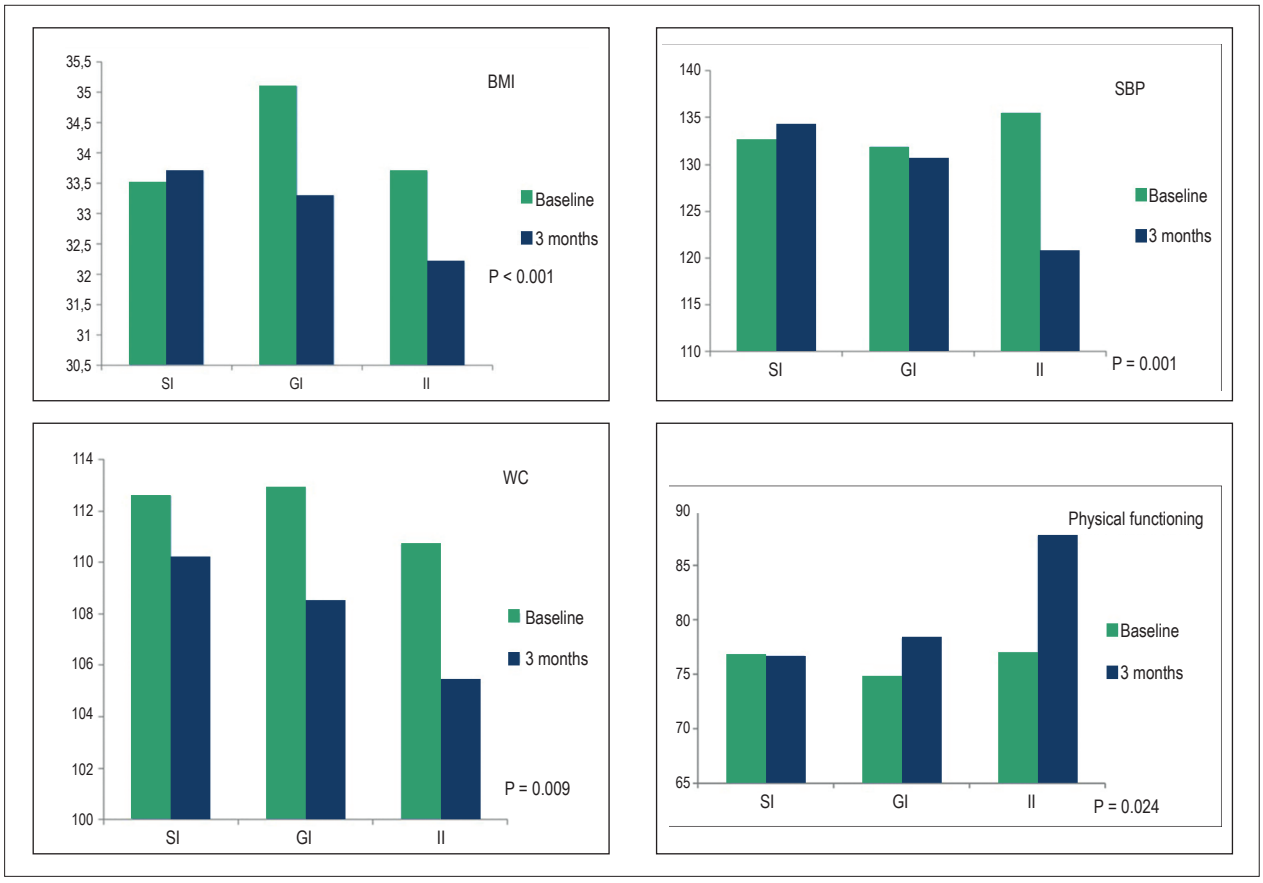

Figure 2 – *BMI, WC, SBP and Physical Functioning measures at baseline and 3 months. BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; SI: Standard Intervention; GI: Group Intervention; II: Individual intervention.*

Table 3 – Comparisons between the 3 groups at 3 and 9 months in the QOL domains by ANCOVA

Variables	SI		GI		II		p	p *
	Month 3 (n=19)	Month 9 (n=17)	Month 3 (n=25)	Month 9 (n=21)	Month 3 (n=28)	Month 9 (n=20)		
SF-36								
PF	76.7±3.5	76.8±4.5	78.3±3.0	78.5±4.1	87.7±2.8	75.3±4.1	0.024	0.865
RP	83.6±5.3	86.5±9.0	92.1±4.7	73.7±8.1	88.4±4.4	82.7±8.2	0.488	0.543
BP	72.8±5.0	70.5±5.4	73.3±4.4	65.9±4.8	79.3±4.2	61.7±5.1	0.511	0.513
GH	79.6±2.8	79.5±3.9	78.0±2.5	76.2±3.5	85.8±2.3	78.8±3.6	0.057	0.799
VT	69.5±3.1	66.9±4.5	71.2±2.7	64.3±4.1	77.6±2.6	68.9±4.2	0.096	0.732
SF	84.2±4.2	78.9±5.3	87.2±3.6	78.5±4.8	92.7±3.5	81.2±5.1	0.272	0.922
RE	88.8±6.8	79.6±7.3	80.2±5.9	87.9±6.6	88.4±5.6	81.7±6.7	0.522	0.676
MH	77.8±3.0	72.9±3.9	76.1±2.6	77.3±3.5	82.7±2.5	75.1±3.6	0.163	0.708
PCS	49.2±1.5	50.3±2.3	50.6±1.3	46.7±2.0	51.8±1.3	47.2±2.2	0.444	0.477
MCS	53.2±1.8	49.8±2.2	52.2±1.6	52.5±2.0	55.2±1.5	52.4±2.1	0.377	0.606

*p: statistical significance at 3 months; p *: statistical significance at 9 months; SI: standard intervention; GI: group intervention; II: individual intervention; SF-36: Medical Outcome Study Short Form General Health Survey; PF: physical functioning; RP: Rrle-physical; BP: bodily pain; GH: general health; VT: vitality; SF: social functioning; RE: role-emotional; MH: mental health; PCS: physical component summary; MCS: mental component summary.*

Table 4 – Averages of the SF-36 scores of individuals with depression (DEP) and without depression (N-DEP) and with anxiety (ANX) and without anxiety (N-ANX)

Variables (n=72)	DEP	N-DEP	p	ANX	N-ANX	p
SF-36						
Physical functioning	73.1±5.3	77.0±2.3	0.502	75.0±3.2	77.0±2.9	0.643
Role-physical	54.7±8.9	87.7±3.3	0.002	76.7±5.2	82.9±5.0	0.388
Bodily pain	52.2±4.2	70.2±2.9	0.001	59.1±3.5	71.3±3.5	0.016
General health	65.7±4.8	74.6±2.2	0.107	70.9±2.8	73.8±2.9	0.471
Vitality	45.9±5.3	63.6±2.8	0.007	51.7±3.6	65.3±3.4	0.008
Social functioning	64.4±6.9	85.0±2.2	0.011	72.3±4.4	86.2±2.5	0.009
Role-emotional	45.8±10.0	81.5±4.1	0.004	61.1±7.5	82.5±4.4	0.018
Mental health	53.5±5.5	75.4±2.1	0.001	61.7±3.8	76.9±2.4	0.001

t test; SF-36- Medical Outcome Study Short Form General Health Survey.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by CNPq.

Study Association

This article is part of the thesis of Doctoral submitted by Patrícia Pozas Saboya, from Pontifícia Universidade Católica do Rio Grande do Sul.

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Original Article

Compound Heterozygous *SCN5A* Mutations in a Toddler - Are they Associated with a More Severe Phenotype?

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Abstract

Compound heterozygosity has been described in inherited arrhythmias, and usually associated with a more severe phenotype. Reports of this occurrence in Brugada syndrome patients are still rare. We report a study of genotype-phenotype correlation after the identification of new variants by genetic testing. We describe the case of an affected child with a combination of two different likely pathogenic *SCN5A* variants, presenting sinus node dysfunction, flutter and atrial fibrillation, prolonged HV interval, spontaneous type 1 Brugada pattern in the prepubescent age and familiar history of sudden death.

Introduction

Brugada Syndrome is a potentially lethal cardiac channelopathy. Diagnosis is challenging in most cases and is mainly based on clinical history and electrocardiographic patterns. The disease is often diagnosed during adulthood and rarely in children.[1]

More than 300 different mutations associated with Brugada Syndrome[2] have been described in the *SCN5A* gene that encodes the cardiac sodium channel. Around 20-30% of Brugada Syndrome patients harbor a putative causal mutation in this gene.[3]The alpha subunit of the sodium channel is associated with atrial and ventricular excitability. Despite the clear causal relationship between *SCN5A* mutations and the Brugada Syndrome phenotype, there are clinical variability of the phenotype including, besides the full-blown Brugada Syndrome set of signs and symptoms, atrial fibrillation, sick sinus syndrome, long QT syndrome, dilated cardiomyopathy and a range of overlap syndromes.[4,5]

While compound heterozygosity has been described in a number of monogenic heart disorders[5] including inherited arrhythmias, and usually associated with a more severe phenotype, the occurrence in Brugada syndrome patients are still under investigation. In this paper, we describe a case of an affected child presenting a combination of two different *SCN5A* pathogenic mutations.

Keywords

Brugada Syndrome; Sinoatrial Node / abnormalities; Arrhythmias, Cardiac; Genetic Testing; Heredity

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Manuscript received: July 19, 2016; revised manuscript: July 19, 2016; accepted October 10, 2016.

DOI: 10.5935/abc.20170006

Family study

The boy presented with palpitations and syncope at age 4 due to a wide QRS tachycardia (Figure 1, A). There was no structural heart disease by echocardiogram and cardiac resonance imaging. The electrophysiological study resulted in a slight increase in the HV interval (62ms), without induction of ventricular arrhythmia. The patient was treated with oral quinidine due to its vagolytic effect before Brugada Syndrome suspicion. External loop recorder showed paroxysmal 2:1 atrial flutter, associated with the symptom of diaphoresis, and asymptomatic sinus pauses of 3.2 up to 4.6 seconds unrelated to the atrial flutter (Figure 1, B). Then an atrioventricular pacemaker was implanted. During the following three years, the child remained asymptomatic. At age 8, he presented a Brugada type 1 electrocardiography (ECG) pattern (Figure 1, C), several episodes of atrial fibrillation, without spontaneous ventricular arrhythmia.

His paternal uncle had atrial fibrillation and died suddenly at age 34, after dinner. His parents had a normal electrocardiogram, although the ajmaline test induced a Brugada type 1 ECG pattern (Figure 1, D).

SCN5A bidirectional Sanger sequencing revealed a compound heterozygosity from paternal (NM_001099404:c.1198 G>A, p. G400R) and maternal (NM_001099404: c.4382 C>G, p.T1461S) inheritance. Both variants were *likely pathogenic*, according to the American College of Medical Genetics and Genomics Guidelines for the interpretation of genetic variants.[6] Family history and genetic results are summarized in Figure 2.

Discussion

In this report, we describe an unusual case of a toddler presenting with sinus node dysfunction, flutter and atrial fibrillation, atrioventricular block, prolonged HV interval and family history of sudden death, probably due to mutations in the SCN5A gene, which, in this case, was characterized as a compound heterozygote (p.G400R and p.T1461S).

This report is original in that it presents a case of a boy at prepubescent age with a distinct clinical presentation – a combination of conduction system disturbances, atrial tachyarrhythmias, Brugada ECG pattern, and two novel genetic variants.

Interestingly, the initial resting ECGs of the index case and the family were normal (except for the first-degree atrioventricular block of the father), but the case follow-up and provocative tests performed on his parents revealed type 1 Brugada electrocardiographic pattern.

