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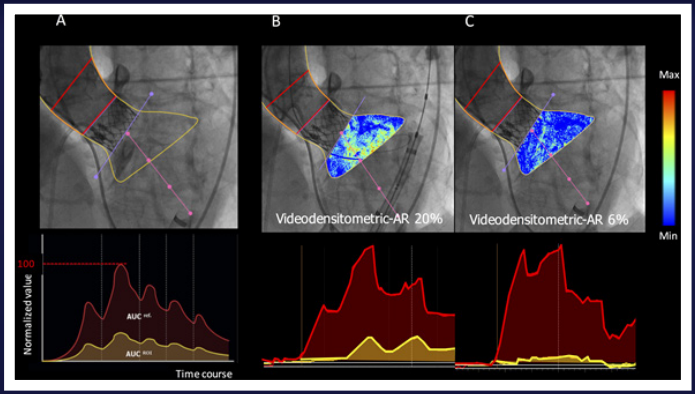

Figure 2 – *Videodensitometric assessment of aortic regurgitation. A) Delineation of the aortic root (reference region: red area in the aortography) and the subaortic one third of LV (ROI: yellow area in the aortography) are shown by the analyser. The time-density curves are provided for both ROI (yellow) and reference (red) regions, and the AUC is automatically computed by the software time-density integrals. VD-AR corresponds to the relative AUC, which is automatically calculated as the ratio of the relative AUC in the ROI (yellow) to that in the reference area (red). Theoretically, the value of VD-AR ranges from 0 to 1. B) An example of VD-AR measurement before BPD. C) An example of VD-AR measurement after BPD. Reproduce and adopted from Tateishi et al. EuroIntervention 2016[14] Page 195*

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Journal of Brazilian Society of Cardiology
REVISTA DA SOCIEDADE BRASILEIRA DE CARDIOLOGIA - Publicada desde 1948

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Microcirculation and Cardiovascular Diseases

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Human microcirculation has some aspects that make it unique in its capacity to adjust the supply of oxygen and nutrients to the metabolic demands of all cells throughout the body by adjusting vascular tone and releasing different vasoactive substances.[1] Endothelium-dependent vasodilation response in humans may vary according to age, gender, the vascular bed involved and the presence of atherosclerotic disease. Vascular smooth muscle cells undergo different hyperpolarization and relaxation when exposed to nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factors (EDHF), among others, as a result of the factors described above.[2]

In the last decade, the importance of assessing microvascular function has become evident in research on the pathophysiology of cardiovascular disease and cardiovascular risk stratification.[3] In this context, cutaneous microcirculation has been considered an accessible and representative vascular bed for assessing microvascular reactivity.[4] Indeed, there is evidence of an association between cutaneous microvascular reactivity and the microcirculatory function in different vascular beds, concerning both the underlying mechanisms and the intensity of the endothelium-dependent vasodilation response.[4] Therefore, assessing cutaneous microvascular reactivity has been proposed as a prognosis marker both for chronic disease and for the action of drugs related to the microvascular endothelial function.[5]

Microcirculation assessment in humans was initially performed using invasive techniques such as coronary angiography; however, the evolution of imaging techniques has made it possible to diagnose microcirculatory abnormalities in cardiovascular disease using non-invasive methods that range from ultrasound techniques such as stress echocardiogram and myocardial perfusion scintigraphy (neither directly assesing myocardial blood flow, as both are used to detect ischemia – which, in the absence of obstructive epicardial coronary disease, is considered evidence of microvascular disease) to more expensive techniques such as positron emission tomography (PET). The prognostic importance of myocardial microvascular dysfunction has been acknowledged, which has boosted studies on the subject, though it is not possible so far to directly visualize its structural abnormalities, which can only be assessed through myocardial flow or coronary flow reserve (CFR).[6]

CFR, or the ratio of hyperemic myocardial blood flow – i.e., at peak stress – to myocardial blood flow at rest,[7] evaluates the whole hemodynamics of coronary circulation, from epicardial arteries to microcirculation, including endothelial and vascular smooth muscle function.[7] Reduced CFR has been shown to be an independent predictor of mortality, also in patients with normal epicardial coronary arteries.[8] CFR can be examined by PET,[9] but because that method is not largely available, CFR exams have recently become possible by means of myocardial scintigraphy (SPECT) using solid-state gamma cameras such as the telluride-cadmium-zinc (CZT) type, which provide higher sensitivity and better temporal and spatial resolutions.[10]

As mentioned earlier, the systemic microcirculatory function can be examined using techniques for measuring the microvascular flow in the skin in a non-invasive way. In the clinical context, the methods most commonly used are based on laser light, including laser Doppler imaging and laser speckle contrast imaging (LSCI).[11] These techniques are usually associated with physiological or pharmacological stimuli that allow assessing endothelium-dependent (or independent) microvascular reactivity.[12] The physiological stimulus is usually forearm post-occlusive reactive hyperemia, which induces an endothelium-dependent vasodilation response. The most commonly used pharmacological stimulus is the cutaneous infusion of acetylcholine (endothelium-dependent vasodilation) or sodium nitroprusside (endothelium-independent vasodilatation), both through micro-iontophoresis.[12]

In this context, we have recently conducted a study using the LSCI technique where we demonstrated that the endothelium-dependent vasodilation response of cutaneous microcirculation is reduced in patients with early-onset coronary artery disease compared to healthy individuals[12]. In addition, the microvascular response related to vascular smooth muscle dilatation was also reduced in those patients, in parallel with significant increases of carotid intima-media thickness.[12] In another study, we demonstrated that the systemic microvascular endothelial function is similarly compromised in patients with ischemic heart disease or chronic Chagas heart disease.[13]

Recently, we adapted the LSCI technique for noninvasive assessment of penile microvascular reactivity.[14] In that study, we demonstrated that LSCI can be used to assess the effects of type 5 phosphodiesterase inhibitors on penile microcirculation in patients with hypertension and erectile dysfunction.[14]

Microcirculation rarefaction has been associated with cardiovascular and metabolic diseases, including hypertension, diabetes, obesity and metabolic syndrome.[15]

Keywords

Microcirculation/physiology; Humans; Cardiovascular Diseases/physiopathology; Endothelium, Vascular/pysiology; Vasodilatation/physiology; Risk Factors; Diagnostic Imaging.

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Change in the microvascular function in the skin has also been shown to correlate with an increased risk of coronary artery disease.[16] In addition, the rarefaction of microcirculation in capillary beds is related to target organ damage, which was suggested by the existence of an association between myocardial disease and the reduction of capillary density, as well as another association between left ventricular hypertrophy and cutaneous microvascular dysfunction, regardless of the level of systemic arterial pressure.[17,18] In a recent study, we demonstrated that cutaneous capillary density, as well as endothelium-dependent capillary recruitment, are reduced in patients with early-onset coronary artery disease.[19]

Therefore, the early detection of subclinical cardiovascular disease through the assessment of microcirculatory density and reactivity non-invasive techniques could represent an opportunity for early intervention, and consequently, prevention of cardiovascular events.[20] Moreover, microcirculation assessment could be useful to evaluate the chronic effects of cardiovascular drugs, making it attractive not only in research contexts but also in clinical practice.

Thus, we believe that microcirculation assessment will be increasingly employed in cardiovascular practice, both for diagnostic and prognostic purposes and for testing novel therapeutic interventions.

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Cryptogenic Acute Ischemic Stroke: Assessment of the Performance of a New Continuous Long-Term Monitoring System in the Detection of Atrial Fibrillation

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Abstract

Background: Long-term monitoring has been advocated to enhance the detection of atrial fibrillation (AF) in patients with stroke.

Objective: To evaluate the performance of a new ambulatory monitoring system with mobile data transmission (PoIP) compared with 24-hour Holter. We also aimed to evaluate the incidence of arrhythmias in patients with and without stroke or transient ischemic attack.

Methods: Consecutive patients with and without stroke or TIA, without AF, were matched by propensity score. Participants underwent 24-hour Holter and 7-day PoIP monitoring.

Results: We selected 52 of 84 patients (26 with stroke or TIA and 26 controls). Connection and recording times were 156.5 ± 22.5 and 148.8 ± 20.8 hours, with a signal loss of 6,8% and 11,4%, respectively. Connection time was longer in ambulatory (164.3 ± 15.8 h) than in hospitalized patients (148.8 ± 25.6 h) (p = 0.02), while recording time did not differ between them (153.7 ± 16.9 and 143.0 ± 23.3 h). AF episodes were detected in 1 patient with stroke by Holter, and in 7 individuals (1 control and 6 strokes) by PoIP. There was no difference in the incidence of arrhythmias between the groups.

Conclusions: Holter and PoIP performed equally well in the first 24 hours. Data transmission loss (4.5%) occurred by a mismatch between signal transmission (2.5G) and signal reception (3G) protocols in cell phone towers (3G). The incidence of arrhythmias was not different between stroke/TIA and control groups. (Arq Bras Cardiol. 2018; 111(2):122-131)

Keywords: Atrial Fibrillation; Stroke; Electrocardiography, Ambulatory; Cell Phone; Ischemic Attack, Transient.

Introduction

Atrial fibrillation (AF) is the main predictive factor of stroke.[1] Many studies have suggested that frequent short runs of atrial tachycardia (AT) or supraventricular extrasystoles (SVES) may yield early left atrial remodeling and predict AF and increased risk for stroke.[2-4] The risk for stroke is independent of clinical presentations of AF and recent studies have shown that in up to 30% of the cases, arrhythmia is diagnosed before, during or following an ischemic event.[5]

The diagnosis of AF requires documentation, and the detection of paroxysmal AF may be challenging.[6] By convention, the diagnosis of AF requires a minimum duration of 30 seconds.[7] The prognostic value of short episodes of AF is still debatable, and some authors have suggested that their occurrence may not be a benign condition.[8] Detection of paroxysmal AF has been performed by different monitoring techniques, and the importance of its early detection is due to the fact that the prompt initiation of anticoagulation significantly reduces the risk of stroke recurrence by up to 40%.[8-10] The American Heart Association and the Stroke Association recommend a long-term electrocardiographic monitoring of 30 days for the diagnosis of AF in post-cryptogenic stroke (class IIa; level of evidence C). Further evidence in support of this recommendation and for the establishment of the role of short AF episodes is still needed.[11,12]

The aim of this study was to evaluate the performance of a new ambulatory electrocardiographic monitoring system using cell phone transmission in the diagnosis of AF during the acute phase of stroke or transient ischemic attack (TIA) and compared it with 24-hour Holter, and to evaluate the incidence and the type of supraventricular arrhythmias in patients with and without stroke/TIA in its acute phase.[13]

Methods

Subjects: patients with recent (less than 15 days of the event) stroke/TIA were enrolled based on clinical and imaging findings. Stroke was classified as cryptogenic based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST).[14] Ambulatory patients without stroke/TIA, but with risk factors for these events (control group) were also included, and both groups had normal sinus rhythm at electrocardiography (ECG) and no history of AF or atrial flutter (AFL).

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Exclusion criteria were previous AF or AFL or admission electrocardiogram showing any of these conditions, hemorrhagic stroke, age younger than 18 years, residence in areas with no mobile phone coverage, need for intensive care due to severity of disease or difficult management of disease, sequela of neurologic injury, and patients with important cognitive impairment that could negatively affect the ability to understand the instructions related to the use of the devices. Patients with suspected stroke/TIA were seen at two medium-sized public hospitals in the city of Curvelo, Minas Gerais, Brazil, between August 2016 and April 2017. Control patients were enrolled during outpatient visits. Patients' follow-up and therapeutic approach were left to the assistant physicians' discretion. Patients or legal caregivers were invited to participate in the study, which was approved by the research ethics committee of University Hospital of São José/FELUMA, and all participants signed an informed form.

Measurement tools: the diagnosis of stroke/TIA was confirmed by computed tomography (CT) and/or magnetic resonance imaging (MRI), and classified for etiologies using the TOAST[14] criteria. CT and MRI tests were performed by radiologists experienced in the Siemens Somatom Spirit or Toshiba Asteion4 CT scanners and the GE Optima MR360 1.5T.

Demographic and clinical data: data of age, sex, skin color, place of residence, anamnesis, previous diseases, family history, weight, height, traditional cardiovascular risk factors, and $CHADS_2$ and CHA_2DS_2-VASc scores were collected, and cardiologic and neurologic tests were also performed.

Complementary tests: 12-lead ECG, transthoracic echocardiography, Doppler examination of carotid and vertebral arteries, chest X-ray (posterior-anterior and lateral views), laboratory tests including complete blood test, urea, creatinine, glucose, transaminases, GGT, potassium, sodium, TSH, free T4, cholesterol (total and fractions), triglycerides, prothrombin time (PT) and partial thromboplastin time (PTT).

Heart rhythm monitoring: during the first week after clinical diagnosis and notification of cryptogenic ischemic stroke or TIA, heart rhythm was monitored by three-channel Holter 24h recorders (DMS 300-8 and DMS 300-9) and analyzed simultaneously with the DMS CardioScan II software (DM Software Inc. Stateline, NV, USA) and electrocardiography (Policardiógrafo IP®, PoIP) (eMaster, Belo Horizonte, MG, Brazil).

PoIP monitoring: PoIP monitors independently collect and transmit electrocardiographic data at real time using the General Packet Radio Services/ *Enhanced Date Rates* for GSM Evolution (GPRS/ EDGE); data are then stored in the cloud. We used the Brazilian cell phone provider *Vivo* for transmission of the data to the PoIP web portal, and the Mozilla Firefox was used as the web browser for analysis of the data. PoIP offers a "Portal de Exames", an app that enables monitoring of different PoIP devices as well as the access to laboratory tests by individual access credentials (Figure 1). Six electrodes were arranged so that frontal plane leads could be monitored beyond V_1-V_2. Patients and family members were instructed and trained for the monitoring technique, quality of transmission signal, battery charge and charging of the lithium-based batteries. The monitoring was closely controlled via internet by the responsible staff members for the correct use of the device, and quality of the electrode contacts; if necessary, family or caregivers were informed about inadequate system operation or the quality of data transmission.

Procedures: Each participant received an electrode pack and a leaflet with a thorax illustration indicating electrodes' colors and correct positioning for replacement. All electrocardiographic recordings were analyzed by the same investigator (RSF), a cardiologist experienced in ambulatory electrocardiography, and all electrocardiographic tracings considered indicative of AF or tachycardia were reviewed by a second investigator (EBS), a cardiac electrophysiologist.

For analysis of PoIP findings, the results were accessed via internet and examined for AF/AFL every 12 hours or every time the monitor button was pressed by the patient/caregiver. Every 24-hour period, all data transmitted by PoIP were exported and reviewed offline, and quantitative analysis of arrhythmias registered. In this analysis, we considered – number of (single or in pairs) SVES, number of nonsustained atrial tachycardia (AT) episodes greater than three consecutive premature atrial complexes and shorter than 30 seconds, sustained AT longer than 30 seconds and number of AF episodes longer or shorter than 30 seconds.

Statistical analysis: categorical variables were expressed as counts and percentages and numerical variables as mean ± standard deviation (SD). Data normality assumptions were verified with the Shapiro-Wilk test. Associations between categorical variables were assessed by Fisher's exact test or the chi-square test of independence. Comparisons of two groups between independent samples were made by the Wilcoxon test, the Mann-Whitney test or the Student's t-test, as appropriate. Analyses were performed using the free R software version 3.3.2 at 5% level of significance. Initial cohort was composed of 58 patients with stroke/acute TIA and 26 controls. For selection of patients with similar characteristics for the groups of interest, we used the propensity score matching (PSM) method. A logistic regression was constructed to estimate the probability of belonging to the stroke/TIA group, considering the following predicting variables – sex, age and $CHADS_2$ corrected by subtracting two points in patients with stroke/TIA. PSM enabled the selection of 26 patients with stroke/TIA matched with controls by the probabilities obtained from the logistic model, so that the analysis of the cohort yielded 52 patients (26 with stroke/TIA and 26 controls) (Figure 2). Sample power to verify the difference between the recording period on the first day of Holter and PoIP use (23.7 ± 1 and 20 ± 3.2h, respectively) was greater than 80%.

Results

Our sample was composed of 52 patients, equally allocated into stroke/TIA and control groups (Figure 2). More than half of patients (51.9%) were men, mean age was 70.7 ± 10.5 years, with 73.1% of patients aged 65 years or older. Mean BMI was 25.5 ± 5.6 kg/m^2, 21.2% were smokers and 19.2% alcohol consumers. Mean corrected CHADS2 and CHA2DS2-VASc scores were 1.8 ± 0.8 and 3.3 ± 1.2, respectively.

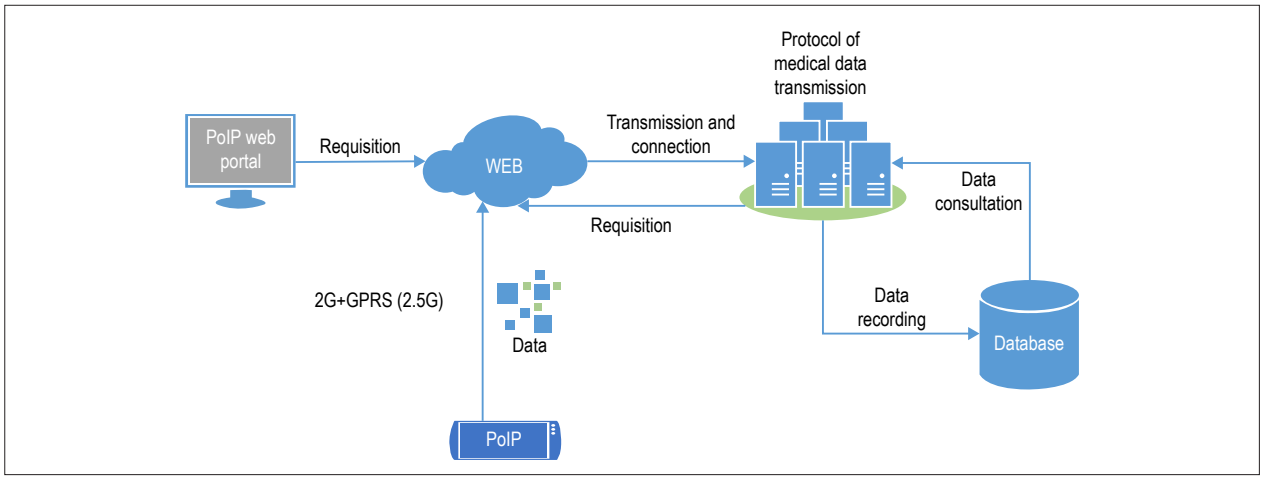

Figure 1 – *Conceptual diagram of PoIP – as can be seen in the diagram, PoIP uses the concept of real-time transmission of the data by the EDGE technology. Wireless data transmission is performed by standard protocol to internet access in mobile devices by GPRS-EDGE – Generic Packet Radio Service, commonly known as 2.5G*

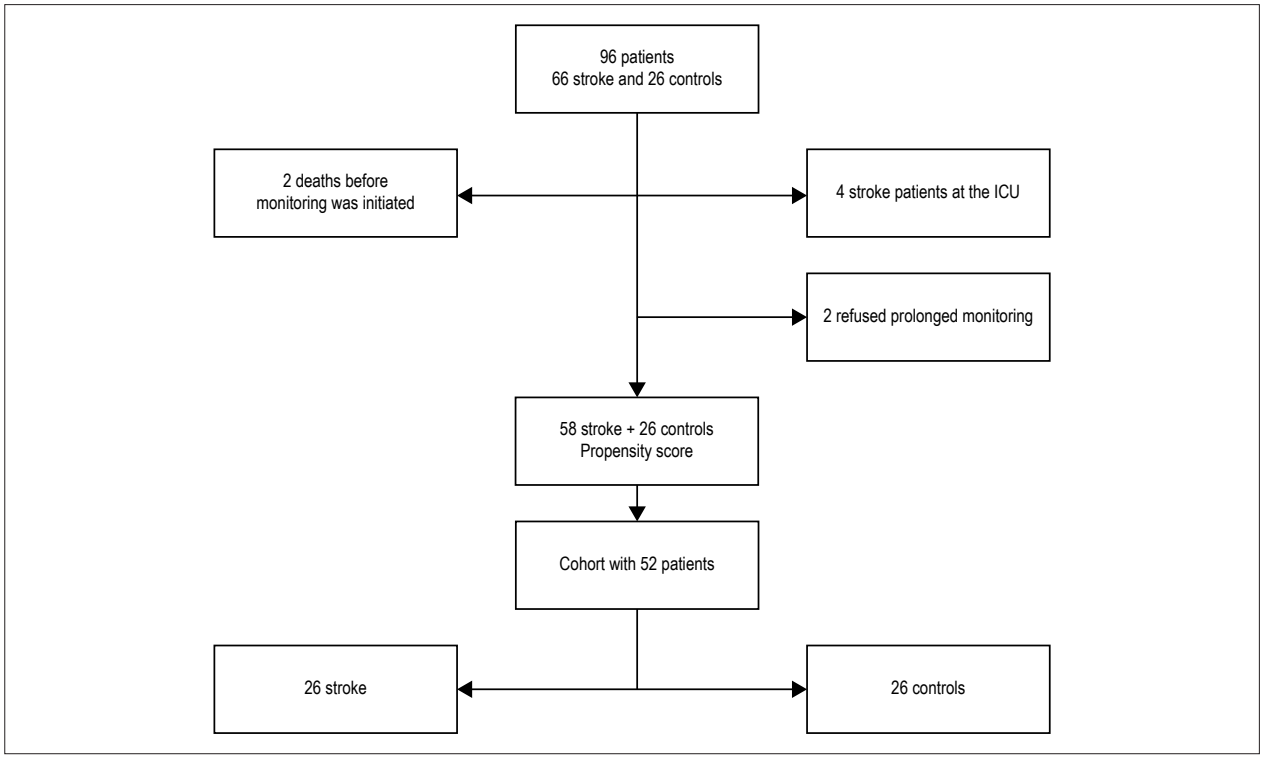

Figure 2 – *Flowchart depicting selection of the study groups*

The most frequent comorbidities were arterial hypertension (84.6%) and diabetes mellitus (51.9%). Among control patients, a significantly higher (p = 0.03) proportion of smokers was found in stroke patients aged 65 years or older (p = 0.04). No other difference was found between the groups (Table 1).

Complementary tests

Echocardiography: the only statistically significant difference between the groups was a lower (although within normal range) ejection fraction (p = 0.04) values in the stroke/TIA group. *Clinical analysis*: the only statistically significant difference was found in free T4 (p = 0.03), which was higher in stroke/TIA, but also within the normal range. No other difference was found between the groups.

Data transmission analysis

Mean recording period was 23.5 ± 0.6 hours by Holter monitoring and 148.8 ± 20.8 hours by PoIP, with no significant

Table 1 – Patients' characteristics by study groups

Variables	Sample (n = 52)	stroke/TIA (n = 26)	Controls (n = 26)	P-value
Clinical data				
Male sex	27 (51.9%)	14 (53.8%)	13 (50%)	1.000[Q]
Age (years)	70.7 ± 10.5	70.9 ± 11.4	70.6 ± 9.7	0.917[T]
≥ 65 years	38 (73.1%)	20 (76.9%)	18 (69.2%)	0.755[Q]
White race	40 (76.9%)	17 (65.4%)	23 (88.5%)	0.100[F]
BMI (kg/m^2)	25.5 ± 5.6	25.6 ± 4.2	25.4 ± 6.9	0.498[W]
> 30 kg/m^2	11 (21.2%)	3 (11.5%)	8 (30.8%)	0.173[F]
Smoking	11 (21.2%)	9 (34.6%)	2 (7.7%)	0.038[F]
< 65 years	3 (21.4%)	2 (33.3%)	1 (12.5%)	0.539[F]
≥ 65 years	8 (21.1%)	7 (35%)	1 (5.6%)	0.045[F]
Alcohol consumption	10 (19.2%)	7 (26.9%)	3 (11.5%)	0.291[F]
Corrected $CHADS_2$	1.8 ± 0.9	1.8 ± 1	1.9 ± 0.8	0.831[W]
Corrected CHA_2DS_2-VASc	3.3 ± 1.2	3.3 ± 1.3	3.3 ± 1.2	0.598[W]
Comorbidities				
Arterial hypertension	44 (84.6%)	23 (88.5%)	21 (80.8%)	0.703[F]
Diabetes mellitus	27 (51.9%)	13 (50%)	14 (53.8%)	1.000[F]
Previous stroke[2]	6 (11.5%)	6 (23.1%)	-	
Previous TIA[2]	6 (11.5%)	6 (23.1%)	-	
Coronary insufficiency	5 (9.6%)	2 (7.7%)	3 (11.5%)	1.000[F]
Congestive heart failure	5 (9.6%)	3 (11.5%)	2 (7.7%)	1.000[F]
Kidney failure	2 (3.8%)	2 (7.7%)	-	
Echocardiography				
Aorta (mm)	32.6 ± 3.9	33.6 ± 4.3	31.6 ± 3.3	0.079[T]
Left atrium (mm)	36.9 ± 4.5	36.3 ± 4	37.6 ± 4.9	0.296[T]
Ejection fraction (%)	63.6 ± 10.3	61 ± 11.3	66 ± 8.9	0.049[W]
Interventricular septum (mm)	10.3 ± 1.4	10.7 ± 1.4	10 ± 1.3	0.086[W]
RV posterior wall (mm)	9.9 ± 1.3	10.1 ± 1.4	9.8 ± 1.3	0.356[W]
Laboratory data				
Glucose (mg/dl)	113 ± 57.5	125.5 ± 76.6	100.5 ± 23.5	0.098[W]
Glycated hemoglobin (%)	6.1 ± 0.7	6.1 ± 0.8	6.1 ± 0.7	0.848[T]
Creatinine (mg/dl)	1.01 ± 0.38	1.06 ± 0.44	0.96 ± 0.31	0.614[W]
HDL (mg/dl)	53.1 ± 15.7	48.8 ± 11.8	57.1 ± 18.1	0.059[T]
LDL (mg/dl)	87.2 ± 30.8	91.1 ± 33.8	83.3 ± 27.7	0.376[T]
Triglycerides (mg/dl)	142.8 ± 96.3	112.4 ± 42.9	171.9 ± 122.4	0.060[W]
TSH (nU/L)	3 ± 3.21	2.70 ± 2.39	3.22 ± 3.82	0.459[W]
Free T4 (ng/dl)	1.03 ± 0.23	1.10 ± 0.25	0.96 ± 0.20	0.038[T]

Numerical variables are expressed as mean ± standard deviation; TIA: transient ischemic attack; [1]Corrected $CHADS_2$ and CHA_2DS_2-VASc scores represent the subtraction of two points from the original scores in the stroke group; [2] previous stroke and TIA were found only in the stroke group (exclusion criteria for controls); [F]exact Fisher's test; [Q]chi-square test of independence; [W] Wilcoxon Mann-Whitney test and [T] Student's t-test for independent samples

difference between the groups, despite higher transmission loss for artifacts among PoIP control subjects. PoIP signal losses were caused by loss of connection (6.8%) and recording signal loss in the server (Table 2).

In the first 24 hours, longer period was required for Holter recording (23.5 ± 0.6 hours) as compared with PoIP (19.2 ± 3.4 hours) ($p < 0.001$).

In the stroke/TIA group, PoIP monitoring was started after 5.4 ± 2.7 days of stroke/TIA during hospitalization, and a shorter connection ($p = 0.02$) and recording period was observed with PoIP (Table 3).

Arrhythmias

AF was detected in one patient by Holter monitoring and in 6 patients by PoIP in the stroke/TIA group, and in only one control by PoIP. Regarding other supraventricular arrhythmias, further cases of nonsustained AT and frequent AT or SVES were identified by Holter monitoring in patients aged 65 years or older in the stroke/TIA group ($p = 0.04$ and 0.04, respectively). In two cases, differential diagnosis of AT and nonsustained AF required revision by the two observers (RFS and EBS). It is worth mentioning, however, that patients who had AF also had AT, and therefore, a misinterpretation of electrocardiographic tracings would not affect the results, due to the occurrence of both conditions in the same patient.

PoIP monitoring revealed that there were no significant differences between the groups regarding tachycardia (Table 4), and all patients with AF also had AT.

Comparisons between Holter and PoIP results showed a higher proportion of AT identified by PoIP in both stroke/TIA ($p = 0.004$) and control ($p = 0.02$) groups. Also, PoIP monitoring revealed a higher proportion of patients with frequent AT or SVES in the stroke/TIA ($p = 0.01$) and control ($p = 0.02$) groups considering total monitoring period, but no difference was found between the groups in the first 24 hours.

Discussion

In the present study that included 52 patients older than 59 years, prolonged rhythm monitoring was performed in 26 patients with acute cerebrovascular events, and initiated only 5 days (mean) after the event. The main findings were high prevalence of arterial hypertension and diabetes mellitus, some connectivity problems and problems related to PoIP signals' recording, and similar profile of cardiac arrhythmias between the study groups.