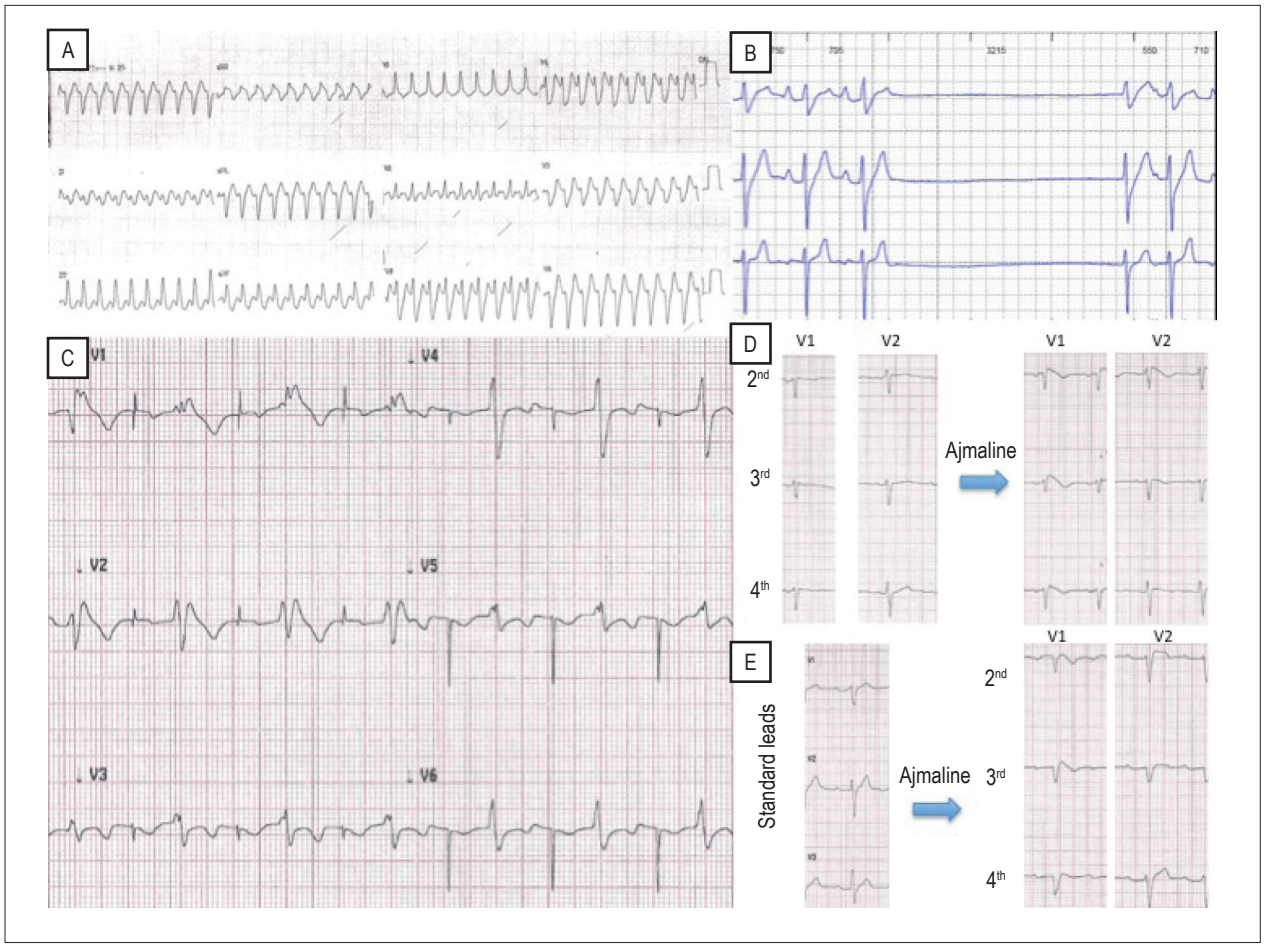

Figure 1 - *Recording of clinical history. A) wide QRS tachycardia at age 4; B) sinus pauses; C) electrocardiogram of the proband in right upper precorial leads after 3 years of follow-up, at age 8; D) electrocardiogram in right upper precorial leads: ajmaline challenge (mother). E) electrocardiogram in standard leads: ajmaline challenge (father).*

We hypothesize that, in this case, the severe phenotype manifested since childhood may be the result of the combination of both mutations. The index case had an overlapping syndrome, and the spontaneous type 1 Brugada pattern was detected at prepubescent age, which is uncommon.[1] The family presentation suggests the incomplete penetrance and variable expressivity of the mutation.[7]

Compound mutations are rare conditions.[8-10] Medeiros-Domingos et al.[8] described a child with progressive cardiac conduction system disease, monomorphic ventricular tachycardia in a febrile state, compound mutation inherited from the mother (*SCN5A* gene, mutation p.R34fs*62), and a prolonged QT interval inherited from the father (*SCN5A* gene, mutation p.R1195H), revealed by the functional analysis. Robyns et al.[9] showed a compound mutation also in the *SCN5A* gene, which was actually a combination of a mutation and a new variant that seemed to evoke a severe phenotype, including spontaneous atrial tachyarrhythmia at young age.

According to our research, the p.G400R and p.T1461S are new variants; the absence of these variants in the Exome Aggregation Consortium, in addition to the in silico analysis of the variants by pathogenicity prediction programs (Mutation Taster, SIFT e Polyphen 2), and familial cosegregation of the disease (including the response to the ajmaline test) indicate the classification of these variants as *likely pathogenic*.[6] Besides, another amino acid substitution in *SCN5A* gene, at the same residue (p.G400A) has been previously reported to cause electrical storm after myocardial infarction. Although the American College of Medical Genetics guidelines provide good genetic evidence for the mutation status of each variant, functional studies assessing the combined effects of these variants would be of benefit.

Conclusion

The wide phenotypic expression of the *SCN5A* mutation remains a challenge. Additional genetic variation is one of the explanations for the low penetrance and variable clinical expression of the disease. We described variants and also their responses to the ajmaline test, which can indicate its pathogenic role.

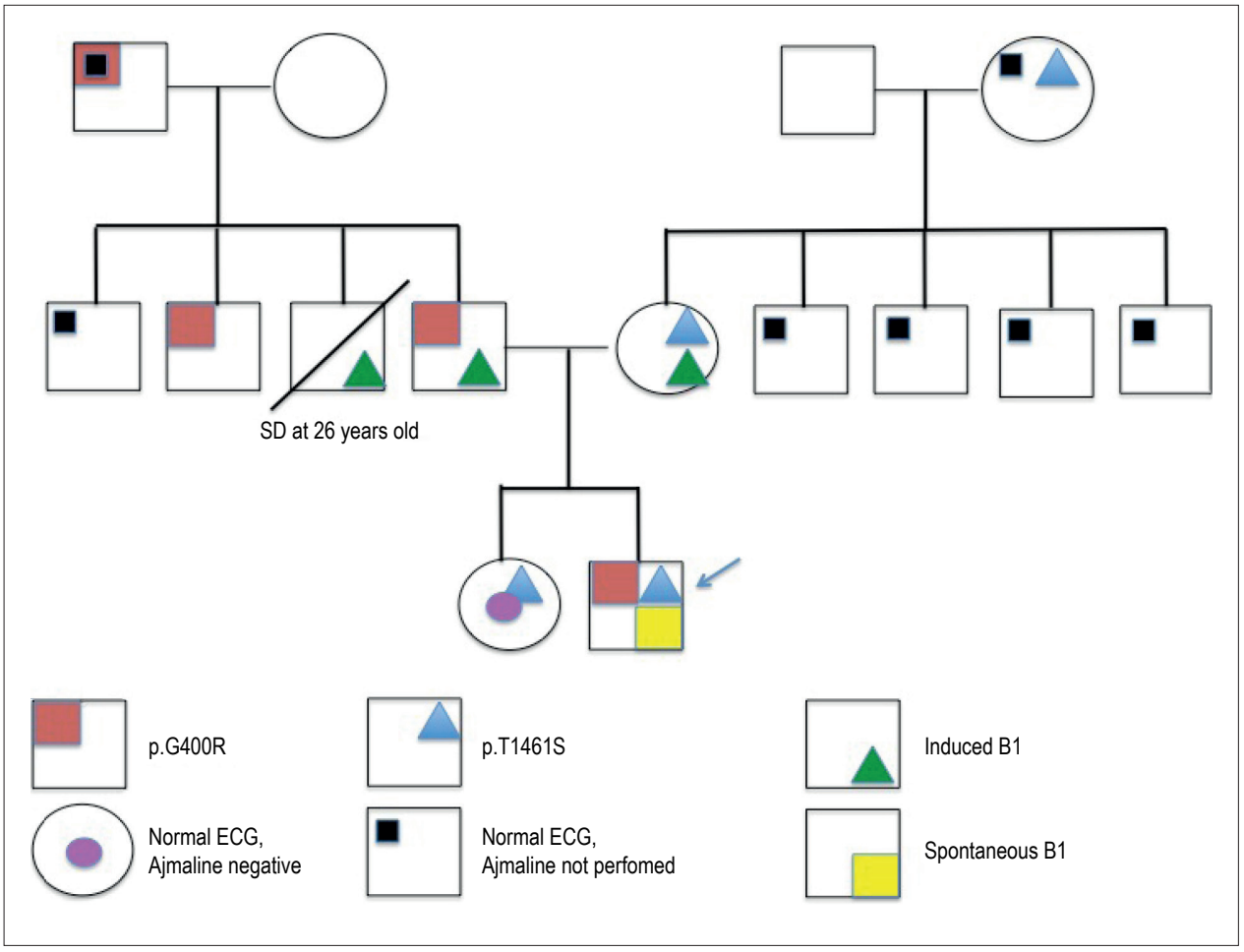

Figure 2 – *Brugada type 1 pattern; SD: sudden death; ECG: electrocardiography.*

SCN5A compound mutations seem to lead to severe clinical and electrocardiographic manifestations. However, further studies are warranted to describe the long-term consequences of harboring compound mutations of the *SCN5A*.

Author contributions

Conception and design of the research: Sacilotto L, Epifanio HB, Scanavacca MI; Acquisition of data: Sacilotto L, Epifanio HB, Wulkan F, Gremen T,; Analysis and interpretation of the data: Sacilotto L, Epifanio HB, Darrieux FCC, Wulkan F, Gremen T, Pereira AC, Scanavacca MI; Writing of the manuscript: Sacilotto L, Darrieux FCC; Critical revision of the manuscript for intellectual content: Sacilotto L, Darrieux FCC, Hachul DT, Pereira AC, Scanavacca MI.

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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Cardiac Cachexia: Perspectives for Prevention and Treatment

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Abstract

Cachexia is a prevalent pathological condition associated with chronic heart failure. Its occurrence predicts increased morbidity and mortality independent of important clinical variables such as age, ventricular function, or heart failure functional class. The clinical consequences of cachexia are dependent on both weight loss and systemic inflammation, which accompany cachexia development. Skeletal muscle wasting is an important component of cachexia; it often precedes cachexia development and predicts poor outcome in heart failure. Cachexia clinically affects several organs and systems. It is a multifactorial condition where underlying pathophysiological mechanisms are not completely understood making it difficult to develop specific prevention and treatment therapies. Preventive strategies have largely focused on muscle mass preservation. Different treatment options have been described, mostly in small clinical studies or experimental settings. These include nutritional support, neurohormonal blockade, reducing intestinal bacterial translocation, anemia and iron deficiency treatment, appetite stimulants, immunomodulatory agents, anabolic hormones, and physical exercise regimens. Currently, nonpharmacological therapy such as nutritional support and physical exercise are considered central to cachexia prevention and treatment.