The most frequent comorbidities were arterial hypertension (84.6%) and diabetes mellitus (51.9%), with similar distribution between the groups studied. This result was expected, since these variables were used in the PSM model, and both comorbidities are also included in the CHADS2 and CHA2DS2-VASc scores. Although these scores provide simple methods for predicting an individual risk of ischemic stroke, the risk estimated by these instruments represent only part of the overall risk (statistical agreement of 0.5). In other words, not all patients with a CHADS2 score equal to 0 or 1 have a low risk, and hence the clinical decision not to anticoagulate patients based only on this score may be erroneous. Despite the higher specificity of a CHA_2DS_2-VASc score ≥ 2, this still underestimates the risk.[15]

For this reason, we analyzed with particular interest the higher prevalence of smoking in stroke/TIA patients ($p = 0.038$), especially among patients older than 65 years ($p = 0.045$). A recent meta-analysis showed that smoking is associated with a modest increase in AF, and that quitting smoking reduces but not eliminates the associated risk of the disease.[16-18] Nevertheless, the addition of smoking to the score does not improve the risk prediction of stroke or TIA.[19]

Monitoring by mobile phone

Although PoIP and Holter monitoring systems had similar performance in the first 24 hours, there were problems with signal connection and transmission during PoIP monitoring. Loss of connection with the cell phone provider accounted for 6.8% of total monitoring time, shorter recording time in the server and lower data losses due to artifacts (Table 2). Loss of connectivity was greater in hospitalized (stroke) patients ($p = 0.024$).

For better interpretation of this result, we measured the strength of the provider signal using the Network Monitor®

Table 2 – Monitoring period (hours) by study groups

Variables	Sample (n = 52)	Stroke/TIA (n = 26)	Controls (n = 26)	P-value
Holter				
Recording time	23.5 ± 0.6	23.4 ± 0.8	23.5 ± 0.4	0.948
Loss (artifacts)	0.6 ± 1.4	0.6 ± 1.7	0.6 ± 1	0.162
PoIP				
Connection period	156.5 ± 22.5	148.8 ± 25.6	164.3 ± 15.8	0.024
Recording time on the first day	19.2 ± 3.4	19.1 ± 2.5	19.2 ± 4.2	0.514
Recording period	148.8 ± 20.8	143.9 ± 23.3	153.7 ± 16.9	0.080
Loss (artifacts)	50.9 ± 26.2	45.6 ± 26.3	56.1 ± 25.5	0.081

Wilcoxon Mann-Whitney test for independent samples; monitoring period had been planned to be up to 24 hours by Holter andu p to 168 hours (7 days) by PoIP. Comparison of recording periods between Holter and PoIP on the first day: $p < 0.001^W$

Table 3 – Holter monitoring results by study groups

Variables	Stroke/TIA (n = 26)	Controls (n = 26)	p-value[F]
Atrial fibrillation (< 30 seconds)	**1 (3.8%)**	**-**	**-**
Atrial tachycardia (AT)	**16 (61.5%)**	**9 (34.6%)**	**0.095**
< 65 years	1 (16.7%)	2 (25%)	1.000
≥ 65 years	15 (75%)	7 (38.9%)	0.047
Frequent SVES*			
< 65 years	5 (19.2%)	3 (11.5%)	0.703
≥ 65 years	5 (25%)	3 (16.7%)	0.697
Frequent AT or SVES	**17 (65.4%)**	**10 (38.5%)**	**0.095**
< 65 years	1 (16.7%)	2 (25%)	1.000
≥ 65 years	16 (80%)	8 (44.4%)	0.042
Ventricular tachycardia	**6 (23.1%)**	**5 (19.2%)**	**1.000**
< 65 years	-	1 (12.5%)	-
≥ 65 years	6 (30%)	4 (22.2%)	0.719
Frequent SVES	**6 (23.1%)**	**7 (26.9%)**	**1.000**
< 65 years	-	1 (12.5%)	-
≥ 65 years	6 (30%)	6 (33.3%)	1.000

*SVES: supraventricular extrasystoles; [F]Fisher's exact test; *frequent SVES was defined as > de 30 events/hour*

Table 4 – POIP monitoring results by study groups

Variables	Stroke/TIA (n = 26)	Controls (n = 26)	p-value[F]
Atrial fibrillation (< 30 seconds)*	**6 (23.1%)**	**1 (3.8%)**	**0.099**
First 24h	2 (7.7%)	1 (3.8%)	1.000
Atrial tachycardia	**22 (84.6%)**	**18 (69.2%)**	**0.324**
< 65 years	4 (66.7%)	5 (62.5%)	1.000
≥ 65 years	18 (90%)	13 (72.2%)	0.222
First 24h	12 (46.2%)	14 (53.8%)	0.782
Frequent SVES **	**4 (15.4%)**	**6 (23.1%)**	**0.727**
< 65 years	-	1 (12.5%)	-
≥ 65 years	4 (20%)	5 (27.8%)	0.709
First 24h	2 (7.7%)	6 (23.1%)	0.249
Frequent atrial tachycardia or SVES	**22 (84.6%)**	**19 (73.1%)**	**0.499**
< 65 years	4 (66.7%)	5 (62.5%)	1.000
≥ 65 years	18 (90%)	14 (77.8%)	0.395
First 24h	12 (46.2%)	14 (53.8%)	0.782
Ventricular tachycardia	**7 (26.9%)**	**7 (26.9%)**	**1.000**
< 65 years	-	2 (25%)	-
≥ 65 years	7 (35%)	5 (27.8%)	0.734
First 24h	3 (11.5%)	4 (15.4%)	1.000
Frequent ventricular extrasystoles	**8 (30.8%)**	**7 (26.9%)**	**1.000**
< 65 years	1 (16.7%)	1 (12.5%)	1.000
≥ 65 years	7 (35%)	6 (33.3%)	1.000
First 24h	6 (23.1%)	6 (23.1%)	1.000

*SVES: supraventricular extrasystoles; [F]Fisher's exact test; *all cases identified in patients aged ≥65 years; **frequent SVES was defined as > de 30 events/hour*

Table 5 – Comparisons between Holter and POIP monitoring results

Variable	Holter	POIP	p-value[F]
Atrial fibrillation (< 30 seconds)	**1 (1.9%)**	**7 (13.5%)**	**0.060**
AVC/AIT	1 (3.8%)	6 (23.1%)	0.099
Controls	-	1 (3.8%)	-
First 24h	1 (1.9%)	3 (5.7%)	0.618
Atrial tachycardia	**25 (48.1%)**	**40 (76.9%)**	**0.004**
AVC/AIT	16 (61.5%)	22 (84.6%)	0.116
Controls	9 (34.6%)	18 (69.2%)	0.025
First 24h	25 (48.1%)	26 (50%)	1.000
Frequent SVES*	**8 (15.4%)**	**10 (19.2%)**	**0.796**
AVC/AIT	5 (19.2%)	4 (15.4%)	1.000
Controls	3 (11.5%)	6 (23.1%)	0.465
First 24h	8 (15.4%)	8 (15.4%)	1.000
Frequent atrial tachycardia or SVES	**27 (51.9%)**	**41 (78.8%)**	**0.007**
Stroke/TIA	17 (65.4%)	22 (84.6%)	0.199
Controls	10 (38.5%)	19 (73.1%)	0.025
First 24h	27 (51.9%)	26 (50%)	1.000
Ventricular tachycardia	**11 (21.2%)**	**14 (26.9%)**	**0.647**
Stroke/TIA	6 (23.1%)	7 (26.9%)	1.000
Controls	5 (19.2%)	7 (26.9%)	0.743
First 24h	11 (21.2%)	7 (13.5%)	0.438
Frequent ventricular extrasystoles	**13 (25%)**	**15 (28.8%)**	**0.825**
Stroke/TIA	6 (23.1%)	8 (30.8%)	0.755
Controls	7 (26.9%)	7 (26.9%)	1.000
First 24h	13 (25%)	12 (23.1%)	1.000

*SVES: supraventricular extrasystoles; [F]Fisher's exact test; *frequent SVES was defined as > de 30 events/hour*

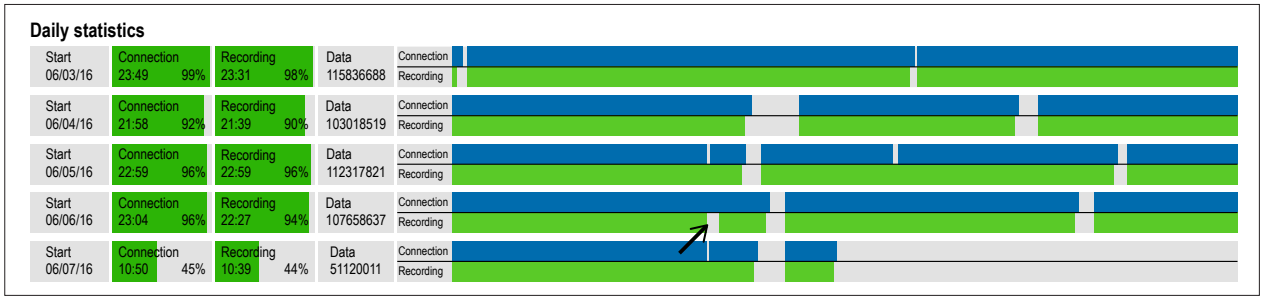

Figure 3 – *PoIP provides daily statistics of connection (blue line) and recoding (green line) data of signal transmission in the server. It is of note that connection and transmission percentages are very similar to each other (day 3/6: 99% and 98%, day 4/6: 92% and 90%, day 5/6: 96% and 96%). Small losses occurred, as on 6/6/2016, when there was a brief period when signal was transmitted but not recorded in the server (arrow)*

software in the ward facilities. We found a high signal variation depending on the site where the measurements were obtained – in the entrance, in the middle and in the ward exit, the signal velocity was 1.6, 12.3 and 0.3 Mbs, respectively, and signal strength was 60, 70 and 20%. Such high signal variation may explain signal losses during the monitoring of patients hospitalized in these areas, which would be lower in the outpatient department.

In addition, transmission losses may occur even in cases of adequate connectivity between PoIP and the mobile phone provider, due to instability of the mobile phone network. During these unstable periods, PoIP remains connected to the provider, and data transmission is restored when connection is recovered (Figure 3). Although such instability periods, are usually short, in our study, they accounted for 11.4% of total monitoring time, *i.e.* approximately 19 hours a week

per patient (Table 2). Also, we found that after the repair of transmission towers and antennas, signal reception was changed from 2.5G (GPRS General Packet Radio Service) to 3G, which negatively affects data transmission. Updating of the technology from 3G to 4G would resolve this issue, as well as reduce the energy expenditure with data package transmission, resulting in optimization of rechargeable battery duration, reduction of charging time and improving monitoring performance.

Greater data loss due to artifacts was seen in control subjects in the PoIP group, which may be justified by the greater freedom of movement of patients in ambulatory treatment.

Arrhythmias detected by PoIP (firs 24 hours) compared with Holter-24

In the first 24 hours, no difference in arrhythmias was observed (AT, SVES, SVES + AT). Despite the longer monitoring period by Holter recordings, all AT runs and the three episodes of AF (2 in the stroke and 1 in the control group).

Twenty-four hour Holter compared with prolonged monitoring

Comparison between Holter and PoIP monitoring results showed a higher proportion of frequent AT and SVES detected by PoIP monitoring in both stroke/TIA and control groups, which was expected by its longer monitoring period.

Comparison of arrhythmias detected in stroke group and controls

No significant difference was found in the occurrence of AT or nonsustained AF, in the comparison between patients with cryptogenic stroke and a control group matched by sex, age and corrected $CHADS_2$. We report a high prevalence of atrial arrhythmias in 52 patients, including 40 with AT and 7 with AF. In stroke/TIA group, proportion of AF was 23.1% in patients monitored by PoIP, and 3.8% in those monitored by Holter, which is in agreement with the literature (Tables 3, 4 and 5).[20] Some studies have suggested that and additional 24-hour period of monitoring would increase the percentage of new diagnoses of paroxysmal AF in 2-4% stroke patients.[21,22] This confirms the efficacy of prolonged ambulatory ECG in patients at risk of AF and may generate a clinically significant diagnostic yield.[23]

Studies have highlighted the association of frequent SVES and AT with increased risk of stroke.[2,3,4,24-27] Studies involving long-term heart rhythm monitoring in patients with previous stroke/TIA have reported a paroxysmal AF prevalence of 5-20%.[20,28,30-33]

In our study, all AF episodes lasted less than 30 seconds. Although an AF episode ≥ 30 seconds is used as a parameter for the diagnosis of AF,[7] some authors have suggested that short AF episodes have an impact on the risk of stroke/TIA or systemic thromboembolism.[10,33]

One important finding was the lack of difference in the prevalence of atrial arrhythmias between patients with and without stroke or TIA, at similar risk for these conditions. This finding suggests that the atrial arrhythmias detected may be an epiphenomenon. Kottkamp and other authors[15,34] have suggested the presence of a thrombogenic fibrotic atrial cardiomyopathy, with risk for embolic events with no causal connections with atrial arrhythmias. Contractile changes would be responsible for the increased thrombogenic risk during sinus rhythm, in addition to interatrial block and sinus node dysfunction. Even ablation of AF would not be able to impede the progression of fibrotic process.[34] Factors like diabetes, hypertension, age, among others, would be involved in myocardial damage. In our sample, more than 80% of patients had arterial hypertension and more than 50% were diabetic. Non-invasive detection of atrial fibrosis is currently limited to MRI techniques, not available in clinical practice.[34] In this context, AF would be a manifestation of atrial structural changes, and thereby increasing the risk of embolic events.

None of our patients with stroke/TIA had AF before or during stroke. In fact, AF may be detected in only a minority of the cases and may take months, as shown by the TRANDS, ASSERT and IMPACT studies, which included patients with implantable continuous monitoring devices.[35-37]

The paradigm used in most studies is that AF detection would be just a matter of time, but even in a one-year follow up, AF is detected in less than half of patients with cryptogenic stroke. This is a pioneering study in monitoring patients at similar stroke and TIA risk, by including a group with stroke and a control group without the disease. The finding that the incidence of atrial arrhythmias was not different between both groups is consistent with the hypothesis that a factor other than arrhythmia may be involved in the risk for stroke; one possibility is fibrotic atrial cardiomyopathy.

Study limitations

The sample size was insufficient to evaluate individual risk factors. Discrimination between short runs of atrial tachycardia and AF may be difficult, even to an experienced electrophysiologist. P-waves in ambulatory monitoring systems may not be clearly identified as compared with conventional 12-lead ECG. Nevertheless, analysis of isolated episodes and analysis of more than one arrhythmia episode yielded similar results, since all patients that had short AF episodes also had AT.

Mobile phone services currently available still have limited coverage, with absent or deficient signal strength, and unstable transmission velocity, which altogether, negatively affect PoIP data collection. Due to frequent repairs of problems caused by electrical discharges in cell phone towers, access to GPRS may be lost, thereby affecting signal reception, which may be solved by implementation of the 4G technology.

Conclusions

Holter and PoIP showed comparable results in the first 24 hours. The shorter monitoring period was caused by a low signal strength. Data transmission loss in hospitalized patients resulted from a mismatch between the protocol of signal transmission in the cell phone tower (3G) and the signal effectively transmitted (2.5G), which can be mitigated by the adoption of a 4G technology. The incidence of arrythmia was not different between stroke and control groups.

Author contributions

Conception and design of the research, acquisition of data and critical revision of the manuscript for intellectual content: Sampaio RF, Gomes IC, Sternick EB; Analysis and interpretation of the data: Gomes IC; Writing of the manuscript: Sampaio RF, Sternick EB.

Potential Conflict of Interest

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

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Study Association

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Hospital Universitário São José/FELUMA under the protocol number CAAE=35481114.0.0000.5134. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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Atrial Fibrillation and Cryptogenic Thromboembolic Events

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Short Editorial regarding the article: Cryptogenic Acute Ischemic Stroke: Assessment of the Performance of a New Continuous Long-Term Monitoring System in the Detection of Atrial Fibrillation

Annual stroke rates are extremely high, affecting around 15 million individuals worldwide, generating major public health and economic impact. Approximately 25% of stroke cases do not have a determined etiology, thus being denominated cryptogenic stroke (CS).[1] Cryptogenic strokes do not have a definite cause; their identification occurs by exclusion, when they are not attributable to definite cardioembolism, large-vessel atherosclerosis of and small-vessel disease, despite extensive vascular, cardiac or serological investigation.[2]

CS rates vary significantly, depending on the degree of diagnostic investigation. Considering that most CS cases have an embolic origin, a new terminology has been recently created for non-lacunar cryptogenic ischemic strokes: "Embolic Stroke of Undetermined Source".[3]

Approximately one-third of patients with CS have a new ischemic episode in 10 years,[4] of which 63% are once again classified as cryptogenic.[5] Possible causes for this recurrence, despite the primary event, are paroxysmal atrial fibrillation (AF), arterial thromboembolism, patent foramen ovale, structural heart disease or less common etiologies, such as thrombophilias. AF detection after a CS or ESUS offers the opportunity to reduce the risk of stroke recurrence by prescribing an oral anticoagulant.[6] Without this diagnosis, the treatment for CS and ESUS consists only of platelet antiaggregation.[7]

Detection of subclinical atrial fibrillation in cryptogenic and embolic stroke of undetermined source

The use of long-term monitoring dramatically improved the ability to detect short, rare, and asymptomatic AF periods in stroke patients. The EMBRACE study evaluated 572 patients with ischemic stroke in the last 6 months, with no AF diagnosis, with randomization for 30-day continuous monitoring (287 patients) vs. 24-hour Holter (285 patients).

The AF detection rates (> 30 seconds) were 16.1% in the long-term monitoring group vs. 3.2% in the Holter group.[8] Similarly, when Implantable Monitors (IM) were used, as in the CRYSTAL-AF (Cryptogenic Stroke and underlying Atrial Fibrillation) study, AF detection rates using IM were higher than the standard detection rates during a long-term follow-up: 8.9%, 12.4% and 30% vs. 1.4%, 2.0% and 3% in the period of 6, 12 and 36 months.[9]

In this issue, Sampaio et al.[10] published an article on the evaluation of a continuous monitoring device (PoIP) when compared to 24-hour Holter in the diagnosis of atrial arrhythmias in patients with and without stroke, or transient ischemic attack (TIA), and without AF. Episodes of AF were detected in the group of patients with a history of stroke / TIA in 23.1% of patients in the PoIP group and in 3.8% of patients in the Holter group. Lower recording times were also observed in the first 24 hours in the PoIP group vs. Holter group. Atrial tachycardia rates were higher in patients in the stroke group when compared to controls. Significant loss of signal was observed in the PoIP group, of 11.4% due to network instability and different types of signal-sending technology, GPRS vs. 3-4G.

Even with a limited number of patients, the incidence of AF was higher in the long-term monitoring group, although it did not reach statistical significance. However, for this type of monitoring, we need to improve the quality of data transmission, the stability of networks and the technologies used for sending and receiving signals, aiming at lower losses and better quality of the received data.

Association between atrial fibrillation, cryptogenic and embolic stroke of undetermined source

Recently, several studies have evaluated the association of atrial tachyarrhythmias diagnosed in implantable devices with the risk of thromboembolic events. The MOST study[11] showed that the detection of periods > 5 minutes of atrial heart rate > 220 bpm was associated with a six-fold increase in the risk of AF and a 2.8-fold increase in the risk of death or stroke in these patients with AF. The TRENDS study[12] showed that patients with episodes of AF / AT > 5.5 hours / day had an increased risk of thromboembolism (hazard ratio = 2.2), when compared to those with AF / AT burden of zero. Similarly, the ASSERT study demonstrated that the presence of atrial heart rate > 190 bpm for a period of time > 6 minutes was associated with a 5.6-fold increase in the development of AF and 2.5-fold increase in new episodes of stroke or systemic thromboembolism.[13] A more recent analysis of this study showed that high-frequency atrial episodes lasting > 24 hours increased the risk of ischemic stroke and systemic embolism to 3.1%/year – a risk comparable to that of clinical AF.[14]

Although the high atrial rate with an increased number of embolic episodes is well documented, the temporal and causal association require further elucidation. A sub-analysis of the TRENDS study demonstrated the presence of tachyarrhythmias prior to the embolic event in only 50% of the patients; 73% of them did not have tachyarrhythmias in the 30-day period before the embolic event. Also, the ASSERT study corroborated the results by showing AF rates in 51% of patients with thromboembolism, but only 8% of them had AF in the 30-day pre-stroke period.[15] The evaluation of these studies suggest that the presence of AF could be simply a marker of thromboembolic risk and be indirectly associated with the occurrence of thromboembolism through a more complex mechanism than the previously expected one.[16]

Keywords

Atrial, Fibrillation; Stroke; Thromboembolism; Foramen Ovale,Patent.

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Persistent Inflammatory Activity in Blood Cells and Artery Tissue from Patients with Previous Bare Metal Stent

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Abstract

Background: Studies have pointed out a higher mortality after coronary artery bypass surgery (CABG) in patients with stent.

Objective: To evaluate inflammatory markers in peripheral blood cells and in coronary artery tissue samples obtained during CABG in patients with stent compared to controls.

Methods: The case series consisted of two groups, one with previous stent implantation (n = 41) and one control (n = 26). The expression of the LIGHT, IL-6, ICAM, VCAM, CD40, NFKB, TNF, IFNG genes was analyzed in peripheral blood cells collected preoperatively. The coronary artery was evaluated for: interleukin-6, ICAM, VCAM, CD40, NFKB, TNF-alpha and IFN-gamma by immunohistochemistry. A total of 176 tissue samples were grouped for analysis in: A1- arteries with stent (n = 38); A2- native arteries from patients with stent in another artery (n = 68); and A3- arteries without stent from controls undergoing routinely CABG surgery (n = 70). A significance level of 0.05 was adopted.

Results: Patients with stent showed higher TNF (p = 0.03) and lower CD40 gene expression (p = 0.01) in peripheral blood cells than controls without stent. In coronary artery samples, the TNF-alpha protein staining was higher in the group A1, not only in the intima-media layer (5.16 ± 5.05 vs 1.90 ± 2.27; p = 0.02), but also in the adipose tissue (6.69 ± 3.87 vs 2.27 ± 4.00; p < 0.001). Furthermore, group A1 had a higher interleukin-6 protein staining in adipose tissue than group A3 (p = 0.04).

Conclusion: We observed a persistently higher systemic TNF expression associated with exacerbated TNF-alpha and interleukin-6 local production in patients with stents. This finding may contribute to a worse clinical outcome. (Arq Bras Cardiol. 2018; 111(2):134-141)

Keywords: Percutaneous Coronary Intervention; Blood Cells; Inflammation; Stents; Polymerase Chain Reaction; Immunohistochemistry; Tumor Necrosis Factor-alpha; Interleukin-6.

Introduction

Retrospective studies have suggested that coronary artery bypass grafting (CABG) surgery after percutaneous coronary intervention (PCI) can impair short and long-term outcomes.[1-7] Previous studies have demonstrated that PCI is associated with higher in-hospital mortality, despite the lower risk profile of PCI patients,[3] but there is no consensus in the literature.[8] An analysis of the MASS study[9] has shown that patients who underwent PCI treatment were more likely to develop progression in native coronary arteries, than those undergoing CABG or medical treatment.

During PCI, a focal inflammatory reaction occurs with plaque rupture caused by stent implantation, but there are still controversies if this reaction persists in the long term. There is scarce information about a persistent systemic inflammatory reaction or tissue mediators in coronary artery after stent implantation, as well as about the comparison between coronary arteries with stent, coronary arteries without stent but with stent in another artery and controls patient.

The CABG allows a unique opportunity to collect coronary artery specimen to evaluate local inflammatory reaction long after stent implantation. This study aims to evaluate inflammatory genes expression in peripheral blood cells and inflammatory protein localization in coronary artery tissue obtained during CABG from patients with and without previous stent implantation. It is worth mentioning that CABG represents a unique opportunity to obtain a tiny coronary artery tissue sample to evaluate local inflammatory in humans. Nowadays, patients who receive previous bare metal stent (BMS) implantation and need CABG surgery later represent a significant number of cardiology hospital patients, especially in developing countries. Our results can contribute to clarify the involvement of persistent local and systemic inflammation in the later phase of stent restenosis.

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Methods

Case series

The patients admitted to elective CABG with previous stent implantation were consecutively included in this study after signing informed consent. The protocol was approved by the Dante Pazzanese Institute of Cardiology ethics committee (Protocol 4059-2011).

This study included 67 patients as follows: 41 patients with previous intracoronary BMS implantation and 26 patients without stent implantation submitted to elective CABG. All patients had stable angina and more than 6 months of the stent implantation, aiming to exclude ongoing restenosis. The exclusion criteria consisted of emergency surgeries, acute coronary syndromes and chronic renal failure in dialysis because of chronic inflammatory reaction.

Peripheral blood sample was collected preoperatively from the antecubital vein, using PAXgene tubes (PreAnalytiX®, BD Company, UK) for systemic gene expression analysis.

During CABG surgery, a tiny fragment of the bypassed coronary artery was obtained at the arteriotomy site, usually 10 mm after the stent implantation site, to evaluate local inflammation markers. All tissue samples were immediately immersed in buffered formalin for further paraffin-embedded block preparation. Some arterial fragments obtained were not adequate for histological analysis, thus, 176 artery samples were included and grouped as follows: A1- arteries with stent (n = 38); A2- native arteries from a patient with a stent in another artery (n = 68); and A3- arteries from patients without previous stent placement (n = 70).

RNA isolation, reverse transcription and real-time polymerase chain reaction (PCR)

Total RNA was extracted from peripheral blood collected in PAXgene tubes (PreAnalytiX®, BD Company, UK) using PAXgene blood RNA kit (QIAGEN GmbH, Hilden, Germany), being then quantified using Qubit® 2.0 fluorometer (Life Technologies, Inc., Grand Island, NY). The RNA integrity was performed using Tapestation® 2200 and R6K Screen Tape (Agilent Technologies, Inc. UK). The cDNA was transcribed from 200 ng of total RNA using High Capacity RNA-to-cDNA Master Mix (Applied Biosystems, Foster City, USA). Real-time qPCR was carried out in Rotor-Gene® detection system using the TaqMan® Fast Multiplex PCR kit (QIAGEN GmbH, Hilden, Germany) and primers from Applied Biosystem commercially designed for TaqMan® qPCR (Applied Biosystems, Foster, CA, USA).

The expression of the following genes was evaluated: *LIGHT* (Hs00542477_m1); *IL-6 (*Hs00985639_m1); *ICAM (*Hs00164932_m1); *VCAM (*Hs00174239_m1); *CD40 (*Hs01002913_g1); *NFKB (*Hs00231653_m1); *TNF* (Hs01113624_g1); *IFNG (*Hs00989291_m1) and *GAPDH* (Hs00266705_g1). For all genes, we constructed standard curves and determined the slope to calculate the PCR efficiency. Almost equal efficiency for all primer/probe systems was observed. All samples were tested in duplicate using *GAPDH* as a reference gene, which was previously chosen between the six most common endogenous myocardium genes using geNorm algorithm.[10] The samples amplified after 40 cycles of PCR were considered negative and excluded from further statistical analysis. The expression of the reference *GAPDH* gene was applied for data normalization, and the relative expression of each mRNA was calculated using the $2^{-\Delta\Delta CT}$ method.[11]

Immunohistochemistry staining

Firstly, 4-μm-thick formalin-fixed-paraffin-embedded artery samples were sectioned and fixed in silanized slides, followed by dewaxing at 70°C, in the oven, for 1 hour, and immersion in three xylene baths for 10 minutes. They were then rehydrated in decreasing concentrations (100%, 90%, 75%) of ethyl alcohol. Antigen retrieval was performed using the Trilogy® buffer (Cell Marque, California, USA) at a Decloaker equipment at 90°C for 40 minutes (Biocare Medical, CA, USA). The specific blocking reagents (Erviegas EasyPath, DuraEdge, USA) were applied for endogenous peroxidase and protein blocking. In the next step, the slides were incubated with respective primary antibodies previously titrated and diluted in universal thinner (Erviegas EasyPath, DuraEdge, USA). Primary antibodies against interleukin-6 (IL-6) (ab6672), ICAM (ab2213), VCAM (ab106777), TNF-alpha (ab1793), IFN-gamma (ab9657), CD40 (ab58612), and NFKB (ab16502) from Abcam (Cambridge, MA, UK) were used. The immunoperoxidase reaction was detected using Mach4 Kit Universal HRP Polymer + DAB (Biocare Medical, California, USA), and, finally, the slides were stained with Harris hematoxylin (Erviegas EasyPath, DuraEdge, USA) and assembled by synthetic resin Erv-Mount (Erviegas EasyPath, DuraEdge, USA). The positive control of immunohistochemistry reaction was performed using tissues that have the same constructive antigens as the antigen of interest. After immunohistochemistry processing, the slides were scanned through a Scanscope CS System unit (Aperio Technologies, Inc., CA, USA), with an objective 20x Olympus UPlanSApo with specifications 20x/0.75 attached to the scanner, generating image files in svs format. Scanned images were analyzed using the Aperio ImageScope viewing software (Aperio Technologies, Inc., CA, USA) that reports the percentage of positively stained area in relation to the total tissue area.