Introduction

Heart failure is an important public health issue due to a high prevalence, severity of clinical manifestations and poor prognosis. Statistical data from the United States estimate that 5.7 million Americans over 20 years of age have heart failure; this is expected to increase by approximately 46% between 2012 and 2030, resulting in over 8 million adults with heart failure.[1]

Heart failure is caused by structural and functional abnormalities in the heart leading to impaired ventricular ejection and/or filling capacity. In Brazil, the main causes of heart failure are myocardial ischemia, systemic arterial hypertension, dilated cardiomyopathy and Chagas' disease, and valve disease.[2] Following cardiac injury, the ensuing molecular, structural, and functional ventricular changes are known as cardiac remodeling. This process is accompanied by cardiac and systemic neurohormonal and inflammatory activation, which adversely affects the heart in a vicious cycle and jeopardizes different organs and systems.[3] In recent decades, it has become clear that pathological changes involve not only the cardiovascular system, but also the renal, neuroendocrinological, immunological, hematologic, gastrointestinal, and musculoskeletal systems, as well as the nutritional status. Currently, experimental and clinical studies have focused on the physiopathology of heart failure-related systemic complications in order to establish treatments to improve quality of life and increase survival.

Cachexia is a prevalent and important pathological condition associated with chronic heart failure. Its occurrence predicts reduced survival, independent of relevant variables such as age, heart failure functional class, ejection fraction, and physical capacity.[4] We evaluate studies on heart failure-induced cachexia and discuss different therapies for its prevention and treatment.

Cardiac cachexia definition

Cachexia has been defined as at least 5% edema-free body weight loss in the previous 12 months (or a body mass index < 20 kg/m^2) in patients with chronic illness and at least three of the following clinical or laboratory criteria: decreased muscle strength, fatigue, anorexia, low fat-free mass index and abnormal biochemistry characterized by increased inflammatory markers [C-reactive protein, interleukin (IL)-6], anemia (Hb < 12 g/dL), or low serum albumin (< 3.2 g/dL).[5] As heart failure is an inflammatory disease, Anker et al.[6] proposed that cardiac cachexia should be diagnosed when body weight loss is > 6% regardless of other criteria and in the absence of other severe diseases. More recently, investigators have used a body weight loss cutoff > 5% to characterize cardiac cachexia.[7,8] It should be pointed out that cachexia is different from malnutrition or anorexia, which can both easily be reversed with adequate nutrition.[5] Currently, several biomarkers have been studied to help diagnose cardiac cachexia.[9] Muscle wasting is an important component of cachexia. It often precedes cachexia development and may also predict poor outcome in heart failure.[10] Differently from cachexia, muscle loss diagnosis depends on the laboratory evaluation of muscle mass, such as dual energy X-ray absorptiometry (DEXA), computed tomography and magnetic resonance imaging.[11] Muscle wasting may also be suggested by poor performance during spiroergometry, 6-min walking test, gait speed, or handgrip strength.[11]

The importance of cachexia in heart failure prognosis became more evident after the description of the reverse epidemiology of obesity in this condition. In healthy people, increased body mass index is associated with an elevated risk

Keywords

Heart Failure; Muscle Wasting; Physical Exercise; Prognosis; Nutrition; Anemia.

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Manuscript received February 05, 2016; revised manuscript May 13, 2016; accepted May 23, 2016.

DOI: 10.5935/abc.20160142

of developing cardiovascular disease. However, body mass index was positively correlated with survival in heart failure patients.[12] In a meta-analysis of nine observational studies, mortality was lower in overweight and obese heart failure patients.[13] The mechanisms involved in both the obesity paradox and the cachexia-induced worse prognosis are not completely clear.[14]

Cardiac cachexia prevalence varies between 8 and 42% according to cachexia definition and the study population.[6,7,15] Anker et al.[6] observed that 34% of heart failure outpatients had a ≥ 6% body weight loss during 48 months of follow-up. More recently, in optimally-treated nondiabetic outpatients, a > 5% body weight loss was observed in 10.5%.[7]

The etiology of heart failure-associated cachexia is multifactorial and the underlying pathophysiological mechanisms are not well established.[16] Important factors include food intake reduction, gastrointestinal abnormalities, immunological and neurohormonal activation and an imbalance between anabolic and catabolic processes.[16,17]

Clinical consequences of cachexia

The clinical consequences of cachexia depend on both weight loss and systemic inflammation, which accompany cachexia development. Severe body weight loss, even in the absence of systemic inflammation, is associated with deleterious effects on most organs and systems. Tissue loss from three compartments, lean tissue, fat mass, and bones, is usually found.[7] In skeletal muscles, an imbalance between protein synthesis and breakdown leads to molecular changes and muscle atrophy, with decreased strength and daily activity impairment.[18-23]

The cardiac consequences of cachexia have been studied in heart disease-free conditions, such as cancer and undernutrition.[24-30] In cachectic individuals, left ventricular mass correlated with lean body mass, showing that the heart is subjected to similar consequences to those in lean tissue during cachexia.[31] In experimental animals, cancer cachexia induced cardiac dysfunction and molecular changes characteristic of the pathologic remodeling process with reduced anabolic pathway signaling.[24,25] We observed that severe food restriction induces mild ultrastructural, morphological, and functional changes in normal rat hearts, which are exacerbated by hemodynamic overload in hypertensive rats.[32-37] Therefore, the occurrence of cachexia can further impair cardiac changes and heart failure in a fatal vicious cycle. Cachexia can also exacerbate heart failure-associated anemia and gastrointestinal changes.[38]

Cachexia prevention and treatment

As cardiac cachexia is multifactorial, it has been difficult to develop a specific therapy for its prevention and treatment.[11] Since skeletal muscle wasting can precede cachexia, preventive strategies have been largely directed towards muscle mass preservation.[39] Different options have been described, mostly evaluated in small clinical studies or experimental settings. These include nutritional support, neurohormonal blockade, reduced intestinal bacterial translocation, anemia and iron deficiency treatment, appetite stimulants, immunomodulatory agents, anabolic hormones, and physical exercise regimes (Table 1).[11] Currently, nonpharmacological therapy such as nutritional support and physical exercise has been considered as the basis for cachexia prevention and treatment.[40]

Nutritional support

Non-obese patients with stable heart failure often have inadequate food intake.[41] Therefore, nutritional support is recommended to obtain and maintain a body weight within or a little below the normal range without edema. Currently, there is no specific recommendation for protein and energy intake. The ingestion of 35 kcal/kg/day was shown to be safe and effective in increasing lean mass in heart failure patients.[42] Some authors have recommended a caloric intake of at least 31.8 kcal/kg/day.[41] Nutritional support should be started with small amounts and slowly increased until desired body weight is reached. Excess energy intake increases catecholamine and insulin plasma concentrations causing physiological stress. An increase in insulin levels induces renal sodium and

Table 1 – Cardiac cachexia: perspectives for prevention and treatment

Nonpharmacological approach
Nutritional support
Physical exercise
Drug therapy approach
Treatment clinically useful
Neurohormonal blockade
Reduction in intestinal bacterial translocation by peripheral edema control
Anemia and iron deficiency correction
Experimental use only
Supplementation of essential amino acids
Supplementation of branched-chain amino acids
Appetite stimulants
Immunomodulatory agents (pentoxyphylline, thalidomide, statins, methotrexate, N-acetylcysteine, T-cell activation inhibitors, chemokine antagonists, interleukin-10, interleukin-1 receptor antagonists)
Anabolic hormones (testosterone, growth hormone release-inducing, growth hormone)
Several mechanisms: myostatin inhibitors and antagonists, bortezomide, lipopolysaccharide bioactivity inhibitors, and melanocortin blockers

water reabsorption and may decompensate cardiac failure. Therefore, patients should be advised to evaluate their body weight daily and tailor diuretic therapy. Protein intake should follow recommendations for healthy people and may be increased in cases of protein loss by intestinal malabsorption or nephropathy. However, a small trial showed that high-caloric protein-rich oral nutritional supplement improved body weight and reduced inflammatory markers.[43] Sodium intake depends on heart failure functional class, being more restricted (0.5 to 2 g/day) in severe cases; this is when patients need to be educated on food sodium content. Chronic and vigorous use of diuretics can deplete potassium and magnesium levels. With increased carbohydrates and amino acids intake and increased insulin levels, there is a shift in potassium, magnesium and phosphorus from extracellular to intracellular compartments, thus decreasing plasma concentrations of these electrolytes which can induce cardiac arrhythmias and sudden death.

There is no specific recommendation for micronutrients in heart failure. Reduced food intake and chronic use of diuretics can cause water-soluble vitamin deficiency. Thiamine needs particular attention as a deficiency may impair cardiac function.[44] Intestinal malabsorption can reduce plasma levels of the soluble vitamins A, D, E, and K.[44] As liver congestion and ascites cause food intake intolerance, meals should be frequent and small. It should be stressed that, despite the importance of nutritional support, it has not been established whether adequate protein-energy intake can reverse nutritional status in chronic heart failure.[45] Furthermore, increased food intake may compensate some weight loss, but it can change tissue distribution towards increased fat mass, particularly when muscle loss is present.[46] Therefore, to preserve or recover muscle mass, nutritional support should be combined with physical exercise.

Recent small studies have suggested that alterations in specific diet components may be useful in cardiac cachexia. For example, supplements of essential amino acids improved nutritional and metabolic status in most muscle-depleted heart failure patients.[45] Supplementation of branched-chain amino acids, which consist of leucine, isoleucine, and valine, preserved body weight, skeletal muscle mass, and cardiac function in rats;[47] however, it failed to benefit heart failure patients.[48]

Neurohormonal activation blockade

Chronic heart failure is characterized by sustained cardiac and systemic activation of the renin-angiotensin-aldosterone and adrenergic nervous systems which, in the long term, impairs ventricular remodeling. Therefore, blockade of these systems is recommended for all heart failure patients with reduced ejection fraction.[2,49] The heart failure control with neurohormonal blockade can reverse cachexia independently of nutritional support.

However, neurohormonal activation is also directly involved in skeletal muscle atrophy. The effects of angiotensin II can be prevented by angiotensin-converting enzyme inhibitors (ACEi) and angiotensin 1 receptor blockers. More recently, angiotensin II has been shown to play a role in cachexia and skeletal muscle wasting through different mechanisms such as increased oxidative stress and protein breakdown; impaired energy balance; reduced appetite via alteration in orexigenic/anorexigenic neuropeptides in the hypothalamus; and inhibition of satellite cell function and muscle regeneration.[50,51] Administration of ACEi enalapril decreased the risk of weight loss in heart failure patients.[6] One may argue that as angiotensin II antagonism improves cardiac remodeling and ventricular function, it would also reduce the risk for cachexia development. Thus, neurohormonal blockade was also examined in cancer cachexia. In tumor-bearing rats, angiotensin and aldosterone antagonism as well as adrenergic nervous system blockade attenuated body weight and lean mass loss.[25] In a phase III clinical trial, ACEi imidapril prevented weight loss in patients with cachexia caused by non–small cell lung cancer and colorectal cancer but not by pancreatic cancer. When data were combined however, weight loss prevention did not reach statistical significance.[51] Future studies are needed to elucidate the role of neurohormonal blockade in different causes of cachexia.

Reduction in intestinal bacterial translocation

Heart failure patients with peripheral edema present increased intestine wall thickness, which suggests bowel wall edema.[38] Among echocardiographic parameters, the combination of right ventricular dysfunction and elevated right atrial pressure provided the best discrimination between cachectic and non-cachectic patients.[52] Furthermore, cardiac cachexia was associated with intestinal congestion irrespective of heart failure stage and cardiac function.[52] Heart failure patients also have a reduction in intestinal blood flow and an increase in juxtamucosal bacterial growth.[53] These abnormalities lead to intestinal bacterial translocation and systemic immune activation.[53,54] Bacterial endotoxins, also known as lipopolysaccharides, are potent inducers of pro-inflammatory substances such as tumor necrosis factor (TNF)-α. As intensive diuretic therapy normalized increased endotoxin levels in heart failure patients with peripheral edema,[54] patients should have as little edema as possible by using one or a combination of diuretics.[49] Despite experimental studies showing antibiotic therapy decreases intestinal bacterial translocation, it is not established whether microflora modulation is safe or useful in reducing systemic immune activation in heart failure. Therefore, this approach is not currently recommended.[55]

Anemia and iron deficiency treatment

The prevalence of anemia in heart failure ranges from 4% to 55%, according to study population and anemia definition.[56] Anemia is associated with increased mortality, hospitalization, and impaired quality of life.[57] Anemia etiology in heart failure is multifactorial. Iron deficiency is present in about half of heart failure patients, independent of the presence of anemia.[56] Both anemia and iron deficiency are associated with reduced exercise tolerance.[58] As decreased exercise capacity is related to a reduced skeletal muscle mass, anemia and iron deficiency may be involved in cachexia development. Diagnostic evaluation for reversible causes

of anemia and subsequent treatment is appropriate in all patients. Currently, several heart associations suggest that iron deficiency should be routinely checked in all heart failure patients and corrected if present.[56] Intravenous iron preparations are safe and effective in treating iron deficiency;[58] little information is available on the effectiveness of oral iron.[56] Intravenous iron correction of iron deficiency was associated with improved functional status.[59] As erythropoiesis stimulating agent darbepoetin alpha failed to improve clinical outcomes in heart failure patients with mild-to-moderate anemia,[60] this class of drug is not recommended for treating heart failure-associated anemia.

Perspectives for future treatment of cachexia

Several pharmacological agents have been tested in experimental and clinical settings for preventing and treating cardiac cachexia. However, they currently represent perspectives for the future and are not recommended for clinical use.

Appetite loss is a common finding in cardiac cachexia and its origin is multifactorial.[15] Although appetite stimulants such as megestrol acetate have been used in other cachectic conditions, they are not approved for cardiac cachexia.

As previously stated, chronic cardiac failure is followed by immunologic activation, which plays an important role in cachexia development. Therefore, several immunomodulatory agents have been tested in heart failure. Tumor necrosis factor (TNF)-α antagonists etanercept and infliximab were tested in large clinical trials with neutral or negative results.[61] Pentoxyphylline and thalidomide, also considered immunomodulatory agents, were used in small trials with neutral or favorable results.[62,63] Other immunomodulatory drugs such as statins, methotrexate, N-acetylcysteine, T-cell activation inhibitors, chemokine antagonists, IL-10, and IL-1 receptor antagonists have been tested in experimental studies.[19,64]

Anabolic hormones have also been examined to preserve and/or increase muscle mass. Testosterone levels decrease with age; this phenomenon being faster in heart failure men than in their healthy male counterparts.[65] Low concentration of testosterone was related to increased risk of death, independently of left ventricular function or functional capacity.[39,65] In skeletal muscle, testosterone increases protein synthesis, reduces protein breakdown, and stimulates proliferation and differentiation of satellite cells, thus increasing muscle mass and strength, and improving exercise capacity.[39] Therefore, androgen deficiency may be involved in the imbalance between anabolic and catabolic processes and contribute to heart failure-induced muscle wasting and cachexia.[65] Testosterone supplementation was evaluated in small randomized double-blind studies including elderly men[66] and women[67] with heart failure. As testosterone improved functional capacity and muscle strength, it was hypothesized that it could be safe and useful in heart failure and cardiac cachexia.

Growth hormone release-inducing (Ghrelin) increases adiposity and food intake by modulating neural circuits that control food intake, energy expenditure, and reward.[68] Ghrelin has been evaluated in small trials in different cachectic conditions.[15] In heart failure, repeated Ghrelin administration improved exercise capacity and muscle wasting, suggesting that Ghrelin and its receptor agonist anamorelin may be an attractive approach for future investigation.[68,69] Growth hormone (GH) also have the potential to improve muscle mass and functional capacity.[70] However, as their effects are not completely established in heart failure patients,[71,72] additional research is needed to clarify the role of GH in cardiac failure and cachexia.

Currently, several drugs such as myostatin inhibitors and antagonists, bortezomide (an ubiquitin-proteasome route inhibitor), lipopolysaccharide bioactivity inhibitors, and melanocortin blockers have been investigated with the purpose of preserving and/or increasing muscle mass in cardiac cachexia.[9,20,22]

Physical Exercise

Physical exercise is the most promising option for treating muscle wasting in several diseases. Current heart failure guidelines strongly recommend regular physical exercise for stable patients to prevent and/or attenuate cardiac remodeling and skeletal muscle alterations.[2,49,73] Clinical and experimental studies have shown that aerobic exercise improves cardiac remodeling and ventricular function, and increases functional capacity and quality of life.[74-76] In skeletal muscle, exercise training reduces oxidative stress, activation of the ubiquitin-proteasome system, expression of myostatin and proinflammatory cytokines, sympathetic nerve activity and peripheral vasoconstriction, reestablishes expression of proteins involved in sarcoplasmic calcium handling, and prevents capillary rarefaction and atrophy.[77-79]

Other exercise modalities have also shown promising results in heart failure. For example, a resistance exercise program improved functional capacity[48] and a combination of hydrotherapy with endurance training improved exercise tolerance and hemodynamic profile of heart failure patients.[81] Additionally, high-intensity aerobic exercise was safe and superior to moderate-intensity aerobic training in increasing maximal oxygen consumption.[82] Therefore, additional studies are needed to establish the best training protocol relating to exercise type, intensity, duration, and frequency to improve outcomes in cardiac cachexia.

Conclusion

Cachexia plays an important role in morbidity and mortality in heart failure patients. Understanding the pathophysiological mechanisms that cause cachexia is an essential step to developing pharmacological and non-pharmacological strategies aimed at effectively preventing and treating heart failure-induced cachexia before significant body weight and muscle wasting occurs. Currently, nonpharmacological therapy such as nutritional support and physical exercise are the basis for cachexia prevention and treatment.

Acknowledgement

We are grateful to Colin Edward Knaggs for English editing. Financial support was provided by CNPq (306857/2012-0, 306845/2012-1, and 479085/2013-7), FAPESP (2012/50512-5, 2014/21972-3), CAPES, and PROPe, UNESP.

Author contributions

Conception and design of the research: Okoshi MP, Capalbo RV, Romeiro FG, Okoshi K; Acquisition of data: Capalbo RV, Okoshi K; Analysis and interpretation of the data: Okoshi MP, Capalbo RV; Obtaining financing and Critical revision of the manuscript for intellectual content: Okoshi MP, Okoshi K; Writing of the manuscript: Okoshi MP, Capalbo RV, Romeiro FG.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by FAPESP and CNPq.

Study Association

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

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The CRISPR/Cas9 System and the Possibility of Genomic Edition for Cardiology

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Introduction

Cardiovascular diseases (CVD) and their associated pathologies are among the greatest causes of morbidity and mortality, entailing approximately 17.3 deaths a year.[1] This class of pathology as a whole has a multi-factor etiology. Its possible prognoses lead to public health issues, with its incidence being related to behavioral, metabolic and genetic risk factors.[2] In spite of the fact that the treatments established for the CVDs and their possible prognoses decrease the rhythm of progression of the illness, the need to develop therapeutic approaches able to reverse the pathology and its complications is growing.

The progresses in the fields of molecular and cellular biology have allowed the elucidation of molecular pathways and genetic causes involved in the establishment and progression of the CVDs, outlining a new viewpoint with regard to the prevention, treatment and possible outcomes of this pathological class. Recent discoveries, both experimental and those obtained by means of bioinformatics tools, regarding the molecular bases of cardiovascular dysfunctions, have been pointing to considerable therapeutic targets.[3] However, most of these targets cannot be pharmacologically manipulated, which makes them potential candidates for genic therapy, such as the factors involved, for example, in angiogenesis, apoptosis and endothelial dysfunction.[4] Within such context, gene manipulation may help suppress genetic factors connected to the incidence of the CVDs, as well as to mitigate the clinical complications caused by ischemic and occlusive events. Thus, the development and improvement of genome edition tools allow the creation of therapies focused in the genetic risk factors to cardiovascular damage and fundamental morphophysiological issues caused by the CVDs. In such context, the system formed by clustered regularly interspaced short palindromic repeats (CRISPR), and its CRISPR associated protein-9 (Cas9), stands out due to how easy it is to use it, its high specificity, easy in vitro and in vivo manipulation, in addition to the possibility of simultaneously editing multiple targets. Given the genomic complexity that intervenes in the CVDs, we shall indicate herein certain possibilities of applying the CRISPR/Cas9 tool in Cardiology.

Keywords

Cardiovascular Diseases / mortality; Morbidity; Risk Factors; Prevention & Control; Molecular Biology; Genomics.

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Manuscript received July 15, 2016, revised manuscript October 13, 2016, accepted October 13, 2016.

DOI: 10.5935/abc.20160200

Unraveling the CRISPR/Cas9 system

Developed from molecular organisms of the bacterial immune system, the CRISPR system allows the edition of the genome by means of splicing of the DNA by an endonuclease (Cas9), guided based on an RNA sequence, which is able to pair up with the bases of a target sequence (Figure 1).[5] The CRISPR genetic structure, in the bacterial system, is made up of clustered regularly interspaced short palindromic repeats. The repeats and the spacers (which may contain interspacing viral sequences), when transcribed, form the transactivator RNA (or guide RNA), which serves to direct the Cas9 enzyme, a nuclease, to the target (in this case, the parasite virus sequence). Taking advantage of this strategy, both the Cas9 protein and the guide RNA can be introduced in vitro into other cells and directed to specific places in the genome, for them to cause breaks to the double strand. After this splicing, the intrinsic molecular machinery of the organism, responsible for the correction of errors in the genome, is used to alter the DNA sequence, adopting the modification. The system can thus be used both to repair mutations (restoring genic function) and to introduce new mutations (causing the genic "knockout"). Therefore, by conciliating sophisticated molecular and biotechnological techniques, the CRISPR/Cas9 system was proposed for application on genomic editing and is currently commercially available for thousands of targets.[6] Both the RNA and the Cas9 protein, produced in vitro, can be delivered to the cells using different mechanisms, such as the use of vectors or chemical agents.