Statistical analysis

Continuous variables are reported as mean and standard deviation or median and interquartile interval, depending on the assumption of normality. Categorical variables are reported as absolute and relative frequency. Values between groups were compared by unpaired Student *t* test after testing for normal distribution by KS test; otherwise, nonparametric Mann-Whitney U tests were used. Fisher exact or chi-square test was used for categorical variables with nominal scales. For comparison of artery tissue markers, Kruskal-Wallis test (or ANOVA, assumption of normality) was used, and, for non-parametric multiple comparisons, Tukey's test. A p-value lower than 0.05 was considered statistically significant. The SPSS version 19 was used.

To detect 3 units with standard deviation of 4, 80% test power, and 5% alpha, the sample size calculation is 105 cases.

Results

Clinical characteristics of study group

The frequencies of clinical characteristics (Table 1), such as sex, diabetes, dyslipidemia, smoking, previous stroke and heart attack, are similar in both groups. However, the stent group was younger and had a higher prevalence of ventricular dysfunction, characterized by an ejection fraction lower than 50%. The control group had a higher blood platelet count (269,560 ± 74,461) than the stent group (237,355 ± 70,831), but with no statistical significance (p = 0.12). All patients were on statins and acetylsalicylic acid treatment.

The time between stent implantation and CABG was over 6 months. Nine patients (22%) had stent implanted within 6 and 12 months, 25 patients (61%) had only one stent, and 16 patients (39%) had two or more stents implanted.

Gene expression in peripheral blood cells by real-time PCR

Total RNA from peripheral blood cells was obtained and the expression of the following genes was evaluated: *LIGHT*, *IL-6*, *ICAM*, *VCAM*, *CD40*, *NFKB*, *TNF*, *IFNG* and *GAPDH*. Of eight genes, the expression of only two differed between the stent and control groups: the expression of *TNF* was significantly higher (p = 0.0308) in the stent group (Figure 1-f) and that of *CD40* was higher in the control group (p = 0.0106) (Figure 1-a). No difference was detected in the expression of *IL-6*, *IFNG*, *LIGHT*, *NFKB*, *ICAM* and *VCAM* genes (Figure 1).

Quantitative analysis by immunohistochemistry

The quantification of proteins staining by immunohistochemistry is presented in Table 2 and illustrated in Figures 2 and 3. The TNF-alpha staining in the arteries of the adipose tissue was higher in group A1 than in group A2 (6.69 ± 3.87 vs 2.27 ± 4.00; p < 0.001) (Figure 2-a). In addition, group A1 had higher TNF-alpha staining in the intima-media region than group A3 did (5.16 ± 5.05 vs 1.90 ± 2.27; p = 0.023) (Figure 2-b). A large amount of TNF-alpha was detected in the cytoplasm of inflammatory cells and around the lipid core (Figure 3-C and D).

Higher IL-6 was detected in arteries of the adipose tissue of group A1 than in those of group A3 (2.29 ± 1.96 vs 0.28 ± 0.33; p = 0.048) (Figure 2-c), also observed under the microscope (Figure 3-E and F). There was no difference between groups in the quantification of the CD40, ICAM, VCAM, NFKB and IFN-gamma staining.

On histological examination, we identified major histocompatibility complex class II (MHCII) positive cells surrounding the lipid core, probably macrophages (Figure 3-A and B). These infiltrate cells also stained for TNF-alpha, being detected in adipose tissue and in intima layer (Figure 3-C and D). Fewer IL-6-positive infiltrate cells were also observed in the same layer of artery tissue (Figure 3-E and F).

Discussion

This study analyzed both the gene expression in peripheral blood cells and the protein localization in coronary artery tissues, to evaluate simultaneous systemic and local inflammation. A persistently higher *TNF-alpha* systemic expression was observed in peripheral blood cells, in addition to a local exacerbated TNF-alpha and IL-6 production in coronary arteries.

Our study evaluated, in stable patients, local coronary and systemic inflammation after stent implantation as compared to controls. All patients included had undergone PCI more than 6 months before and had CABG indication. Regarding stent implantation time, 9 of 41 patients (22%) were within one year

Table 1 – Biodemographic data of the studied groups: with previous stent implantation and controls

Variables	Stent Group	Control Group	p value
Cases	41	26	
Age (years)	60.2 ± 7.1	6.3 ± 8.69	0.004
Female (%)	13 (31.3)	8 (30.8)	0.58
Blood platelet count	237.355 ± 70.831	269.560 ± 74.461	0.12
Hypertension (%)	35 (85.4)	22 (84.6)	0.60
Diabetes (%)	17 (42.5)	8 (36.4)	0.42
Current smoking (%)	4 (10.5)	3 (14.3)	0.48
Stroke (%)	2 (5.3)	0	0.43
CKD (%)	2 (5.3)	0	0.43
Dyslipidemia (%)	25 (62.5)	16 (64)	0.56
MI (%)	14 (35.9)	5 (22.7)	0.22
LVEF < 50% (%)	14 (41.2)	2 (9.1)	0.009
ASA (%)	41 (100)	26 (100)	1.00
Statins (%)	41 (100)	26 (100)	1.00

CKD: chronic kidney disease; MI: myocardial infarction; LVEF: left ventricular ejection fraction; ASA: acetylsalicylic acid. Age and blood platelet count were expressed as mean ± standard deviation. Sex, hypertension, diabetes, smoking, stroke, CKD, dyslipidemia, MI, LVEF < 50%, ASA use, and statin use are expressed in numbers and percentages of the patients studied. The statistical significance level adopted was p < 0.05.

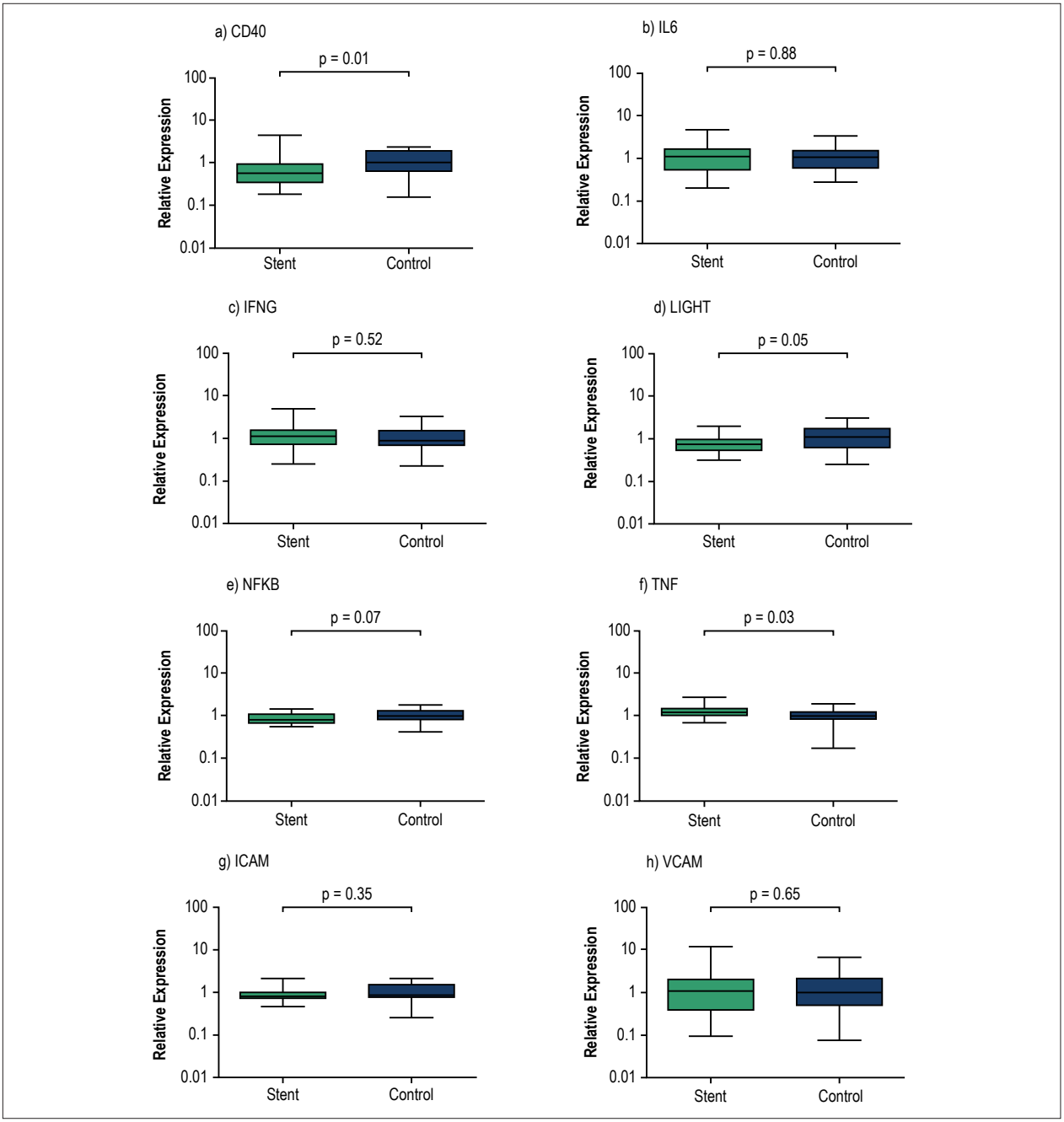

Figure 1 – *Expression of inflammatory genes in peripheral blood. The expression of the genes CD40 (a), IL-6 (b), INFG (c), LIGHT (d), NFKB (e), TNF (f), ICAM (g) and VCAM (h) was evaluated by real-time PCR using GAPDH gene as internal control to calculate relative expression ($2^{-\Delta\Delta CT}$). The patients with coronary stent implantation (n = 35) were compared with controls (n = 25) using nonparametric Mann-Whitney U test. The difference is considered significant for p-values < 0.05.*

from PCI, and 32 patients (78%) had it more than one year before. A former study showed that the inflammatory reaction inherent to PCI no longer exists in this period.[12] No patient with drug-eluted stent (DES) was enrolled in the present study. On the basis of previous literature, a higher number of T lymphocytes and macrophages is observed in DES lesions than in BMS lesions, suggesting the mechanism of restenosis after DES implantation may be different from that observed after BMS implantation.[13]

In this study, the gene expression in peripheral blood cells and the protein localization in coronary artery tissues were carried out to evaluate systemic and local inflammation, respectively. It is worth mentioning that CABG surgery represents a rare opportunity to obtain coronary tissue samples for research without harm to patients, that is why few previous studies worked with this kind of biological sample. In most of them, samples were obtained from the atherosclerotic plaque by endarterectomy.[14,15]

Table 2 – Quantification of CD40, ICAM, VCAM, MHC-II, TNF-alpha, NFKB, IL-6 and IFN-gamma proteins in arterial intima-media layer, adventitia and adipose tissue by immunohistochemistry

Protein	Artery layers	Group A1			Group A2			Group A3			p value (α = 0.05)			
		n	mean	Std. dev	n	mean	Std. dev	n	mean	Std. dev	3 groups	1 *vs* 2	1 *vs* 3	2 *vs* 3
CD40	intima-media	16	1.37	2.02	27	1.51	1.73	23	1.11	1.56	0.55	ns	ns	ns
	Adventitia	13	0.70	0.77	26	0.82	0.71	19	1.27	1.66	0.58	ns	ns	ns
	Adipose tissue	3	0.73	0.12	6	0.62	0.58	9	0.58	0.74	0.45	ns	ns	ns
ICAM	intima-media	18	3.27	3.00	27	3.47	3.77	20	2.81	4.23	0.16	ns	ns	ns
	Adventitia	14	4.07	3.67	25	4.22	4.82	19	3.99	5.39	0.76	ns	ns	ns
	Adipose tissue	1	5.93	-	7	1.99	1.37	5	1.52	1.64	0.22	ns	ns	ns
VCAM	intima-media	14	11.88	16.01	24	10.33	9.64	23	7.88	6.12	0.76	ns	ns	ns
	Adventitia	11	4.69	8.39	19	4.09	4.74	21	2.56	2.26	0.68	ns	ns	ns
	Adipose tissue	2	4.31	5.28	4	1.76	1.22	4	0.32	0.36	0.10	ns	ns	ns
MHC II	total area	7	0.74	0.59	8	0.47	0.12	30	0.75	0.52	0.307	ns	ns	ns
TNF-alpha	intima-media	15	5.16	5.05	24	3.11	3.01	21	1.90	2.27	0.03	ns	0.023	ns
	Adventitia	14	4.05	2.82	21	2.28	2.24	20	3.57	5.95	0.10	ns	ns	ns
	Adipose tissue	4	6.69	3.88	4	1.27	0.84	9	2.27	4.00	0.05	0.001	ns	ns
NFKB	intima-media	14	1.11	1.14	24	0.93	0.88	20	0.92	1.07	0.96	ns	ns	ns
	Adventitia	14	0.64	0.61	21	0.83	0.78	19	0.76	0.55	0.65	ns	ns	ns
	Adipose tissue	2	1.18	0.87	6	0.52	0.44	6	1.63	2.58	0.66	ns	ns	ns
Interleukin-6	intima-media	16	1.15	1.12	23	1.27	1.61	21	0.66	0.88	0.17	ns	ns	ns
	Adventitia	15	1.65	2.04	20	1.65	2.02	21	0.91	0.74	0.69	ns	ns	ns
	Adipose tissue	4	1.12	1.03	3	2.29	1.96	11	0.28	0.33	0.01	ns	0.061	0.048
IFN-gamma	intima-media	14	0.67	0.70	25	0.66	0.69	22	0.56	0.84	0.36	ns	ns	ns
	Adventitia	12	0.52	0.41	20	0.59	0.54	21	0.40	0.46	0.36	ns	ns	ns
	Adipose tissue	3	0.54	0.53	4	0.14	0.10	7	0.10	0.11	0.13	ns	ns	ns

The comparison was performed among groups (Kruskal-Wallis test or ANOVA): A1 (arteries with stent), A2 (native arteries from patients with a stent in another artery), and A3 (control, patients without previous stent placement). The statistical significance level adopted was $p < 0.05$.

Blood Analysis

The analysis of mRNA expression from circulating blood cells pointed out a significant higher expression of the *TNF* gene in the group with previous stent implantation than in controls (Figure 1-f), suggesting a greater activation of this gene in leukocytes from stented patients. This gene encodes a pleiotropic cytokine involved in a broad range of biological activities, including inflammation, cell survival, cell proliferation, and, paradoxically, cell death.[16]

We also observed a significantly lower *CD40* gene expression in blood cells from the stent group than from controls (Figure 1-a). The CD40 is the receptor for CD40L, being present in platelets. Gerdes et al.[17] have demonstrated in knockout mice for CD40 and ApoE that platelet plays a crucial role in inflammation by stimulating leukocyte and endothelial cells activation, thereby promoting atherosclerosis.

Coronary artery tissue samples

We analyzed the arterial tissue separated in three layers (adventitia, intima-media and adipose tissue) by h*ematoxylin-eosin* and immunohistochemistry staining. It is worth pointing that, although only few samples collected contained adipose tissue, due to the difficulty to obtain all layers in such small fragments, we could discriminate a greater amount of TNF-alpha and IL-6proteins in the adipose tissue from groups A1 and A2 than in that from controls (Figure 2-a and 2-c). The white adipose tissue is considered to be an endocrine gland, and the main feature is insulin and leptin resistance, as well as the production of inflammatory cytokines (TNF-alpha and IL-6) and monocyte chemoattractant protein,[18,19] which are involved in atherogenesis.[20,21] Interestingly, on the histological study, we observed activated immune cells, with MHCII expressed in membrane (Figure 3), surrounding the lipid core. Furthermore, these cells were colocalized with TNF-alpha and IL-6 staining, suggesting a greater inflammatory response in the adipose tissue around the artery from individuals with previous stent placement.

The TNF-alpha protein was also expressed in higher quantity in intima-media layer from group A1 than from groups A2 and A3 (Figure 2-b). Probably immune cells migrated from circulation to that layer, mainly macrophages, which

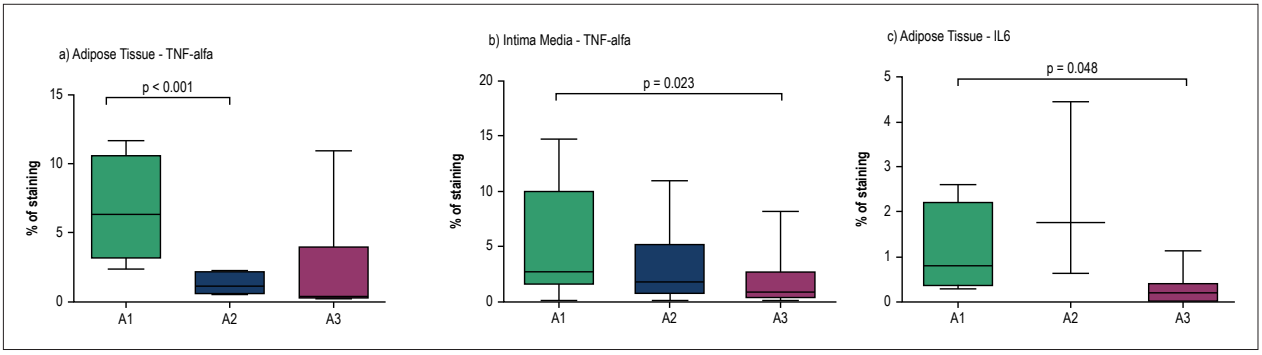

Figure 2 – *Comparison of the staining of TNF-alpha and IL-6 proteins in different layers of arterial tissue. The analysis was performed comparing three groups: A1 (arteries with stent), A2 (native arteries from patient with a stent in another artery) and A3 (control, patients without previous stent placement). TNF-alpha protein staining was higher in the adipose tissue of group A1 (6.69 ± 3.87 vs 2.27 ± 4.00; p < 0.001) (a), as well as in the intima-media layer (5.16 ± 5.05 vs 1.90 ± 2.27; p = 0.02) (b). The IL-6 protein staining was also higher in the adipose tissue from group A1 than that from group A3 (2.29 ± 1.96 vs 0.28 ± 0.33; p = 0.048) (c). The Kruskall-Wallis test and nonparametric Tukey's multiple comparisons test were used for statistical analysis. The difference is considered significant for p-values < 0.05.*

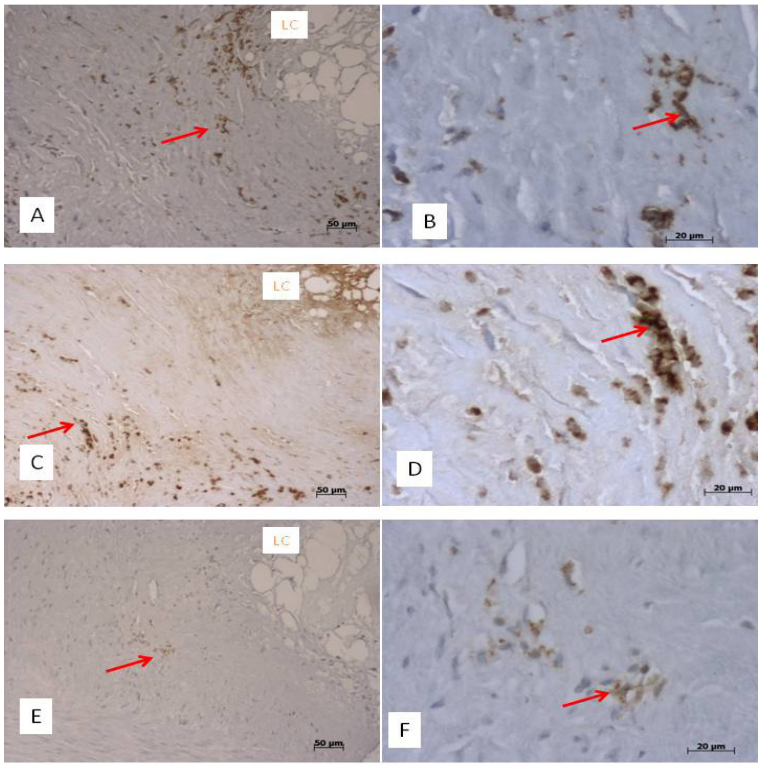

Figure 3 – *Panoramic (left side) and high-power view (right side) of immunostained arterial intima-media layer from individuals with previous stent implantation. Panels A and B show MHCII-positive cells, with morphology of macrophages (arrows), surrounding the lipid core (LC). Panels C and D show a large amount of TNF-alpha in the cytoplasm of inflammatory cells (arrows) and in the lipid core (LC). Panels E and F exhibit fewer inflammatory cells positive for IL-6 protein in similar sites (arrows).*

are responsible for the production of that cytokine. Those are also MHCII-positive cells, primarily responsible for presenting antigenic peptides to T cells of the immune system.

Interleukin-6 is a multifunctional cytokine playing a central role in inflammation and tissue injury.[22] Interleukin-6 activates platelet receptor GPIIb/IIIa and leukocyte-platelet interaction, thus favoring the prothrombotic and atherogenic formation. Previous studies have shown that the increased circulating IL-6 is associated with the risk of coronary restenosis and *de novo* coronary artery lesions,[23] as well as the severity of stenosis.[24] The increase in IL-6 mRNA and protein has been observed in human arterial atherosclerotic wall. In this study, our data showed a significant higher amount of IL-6 protein in the coronary tissue of patients with previous stent placement than in that of controls, suggesting that local arterial inflammation is intensified by stent placement.

Our finding indicated the presence of persistent systemic and local chronic inflammation in individuals with previous stent implantation and can probably contribute to the worst outcome described in a previous meta-analysis study.[7]

It is known that persistent inflammatory response may result in several complications, such as atherosclerotic plaque formation in arterial vessels. In a substudy of the MASS II Trial,[9] consecutive angiographic results were compared with the progression of coronary artery atherosclerosis in medical treatment (MT), CABG and angioplasty. The authors have observed a greater progression in at least one native vessel in angioplasty patients than in CABG and MT patients, concluding that angioplasty treatment has the worst progression in native coronary arteries, especially in the left anterior descending territories. Our result also showed inflammation process in native arteries from individuals with previous stent implantation.

Limitations

This study was limited to BMS. We had very few DES with restenosis and CABG indication. Because of the tiny size of the samples, only a small number of adipose tissue samples was obtained in group A2. Unfortunately, because of the tiny size, many samples collected were inadequate for analysis, and some had insufficient material for analysis. It is worth mentioning that the surgeon primarily ensures the patient's safety.

The arterial sample was collected at the least affected segment, for best surgical results in graft implantation, distal to the stent, place of possible less inflammation and less affected by the stent.

Restenosis is associated with a local and systemic inflammatory reaction that could be related to obstructive lesions in stented arteries. However, only nine patients were operated upon in less than 365 days, and the arterial samples were taken at least 10 mm after the stent implantation, which reduces its influence on the results.

Statin, which has known anti-inflammatory activity, can influence partially on this result, but it was minimized because all patients were under this therapeutic procedure that has a class I indication.

Conclusion

In conclusion, the persistently higher systemic expression of *TNF* in association with the local exacerbated TNF-alpha and IL-6 production in coronary arteries with previous BMS implantation may contribute to worse clinical outcomes after CABG surgery.

Author contributions

Conception and design of the research: Farsky PS, Hirata MH, Lima PHO, Lin-Wang HT; Acquisition of data: Farsky PS, Arnoni RT, Almeida AFS, Issa M, Lima PHO, Higuchi ML, Lin-Wang HT; Analysis and interpretation of the data: Farsky PS, Hirata MH, Arnoni RT, Almeida AFS, Issa M, Lima PHO, Higuchi ML, Lin-Wang HT; Statistical analysis: Lin-Wang HT; Obtaining financing: Farsky PS, Lima PHO; Writing of the manuscript: Farsky PS, Lin-Wang HT; Critical revision of the manuscript for intellectual content: Farsky PS, Hirata MH, Arnoni RT, Almeida AFS, Issa M, Lin-Wang HT.

Potential Conflict of Interest

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

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Study Association

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Instituto de Cardiologia Dante Pazzanese under the protocol number 4059-2011. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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Persistent Inflammatory Activity in Blood Cells and Artery Tissue from Patients with Previous Bare Metal Stent

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Short Editorial regarding the article: Persistent Inflammatory Activity in Blood Cells and Artery Tissue from Patients with Previous Bare Metal Stent

The interesting article by Farsky et al.[1] examined the persistence of inflammatory activity after bare metal stent implantation in patients later submitted to coronary artery bypass grafting (CABG).

The authors evidenced a higher systemic expression of the gene that encodes tumor necrosis factor-alpha (TNF-alpha) in peripheral blood cells, as well as a higher tissue expression of TNF-alpha and interleukin-6 (IL-6) in patients with previous bare metal stent implantation as compared to those of revascularized individuals without previous percutaneous stent placement.

Those authors concluded that, even several months or years after stent implantation, there were markers of persistent inflammatory activity, which could be associated with less favorable outcome of CABG.

The relationship between inflammatory markers and coronary restenosis after stent placement has been recognized for years.[2] There are few reports on the local inflammatory characteristics expressed by tissue markers in samples obtained during CABG.

The increase in circulating IL-6 levels has been associated with the increase in coronary events. A study of Mendelian randomization involving 40 studies and 133449 patients has shown that polymorphism of the gene encoding the IL-6 receptor was related to a significant reduction in the incidence of coronary events, suggesting a causal role in atherosclerosis.[3] Another meta-analysis of genetic data has confirmed the causal role of IL-6 in atherothrombosis.[3] Those two studies have suggested that the IL-6-mediated inflammatory pathway, from its interaction with the receptor, is involved in cardiovascular events.

The TNF-alpha, another biomarker of higher expression evidenced in the study by Farsky et al.,[1] seems to be implicated in atherosclerotic plaque instability.[4]

Despite the substantial advance in surgical and percutaneous procedures, as well as in the clinical therapy involving new antiplatelet, anticoagulant, lipid-lowering, anti-hypertensive and anti-hyperglycemic agents, the residual risk remains elevated and new anti-inflammatory therapies have been proposed.[5]

The CANTOS study,[6] a prospective, randomized, placebo-controlled clinical trial, involving post-myocardial infarction patients who maintained elevated high-sensitivity C-reactive protein levels, has shown that treating inflammation with the monoclonal antibody canakinumab reduced inflammatory markers and cardiovascular events during clinical drug treatment.

Those data show the relevance of the findings of the study by Farsky et al.[1] and suggest that patients receiving bare metal stents might need additional anti-inflammatory therapy. However, new prospective studies of efficacy and safety, in addition to lower cost of that therapy, are required to its definitive incorporation into clinical practice.[7]

Keywords

Myocardial Revascularization; Stents, Inflammation; Coronary Restenosis; Anti-Inflammatory Agents/therapy.

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Prognostic Impact of Iron Metabolism Changes in Patients with Acute Coronary Syndrome

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Abstract

Background: Iron metabolism disorders have been associated with an increased risk of cardiovascular events. However, the prognostic impact on patients (pts) with acute coronary syndrome (ACS) has yet to be clarified.

Objective: To determine the prognostic value of serum iron and ferritin levels in pts with ACS in the short and long-term.

Methods: Consecutive pts admitted to a coronary care unit with a diagnosis of ACS, for a period of 2 years, were evaluated. The population was divided into tertiles of serum iron and ferritin distribution. The primary adverse events were the occurrence of in-hospital death or heart failure (HF) and death or HF at 1 year of follow-up.

Results: We studied 280 pts (73% males; mean age 68 ± 13 years). The mean levels of serum iron and ferritin were 59 ± 34 mcg/dL and 205 ± 185 ng/mL, respectively. Patients included in the 1st tertile of serum iron (≤ 40 mcg/dL) had a higher rate of adverse events, in-hospital and after 1 year. Lower and higher levels of ferritin (1st and 3rd tertiles, ≤ 110; >219 ng/ml, respectively) were associated with a higher incidence of HF during hospitalization and death at 1 year. A ferritin value >316 ng /mL was an independent risk factor for death at 1 year (adjusted OR: 14; 95%CI: 2.6 to 75.9).

Conclusion: In this population, iron metabolism alterations were associated with a higher rate of adverse events and higher ferritin levels constituted an independent mortality predictor in the long-term. (Arq Bras Cardiol. 2018; 111(2):144-150)

Keywords: Acute Coronary Syndrome; Iron Metabolism Disorders; Prognostic.