The most simple applicability of the CRISPR system is connected to changing single or certain bases in genes with a well-defined allelic relationship. It is important to stress that this relationship of Mendelian dominance must be taken into account for the genic function to be achieved, both to activate it and to inhibit it. However, bi-allelic modifications have also been successfully obtained[7]. Moreover, the use of the CRISPR/Cas9 has also been proposed for embryonic stage in animal models, where the progeny can generate "founding" organisms (by recombination) containing allelic mutations that lead to the "knockout" effect or with diminished expression.[8] In such context, the CRISPR/Cas9 system is being quickly adopted to edit and modify genomes in several cellular types, including stem cells,[9] and has been giving good results in the edition of human genes.[10] The press recently reported that researchers from the University of Pennsylvania have been given approval from the Food and Drug Administration (FDA) to conduct

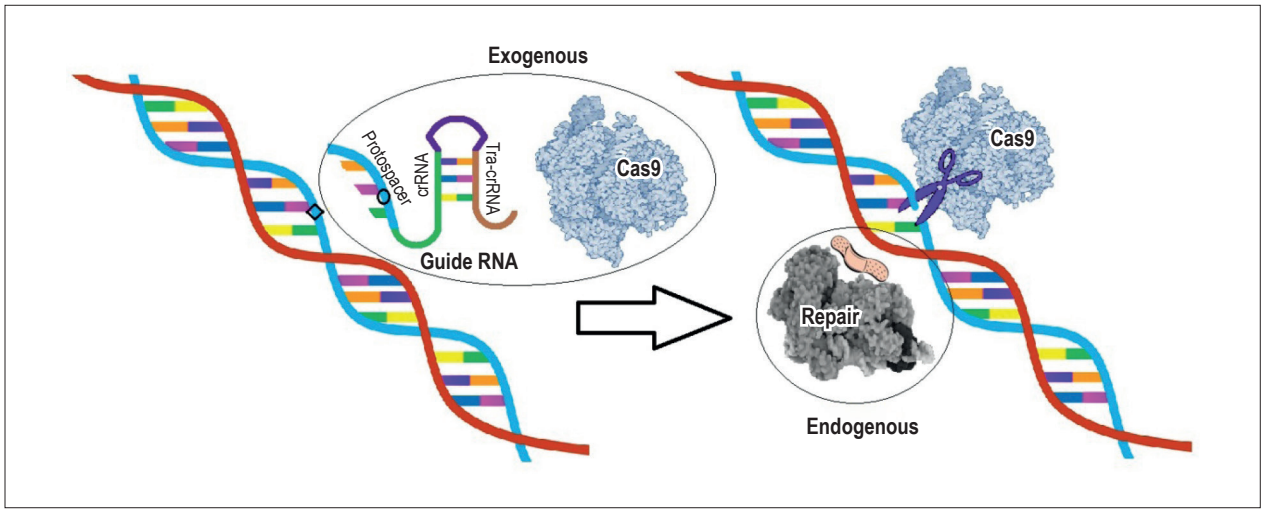

Figure 1 – *CRISPR/Cas9 system - target recognition mechanism. The guide RNA is designed to recognize the target sequence to be modified in the RNA and introduce modifications. When the pairing of nitrogenized bases occurs (due to the annealing of the target sequence with the region of the guide RNA protospacer), some modifications are added (represented hereby the circle) and the Cas9 enzyme is activated, causing breaks to the DNA double strand (where there are pairing flaws due to the mutations introduced). The breaks activate the intracellular repair systems that remake the double strand, accepting the modifications from the guide RNA. The new mutations generally cause flaws in the sequence and generate non-functional proteins. But the mechanism can also be used to correct mutations originally present in the DNA and generate functional proteins.*

a clinical study to begin in 2017, the targets of which are 3 genes involved in cancer. Thus, the question arises regarding the possibility of applying the CRISPR/Cas9 system to such a biologically complex situation as cardiovascular diseases.

How to use the CRISPR/Cas9 system in Cardiology

The first step to suggest the use of the CRISPR/Cas9 system for a certain CVD must be based on an in-depth study of the potential molecular targets involved in the disease. In such scenario, the use of bioinformatics tools and genic sequence banks available online (such as the National Center for Biotechnology Information, NCBI; and the DNA Data Bank of Japan, DBDJ) and of predicted proteins (such as the Universal Protein Resource, UniProt, allocated to the European Molecular Biology Laboratory, EMBL), in addition to single polymorphism banks, SNP (http://www.ncbi.nlm.nih.gov/snp), may assist with the process. Once the targets have been chosen, a detailed analysis of the function of the exons (codification sequences of the genes) must also be carried out. In possession of every information necessary, the guide RNA may be designed and commercially acquired. There are currently several research laboratories that are using the CRISPR/Cas9 tool to edit genes involved in CVD and testing them on cellular systems, conducting pre-clinical trials and scheduling clinical studies. Even though the cardiovascular context is complex, some pathologies are more or less connected to certain genic products, the interaction of which with other molecules is known, as described below, facilitating the feasibility of using the CRISPR/Cas9 system.

One of the great issues with the maintenance of the coronary artery disease (DAC) is the elevation of the LDL, where pharmacological intervention seeks to decrease it by using statins. Given that some patients are intolerant to such substance or do not respond well to it, several researches are being carried out, focusing on inhibiting the Proprotein convertase subtilisin/kexin type 9 (PCSK9), which helps degrade the LDL receptors, which causes an increase to the level of lipoprotein in the blood stream. Through the CRISPR/Cas9 system, Ding et al (2014) introduced a *loss of function* for the PCSK9 gene in the livers of mice, using adenoviruses as "vehicles", and showed a decrease of the cholesterol levels by over 40%.[11] In a study with rabbits, also focused on decreasing the progression of the atherosclerotic plaque, "knockout" animals were developed by genomic edition, by inhibiting several genes, such as Apolipoprotein E (ApoE), CD36, the LDL receptor, leptin, ryanodine receptor type 2 (RyR2), among others.[12] These studies show that the CRISPR/Cas9 system is viable to alter the function of genes connected to CVDs. This favors the exploration of the use of the molecular tool for other mechanisms concerning CVDs.

A good study target for a possible use of the CRISPR/Cas9 is the β-adrenergic system, one of the systems responsible for vasoconstriction/vasodilation and maintenance of the blood pressure and heart rate. Add to that the fact that the renin-angiotensin-aldosterone system also has a crucial role in maintaining the hemodynamic stability. Both systems are regulated by an extensive effector network, such as hormones and peptides, receptors, kinase proteins and other enzymes, working both in outside and inside the cells. In this regard, it would be very interesting to test and appraise the genomic edition tool to assist with the systemic arterial hypertension treatment.

Our group, concurrently with the application of alternative therapies, such as cellular and genic therapy, to treat CVDs, developed the first clinical study in the country to promote angiogenesis, by exogenous expression, by administering a plasmid containing the cDNA related to the vascular

endothelial growth factor (VEGF) in patients with refractory angina, showing that the technique is safe and improves the ventricular ejection fraction.[13] We are currently focusing our efforts on understanding mechanisms that may help with the interventions (surgical, pharmacological, dietetic, etc.) for CVDs, especially for dilated cardiomyopathy (DCM) and ischemic caridiopathies.[14] In collaboration with researchers from the Cancer Institute, we are using the CRISPR/Cas9 system to achieve the inactivation of the function of a tissue-specific kinase MAP, coded by gene TNNI3K, which interact with cardiac troponin I and, when exacerbated, causes a progression of the DCM, leading to heart failure and increasing the risk of death.[15]

Not just the inhibition context, but also the possibility of edition to activate genes in order to stimulate functions connected to, for example, the survival of cardiomyocytes in the after-infarction period, inducement of homing (migration, proliferation and differentiation of stem cells), increase of the level of anti-inflammatory cytokines and metalloproteinases inhibitor proteins (which lead to the pathological ventricular remodeling), in addition to other mechanisms, may be explored with regard to the CVDs. However, due to the multi-factor conditions attributed to the etiology and prognosis of this class of pathologies, the clinical transposition of results obtained through molecular analyses in in vitro cellular systems or animal models, just as with other more innovative approaches, is still a challenge.

In most cases, genetic, environmental and behavioral factors work together to entail a CVD. Even though, in certain cases, likely factors of prediction of the outcomes are observed, it is not yet possible to accurately forecast the influence of the activation/inactivation of genes in relation to the clinical statuses. Lastly, just as with any new technology, the risks, physiological adaptations, implications of the immune response and maintenance of homeostasis, which may be modulated by the CRISPR/Cas9 system, need to be very well assessed. But the possibility of using the new molecular tool in Cardiology can be glimpsed and perhaps, in the near future, come to benefit the population's health.

Author contributions

Writing of the manuscript and Critical revision of the manuscript for intellectual content: Arend MC, Pereira JO, Markoski M

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

M. M. M. is a professor and advisor at the Graduate Program in Health Sciences - Cardiology of IC/FUC.

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Case 1/2017 - Percutaneous Repair of Right Atrioventricular Valve Insufficiency and Blalock-Taussig Shunt after Fontan Operation in Single Ventricle

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Clinical data

Twenty-seven-year old male patient reporting tiredness during exercise for three years, after total cavopulmonary connection with extracardiac conduit fenestration and closure with stiches of the free end of right atrioventricular valve (AV) for severe failure in double inlet left ventricle, pulmonary atresia, and aorta arising from rudimentary right ventricle. The patient had undergone right and left Blalock-Taussig shunts at 17 days and 9 months, respectively, and bidirectional Glenn shunt at 19 years old. The patient had arterial oxygen saturation of 84-88% during exercise and 93% at rest.

Physical exam: eupneic, cyanotic, normal pulse rate, with no jugular vein dilation. Weight: 61 Kg, Height: 163 cm, BP: 110/70 mm Hg, HR: 97 bpm, oxygen saturation = 87%. The aorta was palpable in the supra-sternal notch (grade 2).

In the precordium, the apex beat was palpable at the fourth and fifth interspace and systolic impulses were slightly in the left sternal border (LSB). Accentuated heart sounds; grade 2 systolic murmur in the lower part and end of the LSB; continuous murmur was detected in pulmonary and axillary regions. The liver was not palpable.

Complementary tests

Electrocardiogram showed sinus rhythm, signs of right atrial and ventricular overload. Peaked P waves in lead II, V3-6. QRS complex morphology with R wave amplitude of 10mm in V1 and RS in V6. QRS axis: +120º, T axis: +40º, PA: +55º.

Chest radiograph showed slight to moderate increase in heart area (cardiothoracic index: 0.54), elongated (left ventricular and medial) arches, and increased pulmonary vasculature (Figure 1).

Transesophageal echocardiography (Figure 2) showed *situs solitus* and levocardia, systemic venous drainage at total cavopulmonary connection. Increased right atrium area and interatrial communication. Increased perimembranous ventricular septum in double inlet left ventricle, and aorta arising from a rudimentary chamber at left. Tricuspid annular plane systolic excursion (TAPSE): 9 mm. Ejection fraction calculated by the Simpson's rule method was 47%. Although the right AV had been surgically closed, there was a moderate regurgitation in the medial and anterolateral regions. Left AV with normal opening, and mild regurgitation. Pulmonary valve atresia with no Blalock-Taussig shunt.

Cardiac catheterization showed a mean pulmonary artery pressure of 15 mmHg with total cavopulmonary connection with the fenestrated tube (Figure 1), Blalock-Taussig shunt at left (Figure 2), and severe right atrioventricular regurgitation caused by medial and anterolateral paravalvular leak.

Clinical diagnosis

Double inlet left ventricle, ventriculoarterial discordance and aorta at left, pulmonary atresia, left Blalock-Taussig shunt, cavopulmonary anastomosis with a fenestrated tube, paravalvular and right medial AV insufficiency, which was sutured during the Fontan procedure.

Clinical reasoning

The clinical elements of cyanogenic heart diseases, as of left univentricular type and pulmonary hypoflow after total cavopulmonary are commonly innocent. Tiredness, systolic heart murmurs at the left sternal border and continuous murmur could be signs of right AV valve regurgitation, previously repaired, and continuation of the Blalock-Taussig shunt.

Medical management

In light of the volumetric impact caused by the AV valvular insufficiency at right, in addition to deviation of blood flow through diversion through left Blalock-Taussig shunt, the repair of these residual lesions were found necessary. Since cardiac surgery with extracorporal circulation was considered of high risk, an Amplatzer septal occluder was placed in the right AV opening and closure of the left Blalock-Taussig shunt by percutaneous intervention. The right AV valve was closed using a 30-mm Amplatzer device and an Amplatzer duct occluder (ADO II, number 6) was used for the Blalock-Taussig shunt closure (Figure 2). The immediate recovery was satisfactory, with oxygen saturation greater than 90%, and a modest decrease in the cardiac area (Figure 1). The echocardiography revealed improved ventricular function (65%) with 4.5mm-fenestration, apparent continuous flow at Doppler and posterior desaturation rate of 82%-89%.

Keywords

Heart Defects, Congenital / surgery; Mitral Valve Insufficiency / surgery; Blalock Taussig Procedure; Fontan Procedure.

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DOI: 10.5935/abc.20160201

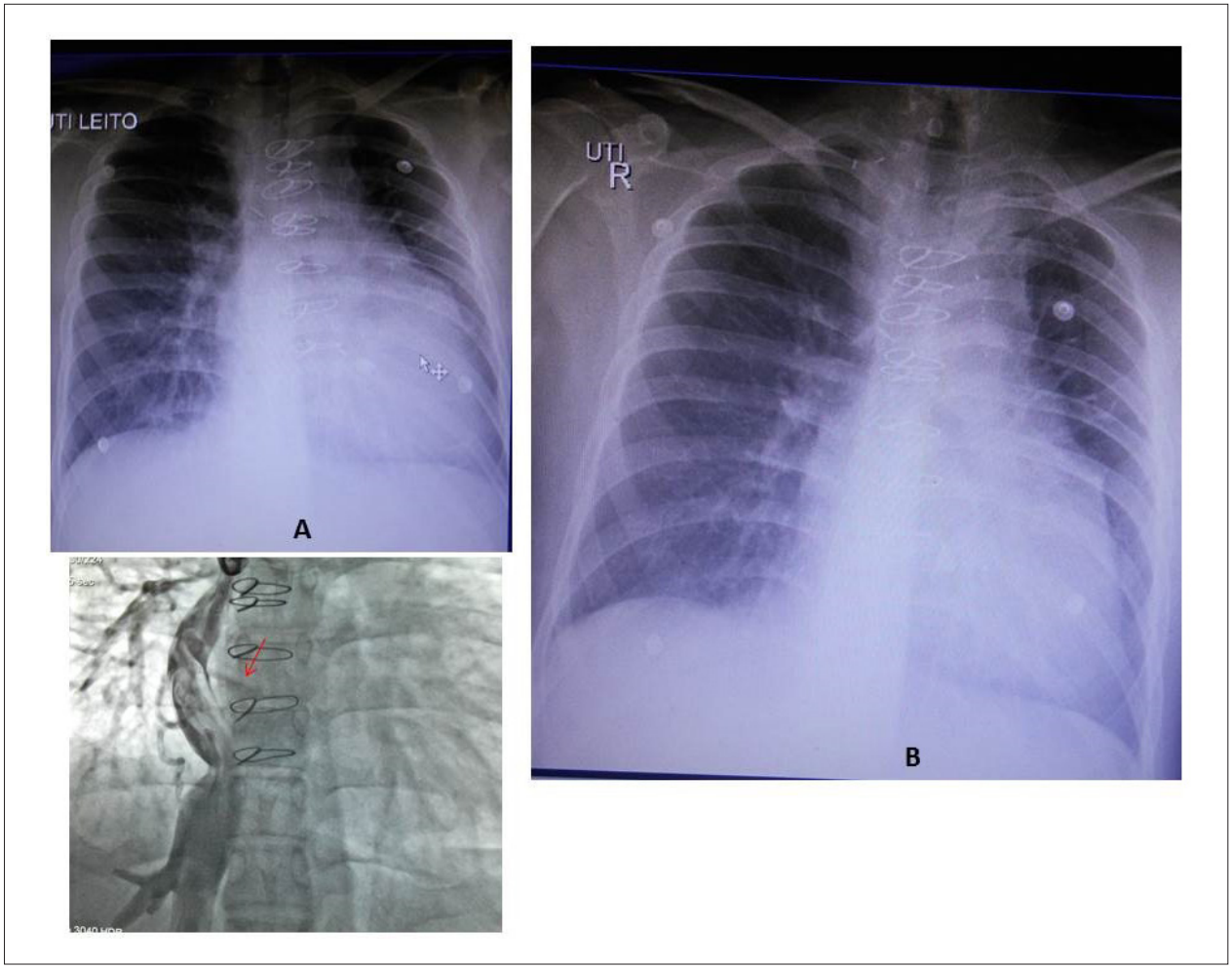

Figure 1 – *Chest radiographs showing increased heart area, elongated left ventricular and medial arches, and increased pulmonary vasculature before (A) and two days after percutaneous closure (B). The medial arch suggests the aorta arising from the left of the right ventricle. The angiography shows the connection between the inferior cava vein and right pulmonary artery with an extracardiac conduit by fenestrated total cavopulmonary connection (arrow).*

Comments

Although the Fontan surgery is a palliative procedure that involves postoperative risk, it is still a promising approach if the indication criteria are strictly followed. In adults, due to acquired conditions related to heart diseases with long-standing overload, the operative risk is higher (10%). Within this context, the difficulty in establishing the surgical indication lies in acquired conditions such as ventricular dysfunction, anatomic valvular lesions, increased pulmonary pressure, among others. These factors should be counterbalanced with unfavorable clinical progress resulting from chronic hypoxia. In case the postoperative benefits overcome this, the clinical rationale should prioritize elements considered reversible. In the present case, the sutures made on the right AV valve during previous cavopulmonary surgery were removed; an Amplatzer device was placed and the Blalock-Taussig shunt was closed using the Amplatzer device at left. Therefore, it is expected that the patient makes a better progress after the repair of these residual lesions.

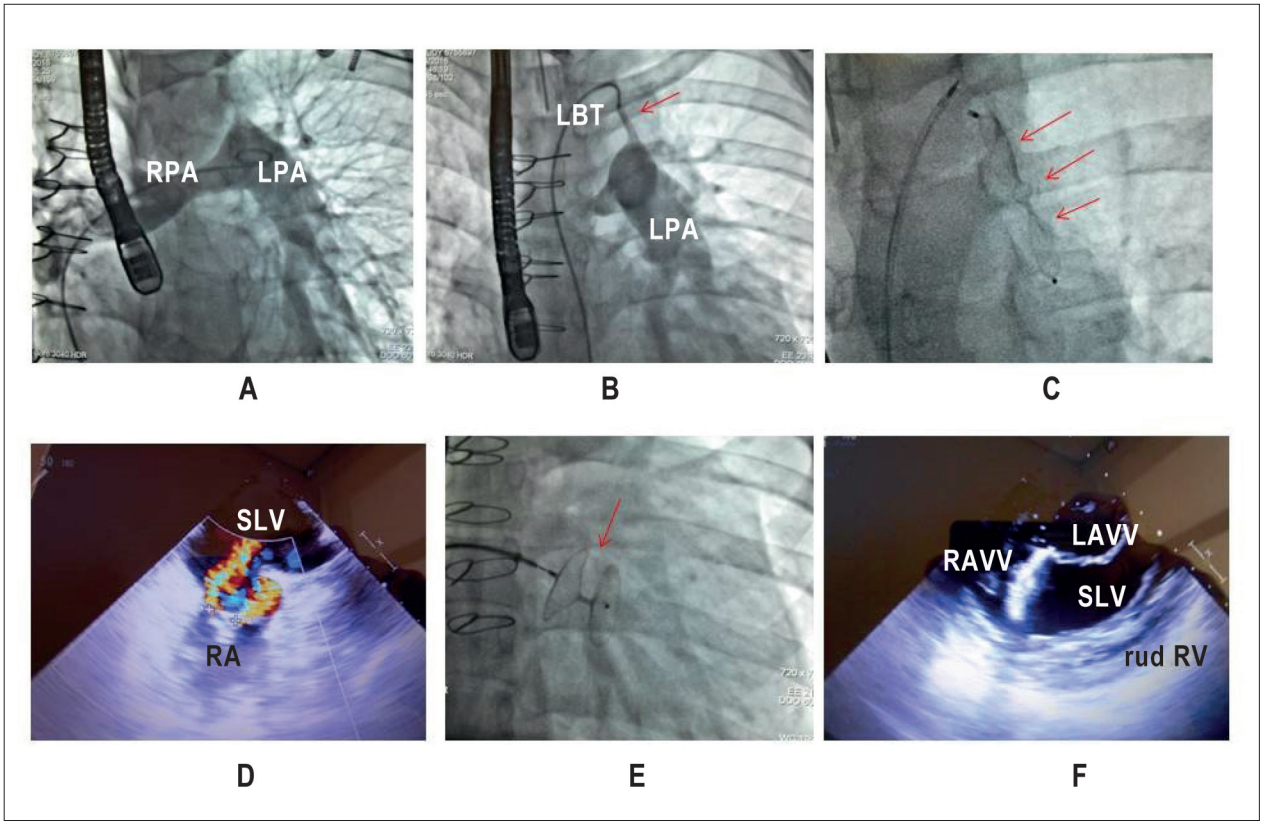

Figure 2 – *Cardiac angiography of pulmonary arterial tree showing the left pulmonary artery (LPA) pulled upwards (A), the Blalock-Taussig contrast medium in LPA (arrow) (B), and total occlusion of the Blalock-Taussig shunt using the Amplatzer device (C). The transesophageal echocardiography shows regurgitation of the right atrioventricular valve (RAVV) to the right (D), its occlusion by the Amplatzer device (E, F), and the wide left atrioventricular valve (LAVV) opening (F). RA: right atrium, RPA: right pulmonary artery, LPA: left pulmonary artery, LBT: left Blalock-Taussig, RAVV: right atrioventricular valve, LAVV: left atrioventricular valve, rud RV: rudimentary right ventricle, SLV: single left ventricle.*

Case Report: Multivessel Coronary Disease Assessment with SPECT ^{99m}Tc-Sestamibi and Rubidium-82 PET/CT

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Introduction

Coronary angiography (CAG) is the standard diagnostic method for detection of coronary artery disease (CAD). However, it is often necessary to evaluate the expression of a coronary obstruction in relation to myocardial perfusion, before defining the best patient management.

Myocardial perfusion scintigraphy with technetium-99m-Sestamibi (^{99m}Tc-sestamibi) allows early detection and evaluation of disease extension and cardiovascular risk in patients with suspected or established CAD, helping in decision-making regarding the start and type of therapy to be implemented.[1] This method has been widely used, but shows difficulties in some situations such as balanced multivessel disease, in which the proportional flow distribution in the myocardial regions can hinder ischemia detection. In such cases, additional assessment data, such as evaluation of contractility, decrease in left ventricular ejection fraction (LVEF) under stress, electrocardiographic alterations or symptoms during stress, dilation of the left ventricle (LV) cavity under stress can provide evidence of ischemia, indicating further diagnostic investigation.

Noninvasive imaging using Positron-Emission Computed Tomography (PET-CT) allows the acquisition of myocardial perfusion imaging with better quality than conventional equipment, in addition to estimating quantitative measures of myocardial blood flow at rest and under stress, as well as of coronary reserve.

We report the case of a patient with multivessel CAD referred for evaluation of myocardial perfusion, which was carried out through the two methods (Figure 1).

Case Report

Female patient, 63 years old, reported chest burning pain and dyspnea on exertion for 2 years and was submitted to CAT, which detected CAD (Figure 2). She had hypertension, dyslipidemia, insulin resistance, heart failure and dilated cardiomyopathy to be clarified. On physical examination, the patient was in good general status, eupneic, acyanotic, regular heart rhythm, normal heart sounds with no murmurs, positive pulmonary breath sounds without adventitious sounds, unaltered abdomen and full pulses and with good amplitude, without edema, New York Heart Association (NYHA) functional class I. She was receiving carvedilol, losartan, spironolactone, furosemide, simvastatin, aspirin and clopidogrel. The resting echocardiogram showed significant degree of diffuse myocardial involvement; LV diastolic dysfunction grade 1, moderate-degree mitral regurgitation and ejection fraction of 30%. The baseline electrocardiogram showed inactive areas in the inferior and anterolateral walls, in addition to possible left ventricular overload.