Introduction

Iron is an important micronutrient in cell metabolism, necessary for body homeostasis.[1] Iron deficiency affects more than one-third of the world's population and is often a chronic disease complication (inflammatory bowel disease, chronic kidney disease, Parkinson's disease, rheumatoid arthritis) and plays a role in the sympathetic nervous system activation, as well as in ventricular hypertrophy and dilation.[1]

According to the EMPIRE study, 1 in 3 Portuguese individuals have iron deficiency.[2] Iron deficiency is an important comorbidity factor in chronic heart failure (HF), as well as in HF decompensation periods, regardless of the presence of anemia.[1,3]

The CONFIRM-HF study demonstrated a favorable effect on the functional capacity and quality of life of HF patients, as well as the reduction in the number of hospitalizations for decompensated HF in patients submitted to intravenous iron therapy.[1,3,4]

Meanwhile, the debate between the role of ferritin and iron in the atherosclerosis metabolism persists, and the function of iron metabolism in coronary disease is unclear. Although small, there are studies that consider iron a proatherogenic agent for its role in free radical formation, with consequent oxidative stress at the vascular level.[5,6]

Studies in animals have confirmed that chronic iron administration accelerates thrombus formation.[6] On the other hand, low iron levels may be associated with ischemia and major cardiovascular events (MACE) in patients with acute coronary syndrome (ACS).[5]

In a recent study, Steen et al. failed to establish any association between iron and the risk of myocardial infarction, as well as recurrent ischemic events.[7] Ferritin is considered by some studies as a cytoprotective agent, yet multivariate analyses have shown that low ferritin levels are predictors of 30-day MACE in patients with ACS.[5,8]

Despite the several studies, the controversy over the role of iron in ACS persists, and the true correlation between iron and atherosclerotic disease is yet to be determined.

In the present study, we aim to determine the short- and long-term prognostic value of serum iron and ferritin levels in patients admitted for ACS.

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Methods

Sample

The sample was retrospectively evaluated and consisted of consecutive patients admitted to a Coronary Unit with an ACS diagnosis between June 2011 and June 2013. Patients whose iron profile was not determined during hospitalization were excluded.

Variables

The population was characterized according to their baseline characteristics (age and gender); clinical characteristics (personal history, type of ACS, Killip Class, LV ejection fraction) and laboratory test results (serum levels of creatinine, BNP, hemoglobin), and was grouped according to the tertiles of distribution of serum iron (1st tertile ≤ 40; 2nd tercile > 40 and ≤ 67; 3rd tertile >67 mcg/dL) and ferritin (1st tertile ≤ 110; 2nd tertile < 110 and ≤ 219; 3rd tertile >219 ng/mL).

The cut-off values of serum iron and serum ferritin were, respectively, 60-180 mcg/dL and 10-120 ng / mL, according to the hospital laboratory.

End-point

The short- and long-term prognoses were assessed based on primary adverse events: in-hospital death and 1-year death; in-hospital heart failure (Killip Class ≥ 2 and BNP ≥ 400 pg/mL) and 1-year follow-up (ejection fraction < 50% and NYHA Class ≥ 2). Other secondary endpoints were reinfarction and ischemic cerebrovascular accident at 1 year of follow-up.

Statistical analysis

The program IBM SPSS Statistics, version 20 for Windows 8, was used to perform the statistical analysis. Continuous variables were shown as mean ± standard deviation and compared according to iron and ferritin tertiles by ANOVA. Categorical variables were shown as absolute values and/or percentages and compared using the chi-square test. The associations were considered statistically significant in the presence of a p-value < 0.05. Continuous variables were associated with primary adverse events (Death and HF) through receiver operating characteristic (ROC) curves. The predictive value of iron and ferritin levels over the risk of in-hospital and 1-year adverse events was determined by the odds ratio, with a 95% confidence interval (95%CI).

Results

The baseline, clinical and laboratory characteristics of the total population and according to serum iron and ferritin tertiles are shown in Tables 1 and 2. A total of 280 patients were studied (73% males) with a mean age of 68 ± 13 years. The distribution of serum iron and ferritin levels is shown in figure 1.

The main diagnosis at admission was ST-segment elevation myocardial infarction (STEMI) in 45% (n = 125) and non-ST-segment elevation myocardial infarction (NSTEMI) in 44% (n = 122) of the patients.

Approximately 87% of the patients (n = 244) were admitted with Killip I Class, and only 2.5% (n = 7) were admitted in cardiogenic shock. In 11 (5%) patients, the transthoracic echocardiography showed severe left ventricular systolic dysfunction.

Regarding the short-term prognosis: 1.1% (n = 3) of the patients died during hospitalization and 28% (n = 79) showed evidence of heart failure. Regarding the long-term impact, approximately 7% (n = 19) of the patients died in the first year of follow-up and 12% (n = 33) developed HF criteria during the clinical follow-up (Tables 3 and 4).

The multivariate regression analysis showed that a ferritin value > 316 ng/mL is an independent risk predictor for 1-year death (adjusted OR:14; 95%CI: 2.6-75.9, p = 0.0023). (Table 5)

The survival curves for Death and HF according to iron and ferritin tertiles showed no statistical difference. (Figures 2 and 3)

Discussion

Iron deficiency is a common and clinically relevant heart failure comorbidity, being associated with a worse prognosis. Some studies (CONFIRM-HF) have shown the benefit of iron correction in terms of quality of life and exercise tolerance in patients with HF and decreased systolic function.[1,3]

According to current European recommendations for HF treatment, intravenous iron is indicated with Class IIa in symptomatic patients with decreased systolic function and iron deficiency (serum ferritin levels <100ng / mL or ferritin level between 100-299 ng / mL and transferrin saturation <20%).[3] On the other hand, the role of iron and ferritin is uncertain in the context of atherosclerotic disease and ACS.[5-8]

In this population, the type of ACS, as well as its clinical presentation regarding Killip class and LV systolic function impairment were not statistically influenced by iron or ferritin levels.

Age had a statistical impact on serum iron and ferritin levels, which may be explained by an iron-poor diet, impaired intestinal absorption that increases with age, and the presence of more comorbidities that interfere with iron metabolism.

Lower levels of iron and ferritin were statistically associated with lower Hb levels, as expected, with a mean value of 12 g/dL.

Mean BNP values >450 pg/mL were statistically associated with the 1st tertiles of iron and ferritin, which is consistent with several studies in the HF scenario, in which iron and ferritin deficiencies were found as a frequent comorbidity of HF.[1,3]

Alberto Dominguez-Rodriguez demonstrated that low iron levels may be associated with major cardiovascular events (MACE) in patients with ACS.[5] In this population of ACS patients, alterations in iron metabolism were associated with a higher occurrence of adverse events.

Iron levels ≤ 40 mcg/dL had a negative impact regarding mortality and in-hospital HF, with statistical significance; however, serum iron levels were not an independent risk factor for the occurrence of cardiovascular events.

Table 1 – Basal and clinical characteristics, according to iron and ferritin tertiles

Basal characteristics	Population n = 280	1st iron tertile (≤ 40 mcg/dL)	2nd iron tertile (> 40 or ≤ 67 mcg/dL)	3rd iron tertile (> 67 mcg/dL)	p value	1st ferritin tertile (≤ 110 ng/mL)	2nd ferritin tertile (>110 or ≤ 219 ng/mL)	3rd ferritin tertile (> 219 ng/mL)	p value
Age, years	68 ± 13	69 ± 15	69 ± 12	67 ± 13	< 0.001	73 ± 12	66 ± 14	67 ± 13	0.001
Male gender	204 (73)	63 (23)	73 (26)	68 (24)	0.12	53 (19)	68 (24)	78 (28)	0.12
Personal history									
SAH	186 (66.4)	56 (20)	70 (25)	60 (21)	0.12	69 (25)	58 (21)	57 (20)	0.12
Diabetes mellitus	90 (32)	36 (13)	30 (11)	24 (9)	0.21	32 (11.4)	32 (11.4)	26 (9.3)	0.69
Dyslipidemia	140 (50)	37 (13)	59 (21)	44 (16)	0.87	46 (16)	49 (18)	43 (15)	0.34
History of AMI	44 (16)	10 (3.6)	18 (6.4)	16 (6)	0.21	18 (6.4)	15 (5.3)	11 (4)	0.42
History of HF	9 (3.2)	2 (0.7)	5 (1.8)	2 (0.7)	0.34	2 (0.7)	3 (1.1)	4 (1.4)	0.68
Renal failure	21 (8)	8 (3)	7 (2.5)	6 (2)	0.8	9 (3.2)	6 (2)	6 (2)	0.6
PAD	11 (4)	3 (1)	3 (1)	5 (1.8)	0.7	8 (3)	3 (1)	0	0.7
ACS type									
Unstable angina	12 (4.3)	3 (1)	2 (0.7)	7 (2.5)	0.21	7 (2.5)	3 (1)	2 (0.7)	0.85
STEMI	125 (45)	50 (18)	43 (15.3)	32 (11)		35 (13)	41 (15)	45 (16)	
NSTEMI	122 (44)	36 (13)	41 (15)	45 (16)		41 (15)	40 (14)	40 (14)	
Undetermined AMI	21 (8)	6 (2.1)	6 (2.1)	9 (3.2)		9 (3.2)	10 (4)	2 (0.7)	

Results expressed as n (%) or mean ± median. SAH: systemic arterial hypertension; AMI: acute myocardial infarction; HF: heart failure; PAD: peripheral arterial disease; ACS: acute coronary syndrome; STEMI: ST-segment elevation myocardial infarction; NSTEMI: Non-ST-segment elevation myocardial infarction.

Table 2 – Clinical and laboratory characteristics, according to iron and ferritin tertiles

Clinical presentation	Population n = 280	1st iron tertile (≤ 40 mcg/dL)	2nd iron tertile (> 40 or ≤ 67 mcg/dL)	3rd iron tertile (> 67 mcg/dL)	p value	1st ferritin tertile (≤ 110 ng/mL)	2nd ferritin tertile (>110 or ≤ 219 ng/mL)	3rd ferritin tertile (> 219 ng/mL)	p value
Killip Class I	244 (87)	74 (27)	83 (30)	87 (31)	0.12	73 (26)	88 (31.4)	78 (28)	0.23
Killip Class II	18 (6.4)	11 (4)	4 (1.4)	3 (1)		14 (5)	2 (0.7)	2 (0.7)	
Killip Class III	11 (3.9)	4 (1.4)	4 (1.4)	3 (1)		3 (1)	2 (0.7)	6 (2.1)	
Killip Class IV	7 (2.5)	6 (2.1)	4 (1.4)	0		2 (0.7)	2 (0.7)	3 (1)	
Ejection fraction classification									
LVEF > 50%	157 (69)	42 (15)	58 (21)	57 (20)	0.08	43 (15)	59 (21)	54 (19)	0.7
LVEF 41-50%	23 (10)	16 (6)	3 (1)	4 (1.4)		7 (2.5)	9 (3)	7 (2.5)	
LVEF 30-40%	38 (17)	16 (6)	14 (5)	8 (3)		16 (6)	8 (3)	11 (4)	
LVEF < 30%	11 (5)	4 (1.4)	4 (1.4)	3 (1)		6 (2.1)	2 (0.7)	3 (1)	
Laboratory assessment									
Creatinine, mg/dL	1.08 ± 0.9	1.2 ± 1.2	1.05 ± 0.6	0.9± 1.8	0.9	1.1 ± 0.7	1 ± 0.6	1.1 ± 1.2	0.8
BNP, pg/mL	331.6 ± 499	567 ± 712	266 ± 371	180 ± 212	< 0.001	488 ± 684	204 ± 270	346 ± 427	0.001
Hemoglobin, g/dL	13.1 ± 2	11.8 ± 2.2	13.4 ± 2	14 ± 16	< 0.001	12 ± 2.2	14 ± 1.7	14 ± 2	<0.001

Results expressed as n (%) or mean ± median. LVEF: left ventricular ejection fraction; BNP: brain natriuretic peptide.

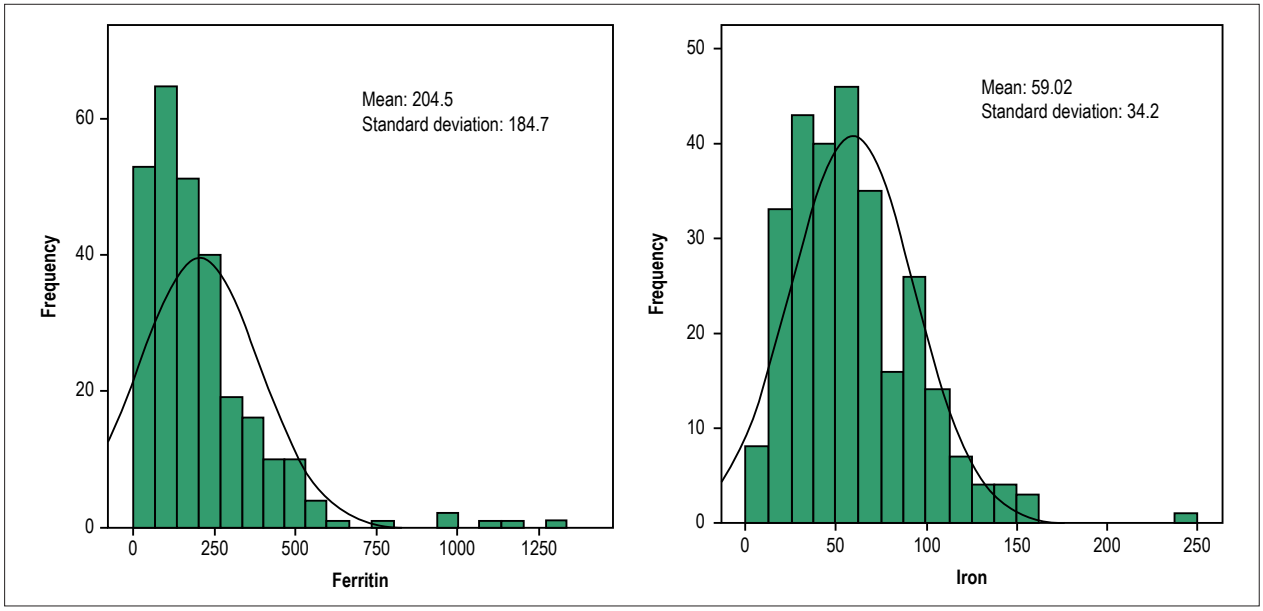

Figure 1 – *Distribution of serum levels of iron and ferritin in the population.*

Table 3 – Short-term and long-term events, according to iron levels

Event	1st iron tertile (≤ 40 mcg/dL)	2nd iron tertile (> 40 or ≤ 67 mcg/dL)	3rd iron tertile (> 67 mcg/dL)	p value
In-hospital death	3	0	0	0.04
Death at 1 year	12	4	3	0.02
In-hospital HF	46	19	14	< 0.001
HF at 1 year	17	9	7	0.08
Reinfarction at 1 year	3	3	2	0.9
CVA at 1 year	0	1	1	0.8

HF: heart failure; CVA: cerebrovascular accident.

Table 4 – Short-term and long-term events, according to ferritin levels

Event	1st ferritin tertile (≤ 110 ng/mL)	2nd ferritin tertile (> 110 or ≤ 219 ng/mL)	3rd ferritin tertile (> 219 ng/mL)	p value
In-hospital death	1	1	1	0.8
Death at 1 year	9	2	8	0.04
In-hospital HF	38	16	25	0.001
HF at 1 year	13	9	11	0.1
Reinfarction at 1 year	4	3	1	0.1
CVA at 1 year	1	1	0	0.5

HF: heart failure; CVA: cerebrovascular accident.

Is ferritin a cytoprotective or atherogenic agent or, on the other hand, is its deficiency a predictor of major cardiovascular events in patients with ACS? The literature results are not in agreement regarding the role of ferritin in atherosclerosis.[3] Patients with ACS and major cardiovascular events (MACE) showed low levels of ferritin versus patients with ACS without adverse events.[5]

In our population, regarding ferritin levels, the 1st and 3rd tertiles were associated with the occurrence of more adverse events, with statistical significance in terms of in-hospital HF and 1-year death. A serum ferritin level of 316 ng/mL was considered an independent risk predictor for 1-year death (adjusted OR:14; 95%CI: 2.6-75.9, p = 0.0023), which

Table 5 – Establishment of independent variables associated with cardiovascular events in the short and long-term.

Variable	Odds ratio	95%CI	p value
Death at 1 year			
Iron ≤ 36 mcg/dL	2.6	0.7-9	0.13
Ferritin > 316 ng/mL	14	2.5-75	0.0027
Hemoglobin ≤ 11.7 g/dL	17	3-102	0.0016
Age > 70 year	21	2-237	0.01
In-hospital death			
Iron ≤ 14 mcg/dL	3.9	1-9	0.99
Ferritin > 104 ng/ml	1.17	2-70	0.99
Heart failure at 1 year			
Iron ≤ 40 mcg/dL	0.9	0.2-3.9	0.9
Ferritin ≤ 157 ng/mL	0.36	0.06-2.1	0.2
In-hospital heart failure			
Iron ≤ 30 mcg/dL	1.8	0.6-5.3	0.3
Ferritin ≤ 116 ng/mL	0.5	0.15-1.8	0.3

Values determined from the Receiver Operating Characteristic curves. 95% CI: 95% confidence interval.

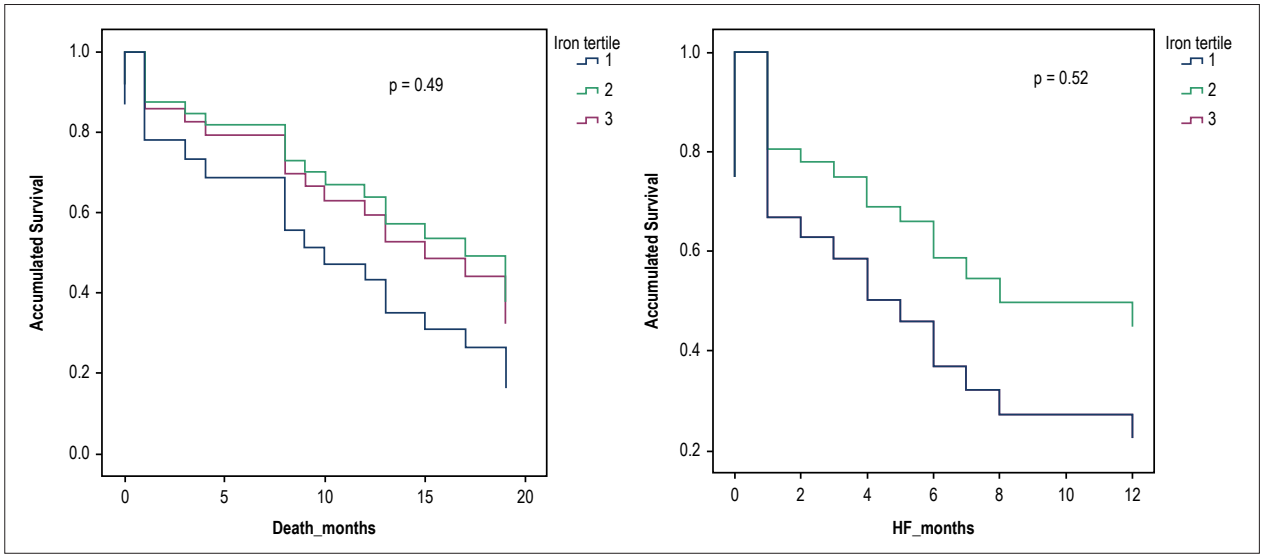

Figure 2 – *Survival curves according to iron tertiles.*

differs from the conclusions of some studies, as previously mentioned. The serum ferritin level was not an independent risk predictor for heart failure.

Limitations

The present study is subject to the limitations associated with all retrospective, non-randomized analyses carried out in a single center.

Conclusion

In this population of patients with ACS, iron metabolism alterations were associated with a higher occurrence of adverse events. Elevated ferritin levels were an independent predictor of long-term mortality. Serum iron levels did not constitute an independent risk factor for the occurrence of cardiovascular events.

Additional studies are required to clarify whether serum iron or ferritin levels constitute a vascular injury/prognostic

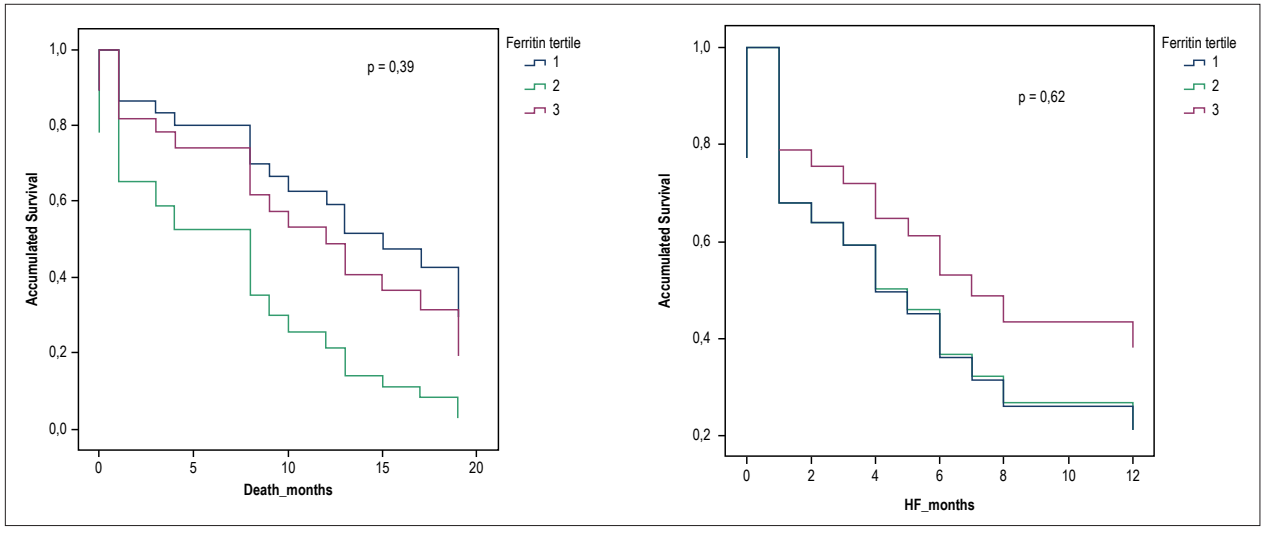

Figure 3 – *Survival curves according to ferritin tertiles.*

marker in ACS patients and establish the "actual" role of iron and ferritin in this type of cardiovascular disease.

Author contributions

Conception and design of the research: Duarte T, Gonçalves S; Acquisition of data: Duarte T, Sá C, Rodrigues R, Marinheiro R, Fonseca M; Analysis and interpretation of the data, statistical analysis and writing of the manuscript: Duarte T; Critical revision of the manuscript for intellectual content: Gonçalves S, Seixo F, Caria R.

Potential Conflict of Interest

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

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Study Association

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

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

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Satisfaction of Emergency Physicians with the Care Provided to Patients with Cardiovascular Diseases in the Northern Region of Minas Gerais

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Abstract

Background: The dissatisfaction of health professionals in emergency services has a negative influence on both the quality of care provided for acute myocardial infarction (AMI) patients and the retention of those professionals.

Objective: To assess physicians' satisfaction with the structure of care and diagnosis at the emergency services in the Northern Region of Minas Gerais before the implementation of the AMI system of care.

Methods: This cross-sectional study included physicians from the emergency units of the ambulance service (SAMU) and level II, III and IV regional hospitals. Satisfaction was assessed by using the CARDIOSATIS-Team scale. The median score for each item, the overall scale and the domains were calculated and then compared by groups using the non-parametric Mann-Whitney test. Correlation between time since graduation and satisfaction level was assessed using Spearman correlation. A p value < 0.05 was considered significant.

Results: Of the 137 physicians included in the study, 46% worked at SAMU. Most of the interviewees showed overall dissatisfaction with the structure of care, and the median score for the overall scale was 2.0 [interquartile range (IQR) 2.0-4.0]. Most SAMU physicians expressed their dissatisfaction with the care provided (54%), the structure for managing cardiovascular diseases (52%), and the technology available for diagnosis (54%). The evaluation of the overall satisfaction evidenced that the dissatisfaction of SAMU physicians was lower when compared to that of hospital emergency physicians. Level III/IV hospital physicians expressed greater overall satisfaction when compared to level II hospital physicians.

Conclusion: This study showed the overall dissatisfaction of the emergency physicians in the region assessed with the structure of care for cardiovascular emergencies. (Arq Bras Cardiol. 2018; 111(2):151-159)

Keywords: Cardiovascular Diseases; Myocardial Infartion; Acute Coronary Syndrome; Epidemiology; Health Profile; Quality Indicators, Health Care; Emergency Medical Services.

Introduction

The recent decades have witnessed a significant reduction in mortality from cardiovascular diseases resulting from the advances in primary prevention and treatment of acute coronary syndrome.[1-4] Despite being a worldwide trend, it is more evident in developed countries, where proper and timely treatment is available.[5] The "*Sistema de Informação de Mortalidade* (SIM) of the *Ministério da Saúde"* (Brazilian Health Ministry Mortality Information System (SIM)) recorded, in 2015, approximately 350 000 deaths from cardiovascular diseases, which, in Brazil, remain the leading cause of proportional mortality, accounting for 27.6% of the deaths in 2015. Additionally, it is the major cause of years of life lost due to premature death.[6]

Of the cardiovascular diseases, acute myocardial infarction (AMI) is the most frequent cause of death (26.0%),[6] and mortality at public healthcare services is higher than at private healthcare services.[7] That difference may be attributed to difficulties experienced by AMI patients to have access to intensive care, reperfusion methods and the therapeutic measures established for AMI.[7,8] Such difficulties can have a negative impact on the satisfaction of emergency healthcare professionals, which might impact negatively the retention of those professionals in regions lacking healthcare structure. The current crisis in emergency services is well known.[9] Thus, assessing the factors related to it, such as the satisfaction of healthcare professionals with healthcare structure, is paramount.

The Northern Region of Minas Gerais comprises 89 municipalities, occupying an area of approximately

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128 000 km², with around 1 594 000 inhabitants. That region differs from the rest of the Minas Gerais state, as it has a human development index close to those of the poorest states in Northeastern Brazil.[10] Similar to the rest of Brazil, specialized healthcare is concentrated in the largest municipality of the region, Montes Claros, and mortality from AMI is very high,[11] motivating the implementation of a project to organize the AMI system of care in the region.

This study aimed at assessing the satisfaction of physicians with the structure of care and diagnosis of public emergency services in the Northern Region of Minas Gerais before the implementation of the AMI system of care in the region.

Methods

Organization of the Care Network for Emergency Services in the Northern Region of Minas Gerais

The care network for emergency services in the Northern Region of Minas Gerais is an integrated network that comprises a regional mobile emergency care service (SAMU, in Portuguese), and micro- and macroregional hospitals. The *"Projeto Estadual de Redes de Atenção"* has categorized the hospitals according to their expertise and their response to two major problems that impact the potential years of life lost: severe trauma and cardiovascular and cerebrovascular emergencies.[12]

SAMU has a macroregional scope, attending 86 of the 89 municipalities of the region, with 7 advanced ambulances (with ambulance driver, nurse and physician), 40 basic ambulances (with an ambulance driver and two nursing technicians) and a rapid interception vehicle. There is only one regulatory center.

The regional hospitals are as follows:

- Level I hospitals: provide several "high-complexity" procedures, such as neurosurgery, vascular surgery and interventional angiography, resuscitation room (red) with mobile radiography and ultrasound, computerized tomography, operating rooms for complex surgeries, heliport with exclusive access, trauma surgical team, transfusion unit, and several differentiated and special hospital beds at intensive care and coronary care units.
- Level II hospitals: located in municipalities with more than 200 000 inhabitants, similar to level I hospitals, except for the absence of angiography, vascular surgery and coronary care units.
- Level III hospitals: located in municipalities with more than 100 000 inhabitants, destined to patients' stabilization until definite transfer to a level I or level II hospital. Their minimum requirements are: emergency healthcare professionals, general surgery, radiology, anesthesiology, transfusion unit and general intensive care unit.
- Level IV hospitals: located in areas that lack healthcare, which are more than 60 minutes away from a reference microregional hospital.[12,13]

Implementation of the AMI System of Care in the Northern Region of Minas Gerais: Minas Telecardio II Project

Minas Telecardio II Project was aimed at implementing and assessing the AMI System of Care in the Northern Region of Minas Gerais and at evaluating its impact on AMI mortality. It was a quasi-experimental study conducted from June 19, 2013 to May 19, 2015 in three steps: (i) establishment of the baseline; (ii) implementation of the AMI Sysem of Care with the mobile tele-electrocardiology system and the new operational flow, in addition to training healthcare professionals of the pre-hospital and hospital emergency services of the region; and (iii) reassessment of the quality indicators for the care provided after the implementation. All phases have been concluded and detailed previously.[14]

The satisfaction of the group of physicians with the structure of care provided to patients with cardiovascular diseases was one of the aspects assessed in the study baseline, being the object of this article.