The patient underwent myocardial perfusion scintigraphy with Sestamibi and rubidium-82 (^{82}Rb), according to previously described protocol and technique.[1] Initially, the resting image was carried out; approximately 2 hours later, the stress imaging was performed (Figures 1 and 2) using dipyridamole as a stress agent. The findings showed greater extension of perfusion alterations in the examination with ^{82}Rb, in addition to coronary reserve alteration in all arterial territories.

Currently, the patient is being followed with optimal medical treatment due to the high risk and the presence of well-developed collateral circulation.

Discussion

The established method for assessing perfusion and myocardial function, with an important role in risk stratification of patients with known or suspected CAD is cardiac SPECT with ^{99m}Tc-Sestamibi. However, some disadvantages of the study related to the presence of image artifacts, the long duration of the examination and the possibility of underestimating ischemia severity in patients with multivessel disease [2] should be considered.

Among the noninvasive methods of LV perfusion and wall motion assessment, PET-CT with ^{82}Rb has shown higher sensitivity and accuracy.[3] This is a positron-emitting radionuclide that has characteristics similar to those of potassium and an ultrashort half-life of 75 seconds.

The advantages of performing tests with ^{82}Rb in PET-CT are: better image quality due to attenuation correction, reduced examination time (approximately 40 minutes), less radiation exposure, and the possibility of quantification of myocardial blood flow and coronary flow reserve.[4,5] Despite the high cost, this test allows the noninvasive evaluation of CAD, providing new data with probable impact on patient management[6] and, eventually, can prevent costly interventions that do not result in clinical improvement.

Keywords

Coronary Artery Disease; Cardiac Catheterization; Myocardial Perfusion Imaging; Rubidium-82; Technetium Tc 99m Sestamibi; Radionuclide Imaging.

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Manuscript received July 03, 2015; revised manuscript November 29, 2015; accepted February 24, 2016.

DOI: 10.5935/abc.20160198

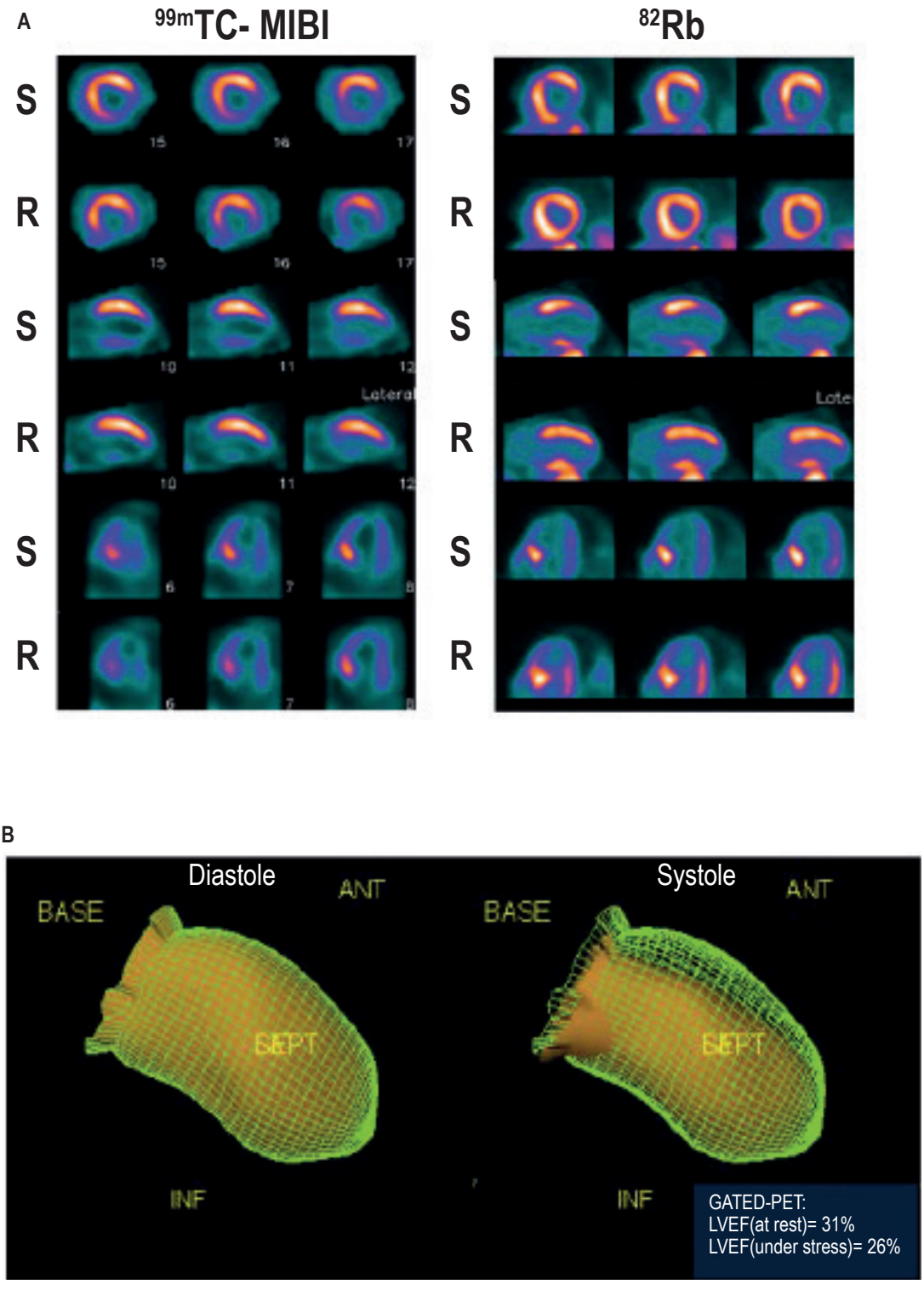

Figure 1 – *A) Myocardial perfusion at rest (R) and stress (S) with technetium-99m-Sestamibi (MIBI) to the right and rubidium-82 (82Rb) to the left. Ischemia can be observed in most prominent inferolateral wall in 82Rb. B) Left ventricular motility study (GATED-PET) shows apical akinesia and severe hypomotility of the left ventricular inferior and septal walls, with a decrease in ejection fraction under stress and the presence of transient ischemic dilation (volumetric ratio between stress and rest of 1.28).*

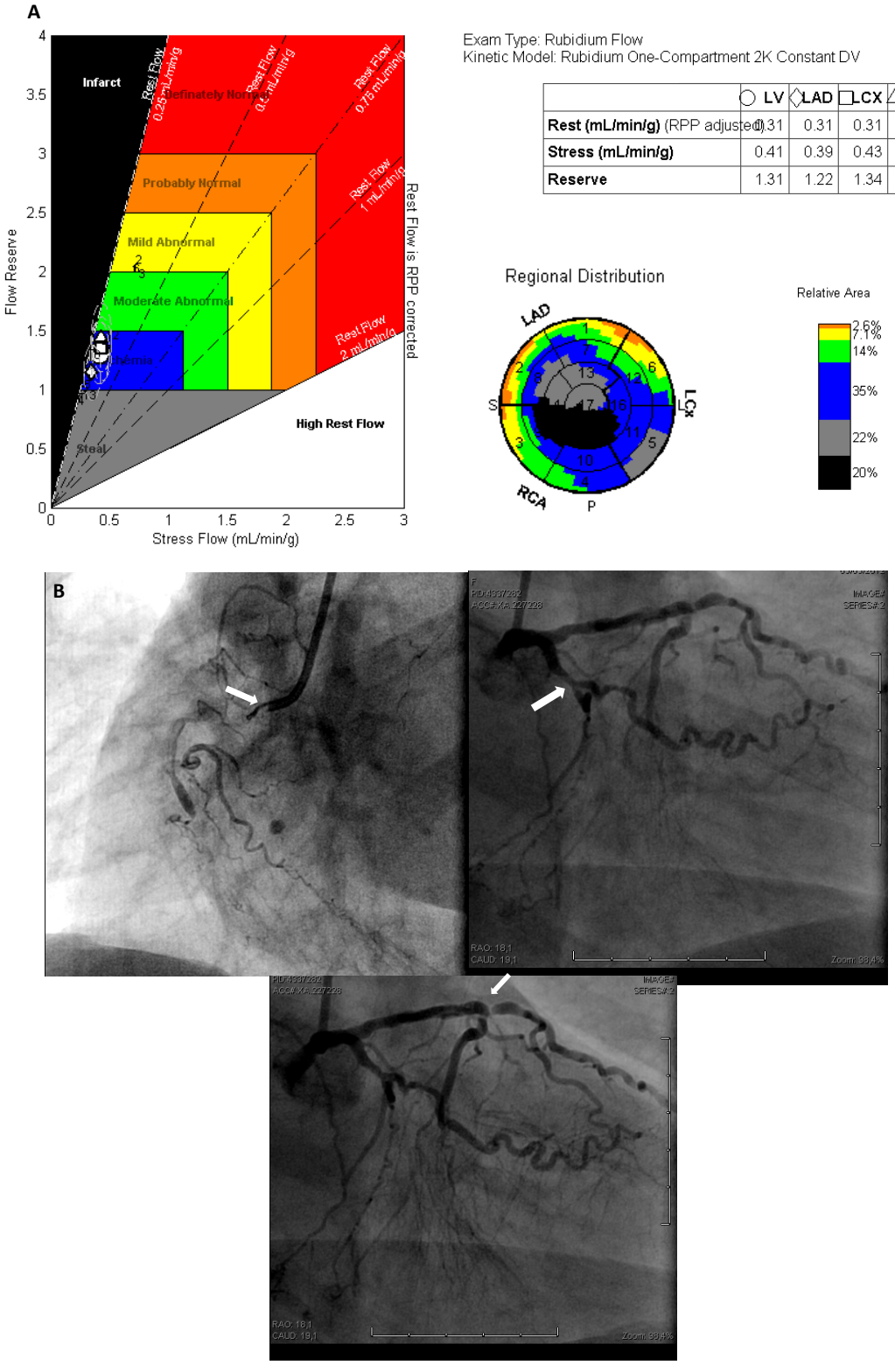

Figure 2 – *(A) Myocardial blood flow measurements (mL/ min/g) at rest and under stress, and coronary reserve in the territories of the left anterior descending (LAD), circumflex (LCX) and right coronary (RCA) arteries obtained with rubidium/positron-emission tomography. Observe the overall reduction in myocardial blood flow and left ventricular (LV) reserve and the territories of the three arteries (reserve <2.0). (B) Coronary angiography showing 100% occlusion in the anterior descending, circumflex and right coronary arteries, as well as the presence of grade 3 collateral circulation of multiple origin to the anterior descending artery, grade 2 in the right coronary artery and grade 3 in the second left marginal artery.*

The quantification of coronary flow reserve with ^{82}Rb is calculated by dividing the blood flow under stress by that at rest, considering the coronary territories of the anterior descending, right coronary and circumflex arteries, as well as that of the LV as a whole. This index provides subsidies to differentiate patients with ischemia in the territory supplied by an artery with less severe stenosis from those with multivessel disease (balanced ischemia) because in these cases the reserve is globally decreased.[1,7]

In a recently published study, the coronary blood flow was considered an independent risk factor for symptomatic patients with normal myocardial perfusion study on PET.[8] Other published studies have shown subclinical abnormalities in myocardial blood flow or coronary flow reserve in different cohorts of patients, including obese, diabetic, smoker, hypertensive and HIV-positive patients,[9,10] with microcirculation disease and dilated hypertrophic cardiomyopathy, which seems to have implications for the prognosis of these patients.

In this present case, the myocardial perfusion scintigraphy with Sestamibi showed a pattern of transient relative myocardial perfusion with Sestamibi, which seems visually less extensive than that observed in the study with ^{82}Rb. Additionally, the quantification of myocardial blood flow and coronary reserve showed alterations in the three arterial territories, characterizing a worse prognosis. If the patient had not been submitted to assessment with ^{82}Rb, further evaluation would be needed for the feasibility study, due to the small degree of transient defect detected by the examination with Sestamibi. This would increase the time of examination and radiation dose received by the patient.

In our country, it is not possible to routinely perform myocardial perfusion imaging with PET-CT and ^{82}Rb due to several factors, such as limited availability of PET-CT equipment and strontium/rubidium generator. However, the technique has great applicability in nuclear cardiology, either with ^{82}Rb or ammonia, particularly in the increase of prognostic information provided by the test, such as in the case of coronary flow reserve.

Author contributions

Conception and design of the research: Meneghetti JC; Analysis and interpretation of the data: Padilha BG; Writing of the manuscript: Sabino D; Critical revision of the manuscript for intellectual content: Giorgi MC, Soares Jr. J, Izaki M.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by FAPESP.

Study Association

This article is part of the thesis of Doctoral submitted by Bruno Gomes Padilha, from INCOR-HCFMUSP.

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Fatty Left Ventricular Cardiomyopathy: An Under-Diagnosed Disease

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During a preoperative cardiac assessment, a 57-year-old asymptomatic female, without known cardiac risk-factors, was found to have LBBB on ECG. Echocardiography revealed dilated cardiomyopathy. To rule-out coronary artery disease, coronary CT-angiography was performed and showed no significant coronary obstruction.

For further evaluation, a cardiac magnetic resonance (CMR) study was performed. A focal area of fatty infiltration was seen in the left ventricular (LV) apex extending from the subendocardial to the subepicardial surfaces (Figure 1A and 1B). This area was suppressed with fat saturation pulse-sequences (Figure 1C), and did not show enhanced signal on delayed-hyperenhancement images (DHE) (Figure 1D). Both LV and LA were dilated; LV systolic function was reduced globally. Additionally, the CMR showed normal RV dimensions, function, and wall-thickness. The fatty area was also seen retrospectively on the cardiac CT (Figure 1E).

A follow-up CMR study after 18 months - using 3 Tesla magnet - showed no significant changes of fat distribution within the LV, and a mild reduction of LV systolic function (ejection fraction =45%) that was similar to the initial study (Figure 1F-I).

There are few differential diagnoses. Firstly, cardiac lipoma, which is usually well-defined and well-encapsulated, and generally produces compression on adjacent cardiac structures. Secondly, fatty deposition following MI is associated with myocardial thinning and scarring on DHE images. Lastly, arrhythmogenic left ventricular cardiomyopathy is a substrate for ventricular arrhythmias and usually involves interventricular septum.

Isolated fatty LV cardiomyopathy is an uncommon clinical entity, and it could be under-diagnosed. With recent technological advancements in CT and MRI, more cases can be detected and investigated.

Author contributions

Conception and design of the research, Acquisition of data, Analysis and interpretation of the data, Statistical analysis, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Elagha A, Fuisz A; Obtaining financing: Fuisz A.

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.

Keywords

Cardiomyopathy, Dilated; Tomography, X Ray-Computed/methods; Ventricular Dysfunction, Left; Lipoma.

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DOI: 10.5935/abc.20160199

Image

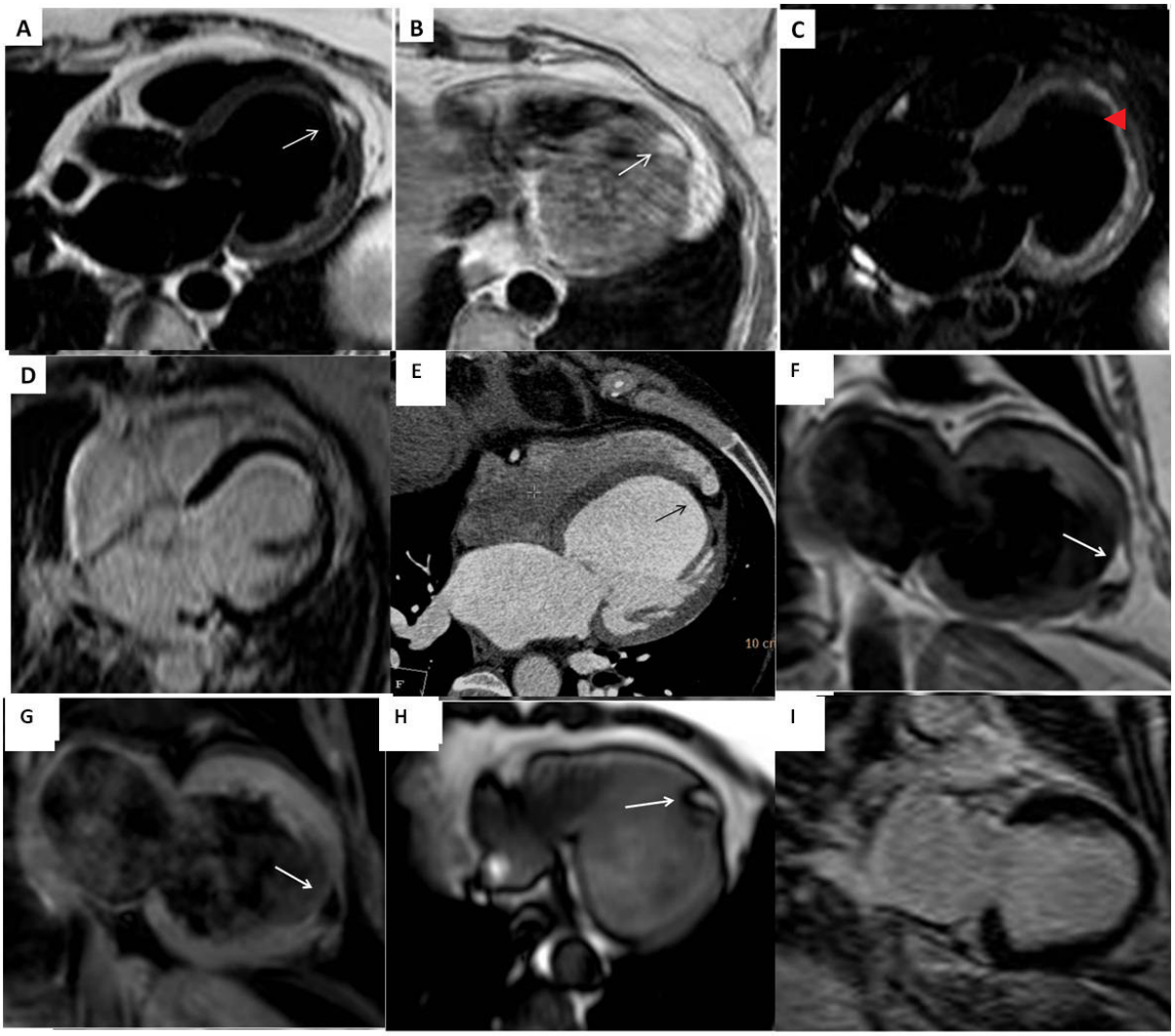

Figure 1 – *MRI axial images of the heart using different pulse sequences to demonstrate myocardial segment of fatty infiltration in the LV apex. The CMR protocol included T1-weighted spin-echo (with-and-without fat-suppression), T2-weighted black-blood image, and DHE after administration of gadolinium. (A) T2-weighted black blood; (B) T1-weighted turbo spin echo; (C) T1-weighted turbo spin echo with fat saturation (showed clear nulling of apical fatty area); (D) DHE technique showing no evidence of myocardial scar; (E) Multi-detector CT image that shows a focal area of fatty infiltration in the LV apex (black arrow). This area has a negative Hounsfield value indicating its fatty nature. Follow-up 3 Tesla CMR study after 18 months showed no significant changes in fat distribution within LV; (F) Two-chamber T1-weighted turbo spin echo showing the fatty area at the LV apex; (G) Two-chamber T1-weighted turbo spin echo with fat saturation showing clear nulling of apical fatty area; (H) Axial T1-weighted single-shot axial image of the most distal apical portion of LV showing part of the fatty area; (I) DHE technique showing no evidence of myocardial scar; however, the fatty area is not enhanced.*

Recommendation of Early Surgery in Primary Mitral Regurgitation: Pros and Cons

Levent Cerit
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To the Editor,

I have read the article entitled "Recommendation of Early Surgery in Primary Mitral Regurgitation: Pros and Cons" by Rosa et al.[1] with great interest, recently published in Arquivos Brasileiros de Cardiologia 2016; 107: 173-5. The investigators reported that currently, the recommendation of mitral surgery for asymptomatic patients is very controversial, since the indication of valvular intervention for symptoms, left ventricular dysfunction and dilatation, recent onset atrial fibrillation or pulmonary arterial hypertension is well consolidated in literature.[1]

Quantifying the mitral regurgitation (MR) in echocardiographic quantification is used primarily to aid grading as mild, moderate, and severe regurgitation. Cardiovascular magnetic resonance (CMR) is able to quantify MR with high accuracy and reproducibility using a combination of left ventricle (LV) volumetric measurements and aortic flow quantification. Myerson et al.[2] reported that quantifying MR with CMR showed a strong association with the future need for surgery over the subsequent 5 years, demonstrating the potential value of this approach. Previous studies also suggest only moderate agreement between CMR and echocardiography[3,4] and limited reproducibility for quantitative echocardiographic grading.[5] Evaluation of MR with CMR showed a significant relation with the future need for mitral valve surgery and was superior to CMR-derived LV volume and echocardiographic grading of regurgitation. These CMR parameters might prove useful for identifying suitable patients for early mitral valve repair replacement.[2]

In the light of these knowledge, CMR parameters might be beneficial to determine suitable patients for early mitral valve repair/replacement.

Keywords

Mitral Valve Insufficiency / surgery; Magnetic Resonance Imaging / methods; Echocardiography.

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Manuscript received August 20, 2016; revised manuscript October 14, 2016; accepted October 14, 2016.

DOI: 10.5935/abc.20160188

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Letter to the Editor

Reply

To the Editor,

Thank you so much for you interest in our Article "Indicação Cirúrgica Precoce na Insuficiência Mitral Primária: Prós e Contras", published in Arquivos Brasileiros de Cardiologia.

Cardiovascular magnetic resonance (CMR) is a method of great value, primarily regarding the anatomical diagnostic of valvular diseases. Its main application, to the present day, occurs in patients in which a clinical and echocardiographic dissociation, namely cases in which propaedeutics indicates an important valvar disease and the echocardiogram describes such injury as moderate, or vice-versa. In these cases, CMR is a better choice instead of cardiac catheterization together with ventriculography, as it seems less invasive and able to quantify more precisely the cardiac chambers volumes and functions.[1]

Myerson et al.[2] and Enriquez-Sarano et al.[3] articles show how extremely important is to demonstrate the existence of patient subgroups which could have benefited from an early mitral valvular surgery. Nevertheless, the parameters evaluated in this article need validation in future studies due to the work's pattern under discussion (observational study, non-blinded, surgical recommendation based on the patient's doctor opinion).

Thus, our opinion is that one must pay attention when recommending intervention based solely on the CMR parameters. However, we are in agreement as to such data is able to, in connection with other risk markers (BPF, effective regurgitant orifice, among others), add information for the *Heart Team* clinical decision.

Dr. Vitor Emer Egypto Rosa
Prof. Dr. Flávio Tarasoutchi

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