Study design and satisfaction assessment

This is a cross-sectional study. Emergency physicians from SAMU and from the level II, III and IV regional hospitals that comprise the emergency network of the Northern Region of Minas Gerais participated in this study. The eligibility criteria were as follows: i) be a regular registered member at the Regional Council of Medicine; ii) provide care at SAMU and/or emergency centers of Northern Region of Minas Gerais' regional hospitals.

The research team visited all advanced ambulances of SAMU in the region. Due to the long distance between the regional hospitals, which would hinder the evaluation of the physicians' satisfaction in all of them, a random selection was performed by use of probabilistic simple random sampling. Thus, a numerical list was created, and the municipalities were selected, so that there would be one level III or IV hospital per microregion in the sample. Two level III hospitals and five level IV hospitals were selected.

Assessment of the physicians' satisfaction was performed with the CARDIOSATIS-Team scale, specifically developed to evaluate physicians' satisfaction with the care provided to cardiovascular emergencies. It follows the international standards for the creation of tools and has good validity and reliability for the Brazilian context.[15-17] It is a self-administered tool with 11 closed items and 3 open questions. The open questions include information on access to and interest in professional qualification. The closed items include overall satisfaction and two domains: i) *satisfaction with the care provided;* and ii) *satisfaction with the structure of care and diagnosis*. Each item is assessed by use of a five-point Likert scale, where a score of 4 or 5 indicates higher satisfaction, a score of 1 or 2 indicates dissatisfaction, and a score of 3 indicates average satisfaction with the item assessed ('neither').

Each participant received a questionnaire with the scale and filled it out individually, after providing written informed consent. Those procedures were supervised by a previously trained team, which was available for clarifications, checking the professionals' understanding and answering all their doubts.

Statistical analysis

The statistical analysis was performed by using the IBM SPSS software, version 19.0 (IBM Corp, Armonk, NY). Categorical variables were described as absolute and relative frequency, and continuous variables as measures of central trend and dispersion [median and interquartile range (IQR)]. Data distribution was not normal, as assessed by use of the Kolmogorov-Smirnov test, thus, nonparametric tests were used. The statistical analysis was performed for groups (SAMU *versus* non-SAMU) and non-SAMU subgroups (level II hospitals *versus* level III/IV hospitals). Categorical variables were compared by using the chi-square test. The median score for each item, overall scale and domains were calculated and compared by using the nonparametric Mann-Whitney U test to assess the existence of difference, and a 5% significance level was used. The correlation between professional training time and overall satisfaction was assessed by use of Spearman correlation (r_s).

Ethical aspects

This study was approved by the Ethics Committee of Research of the Universidade Federal de Minas Gerais, number 260/09, aligned with the resolution CNS 466/12. All physicians provided written informed consent to participate in the study.

Results

Of the 164 professionals, 137 (83.5%) completed the questionnaire. Of the respondents, 63 (46.0%) provided care at SAMU emergency units, and 74 (54.0%), at hospital emergency services. Among these, 28 (37.8%) worked at level II hospitals, and 46 (62.2%), at level III/IV hospitals.

Table 1 shows the descriptive characteristics of the groups. The median number of years since graduation was 5.3 (IQR 1.8-12.7), and it was similar when comparing physicians working at the SAMU emergency units and those at the hospital emergency services, except for those working at level III/IV hospitals. Most physicians were male (67.9%) and specialized (68.6%), and that proportion was higher at level III/IV hospitals when compared to the proportion of specialists at level II hospitals and SAMU units. The most common medical specialties were internal medicine (29.1%), pediatrics (9.5%), surgery (7.2%) and gynecology and obstetrics (7.2%). No statistically significant difference was observed between the groups regarding the distribution in the different specialties (SAMU vs non-SAMU, $p = 0.168$; level II hospitals vs level III/IV hospitals, $p = 0.214$).

Most respondents showed overall dissatisfaction with the structure of care provided to cardiovascular emergencies in the region, whose median of the overall scale was 2.0 (IQR 2.0-4.0). When assessing *"overall satisfaction"*, the dissatisfaction of SAMU physicians was lower ($p = 0.01$). In addition, the physicians of level III/IV hospitals showed higher *"overall satisfaction"* as compared to those of level II hospitals ($p \leq 0.05$) (Table 2). No statistically significant correlation was observed between professional training time and *"overall satisfaction"* [$r_s = 0.112$, $p = 0.195$].

When assessing the scale domains, slightly higher *"satisfaction with the structure of care and diagnosis"*

Table 1 – Distribution of the physicians according to time since graduation, sex and specialty

Characteristics	Overall total (n = 137)	Non-SAMU (n = 74)			SAMU (n = 63)
		Level II hospitals (n = 28)	Level III/IV hospitals (n = 46)	Non-SAMU total (n = 74)	
Time since graduation (years) (median, IQR)	5.3 (1.8-12.7)	2.3 (1.5-5.0)*	11.0 (2.4-23.2)*	5.5 (1.9-15.3)†	5.3 (1.8-10.7)†
Male sex	93 (67.9)	13 (46.4)	35 (76.1)	48 (64.9)	45 (71.4)
Medical category/specialty					
Generalist	43 (31.4)	12 (42.9)	8 (17.4)	20 (27.0)	23 (36.5)
Specialty	94 (68.6)	16 (57.1)	38 (82.6)	54 (73.0)	40 (63.5)
Internal medicine	40 (29.1)	9 (32.1)	18 (39.1)‡	27 (36.4)‡	13 (20.6)
Pediatrics	13 (9.4)	3 (10.7)	5 (10.8)	8 (10.8)	5 (7.9)
Surgery	10 (7.2)	1 (3.5)	4 (8.6)‡	5 (6.7)‡	5 (7.9)
Gynecology and Obstetrics	10 (7.2)	1 (3.5)	6 (13)‡	7 (9.4)‡	3 (4.7)
Cardiology	4 (2.9)	0 (0)	0 (0)	0 (0)	4 (6.3)
Family Medicine	4 (2.9)	0 (0)	0 (0)	0 (0)	4 (6.3)
Others§	16 (11.6)	2 (7.1)	8 (17.3)‡	10 (13.5)‡	6 (9.5)

SAMU: mobile emergency care service; IQR: interquartile range.
** Comparison of the time since graduation between physicians of level II hospitals and level III/IV hospitals: p ≤ 0.01;*
† Comparison of the time since graduation between SAMU and non-SAMU physicians: p = 0.64;
‡ Two physicians had multiple specialties: one had two specialties (Internal Medicine and Surgery) and the other, three (Anesthesiology, Gynecology and Obstetrics, Labour Medicine). Both worked at a level III/IV hospital;
§ Others: Anesthesiology (3, 1 at SAMU and 2 at level III/IV hospital), Cardiovascular Surgery (2, at SAMU), Thoracic Surgery (2, 1 at SAMU and 1 at level III/IV hospital), Intensive Care Medicine (2, 1 at SAMU and 1 at level III/IV hospital), Neurology (1, at level II hospital), Dermatology (1, at level II hospital), Traffic Medicine (1, at SAMU), Labour Medicine (2, at level III/IV hospital), Orthopedics and Traumatology (1, at level III/IV hospital) and Psychiatry (1, at level III/IV hospital).

Table 2 – Comparison of the satisfaction of physicians (CARDIOSATIS-Team scale) categorized according to the type of emergency service, and result of the comparison test between groups

Domains/Itens of the scale	Overall (n = 137)	Non-SAMU (n = 74)				SAMU (n = 63)	Comparison between SAMU and non-SAMU (p-value)*
		Level II hospitals (n = 28)	Level III/IV hospitals (n = 46)	Non-SAMU total (n = 74)	Comparison between level II hospitals and level III/IV hospitals (p-value)*		
Domain 1: Satisfaction with the care provided (5 items)	2.0 (2.0-4.0)	2.0 (2.0-4.0)	2.0 (2.0-4.0)	2.0 (2.0-4.0)	0.96	2.0 (2.0-4.0)	0.05
Satisfaction with the care provided	2.0 (2.0-4.0)	4.0 (4.0-4.0)	3.5 (2.0-4.0)	4.0 (2.0-4.0)	0.38	2.0 (2.0-4.0)	0.87
Municipality's structure for diagnosis	2.0 (2.0-4.0)	2.0 (2.0-4.0)	2.0 (2.0-3.0)	2.0 (2.0-3.5)	0.49	2.0 (2.0-4.0)	0.03
Structure for managing cardiovascular diseases	2.0 (2.0-4.0)	2.0 (2.0-4.0)	2.0 (2.0-4.0)	2.0 (2.0-4.0)	0.34	2.0 (2.0-4.0)	0.59
Diagnostic accuracy	2.0 (2.0-4.0)	2.0 (2.0-2.0)	2.0 (2.0-4.0)	2.0 (2.0-4.0)	≤ 0.05	2.0 (2.0-4.0)	0.01
Technical support	5.0 (5.0-5.0)	5.0 (5.0-5.0)	5.0 (1.0-5.0)	5.0 (1.0-5.0)	0.50	5.0 (5.0-5.0)	≤ 0.01
Domain 2: Structure of care and diagnosis (6 items)	2.5 (2.0-3.5)	2.0 (2.0-2.0)	2.5 (2.0-3.5)	2.0 (2.0-3.0)	≤ 0.001	3.0 (2.0-4.0)	≤ 0.001
Medical facilities for the diagnosis of cardiovascular diseases	3.0 (2.0-4.0)	1.0 (1.0-2.0)	3.0 (2.0-4.0)	3.0 (2.0-3.0)	≤ 0.001	3.0 (2.0-4.0)	≤ 0.001
Quality of the equipment and materials	3.0 (2.0-3.0)	2.0 (2.0-2.0)	3.0 (2.0-3.0)	3.0 (2.0-3.0)	0.12	3.0 (3.0-4.0)	≤ 0.001
Technology available for diagnosis	2.0 (2.0-3.5)	2.0 (2.0-2.0)	2.0 (2.0-3.0)	2.0 (2.0-3.0)	0.66	2.0 (2.0-4.0)	≤ 0.001
Promptness in diagnosis	2.0 (2.0-3.5)	2.0 (2.0-2.0)	2.0 (2.0-4.0)	2.0 (2.0-3.0)	≤ 0.01	2.0 (2.0-4.0)	≤ 0.001
Adequacy of the service	3.0 (2.0-3.0)	2.0 (1.0-2.0)	3.0 (3.0-3.0)	3.0 (2.0-3.0)	≤ 0.001	3.0 (3.0-4.0)	≤ 0.001
Resolutivity	2.0 (2.0-4.0)	2.0 (2.0-2.0)	2.0 (2.0-4.0)	2.0 (2.0-4.0)	≤ 0.001	3.0 (2.0-4.0)	≤ 0.001
Overall scale (11 items)	2.0 (2.0-4.0)	2.0 (2.0-2.0)	3.0 (2.0-4.0)	2.0 (2.0-3.0)	≤ 0.05	2.0 (2.0-4.0)	≤ 0.001

*Values expressed as median (interquartile range), except when indicated; * Comparative analysis by use of Mann-Whitney U test.*

(median 2.5, IQR 2.0-3.5) was observed as compared to *"satisfaction with the care provided"* (median 2.0, IQR 2.0-4.0). In the domain *"satisfaction with the care provided"*, a significant difference was observed between the groups regarding *technical support*, perceived as worse by the hospital physicians. In the domain *"structure of care and diagnosis"*, the satisfaction of the hospital physicians with *"medical facilities for the diagnosis of cardiovascular diseases"*, *"promptness in diagnosis"*, *"adequacy of the service"* and *"resolutivity"* was lower as compared to that of SAMU physicians. In addition, the satisfaction of physicians of level II hospitals with those same items was lower than that of physicians of level III/IV hospitals. The satisfaction with the *"technology available for diagnosis"* was lower among the hospital physicians as compared to that of SAMU physicians, but did not differ between the two subgroups of hospital physicians.

When comparing SAMU physicians with those working at hospital emergency services, the former showed a higher satisfaction level in both domains (Figure 1, Table 3). When comparing physicians working at level II hospitals with those at level III/IV hospitals, the satisfaction with the care provided was similar. However, when assessing the domain *"structure of care and diagnosis"*, the physicians working at level III/IV hospitals were more satisfied (Figure 2, Table 4).

Discussion

This study involved physicians working in the public emergency services of the Northern Region of Minas Gerais (SAMU and emergency units of hospitals of different levels of complexity). Most of them had a short time since graduation, were male and specialists (68.6%). In addition, most of them expressed overall dissatisfaction with the care provided to cardiovascular diseases. SAMU physicians expressed higher level of satisfaction with the structure of cardiovascular care as compared to those working at the regional hospitals. In both groups, most physicians were satisfied with the *"technical support"* for the management of a patient, while most SAMU physicians were dissatisfied with the *"care provided"* and *"technology available for diagnosis"* (54% for both), and most hospital physicians were dissatisfied with the *"technology available for diagnosis"* (78.4%) and *"promptness in diagnosis"* (70.3%).

The health system of the Northern Region of Minas Gerais is a hierarchical regional emergency care network.[13] Oliveira et al.[18] have reported that the health system would be better considered as a circuit with multiple entry points, in which there is a more suitable place for each patient regarding the required type of care. When referring a patient to an emergency service, SAMU regulatory center physicians should always consider the best option regarding

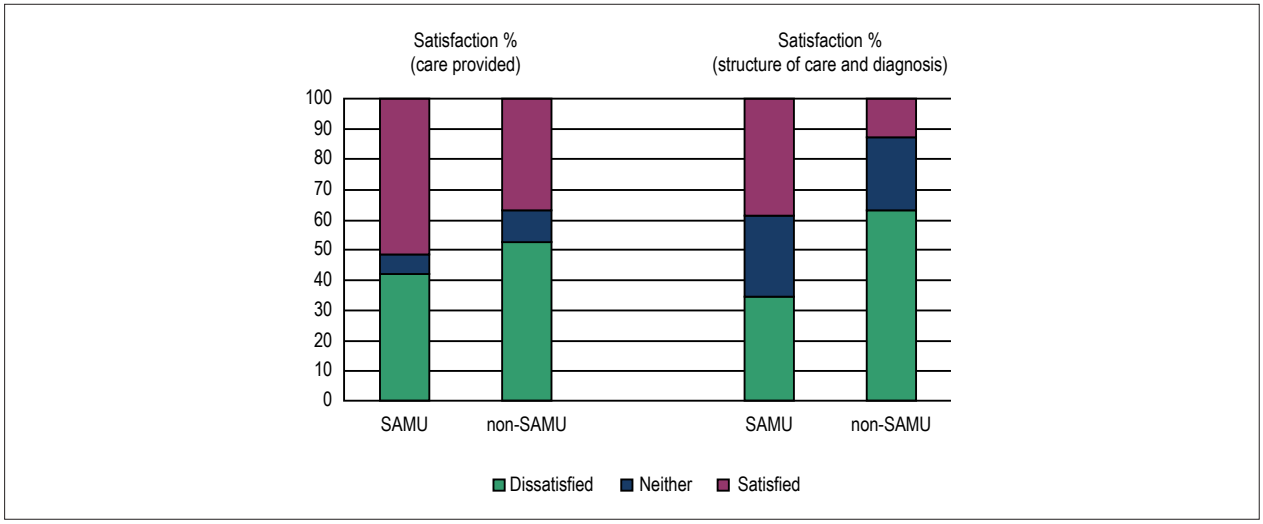

Figure 1 – *Satisfaction of physicians of the mobile emergency service (SAMU) and of hospital emergency services (non-SAMU) according to the domains of the CARDIOSATIS-Team scale.*

Table 3 – Description of the satisfaction level of physicians of the mobile emergency care service (SAMU) and of hospitals (non-SAMU) according to the CARDIOSATIS-Team scale

Domains/Itens of the scale	SAMU (n = 63)			Non-SAMU (n = 74)		
	Dissatisfied (1-2)	Neither (3)	Satisfied (4-5)	Dissatisfied (1-2)	Neither (3)	Satisfied (4-5)
Domain 1: Satisfaction with the care provided (5 items)						
Satisfaction with the care provided	34 (54.0)	1 (1.6)	28 (44.4)	37 (50.0)	7 (9.5)	30 (40.5)
Municipality's structure for diagnosis	29 (46.0)	3 (4.8)	31 (49.2)	49 (66.2)	8 (10.8)	17 (23.0)
Structure for managing cardiovascular diseases	33 (52.4)	7 (11.1)	22 (34.9)	46 (62.2)	7 (9.5)	21 (28.4)
Diagnostic accuracy	25 (39.7)	10 (15.9)	28 (44.4)	44 (59.5)	14 (18.9)	13 (17.6)
Technical support	9 (14.3)	-	52 (82.5)	11 (14.9)	-	52 (70.3)
Domain 2: Structure of care and diagnosis (6 items)						
Medical facilities for the diagnosis of cardiovascular diseases	20 (31.8)	14 (22.2)	29 (46.0)	42 (56.8)	18 (24.3)	13 (17.6)
Quality of the equipment and materials	11 (17.5)	31 (49.2)	21 (33.3)	34 (46.0)	34 (46.0)	5 (6.8)
Technology available for diagnosis	34 (54.0)	9 (14.3)	20 (31.8)	58 (78.4)	7 (9.5)	9 (12.2)
Promptness in diagnosis	30 (47.6)	6 (9.5)	27 (42.9)	52 (70.3)	11 (14.9)	11 (14.9)
Adequacy of the service	10 (15.9)	30 (47.6)	23 (36.5)	40 (54.1)	24 (32.4)	7 (9.5)
Resolutivity	26 (41.3)	11 (17.5)	26 (41.3)	49 (66.2)	11 (14.9)	12 (16.2)

Values expressed as n (%).

the resources available, the location of the teams and proximity.[19] In the Northern Region of Minas Gerais, as SAMU is regionalized, the number of advanced ambulances is limited. Because that number is calculated based only on a population criterion, ignoring the long distances, more often than not the closest advanced support is an emergency center of a regional hospital, independently of the severity of the patient's condition or even of the technical skills of the team.

In the present study, more than 50% of the hospital physicians expressed dissatisfaction with 9 of the 11 items. Those professionals highlighted the inadequacy of the emergency units, which involves the quality of equipment and materials, in addition to the municipalities' limited structure for diagnosis, which reflects on the overall quality of the cardiovascular care provided.

It is worth noting that the physicians of level II hospitals expressed more dissatisfaction than those of level III/IV hospitals

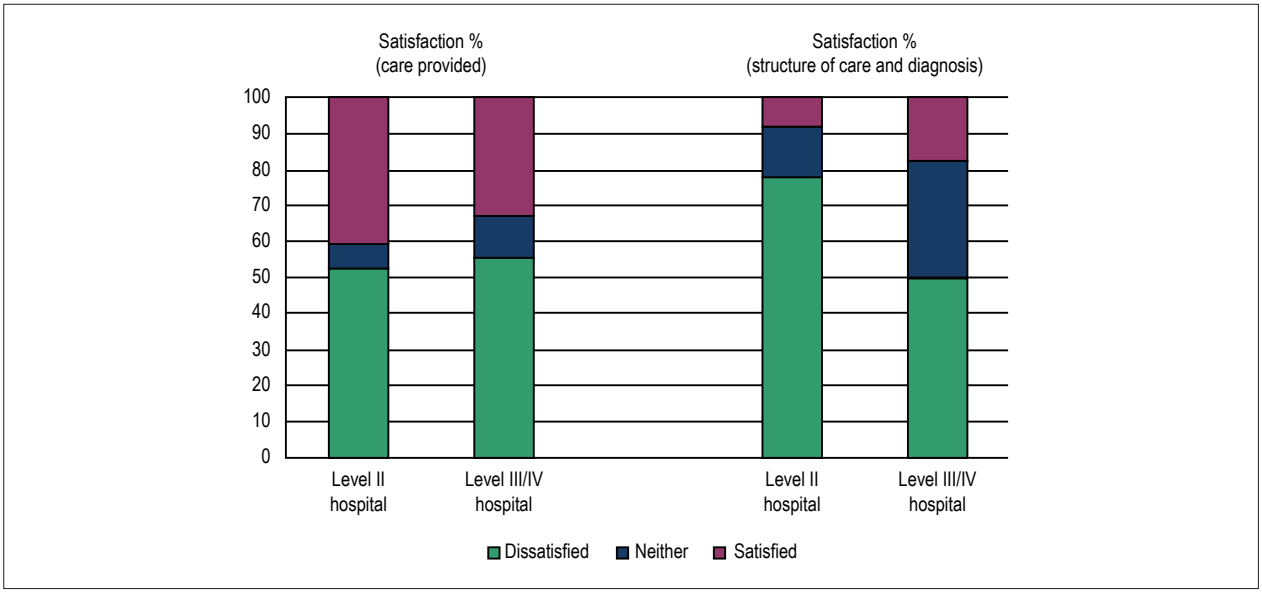

Figure 2 – *Satisfaction of physicians of level II hospitals and those of level III/IV hospitals according to the domains of the CARDIOSATIS-Team scale.*

Table 4 – Description of the satisfaction level of physicians of level II hospitals and level III/IV hospitals according to the CARDIOSATIS-Team scale

Domains/Itens of the scale	Level II hospitals (n = 28)			Level III/IV hospitals (n = 46)		
	Dissatisfied (1-2)	Neither (3)	Satisfied (4-5)	Dissatisfied (1-2)	Neither (3)	Satisfied (4-5)
Domain 1: Satisfaction with the care provided (5 items)						
Satisfaction with the care provided	13 (46.4)	1 (3.6)	14 (50.0)	24 (52.2)	6 (13.0)	16 (34.8)
Municipality's structure for diagnosis	18 (64.3)	2 (7.1)	8 (28.6)	31 (67.4)	6 (13.0)	9 (19.6)
Structure for managing cardiovascular diseases	16 (57.1)	2 (7.1)	10 (35.7)	30 (65.2)	5 (10.9)	11 (23.9)
Diagnostic accuracy	21 (75.0)	4 (14.3)	2 (7.1)	23 (50.0)	10 (21.7)	11 (23.9)
Technical support	9 (32.1)	-	19 (67.9)	18 (39.1)	-	27 (58.7)
Domain 2: Structure of care and diagnosis (6 items)						
Medical facilities for the diagnosis of cardiovascular diseases	24 (85.7)	1 (3.6)	3 (10.7)	18 (39.1)	17 (37.0)	10 (21.7)
Quality of the equipment and materials	15 (53.6)	12 (42.9)	1 (3.6)	19 (41.3)	22 (47.8)	4 (8.7)
Technology available for diagnosis	21 (75.0)	3 (10.7)	4 (14.3)	37 (80.4)	4 (8.7)	5 (10.9)
Promptness in diagnosis	23 (82.1)	3 (10.7)	2 (7.1)	29 (63.0)	8 (17.4)	9 (19.6)
Adequacy of the service	23 (82.1)	3 (10.7)	1 (3.6)	17 (37.0)	21 (45.7)	6 (13.0)
Resolutivity	23 (82.1)	2 (7.1)	2 (7.1)	26 (56.5)	9 (19.6)	10 (21.7)

Values expressed as n (%).

regarding the *structure of care and diagnosis*, such as the *medical facilities for the diagnosis of cardiovascular diseases*, *promptness in diagnosis, adequacy of the service,* and *resolutivity*, even if, by definition, the structure of a level II hospital is better than that of level III/IV hospitals. The number of dissatisfied physicians was higher among those of level II hospitals regarding the domains *"medical facilities for the diagnosis of cardiovascular diseases"*, *"promptness in diagnosis"*, *"adequacy of the service"* and *"resolutivity"*, in which a lower level of dissatisfaction would be expected among physicians of level II hospitals than the ones of level III/IV hospitals. It might be due to the higher expectations of those professionals, because satisfaction is known to relate to both adequacy of the services and individuals' expectations regarding quality care.[16,20]

It is worth noting the high number of physicians without medical residency (31.4%) – generalists – or from medical specialties without specific training in adult cardiovascular emergency (pediatrics and gynecology). The findings of the present study show the importance of promoting continuous education programs in the region to improve the skills of the physicians working at cardiovascular emergency services. The highest satisfaction level with *"technical support"* is positive in that context. In addition, such findings emphasize the need for training in emergency medicine in the medical curriculum. In Brazil, physicians graduate without the necessary work experience in the emergency setting. That has been recognized by the *Associação Brasileira de Educação Médica* (Brazilian Association of Medical Education), which, nevertheless, reports that "most newly graduated physicians end up on work shifts at emergency units or pre-hospital care units", but the "*Diretrizes Curriculares Nacionais"* (National Curriculum Guidelines) do not value that area of medical practice.[21]

The previous "*Diretrizes Curriculares Nacionais"* (National Curriculum Guidelines) for medical education did not include emergency medicine in the required disciplines of the medical internship.[22] The current ones require that at least 30% of the hours of the medical internship be spent in Primary Care and Emergency Care of the Brazilian Unified Health System (SUS), "respecting the minimum of two years of internship".[23] However, the number of hours dedicated to emergency education is still limited in most medical schools in Brazil,[24] which tends to aggravate with the ever-increasing number of medical schools and the scarcity of practice scenarios.

In 2015, emergency medicine was recognized as a medical specialty by the "*Conselho Federal de Medicina*" (Brazilian Federal Council of Medicine), the "*Conselho Nacional de Residência Médica*" (National Council of Medical Residency) and the "*Associação Brasileira de Educação Médica*" (Brazilian Association of Medical Education). Although that qualification in emergency care was being structured during the time this study was being performed, so far there is no official medical education program for pre-hospital care.

Currently, emergency services face great challenges in several realms: scarcity of skilled labor, overcrowded facilities, low quality of care provided to those who most need it high turnover of professionals, and exposure of professionals to risks due to the growth of violence in large cities.[19] Several studies have assessed the organization of emergency services, but data analyzing those professionals' satisfaction are scarce. Another study assessing the physicians' satisfaction with the structure of cardiovascular care has been conducted in the same region, but with professionals working in primary healthcare before and after the implementation of a Telehealth system in cardiology.[16]

Studies have investigated the burnout of physicians. Its frequency among emergency professionals is alarming.[25] Work dissatisfaction is one of the burnout-related factors reported. A study of 771 North American emergency physicians has observed that those reporting stress and burnout as severe problems expressed lower levels of satisfaction with their careers.[26] Another study of 193 North American emergency physicians members of the American College of Emergency Physicians, has reported that dissatisfaction related to clinical autonomy, to challenges in the emergency medicine practice and to stress were significantly associated with high levels of burnout.[27] Our study was not aimed at specifically investigating burnout in that population, but the high dissatisfaction level found indicates the need for specific assessments.

This study has limitations inherent in its cross-sectional design, preventing inference of causality. Other factors might have affected the physician's satisfaction, such as professional acknowledgement, changes in salary and better working conditions, which were not directly measured in this investigation.[16]

The results of the present study are important because they enable managers of pre-hospital and hospital emergency services to reflect, aiming at qualifying the care provided. Negative changes in the mental state of emergency professionals have a adverse impact on their professional performance.[28] The satisfaction with the structure of cardiovascular care of the SAMU or hospital physicians found in this study was extremely important to delineate and implement the AMI system of care. The operational flow was discussed with the managers, the tele-electrocardiogram was installed in the ambulances and the thrombolytic was acquired,[11] however, without the adherence and motivation of the physicians and nurses at the emergency services, the AMI system of care would be doomed to failure. This is a pioneer study in the assessment of the baseline for the implementation of the AMI system of care in Brazil, which can be a model for future implementations. Additionally the results can help the assessment of the quality of the care provided and the planning of training programs, guiding the definition of priorities, mainly for services that provide care for cardiovascular diseases.[16]

Conclusion

This study showed the overall dissatisfaction of emergency physicians in the Northern Region of Minas Gerais with the structure of care provided for cardiovascular emergencies. Most physicians expressed dissatisfaction with the care provided, the structure for managing cardiovascular diseases and the technology available for diagnosis. The dissatisfaction of SAMU physicians was lower as compared to that of the emergency physicians at the regional hospitals, and the dissatisfaction of physicians of level III/IV hospitals was lower as compared to that of physicians of level II hospitals.

Acknowledgments

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Author contributions

Conception and design of the research: Marcolino MS, Oliveira JAQ, Ribeiro AL, Cardoso CS; Acquisition of data: Silva GKM, Dias TD, Marino BCA, Antunes AP; Analysis and interpretation of the data: Marcolino MS, Oliveira JAQ, Silva GKM, Dias TD, Marino BCA, Antunes AP, Cardoso CS; Statistical analysis: Marcolino MS, Oliveira JAQ; Obtaining

financing: Ribeiro AL; Writing of the manuscript: Marcolino MS, Oliveira JAQ, Silva GKM, Dias TD, Cardoso CS; Critical revision of the manuscript for intellectual content: Marcolino MS, Oliveira JAQ, Marino BCA, Antunes AP, Ribeiro AL, Cardoso CS.

Potential Conflict of Interest

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

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Study Association

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais under the protocol number 260/09, resolution 466/12. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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Are We Taking Good Care of Our Patients and Physicians?

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The thorough and well-structured analysis by Marcolino et al.[1] in the article "Satisfaction of emergency physicians with the care provided to patients with cardiovascular diseases in the Extended Northern Region of Minas Gerais", published in this *Arquivos Brasileiros de Cardiologia* issue, highlights important problems to be addressed in the Brazilian medical healthcare.

Although circumscribed to a region, that study would most probably reproduce the reality of several other Brazilian regions, some even with a higher human development index. The dissatisfactions brought up in that study can be easily observed in the daily medical practice in almost all Brazilian cities, notably emergency care in general, and emergency cardiovascular care in particular. In the later, the issue is compounded by the frequent need for combining good and prompt care to yield effectiveness.

One of the major problems detected in the care provided to emergency patients is the physicians' lack of specific training in cardiovascular diseases, which might be the reason of the other finding in the study referred to, the physicians' dissatisfaction with their work.

It is certainly not reasonable to assume that physicians trained in cardiovascular diseases will be available at all less populated regions of Brazil. Grouping the care provided according to complexity, with an agile referral system between units from lower to higher capacity for care, would be desirable, as long as more qualified care conditions would be assured, extending beyond cardiologists, involving trained clinicians and mainly the finally recognized specialists in emergency medicine. Although the emergency medicine specialty already exists in several parts of the world, such as the United States, where the first residency program in the specialty was inaugurated at the Cincinnati University in 1970, it was recognized in Brazil only in 2016.[2] More recently, the Mixed Committee of Medical Specialties (CME), comprising the Federal Council of Medicine (CFM), the Brazilian Medical Association (AMB) and the National Committee of Medical Residency (CNRM), has put their seal of approval on the education program for emergency medicine, which resulted from the commendable initiative of the Brazilian Association of Emergency Medicine (ABRAMEDE).

It is worth noting that, in the study referred to, although specialized physicians predominated in the care provided to cardiovascular emergencies, both at level II, III and IV hospitals and at SAMU (68.6%), most of them had specialized in areas not related to specific care to cardiovascular diseases, such as pediatrics, general surgery, gynecology and obstetrics, and internal medicine, only 2.9% being cardiologists, while the others had not even attended a medical residency program (31.4%).

Our guidelines for the formation of cardiologists recommend a minimum 288-hour training in cardiovascular emergency.[3] Other forms of training less directed to that objective, or even the lack of any training, leave a lot to be desired regarding the quality of the care provided to patients with cardiovascular diseases.

In addition, the study referred to evidenced the dissatisfaction with the structure of care provided at cardiovascular emergency units as an important reason for the physicians' dissatisfaction. However, it is worth highlighting the importance of 'technical support' as one of the items related to physicians' satisfaction, reinforcing the significance of recognizing the area as a relevant element for professional action.

Another element that decisively influences the professional's satisfaction relates to professional and financial appreciation. Although the topic was not directly assessed by use of the CARDIOSATIS scale,[4] it is something to be considered in future studies, even for the desired retention of professionals.

This set of measures should be implemented. The practice of medicine amidst such discontentment is inconceivable, mainly at a time with increasing evidence of the significant loss of quality and amount of life among physicians.[5]

Keywords

Medical Assistance; Cardiovascular Diseases; Ambulatory Care; Ambulatory Care; Emergency Medical Services; Inservice Training; Medical Education / manpower.

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Pioglitazone Induces Cardiomyocyte Apoptosis and Inhibits Cardiomyocyte Hypertrophy Via VEGFR-2 Signaling Pathway

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Abstract

Background: **Pioglitazone has been widely used as an insulin-sensitizing agent for improving glycemic control in patients with type 2 diabetes mellitus. However, cardiovascular risk and protective effects of pioglitazone remain controversial.**

Objectives: **In this study, we investigated whether pioglitazone affects cardiomyocyte apoptosis and hypertrophy by regulating the VEGFR-2 signaling pathway.**

Methods: **Cardiomyocytes were enzymatically isolated from 1- to 3-day-old Sprague-Dawley rat ventricles. Effects of pioglitazone and the VEGFR-2-selective inhibitor apatinib on cardiomyocyte apoptotic rate was determined using flow cytometry, and hypertrophy was evaluated using [^{3}H]-leucine incorporation. The protein expressions of unphosphorylated and phosphorylated VEGFR-2, Akt, P53, and mTOR were determined by Western-Blotting. Analysis of variance (ANOVA) was used to assess the differences between groups.**

Results: **Pioglitazone and VEGFR-2-selective inhibitor apatinib reduced rat cardiomyocyte viability and cardiomyocyte hypertrophy induced by angiotensin II in vitro. Furthermore, in the same in vitro model, pioglitazone and apatinib significantly increased the expression of Bax and phosphorylated P53 and decreased the expression of phosphorylated VEGFR-2, Akt, and mTOR, which promote cardiomyocyte hypertrophy.**

Conclusions: **These findings indicate that pioglitazone induces cardiomyocyte apoptosis and inhibits cardiomyocyte hypertrophy by modulating the VEGFR-2 signaling pathway. (Arq Bras Cardiol. 2018; 111(2):162-169)**

Keywords: **Apoptosis; Myocytes, Cardiac; Cardiomegaly; Heart Failure/physiopathology; Antihypertensive Agents; Thiazolidinediones; Insulin Resistance**

Introduction

Heart failure (HF) is the most common consequence of cardiovascular diseases and the leading cause of cardiovascular mortality worldwide.[1] Basic pathophysiology of HF is cardiac remodeling, which involves a number of cellular changes including cardiomyocyte hypertrophy, loss of cardiomyocytes due to apoptosis, necrosis, fibroblast proliferation and fibrosis.[2] Recent fundamental and clinical studies have demonstrated that diabetes mellitus (DM) drives cardiac remodeling, including myocardial hypertrophy and cardiomyocytes loss, via glucotoxicity and lipotoxicity, eventually resulting in HF.[3] Intensive glucose control was shown to reduce the occurrence of major cardiovascular events including HF, but did not improve the overall survival rate in patients with type 2 DM, compared to patients receiving standard therapy.[3] Thiazolidinediones, including pioglitazone, have been widely used as peroxisome proliferator-activated receptor (PPAR)-γ agonists and insulin-sensitizing agents for improving glycemic control in patients with type 2 DM. However, cardiovascular risks of pioglitazone remain controversial.

One view is that intensive glycemic control with pioglitazone or rosiglitazone increases the risk of HF (OR ≤ 2.1; 95% CI 1.08–4.08) based on meta-analyses of randomized clinical trials.[4-6] Rosiglitazone was more likely to induce HF than pioglitazone.[7] Additionally, animal experiments confirmed the increased risk of HF with pioglitazone treatment, as pioglitazone augmented cardiac damage in isoproterenol-induced HF rat model and induced rat ventricular hypertrophy in acute toxicity experiments.[8,9] However, another point of view is that pioglitazone use does not significantly increase the risk of myocardial infarction or cardiac death, based on the PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive) data,[10] and that pioglitazone can suppress overload-induced cardiac hypertrophy by inhibiting AKT/GSK3β and MAPK signaling pathways.[11]

Vascular endothelial growth factor receptors (VEGFR) are considered critical factors for cardiac hypertrophy and HF.

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Three different subtypes, VEGFR-1, -2, and -3 have been described. Recent studies showed that VEGFR-1 and VEGFR-2 are essential for regression and induction of cardiomyocyte hypertrophy, respectively,[12] whereas VEGFR-3 was shown to be beneficial for the infarcted myocardium by promoting compensatory cardiomyocyte hypertrophy and improving survival.[13] Additionally, VEGFR-2 is involved in the delayed phase of endothelial cell (pulmonary artery and human aortic endothelial cells) barrier dysfunction caused by high levels of 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphatidylcholine oxidation products, contributes to stress fiber formation, and increases phosphorylation of myosin light chains.[14] Pioglitazone decreased the expression of VEGFR-2 in splanchnic tissues and inhibited neoangiogenesis in a rat model of portal hypertension,[15] indicating a possible direct effect on VEGFR-2 expression. Reverse screening approaches (reverse pharmacophore mapping and reverse docking) have been very important methods to discover new cardiovascular disease-related protein targets for pioglitazone. In this study, we used the PharmMapper for reverse pharmacophore mapping. The structure of pioglitazone in mol2 format was submitted to PharmMapper, obtaining 10 targets and poses, which were sorted by decreasing PharmMapper fit score. The top ten PharmMapper fit scores for potential pioglitazone targets showed that VEGFR-2 was the best-ranked potential target, which will be essential for understanding the interaction between pioglitazone and VEGFR-2.

Given the potential link between pioglitazone and VEGFR-2 and their function in cardiomyocyte hypertrophy and apoptosis in the pathophysiology of HF, we investigated, for the first time, whether pioglitazone affects cardiomyocyte hypertrophy and apoptosis by regulating the VEGFR-2 signaling pathway. Furthermore, it may be expected to explore a promising approach for clarifying the potential mechanism for the effect of pioglitazone on cardiovascular outcomes.

Methods

Ethics Statement

All animal experiments were approved by the Institutional Animal Care and Use Committee of the First Hospital of Nanping City.

Molecule preparation

In order to characterize the binding sites in the predicted protein targets we used Genetic Optimization for Ligand Docking (GOLD) suite v5.3. VEGFR-2-inhibitor complex crystallographic structure (Protein Data Bank ID: 3CP9) was selected as the starting structure for predicting the binding site of pioglitazone at VEGFR-2. GOLD uses a genetic algorithm for docking ligands into the binding site of target proteins, with full conformational flexibility of the ligand and partial receptor flexibility. Ligand binding energy was predicted with the ChemScore scoring function and free energy of binding (ΔG) as implemented in GOLD.[16] Solvent molecules were removed from the crystal structure and protein hydrogen atoms were added. Ligand binding site for docking was defined to include amino acids within 10 Å of the coordinates of the inhibitor in the crystal structure. Top 10 docking poses were obtained by terminating the simulation once root mean square deviation (RMSD) between any five ligand poses was reached < 1 Å PyMol v.1.3 and LigPlot+ v.1.4 were used to visualize the results.

Isolation and culture of rat neonatal cardiomyocytes

Cardiomyocytes were enzymatically isolated from 1- to 3-day-old Sprague-Dawley rat ventricles as previously described.[17] A total of twelve rats were used to perform twelve independent experiments in the study. Isolated cardiomyocytes were seeded onto cell culture plates precoated with 10 g/ml of fibronectin and cultured in a medium containing DMEMF-12 with HEPES (Invitrogen, Carlsbad, CA, USA), 5% heat-inactivated horse serum, 100 U/ml penicillin, 10 μg/ml streptomycin, 3 mM pyruvic acid, 2 mg/ml bovine serum albumin, 100 g/ml ampicillin, insulin-transferrin-sodium selenite media supplement (Sigma, St. Louis, MO, USA), 5 g/ml linoleic acid, and 100 M ascorbic acid at 37°C in a humidified atmosphere containing 5% CO_2. For all experiments, cells were cultured at 5×10^4 cells/cm^2 unless otherwise stated.

Cell proliferation assay

Effects of pioglitazone and the VEGFR-2-selective inhibitor apatinib (Apexbio Technology LLC, Houston, TX, USA) on cardiomyocyte proliferation were measured by counting crystal violet-stained cells 24 h after treatment using an automated cell counter (BioRad). Briefly, cardiomyocytes (5×10^4 cells/well) were seeded in 96-well plates and cultured in standard medium for 24 h. After 24 h serum starvation, cardiomyocytes were treated with 0.1 μM angiotensin (Ang) II for 24 h. Pioglitazone (0, 10 or 20μM) and apatinib (2 μM) was added to the culture medium 2 h prior to Ang II administration. For crystal violet staining, the cells were washed twice with 1× phosphate-buffered saline, fixed with 20% methanol for 30 min, and stained with 0.2% crystal violet solution for 30 min at room temperature with gentle shaking. Stained cells were washed with water until a clear background was visible. Crystal violet dye was extracted using 1% SDS and the cells were counted using an automated cell counter.

Detection of apoptosis by flow cytometry

Cardiomyocyte apoptotic rate was determined using flow cytometry with the annexin V-FITC (AV)/propidium iodide (PI) dual staining. Briefly, after treatment, the cells ($1\text{-}5 \times 10^5$/ml) were collected, washed twice with phosphate-buffered saline, and resuspended in 500 μl of binding buffer. Next, the cells were incubated with 10 μL AV and 5 μL PI in the dark at room temperature for 15 min. The apoptotic cells were identified by FCM within 30 min.

Cardiomyocyte hypertrophy

Cardiomyocytes were seeded at a density of 5×10^4 cells/well in 96-well plates and hypertrophy was evaluated using [^{3}H]-leucine incorporation, as previously described.[18] Briefly, 2 h after treatment with pioglitazone (0-20 μmol/l) and apatinib (2 μM), 0.1 μM Ang II was used to stimulate cardiomyocyte hypertrophy and 1 μCi [^{3}H]-leucine was simultaneously added

to each well. After stimulation with Ang II for 60 h, cells were harvested by precipitation with 10% trichloroacetic acid on ice for 30 min before being solubilized with 1 mol/l NaOH overnight at 4°C. The samples were neutralized with 1 mol/l HCl and [^{3}H] levels were determined in scintillation fluid using a β counter to assess [^{3}H]- leucine incorporation.

Western Blot Analysis

Trypsinized cells were lysed in radioimmunoprecipitation assay buffer, homogenized on ice, and centrifuged. Supernatants were resolved via 10% SDS-PAGE and transferred to PVDF membranes (Millipore). The membranes were blocked with Tris-buffered saline (TBS) containing 5% non-fat milk and incubated with the following primary antibodies at 4°C overnight: VEGFR-2 (ab39256; 1:1000; Abcam), phospho-VEGFR-2 (Tyr1175) (#2478; 1:1000; Cell Signaling Technology), Akt (#9272; 1:1000; Cell Signaling Technology), phospho-Akt (Thr308) (#9275; 1:1000; Cell Signaling Technology), P53 (#9282; 1:1000; Cell Signaling Technology), phospho-P53 (Ser15) (#9284; 1:1000; Cell Signaling Technology), Bax (#14796; 1:1000; Cell Signaling Technology), rabbit anti-mTOR (#2972; 1:1000; Cell Signaling Technology), rabbit anti- phosphorylated-mTOR (Ser2448) (#5536; 1:1000; Cell Signaling Technology), and GAPDH (#2118, 1:1000; Cell Signaling Technology). On the following day, the membranes were washed three times with tris-buffered saline with tween (TBST) for 5 min at room temperature and subsequently incubated with anti-rabbit IgG secondary antibodies, for 1 h at room temperature. Following incubation, the membranes were washed with TBST and exposed to an X-ray film. Band intensities on the film were analyzed by densitometry, and the results were normalized to β-actin. GAPDH was used as the protein loading control.

Statistical analysis

Statistical analysis was performed using the SPSS 13.0 software package. Kolmogorov Smirnov test was used to verify the normality of data distribution and Levene test was used to inspect the homogeneity of variance. All data satisfied normal distribution and homogeneity of variance were presented as mean ± SD. One-way ANOVA was used for comparisons between multiple groups, whereas post-hoc Bonferroni test was used for pairwise comparisons. $P < 0.05$ indicated statistical significance.

Results

VEGFR-2 was the optimal potential target for pioglitazone

Top ten PharmMapper fit scores of potential pioglitazone targets shown VEGFR-2 was the best-ranked potential target. To understand the interaction between pioglitazone and VEGFR-2 and assess ligand binding energy, we performed a docking study using GOLD. Optimal binding conformation of the pioglitazone-VEGFR-2 complex is presented in Figure 1A and 1B. ChemScore score and binding energy of pioglitazone-VEGFR-2 complex were comparable to the VEGFR-2-inhibitor complex crystallographic structure. Pioglitazone was predicted to form van der Waals interactions with Val363, Leu428, Cys454, Leu444, Leu310, Phe456, Gly387, Phe383, Val364, and Ile453 and bind to Cys384 and Asp455 with hydrogen bonds. Predicted hydrogen bonds are shown in Fig. 1B. Western blot was conducted to examine the effect of pioglitazone on expression of VEGFR-2 and phospho-VEGFR-2 in rat neonatal cardiomyocytes under hypertrophic stimuli. Pioglitazone treatment decreased VEGFR-2 phosphorylation in rat neonatal cardiomyocytes in a dose-dependent manner (Figure 1C).

Pioglitazone promoted cardiomyocyte apoptosis and inhibited cardiomyocyte hypertrophy induced by Ang II

To validate the effects of pioglitazone, cardiomyocyte viability and Ang II-induced cardiomyocyte hypertrophy were evaluated. Crystal violet staining, a quick and versatile assay for screening cell viability under diverse stimulation conditions,[19] was used to analyze cell viability. Pioglitazone inhibited cardiomyocyte viability in a dose-dependent manner (Figure 2A and 2B, $p < 0.01$), with effective concentrations ranging from 0 to 20 μmol/l. Apatinib also inhibited cardiomyocyte viability (Figure 2A and 2B, $p < 0.01$). Cardiomyocyte apoptotic rate was determined using FCM with AV/PI staining. Apoptotic rates in pioglitazone 20 μM, pioglitazone 10 μM, and apatinib 2 μM groups were significantly higher compared to control (Figure 2C and 2D, $p < 0.01$), with apoptotic rate in the pioglitazone 10 μM group significantly lower than rates in pioglitazone 20 μM and apatinib 2 μM groups (Figure 2C and 2D, $p < 0.01$). Ang II-induced [^{3}H]-leucine incorporation was significantly decreased after pioglitazone or apatinib treatment, indicating that both inhibited cardiomyocyte hypertrophy.

Pioglitazone inhibited cardiomyocyte hypertrophy and promoted cardiomyocyte apoptosis by suppressing VEGFR-2 signaling

Potential mechanisms of pioglitazone-induced inhibition of cardiomyocyte hypertrophy and promotion of cardiomyocyte apoptosis were assessed *in vitro*. Compared to cardiomyocytes under control conditions, pioglitazone significantly increased the expression of Bax and phospho-P53 and decreased the expression of phospho-VEGFR-2 in neonatal rat cardiomyocytes under hypertrophic stimuli (Figure 1C, Figure 3).

To further investigate whether pioglitazone targets VEGFR-2, effects of pioglitazone on the VEGFR-2 expression and VEGFR-2-regulated intracellular signaling were determined in neonatal rat cardiomyocytes, under hypertrophy induced by Ang II. Compared to untreated hypertrophic cardiomyocytes, pioglitazone significantly decreased the expression of phospho-VEGFR-2, phospho-Akt, and phospho-mTOR, which may contribute to cardiomyocyte hypertrophy. Likewise, apatinib significantly decreased the expression of VEGFR-2, phospho-VEGFR-2, phospho-Akt, and phospho-mTOR (Figure 1C, Figure 3).

Discussion

In the present study, we demonstrated that pioglitazone reduced cardiomyocyte viability and hypertrophy induced by Ang II *in vitro*. We further show that pioglitazone increased the expression of Bax and phosphorylated P53, and decreased the

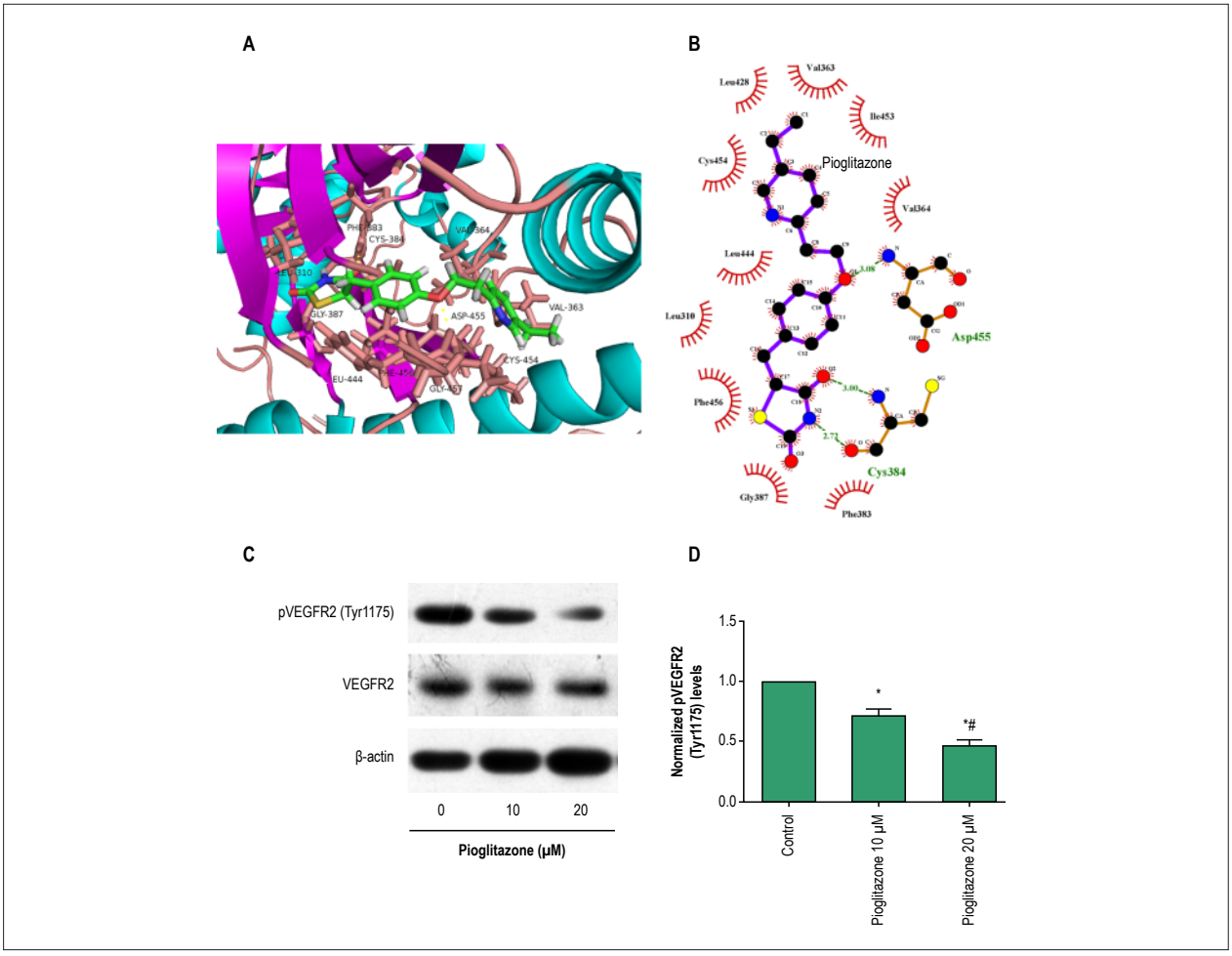

Figure 1 – *VEGFR-2 is the potential target of pioglitazone. A, 3D molecular docking model of pioglitazone with VEGFR-2; green structure, the conformer of pioglitazone. B, 2D molecular docking model of pioglitazone with VEGFR-2; purple structure, the conformer of pioglitazone. H-bonding interactions between the pioglitazone and VEGFR-2 were indicated with green dashed lines. C and D, Representative western bolotting trace of VEGFR-2 and phospho-VEGFR-2 (Tyr1175) protein levels in rat neonatal cardiomyocytes under hypertrophic stimuli, and treated with 0, 10, 20 (μM) pioglitazone for 24 hours, and intensity of the bands in C normalized to β-actin (n = 12 in each group). All data shown are the mean ± SD. *p < 0.01 compared with control; #p < 0.01 compared with pioglitazone 10 μM group, calculated by one-way ANOVA followed by the post hoc Bonferroni test for pairwise comparisons.*

expression of phosphorylated VEGFR-2, Akt, and mTOR *in vitro*. These findings suggest that pioglitazone induces cardiomyocyte apoptosis and inhibits cardiomyocyte hypertrophy through effects on the VEGFR-2 signaling pathway.

Pioglitazone has been widely used to improve glycemic control in patients with type 2 DM. Additionally, the PROactive trial showed that pioglitazone reduced the main secondary composite outcome of cardiovascular death/myocardial infarction/stroke vs. placebo by 43 % in the trial population.[10] These findings indicate that pioglitazone improves vascular function in diabetic patients and non-diabetic patients with insulin resistance and suggesting a possible beneficial effect of pioglitazone treatment on cardiovascular prognosis. Furthermore, a meta-analysis showed that supplementing insulin treatment with pioglitazone in type 2 DM patients with poorly controlled glucose levels could help decrease glucose levels and reduce the daily insulin dose without increasing the risks of myocardial infarction, HF, cardiac death and all-cause death, but at the cost of increasing total cholesterol levels and risks of hypoglycemia and edema.[20]

Given available evidence, pioglitazone treatment appears advantageous in patients with HF. However, pioglitazone was also reported to increase the risk of hospitalization for HF over a 30-day period, even though patients at high risk of HF were unlikely to be prescribed the drug.[21] Furthermore, clinical studies indicated that low dose pioglitazone treatment does not reduce the rate of in-stent restenosis, neointima volume nor atheroma volume in DM patients who have undergone percutaneous coronary intervention with drug-eluting stents.[22] Beyond different methodologies applied in the discussed studies, reasons for contradictory reports on effects of pioglitazone on HF remain unclear. Investigating cardiovascular targets of pioglitazone is a promising approach for clarifying the effect of the drug on cardiovascular outcomes.

Effects of pioglitazone on the cardiovascular system have been previously reported. Pioglitazone attenuated monocrotaline-induced rat right ventricular hypertrophy and fibrosis and decreased cardiomyocyte size.[23] Pioglitazone (2.5 mg/kg) ameliorated systolic and diastolic cardiac dysfunction in a rat model of Ang II-induced

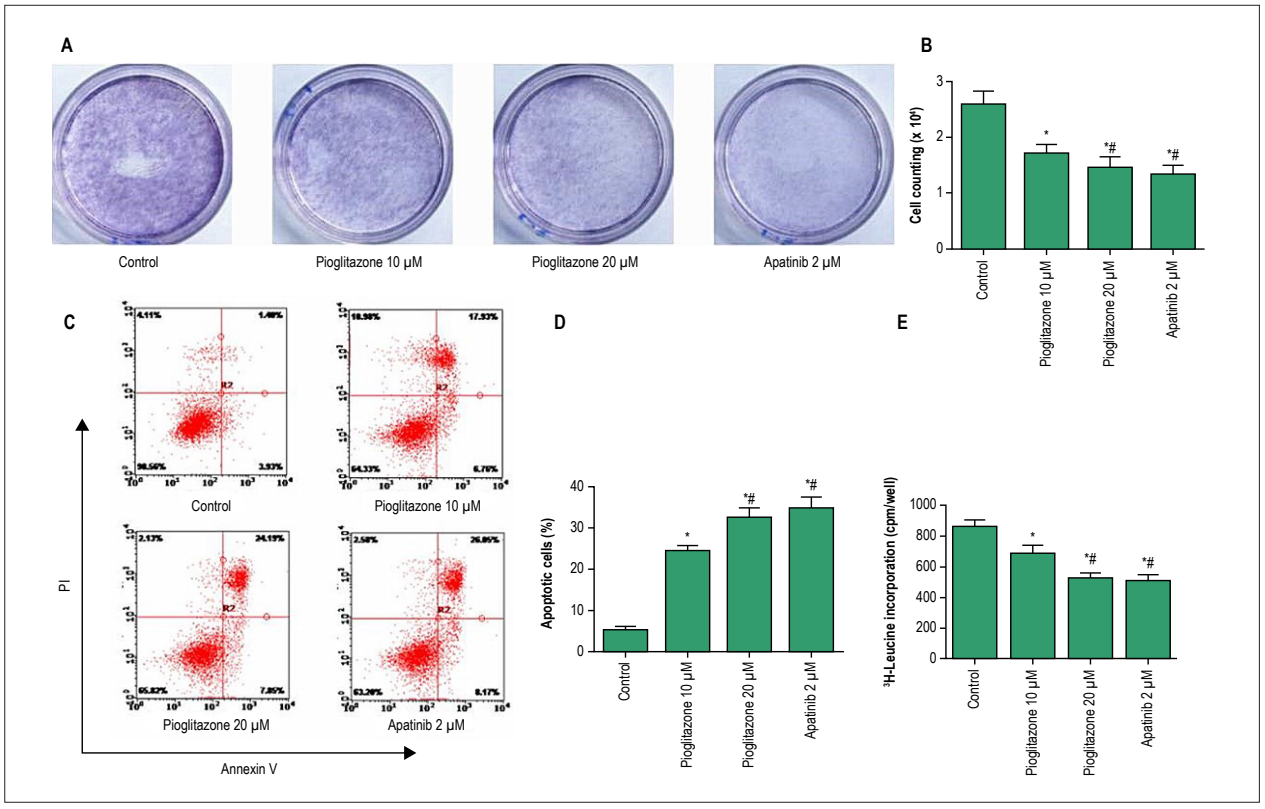

Figure 2 – *Pioglitazone and apatinib induced apoptosis and inhibited hypertrophy of rat neonatal cardiomyocytes (n = 12 in each group). A, Crystal violet staining of rat neonatal cardiomyocytes in response to various concentrations of pioglitazone or apatinib. Both inhibited the viability of rat neonatal cardiomyocytes. B, Cell proliferation was determined using the automated cell counter. C and D, Pioglitazone and apatinib induced neonatal rat cardiomyocytes apoptosis, which was detected by flow cytometry with the annexin V (AV) / propidium iodide (PI) dual staining. Pioglitazone (10, 20μM) and Apatinib (2μM) increased the apoptosis of the cardiomyocytes compared with the control group. E, Angiontensin II-induced [³H]- leucine incorporation following various concentrations of Pioglitazone or Apatinib. All data represent the means ± SD Statistical comparison with control group: *p < 0.01 compared with controls; #p < 0.01 compared withi pioglitazone 10 μM group, calculated by one-way ANOVA followed by the post hoc Bonferroni test for pairwise comparisons.*

hypertension.[24] Furthermore, pioglitazone protected from Ang II-induced cardiomyocyte hypertrophy by inhibiting AKT/GSK3β and MAPK signaling pathways. However, pioglitazone (40 mg/kg) was observed to induce cardiac hypertrophy with increase in plasma volume, without compromising it effects on the metabolic switch in the heart and whole-body insulin sensitivity.[25] These contradicting findings may be caused by differences in administered doses of pioglitazone, as treatment with pioglitazone at supratherapeutic doses was shown to induce cardiotoxicity.[26,27] Chemical proteomics-based analysis of off-target binding profiles for pioglitazone suggested potential sources contributing to efficacy and cardiotoxicity: perturbations in mitochondrial function, cardiac ion channels, and disruption of the cardiac sympathetic signaling.[28] In the present study, pioglitazone induced cardiomyocyte apoptosis and inhibits cardiomyocyte hypertrophy. We inferred that these findings indicate that pioglitazone treatment appears disadvantageous in patients with HF. It was reported increased number of apoptotic cells in the heart of spontaneously hypertensive rats, suggesting that apoptosis might be a mechanism involved in the reduction of myocyte mass that accompanies the transition from stable compensation to HF in this model.[29] Furthermore, cardiac myocyte apoptosis is a more critical determinant during the transition from compensatory cardiac hypertrophy to HF.[30] However, available studies on the mechanisms underlying possible cardiovascular risk effects of pioglitazone on cardiovascular risk factors have been conducted *in vitro* conditions and therefore, prospective cohort studies are needed to confirm these effects.

In this study, reverse screening approaches (reverse docking and reverse pharmacophore mapping) were used to predict potential cardiovascular disease-related protein targets of pioglitazone. Pioglitazone was shown to bind strongly binding to VEGFR-2, suggesting that cardiovascular effects of pioglitazone may be related to the regulation of angiogenesis, neointima formation, and atherosclerosis associated with VEGFR-2-participating pathways.[31-33] VEGFR-2 is a tyrosine kinase receptor that dimerizes upon ligand binding and is activated by trans-phosphorylation.[33] VEGFR-2 activation stimulates downstream signaling, including activation of the c-Raf/MEK/ERK and PI3K/Akt pathways leading to increased cell proliferation, migration, and survival.[33,34] VEGFR-2 is a critical factor in hypertrophic growth of cardiomyocytes.[35,36] We found that pioglitazone directly targeted VEGFR-2 and inhibited phospho-VEGFR-2 expression, suggesting that pioglitazone induces cardiomyocyte apoptosis and inhibits cardiomyocyte hypertrophy in neonatal rats by inhibiting VEGFR-2 signaling. Downstream PI3K/Akt signaling pathway

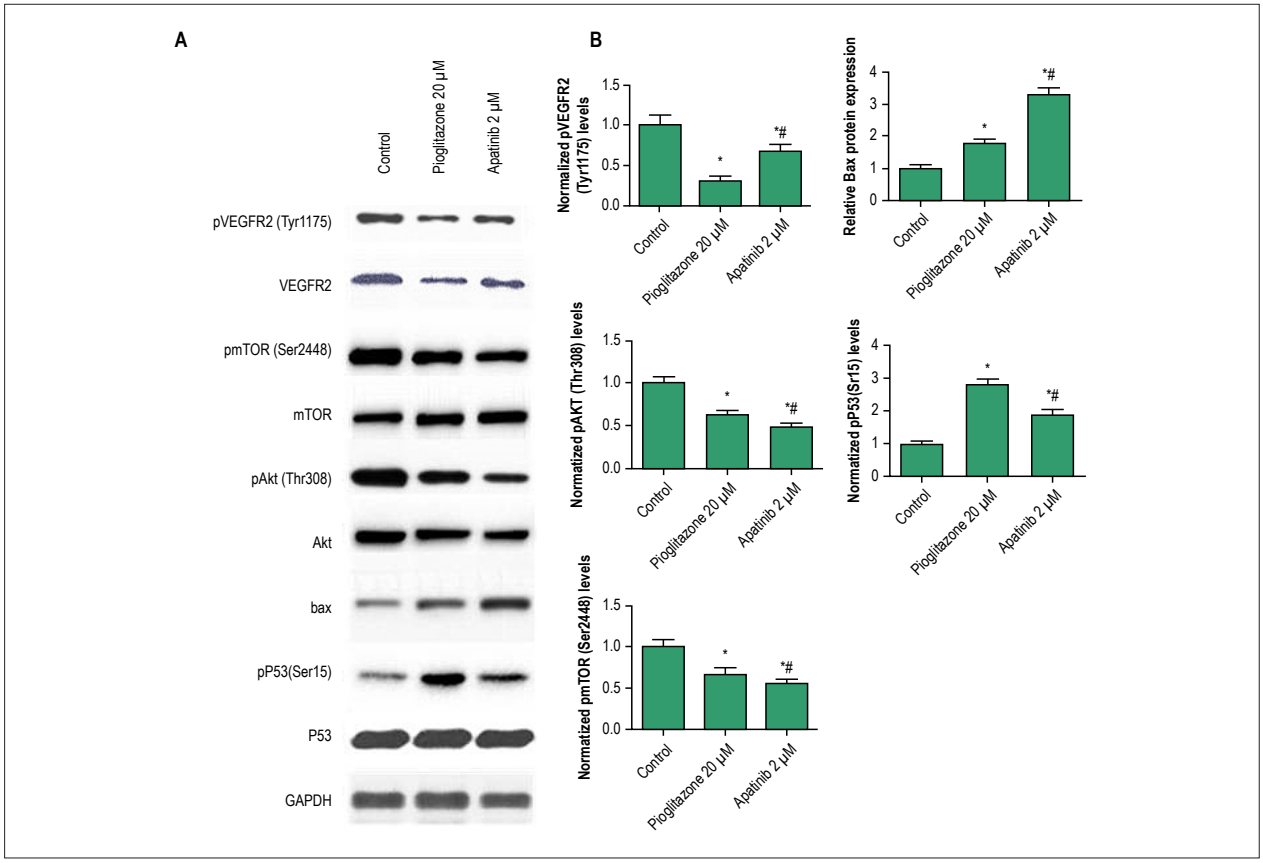

Figure 3 – *Pioglitazone and Apatinib regulate VEGFR-2 signaling in neonatal rat cardiomyocytes under hypertrophy induced by Angiotensin II (n = 12 in each group). A and B, Representative western bolotting trace of phospho-VEGFR-2, VEGFR-2, phospho-mTOR, mTOR, phospho-Akt, Akt, Bax, phospho-P53 and P53 protein levels in rat neonatal cardiomyocytes under hypertrophic stimuli, and treated with pioglitazone (20 μM) or apatinib (2 μM) for 24 hours, and intensity of the above bands in A normalized to GAPDH. All data represent the means ± SD. *p < 0.01 compared with controls; #p < 0.01 compared withi pioglitazone 20 μM group, calculated by one-way ANOVA followed by the post hoc Bonferroni test for pairwise comparisons.*

also participates in survival and hypertrophy of these cells by inhibiting P53-dependent pathways and activating mTOR-dependent pathways, respectively.[37] In this study, pioglitazone and VEGFR-2 inhibitor apatinib increased phospho-P53 and Bax expression in cardiomyocytes and decreased phospho-Akt and phospho-mTOR expression in hypertrophic cardiomyocytes, indicating the connection between pioglitazone and the VEGFR-2 signaling pathway.

Conclusion

In conclusion, these findings indicate that pioglitazone induces apoptosis and inhibits hypertrophy of cardiomyocytes in part by acting on the VEGFR-2 signaling pathway. These findings contribute to understanding cardiovascular risks of pioglitazone.

Author contributions

Conception and design of the research: Zhong W, Chen L; Acquisition of data: Zhong W, Jin W, Wu Y, Luo S, Liang M; Analysis and interpretation of the data and statistical analysis: Zhong W, Jin W, Xu S, Wu Y, Luo S, Liang M;Obtaining financing: Zhong W; Writing of the manuscript: Zhong W, Jin W, Liang M; Critical revision of the manuscript for intellectual content: Zhong W, Xu S, Liang M.

Potential Conflict of Interest

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

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Study Association

This article is part of the thesis of Post-Doctoral submitted by Wenliang Zhong, from Union Hospital of Fujian Medical University.

Ethics approval and consent to participate

This study was approved by the Ethics Committee on Animal Experiments of the First Hospital of Nanping City under the protocol number NP01371.

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VEGFR-2: One of Pioglitazone's Signaling Pathways in the Heart

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Short Editorial regarding the article: Pioglitazone Induces Cardiomyocyte Apoptosis and Inhibits Cardiomyocyte Hypertrophy Via VEGFR-2 Signaling Pathway

Pioglitazone is currently the only commercially available hypoglycemic agent that improves insulin sensitivity. Its mechanism of action involves activation of peroxisome proliferator-activated receptor (PPAR) gamma, a nuclear receptor that alters the transcription of genes involved in glucose and lipid metabolism and in energy balance.[1,2] Hence, pioglitazone increases insulin sensitivity, reduces glucose production by the liver and increases glucose uptake by peripheral tissues.[1,2]

Beneficial effects of pioglitazone include a low risk of hypoglycemia and the improvement of cardiovascular risk factors such as lipid profile and endothelial function.[1,2] The main side effects of the drug include weight gain, especially due to the risk of edema or heart failure, increased risk of bone fractures and its association with prostate cancer, which has been questioned in recent studies.[2] Pioglitazone is relatively potent in reducing glycated hemoglobin A1c levels; however, previous studies have shown no benefit in performing a more intensive control of glucose on cardiovascular mortality as compared with a less intensive control.[3] This is important since cardiovascular diseases are still the most common causes of diabetes.[3]

Also, recent studies have reported beneficial effects of sodium-glucose transporter 2 (SGLT2) inhibitors and glucagon-like peptide-1 (GLP-1) analogues in the secondary prevention of cardiovascular events.[4-7] For this reason, these should be the drugs of choice to be used in combination with metformin in patients with established cardiovascular diseases according to the American Diabetes Association recommendations.[8] Nevertheless, few studies have been conducted on patients with recently diagnosed diabetes and low prevalence of cardiovascular diseases.

In this context, the TOSCA.IT study compared the cardiovascular effects of the addition of pioglitazone or sulfonylureas to metformin in patients with type 2 diabetes.[9] The study showed that, in absence of clinically evident cardiovascular disease, both treatments are suitable options. However, considering the long-term metabolic effects, pioglitazone plus metformin may be considered the therapy of choice, since this was associated with a lower risk for hypoglycemia and a reduction in cardiovascular events by nearly 30%.[9] These findings agree with the beneficial effects of pioglitazone on cardiovascular events reported in the PROactive and PERISCOPE studies.[10,11]

With respect to potential pathophysiological mechanisms of the cardiovascular benefits of pioglitazone, it is believed that, in addition to its metabolic effect in reducing insulin resistance, this thiazolidinedione may have a direct effect on the heart. Experimental studies have already reported the effects of pioglitazone in fibrosis, apoptosis and myocardial hypertrophy.[12-14] In this issue of *Arquivos Brasileiros de Cardiologia*, Zhong et al.[15] investigated whether the effects of pioglitazone on cardiomyocyte apoptosis and hypertrophy occur via vascular endothelial growth factor receptor-2 (VEGFR-2) signaling. VEGFR-2 is a tyrosine kinase receptor that activates intracellular signaling pathways involved in cell proliferation, migration and cycle. First, using the reverse pharmacophore mapping technique, the authors identified VEGFR-2 as the best-ranked potential target for pioglitazone. Then, the authors isolated cardiomyocytes from Sprague-Dawley rats and evaluated the effects of pioglitazone and the VEGFR-2-selective inhibitor apatinib on two outcomes – cardiomyocyte apoptotic rate using flow cytometry and hypertrophy using [^{3}H]-leucine incorporation. Interestingly, the results showed a reduction not only in cardiomyocyte viability but also in cardiomyocyte hypertrophy induced by angiotensin II *in vitro*. Besides, both pioglitazone and apatinab increased the expression of Bax and phosphorylated P53 and decreased the expression of phosphorylated VEGFR-2, Akt, and mTOR in the cardiomyocytes. Studies in the literature are controversial regarding the effects of pioglitazone on cardiomyocyte hypertrophy and apoptosis,[12-14] maybe due to different dosages and models used in the studies. However, in the study in question, the authors suggested that heart failure patients would not benefit from therapy with pioglitazone, since although it attenuated cardiomyocyte hypertrophy, the drug induced apoptosis of these cells.

In addition, although the direct effects of pioglitazone on the heart are still under investigation, Zhong et al.[15] make an important contribution to the field, as suggesting that one of the mechanism of action of pioglitazone is via VEGFR-2. Also, if we consider that there is clinical evidence of the beneficial effects of this hypoglycemic agent on cardiovascular outcomes, further studies should be conducted to better define the role of pioglitazone in cardiovascular diseases in diabetics.

Keywords

Peroxisome Proliferators/adverse effects; Glucose/metabolism; Lipids/metabolism; Pioglitazone, Diabetes Mellitus/drug therapy.

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Physical Exercise and Regulation of Intracellular Calcium in Cardiomyocytes of Hypertensive Rats

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Abstract

Background: Regulation of intracellular calcium (Ca^{2+}) in cardiomyocytes is altered by hypertension; and aerobic exercise brings benefits to hypertensive individuals.

Objective: To verify the effects of aerobic exercise training on contractility and intracellular calcium (Ca^{2+}) transients of cardiomyocytes and on the expression of microRNA 214 (miR-214) in the left ventricle of spontaneously hypertensive rats (SHR).

Methods: SHR and normotensive Wistar rats of 16 weeks were divided into 4 groups –sedentary hypertensive (SH); trained hypertensive (TH); sedentary normotensive (SN); and trained normotensive (TN). Animals of the TH and TN groups were subjected to treadmill running program, 5 days/week, 1 hour/day at 60-70% of maximum running velocity for 8 weeks. We adopted a $p \leq 0.05$ as significance level for all comparisons.

Results: Exercise training reduced systolic arterial pressure in hypertensive rats. In normotensive rats, exercise training reduced the time to 50% cell relaxation and the time to peak contraction and increased the time to 50% decay of the intracellular Ca^{2+} transients. In SHR, exercise increased the amplitude and reduced the time to 50% decay of Ca^{2+} transients. Exercise training increased the expression of miR-214 in hypertensive rats only.

Conclusion: The aerobic training applied in this study increased the availability of intracellular Ca^{2+} and accelerated the sequestration of these ions in left ventricular myocytes of hypertensive rats, despite increased expression of miR-214 and maintenance of cell contractility. (Arq Bras Cardiol. 2018; 111(2):172-179)

Keywords: Hypertension; Exercise; Rats; Calcium Signaling; Intracellular Calcium; Sensing Proteins.

Introduction

Sustained systemic arterial hypertension causes progressive myocardial remodeling. While cardiac function is increased in response to active myocyte hypertrophy in the left ventricle in the compensated phase, left ventricular (LV) remodeling is characterized by the combination of cardiomyocyte hypertrophy and proliferation of other tissues. For example, collagen deposition result in fibrosis of ventricular tissue and consequent myocardial stiffness.[1,2] This has been reported in the initial compensatory phase of hypertension (3-4 months), with preservation of cardiac function.[1] Increased proinflammatory markers (e.g. IL-6; TNF-α) have been found in the left ventricle of spontaneously hypertensive rats (SHR) as young as 5-6 months.[3,4]

The benefits of aerobic training to hypertensive subjects are well established in the literature.[5,6] Animal models of hypertension – SHR in the compensated stage (~ 6-month-old) – have shown the efficacy of this type of training in attenuating systolic dysfunction and restoring ventricular elasticity in female SHR.[7] Aerobic training reduced apoptosis in the myocardium of SHR,[4,8] improved contractile function of cardiomyocytes isolated from the left ventricle, and normalized the expression of proteins involved in the regulation of intracellular calcium cycle, such as the sarcoplasmic reticulum Ca2+-ATPase (SERCA2a) and phospholamban.[9-11]

In physiological or pathological cardiac hypertrophy, expression of microRNAs (miRNAs) related to cardiac remodeling is altered.[12] MicroRNA 214 (miR-214) is involved in cardiac muscle contraction and Ca^{2+} sequestration, due to its negative effect on SERCA-2a expression.[13] With respect to physical exercise, resistance training improved cardiomyocyte contractile function, with increased expression of SERCA2a and decreased miRNA-214 expression in normotensive, infarcted rats.[13,14] However, little is known about miR-214 expression in SHR subjected to aerobic training. Thus, the aim of this study was to evaluate the effects of aerobic training on cardiomyocyte contractility and miR-214 expression in the left ventricle in hypertensive rats.

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Methods

Experimental animals

SHR and normotensive Wistar rats, 16 weeks of age, obtained from the central vivarium of the Biological Science and Health Center of Viçosa Federal University, were divided into four groups (of 13 animals each): sedentary normontensive (SN); trained normotensive (TN), sedentary hypertensive (SH) and trained hypertensive (TH). Sample size was determined by convenience. In each group, 8 animals were used for cardiomyocyte isolation, and 5 for analysis of gene expression. All procedures were carried out following the ethical principles of the Brazilian Society of Laboratory Animal Science (COBEA, *Colégio Brasileiro de Experimentação Animal*), and approved by the Ethics Committee on Animal Experimentation of Viçosa Federal University (CEUA-UFV; approval number 29/2014). The animals were housed in group cages (4 animals per cage) and allowed free access to water and chow at controlled temperature (mean of 22°C) and lighting (12:12 h light–dark cycle).

Protocol of treadmill exercise training and stress test

Before the training was initiated, animals were adapted to the treadmill during a 5-day period, 10min/day, 0º inclination at 5 m/min. After 48 hours, all animals were subjected to an incremental treadmill test for establishment of the maximal running velocity (MRV), starting at 5 m/min, 0º inclination and increments of 3m/min every 3 minutes until exhaustion. Exhaustion was defined as the time point when the animals could not run at the predetermined speed and, at this point, the test was stopped.

The TN and TH groups were subjected to an exercise training program for 8 weeks, 5 days/week (from Monday to Friday). The training started with treadmill running at 5-6 m/min and 0º inclination for 10 minutes on the first day. In the first week, exercise duration was increased in 5 minutes per day and the intensity was maintained. In the second week, duration continued to be increased in 5 minutes/day, but intensity was increased by 2% of MRV per day, so that from the first day of the third week to the end of the eighth week, the animals ran at 60% of MRV (~18-22 m/min) during a 60-minute period per day.

MRVs of each animal were determined before the training started and at the end of the fourth week in both TN and TH groups for measurement of the time to exhaustion (TTE) and definition of the training intensity. Forty-eight hours after the last training session, the tests were repeated in all animals for analysis of the effects of the physical training on running capacity.

During the experimental period, animals of the sedentary groups (SN and SH) were placed on the treadmill 3 days/week (Mondays, Wednesdays and Fridays), 10 minutes/day, 0º inclination at 5 m/min to subject them to similar conditions of the trained groups.

Systolic arterial pressure (SAP) was measured in the beginning and in the end of the experimental period, i.e., 48 hours after the last exercise training session. The measurements were taken in the mornings, without anesthesia, by tail-cuff plethysmography (LE 5001, Panlab, Harvard Apparatus, Spain). Three measurements were performed, and the intermediate value used for analysis.

Isolation of cardiomyocytes

Forty-eight hours after the last exercise session, isolation of LV myocytes was performed as described by Locatelli et al.[15] Briefly, the animals were weighed and euthanized by cervical dislocation. The heart was excised, weighed, cannulated and perfused in the Langendorff's mode with the isolation solution of the following composition (in mM): 130 Na+, 5.4 K+, 1.4 Mg+, 140 Cl−, 0.75 Ca^{2+}, 5.0 Hepes, 10 glucose, 20 taurine and 10 creatine, pH 7.3, at room temperature. The heart was then perfused with the calcium-free solution containing 0.1 mM ethylene glycol-bis (ß-aminoethyl ether)-N, N, N′, N′-tetraacetic acid (EGTA), for 4 to 6 min. The perfusion was then changed to a solution containing 1.0 mg/mL collagenase type II (Worthington, USA) and 0.1 mg/mL protease (Sigma-Aldrich, USA) for 10-15 min. All the solutions were oxygenated (O2 100% - White Martins, Brazil) and maintained at 35°C. After perfusion, ventricles were separated from the atria and weighed. The ventricles were placed in a flask containing 5.0 mL of enzymatic solution (collagenase + protease). The flasks were briefly shaken for 5 min in a water bath at 37°C. Next, tissues were removed from the flasks and the remaining content centrifuged (3,000 rpm for 30s). The supernatant was removed, and the cardiomyocytes suspended in the isolation solution and stored in refrigerator (5ºC) until being used.

Measurements of cell contractility

Contractility of isolated myocytes was measured by evaluation of cell length using the motion edge detector (Ionoptix, Milton, MA-USA) mounted on an inverted microscope (Nikon Eclipse - TS100, Japan), as previously described.[15] Briefly, myocytes were placed in a chamber with a glass coverslip base and bathed with a buffer solution containing (mM) 136.9 NaCl; 5.4 KCl; 0.37 NaH2PO4; 0.57 MgCl2; 5.0 Hepes = 5; 5.6 glucose and 1.8 $CaCl^2$ (pH = 7.4 at room temperature). Cells were visualized on a monitor with a camera (Myocam, Ionoptix, at 240 Hz) attached to a microscope using an image detector system (Ionwizard, Ionoptix). External stimulation was applied at 1.0 Hz (20V) for 5 minutes at room temperature (~25ºC) via platinum electrodes and an electric field stimulator (Myopacer, Ionoptix). Motions of myocyte longitudinal borders were captured by the motion edge detector (Ionwizard, Ionoptix) and stored for posterior analysis. Only myocytes in good conditions, with clear borders and striated sarcomere, relaxed at rest and without voluntary contractions were selected for analysis. Myocyte contractions were analyzed as previously described.[15]

Measurement of intracellular Ca^{2+} transients

Intracellular Ca^{2+} transients in isolated cardiomyocytes were measured using fluorescence imaging (Ionoptix, USA), mounted on an inverted microscope (Nikon Eclipse – TS100, USA) equipped with oil immersion objective lens (S Fluor, 40x, Nikon, USA), as described by Natali et al.[16] Briefly, the myocytes were

incubated with calcium probe (5 μM for 10 minutes) (Fura-2AM, ThermoFisher, Waltham, USA). The ratio of fluorescence emission at 510 nm in response to excitation wavelengths of 340 nm to that in response to 380 nm wavelengths was used as concentration index of intracellular Ca^{2+} transients. The myocytes were electrically stimulated (Myopacer, Field Stimulator, Ionoptix, USA) by a pair of platinum electrodes with a 0.2 ms (20V) supra-threshold pulse, frequency of 1 Hz at room temperature (~25 ºC). Parameters of intracellular Ca^{2+} transients were analyzed using the IonWizard software (IonWizard, 6.3, IonOptix, Milton, USA).

Gene expression analysis

For analysis of gene expression after euthanasia, LV samples were collected and stored at -80ºC. LV total RNA was isolated in 1 mL of Trizol (Invitrogen) following the manufacturer's recommendations and stored at -70ºC. RNA samples were diluted 1:100 with water and analyzed by spectrophotometry at 260-280 nm.

Analysis of the miRNA-214 gene expression was performed using the Taqman MicroRNA Assays (Applied Biosystems) following the manufacturers' recommendations. Gene expression quantification was performed in two stages: first, complementary DNA (cDNA) was obtained from reverse transcription of the total RNA sample using a stem-looped primer for reverse transcription to detect the miRNA analyzed and the TaqMan® MicroRNA Reverse Transcription Kit; second, by real-time polymerase chain reaction, PCR products were amplified from the cDNA samples previously obtained using the TaqMan® MicroRNA Assay and the TaqMan® Universal PCR Master Mix II. The U6 snRNA normalizer was used as control, and analysis was performed using the ABI 7500 Real Time-PCR Systems (Applied Biosystems).

Statistical analysis

Analysis of variance (ANOVA) assumptions regarding homogeneity of variances between the groups and normality of observations were checked and no violations of the assumptions were detected. The variables had normal and continuous distribution. The Levene's test, the chi-square test and the Kolmogorov test were used for analysis. Comparisons between initial and final SAP and TTE in each group were performed by the paired student's t-test. Two-way ANOVA followed by Tukey post-hoc test was used for comparisons between the four groups. Analysis was performed using the SigmaPlot software (Systat Software, Inc., San Jose, CA, USA) and significance level was set at 5%. Results are expressed as mean ± standard deviation (SD).

Results

The physical training program increased TTE of both normotensive and hypertensive animals (Figure 1A). Figure 1B shows running velocity of these animals during training.

As compared with normotensive animals, hypertensive animals showed lower body weight (BW), similar LV weight (LVW), higher LVW/BW, whereas TH animals showed higher ventricular weight (VW)/BW (Table 1). Physical training had no effect on these parameters.

Results of SAP are presented in Figure 2. As compared with pre-training values, although the physical training program had no effect on SAP in normotensive animals, a reduction in SAP was observed in hypertensive animals (Figure 2A).

Cell contractility data are depicted in Figure 3. Contraction amplitude was not affected by hypertension or by physical training (Figure 3 A); and time to peak contraction did not change in any of the groups (Figure 3B). However, physical training reduced time to relaxation by 50% (Figure 3C) in normotensive, but not in hypertensive animals.

Figure 4 shows that after training, $[Ca^{2+}]_i$ amplitude did not change in normotensive animals, but increased in the TH group as compared with SH animals. In addition, physical exercise decreased the time to peak $[Ca^{2+}]_i$ in TN animals as compared with the SN group, but had no effect

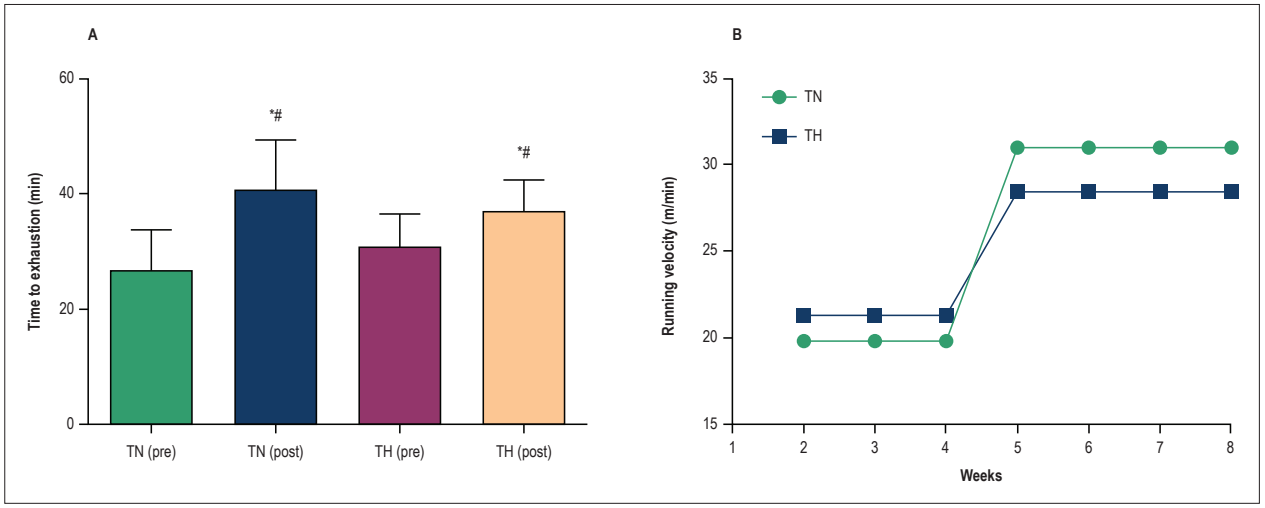

Figure 1 – *(A) Time to exhaustion of normotensive and hypertensive before (pre) and after (post) training. (B) Running velocity during training sessions. TN: trained normotensive; TH: trained hypertensive. Data are expressed as mean ± SD of 8 animals in each group. * compared with TN (pre); # compared with TH (pre) (p < 0.05).*

Table 1 – Body weight and ventricular weight in the study groups

	SN (n = 8)	TN (n = 8)	SH (n = 8)	TH (n = 8)	p-value
BW (g)	411.30 ± 21.51	447.10 ± 43.94	350.60 ± 21.97*&	338.90 ± 30.67*&	0.001
VW (g)	1.62 ± 0.20	1.49 ± 0.25	1.47 ± 0.17	1.66 ± 0.23	0.954
LVW (g)	1.12 ± 0.13	1.08 ± 0.13	1.18 ± 0.14	1.25 ± 0.15	0.265
VW/BW (mg/g)	3.97 ± 0.62	3.46 ± 0.47	4.19 ± 0.45	4.93 ± 0.80*&	0.000
LVW/BW (mg/g)	2.75 ± 0.42	2.42 ± 0.23	3.39 ± 0.39*&	3.72 ± 0.48*&	0.000

*SN: sedentary normotensive; TN: trained normotensive; SH: sedentary hypertensive; TH: trained hypertensive; VW: ventricular weight; LVW: left ventricular weight; n: number of animals; * compared with SN; & compared with TN (p < 0.05).*

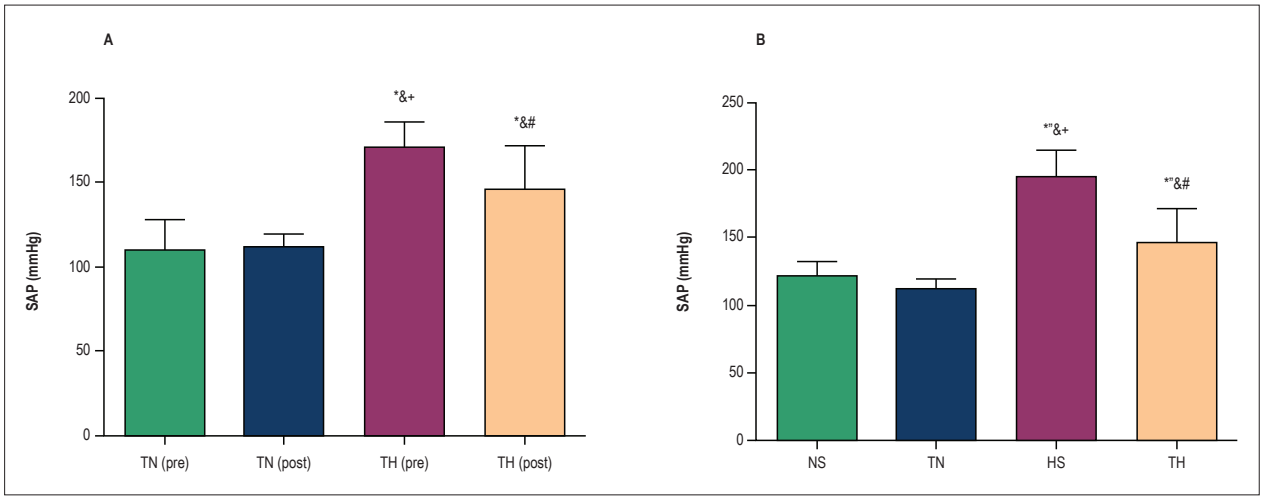

Figure 2 – *Systolic arterial pressure (SAP) in normotensive animals and hypertensive animals. (A) SAP pre-training vs. post-training). (B) Final SAP of the groups. SN: sedentary normotensive; TN: trained normotensive; SH: sedentary hypertensive; TH: trained hypertensive. Data are expressed as mean ± SD of 8 animals in each group. * compared with TN (pre) (A) SN (B); & compared with TN (post) (A) TN (B); # compared with TH (pre) (A) SN (B); * compared with TH-post (A) TH (B) (p < 0.05).*

on this parameter in hypertensive animals. Longer time to a 50% decay of $[Ca^{2+}]_i$ was observed in TN than SN group. In hypertensive animals, however, this parameter was decreased after physical training ($p < 0.05$).

Although the physical training had no effect on mir-214 expression in normotensive animals (Figure 5), increased expression was found in TH as compared with SH and the other groups.

Discussion

In the present study, the authors evaluated the effects of physical activity on contractility and intracellular Ca^{2+} transients in myocytes and miR-214 expression in the left ventricle in hypertensive rats. The results showed that the aerobic training not only reduced SAP in hypertensive animals, but also increased the amplitude of miR-214. No effect on LV myocyte contractility was observed.

The efficacy of the physical training applied in the study groups was confirmed by the higher physical capacity, indicated by the TTE, in trained animals as compared with controls. Such increase in physical capacity in response to aerobic training has been previously demonstrated.[10,17,18] More importantly, physical training reduced SAP in hypertensive animals, and such effect is well established in the literature.[11,19]

Regarding LV cardiomyocyte contractility, although a reduction in the cell relaxation time in response to physical training was observed in normotensive animals, in SHR, cell contractility of trained animals was not different than that in sedentary animals.

Although cardiomyocyte contractility in SHR was not affected by exercise, higher amplitude and shorter 50% decay time of intracellular Ca^{2+} transient levels were seen in trained animals as compared with sedentary controls. This suggests higher availability of Ca^{2+} in the cytosol and faster removal of calcium from the cytosol, which in turn brings about relaxation.[20] These findings corroborate those of another study,[21] that showed increased expression of SERCA2a, which is the main determinant of Ca^{2+} removal from the cytosol into sarcoplasmic reticulum.[20]

With respect to miR-214, which antagonizes the effects of SERCA2a, our results contradict existing data in the literature, since the physical exercise program increased the expression of this microRNA in the left ventricle of hypertensive animals. We expected that aerobic training would reduce the expression of miR-214, which would justify de shorter time to 50% of decay in intracellular concentration of Ca^{2+} transients in LV cardiomyocytes of SHR due to an expected increase in SERCA2a expression. Although SERCA2a expression in the left ventricle was not evaluated in the present study, it was previously demonstrated[14] that the left ventricle of normotensive rats subjected to resistance training showed

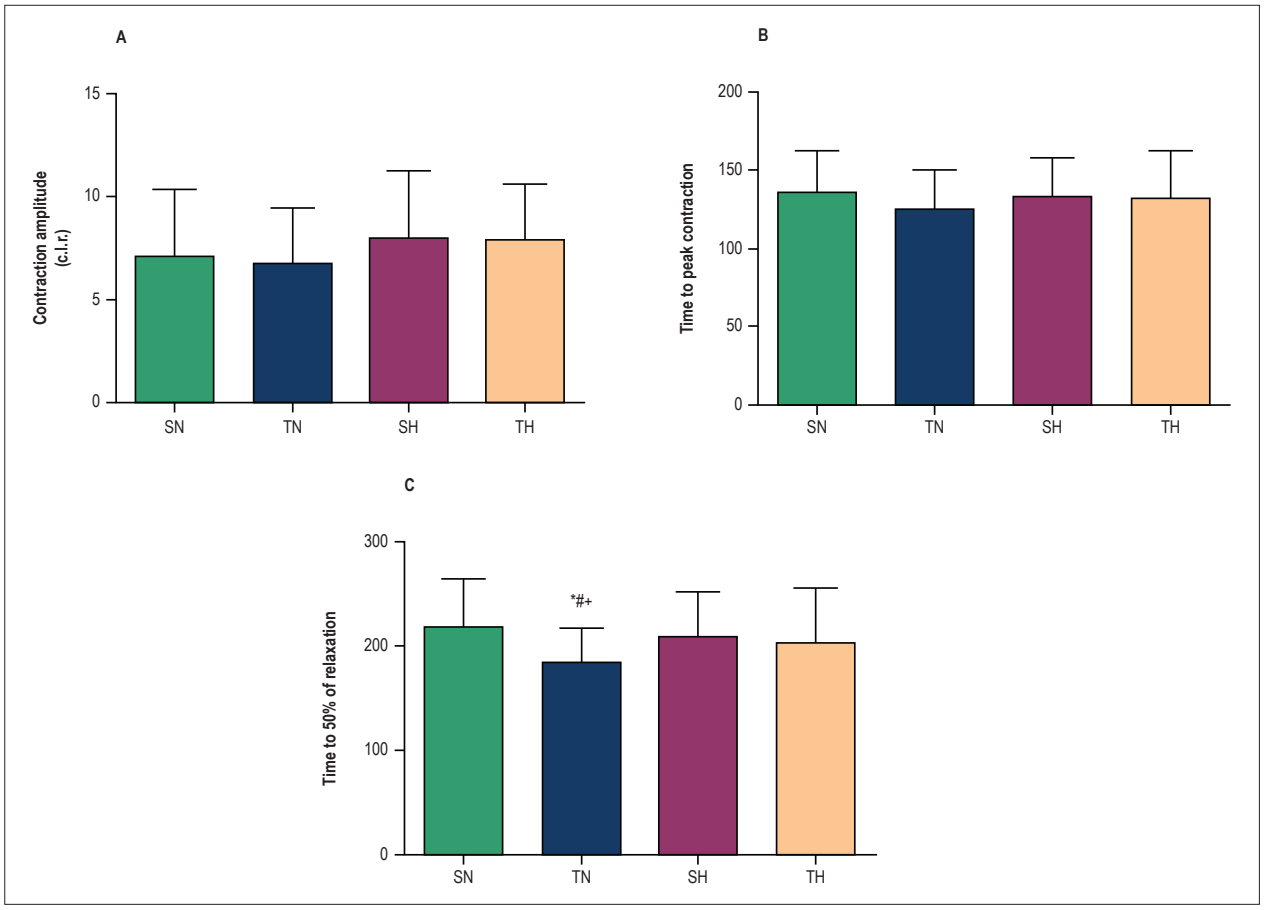

Figure 3 – *Cardiomyocyte contractility in normotensive and hypertensive animals. (A) Contraction amplitude expressed as percentage of change in cell length at rest (%c.l.r.) after electrical stimulation at 1HZ; (B) time to peak concentration; (C) time to 50% of relaxation; SN, sedentary normotensive; TN, trained normotensive; SH, sedentary hypertensive; TH, trained hypertensive. Data as mean ± SD of 60-80 cells in each group. * compared with SN; # compared with SN; + compared with TH (p < 0.05).*

reduced miR-214 expression and increased SERCA2a expression. These adaptations were associated with faster relaxation of myocytes isolated from the left ventricle of trained animals. Similar results showing reduction in miR-214 expression and elevated SERCA2a expression were also reported in infarcted rats subjected to resistance training.[13] Therefore, little is known about the effects of aerobic exercise on hypertensive cardiomyocytes, and further studies are needed to investigate other possible changes associated with intracellular Ca^{2+} regulation in the cardiomyocytes of hypertensive rats subjected to aerobic training.

Conclusion

The aerobic training applied in the present study increased the availability of intracellular Ca^{2+} in the myocytes of the left ventricle of hypertensive rats, despite the increased expression of miR-214 and maintenance of cell contractility.

Author contributions

Conception and design of the research: Rodrigues JA, Prímola-Gomes TN, Natali AJ; Acquisition of data: Rodrigues JA, Soares LL, Leal TF, Nóbrega C, Pedrosa DL, Rezende LMT, Oliveira EM, Natali AJ; Analysis and interpretation of the data: Rodrigues JA, Soares LL, Leal TF, Nóbrega C, Oliveira EM, Natali AJ; Statistical analysis: Rodrigues JA, Soares LL, Leal TF, Nóbrega C, Natali AJ; Obtaining financing: Prímola-Gomes TN, Natali AJ; Writing of the manuscript: Rodrigues JA, Natali AJ; Critical revision of the manuscript for intellectual content: Natali AJ.

Potential Conflict of Interest

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

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Study Association

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Ethics approval and consent to participate

This study was approved by the Ethics Committee on Animal Experiments of the Colégio Brasileiro de Experimentação Animal (COBEA) under the protocol number 29/2014.

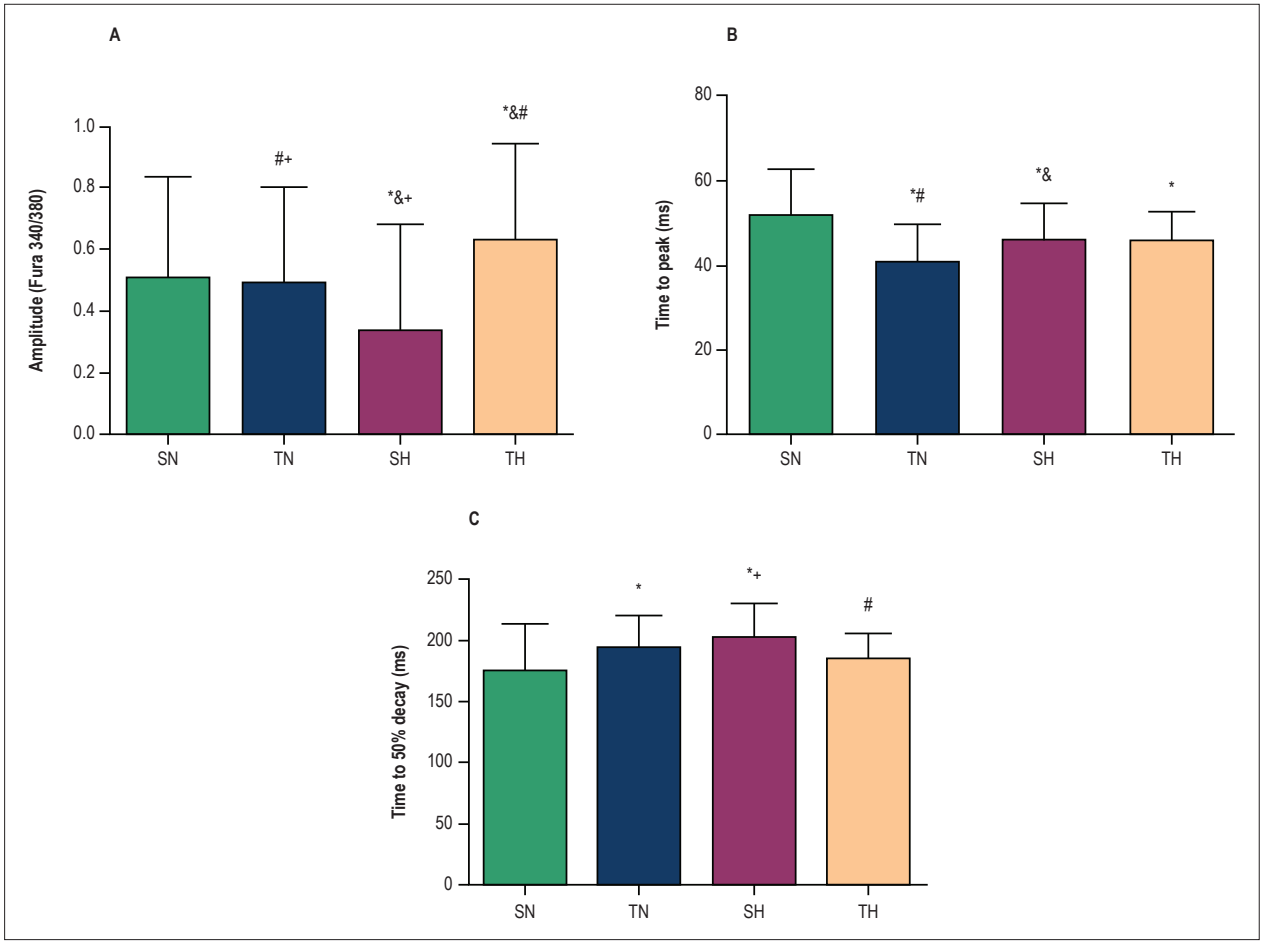

Figure 4 – *Intracellular Ca^{2+} transient levels in cardiomyocytes of normotensive and hypertensive animals. (A) Amplitude of intracellular calcium. (B) Time to peak intracellular calcium. (C) Time to 50% decay of intracellular calcium. SN, sedentary normotensive; TN, trained normotensive; SH, sedentary hypertensive; TH, trained hypertensive. Data as mean ± SD of 40-50 cells in each group. * compared with SN; & compared with TN; # compared with SN; + compared with TH (p < 0.05).*

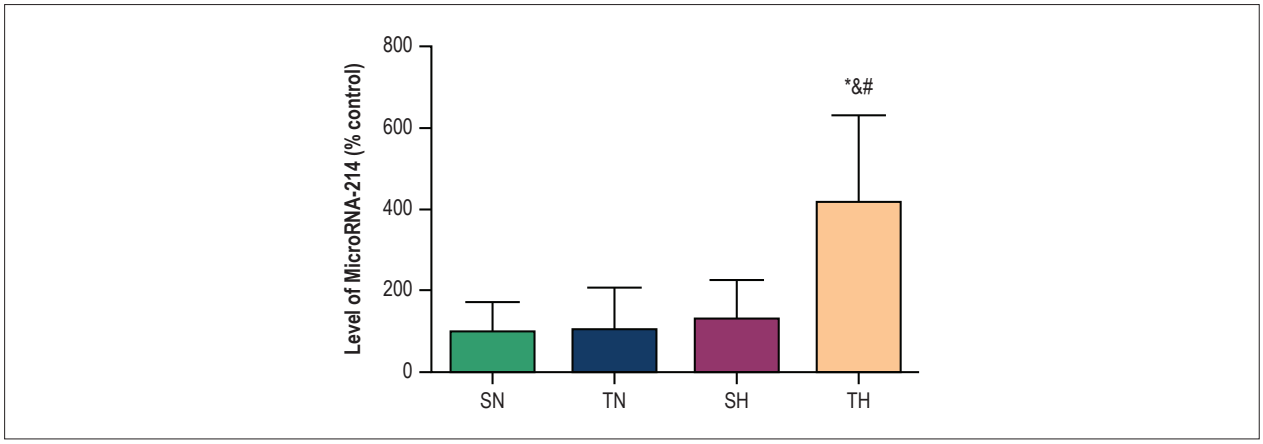

Figure 5 – *MicroRNA-214 expression in the left ventricle in normotensive and hypertensive animals. SN: sedentary normotensive; TN: trained normotensive; SH: sedentary hypertensive; TH: trained hypertensive. Data as mean ± SD of 5 animals in each group. * compared with SN; & compared with TN; # compared with SN (p < 0.05).*

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Hypertension and Exercise: A Search for Mechanisms

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Short Editorial regarding the article: Physical Exercise and Regulation of Intracellular Calcium in Cardiomyocytes of Hypertensive Rats

Arterial hypertension is a chronic disease that affects approximately 40% of the population, with higher incidence at older ages.[1] Arterial hypertension is a risk factor for other cardiovascular diseases, such as heart failure, stroke, atherosclerosis and also chronic renal disease. It is estimated that more than 50% of deaths from coronary diseases and stroke occur in hypertensive patients;[2] for this reason, hypertension produces high costs in health and constitutes a public health problem.[3] In this context, the development of nonpharmacological therapies is a cost-effective strategy with few side effects, that helps in the prevention of comorbidities, such as diabetes and obesity, and increases the cardiovascular risk of the patient. Among nonpharmacological strategies, physical exercise deserves consideration.

Rodrigues et al.,[4] in the study published in this issue of *Arquivos Brasileiros de Cardiologia*, evaluated the effect of moderate aerobic exercise on a treadmill in spontaneously hypertensive rats. The animals ran at 18-22m/min for 60 minutes, five times a week, for eight weeks.[4] The study confirmed the anti-hypertensive effects of aerobic exercise, as already reported previously.[5] More recently, other types of exercise in addition to aerobic training, such as resistance and interval training, have been shown to be promising in preventing hypertension.[6] Prescription of physical exercise for the treatment and prevention of hypertension is well established, and more recent guidelines for the treatment of hypertension strongly recommend exercise as a therapeutic option.[1,2]

Even though no doubt remains about the importance of physical exercise for the management of hypertension, the mechanisms of the beneficial effects have not been fully elucidated. In this regard, the study by Rodrigues et al.[4] proposed to investigate the transient concentration of intracellular calcium as well as the expression of microRNA (miRNA)-214, which is related to regulation of intracellular calcium and Serca-2a expression. The authors observed that physical exercise, in the presence of hypertension, increased the amplitude and decreased decay time of cytosolic calcium, which may suggest a higher availability of intracellular calcium, faster removal of this ion from the cytosol, and consequently, increased cellular relaxation. These results contribute to the understating of biological processes induced by exercises in the cardiomyocytes.

Another interesting result of the study by Rodrigues et al.[4] was that non-hypertensive animals that underwent exercise training did not have any change in miRNA-214 expression whereas hypertensive animals that underwent training showed higher expression of this miRNA. MiRNAs are small RNA fragments that do not encode proteins, and negatively regulate gene expression at a post-transcriptional level. When discovered, miRNAs were believed to be non-functional sequences; however, since the 90's decade, the interest in these molecules has grown and today is known to be involved in the regulation of important biological processes, including physiological and pathological ones.[7] In hypertension, clinical and experimental studies have identified many miRNAs that may be related to the hypertension and its complications,[8] emerging as possible biological markers and therapeutic targets in hypertension.[9]

MiRNAs constitute a complex biological control network – one miRNA can have multiple genes as targets, while one gene can be regulated by many miRNAs.[10] So far, all possible interactions between miRNAs involved in a signaling pathway, as well as the regulatory mechanisms of miRNA functions are unknown. Maybe a miRNA expression panel is a stronger determinant than the expression of one unique miRNA in disease conditions. Despite these uncertainties, the promising role of miRNAs for the future of medicine is unquestionable, be it as a biomarker or as a therapeutic target.

Despite the results of this study, the underlying mechanisms of the beneficial effect of exercise still need to be elucidated.

Keywords

Hypertension/physiopathology; Hypertension/prevention & control; Exercise; Exercise Therapy; MicroRNAs/genetics; Molecular Targeted Therapy.

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Sympathetic Dysautonomia in Heart Failure by ^{123}I-MIBG: comparison between Chagasic, non-Chagasic and heart transplant patients

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Abstract

Background: **Heart failure (HF) is a severe public health problem because of its high morbidity and mortality and elevated costs, thus requiring better understanding of its course. In its complex and multifactorial pathogenesis, sympathetic hyperactivity plays a relevant role. Considering that sympathetic dysfunction is already present in the initial phases of chronic Chagas cardiomyopathy (CCC) and frequently associated with a worse prognosis, we assumed it could be more severe in CCC than in cardiomyopathies of other etiologies (non-CCC).**

Objectives: **To assess the cardiac sympathetic dysfunction (^{123}I-MIBG) of HF, comparing individuals with CCC to those with non-CCC, using heart transplant (HT) patients as denervated heart parameters.**

Methods: **We assessed 76 patients with functional class II-VI HF, being 25 CCC (17 men), 25 non-CCC (14 men) and 26 HT (20 men), by use of cardiac ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy, estimating the early and late heart-to-mediastinum ratio (HMR) of ^{123}I-MIBG uptake and cardiac washout (WO%). The 5% significance level was adopted in the statistical analysis.**

Results: **The early and late HMR values were 1.73 ± 0.24 and 1.58 ± 0.27, respectively, in CCC, and 1.62 ± 0.21 and 1.44 ± 0.16 in non-CCC (p = NS), being, however, higher in HT patients (p < 0.001). The WO% values were 41.65 ± 21.4 (CCC), 47.37 ± 14.19% (non-CCC) and 43.29 ± 23.02 (HT), p = 0.057. The late HMR values showed a positive weak correlation with left ventricular ejection fraction (LVEF) in CCC and non-CCC (r = 0.42 and p = 0.045; and r = 0.49 and p = 0.015, respectively).**

Conclusion: **Sympathetic hyperactivity (^{123}I-MIBG) was evidenced in patients with class II-IV HF, LVEF < 45%, independently of the HF etiology, as compared to HT patients. (Arq Bras Cardiol. 2018; 111(2):182-190)**

Keywords: **Heart Failure; Primary Dysautonomies; Chagas Cardiomyopathy; Myocardial/radionuclide imaging; ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG).**

Introduction

Heart failure (HF) currently represents a public health problem because of its epidemic proportions (worldwide prevalence greater than 23 million individuals), its high morbidity and mortality, and, consequently, high health expenditures.[1]

A large variety of cardiac conditions can result in HF, whose prevalence differs when comparing developed and developing countries.[2] In Brazil, the ischemic etiology accounts for 34.1% of the HF cases, being followed by the Chagasic (21.4%) and hypertensive (13.2%) etiologies, the Chagasic being associated with a worse prognosis.[3-6]

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Previous studies have suggested that the pathogenesis of HF is complex and multifactorial,[7] sympathetic dysautonomia playing a relevant role in the process.[8-11] In the initial phase of HF, the sympathetic nervous system activation would modulate the pump function; however, over time, its action would become deleterious, leading to myocardial remodeling and restructuring, with progressive decline of the cardiac function.[10,12,13] In such patients, sympathetic dysfunction would be characterized by a significant reduction in the presynaptic uptake of norepinephrine, with consequent elevation in its serum levels and reduction in the postsynaptic density of β-adrenoreceptors.[10,13,14]

In chronic Chagas cardiomyopathy (CCC), early impairment of the parasympathetic nervous system has been well established,[15,16] and sympathetic dysfunction, although not totally established, is believed to be present in the initial phases of the disease,[11,17-19] when the cardiac pump function is preserved and potentially associated with malignant arrhythmias and sudden death.[20-22]

Because cardiac autonomous dysfunction is present early in CCC and the incidence of malignant arrhythmias and sudden death is elevated in those patients,[15,16,20-22] this

study was designed aimed at assessing the presence and the magnitude of cardiac sympathetic dysfunction in Chagasic patients with HF. Chagasic *versus* non-Chagasic patients were compared, and heart transplant (HT) patients were considered as the denervated heart pattern (known to be abnormal).[23] Cardiac ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy was used to assess the patients, because it properly evaluates cardiac sympathetic dysfunction,[12,13,24,25] providing relevant parameters to understand the progression of HF.[12,13,24]

Methods

This is a cross-sectional study of 76 patients selected from the Heart Failure and Heart Transplant outpatient clinic of our institution from March 2014 to February 2016.

The eligibility criteria for individuals with HF were: age over 18 years; left ventricular ejection fraction (LVEF) ≤ 45%; confirmed non-Chagasic or Chagasic etiology (positivity for Chagas disease confirmed by use of two different serological techniques) associated with left ventricular systolic dysfunction;[26] and accepting to participate in the study. In addition, for individuals with HF submitted to HT (comparison group or denervated heart model),[23] time from HT shorter than 12 months was required. Patients with diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease, Parkinson disease, non-sinus heart rhythm or implantable pacemaker were excluded.

The patients were studied prospectively, divided into three groups: CCC group - 25 patients with CCC (mean age, 53.3 ± 9.2 years; 17 males); non-CCC group - 25 patients with heart disease etiologies other than CCC (56% idiopathic, 36% ischemic, and 8% post-partum cardiomyopathy; mean age, 43.3 ± 12 years; 14 males); and HT group - 26 patients previously submitted to HT within less than 12 months (mean, 6.5 ± 3.8 months), with mean age of 47.3 ± 13.1 years, being 20 of the male sex. All patients provided written informed consent, which had been approved by the Ethics Committee of the institution, according to the Declaration of Helsinki. All patients underwent clinical control during the study period.

Clinical, electrocardiographic (ECG at rest) and echocardiographic data were collected by the same researcher. Echocardiography was performed using the Phillips iE33® ultrasound device (Phillips Medical, Andover, MA, USA), LVEF being estimated by using Simpson's formula.[27] Planar scintigraphy of the myocardial innervation was performed by use of slow intravenous administration of 111 MBq/3 mCi ^{123}I-MIBG (IPEN/CNEN), with anterior image acquisition of the chest after 15 minutes and 180 minutes on a Hawkeye® gamma-camera (GE healthcare, Milwaukie, USA), 10 min/frame, ^{123}I photopeak of 159 KeV, window of 20%, and low-energy high-resolution collimator (LEHR). The heart region of interest (ROI) was drawn encompassing the entire left ventricle, while that of the superior mediastinum encompassed a square ROI of 12x12 pixels. Early and late cardiac uptakes were estimated by use of the ratio between the radioactive counts of the heart and mediastinal ROIs on early and late imaging (early HMR and late HMR, respectively). The cardiac washout rate (WO%) of ^{123}I-MIBG was calculated using the formula: (early heart uptake early mediastinal uptake) (late heart uptake late mediastinal uptake) / (early heart uptake early mediastinal uptake) x 100, without considering radioactive decay, and expressed as percentages[28] (Figure 1). Two nuclear physicians analyzed separately the images, with 98% of interobserver agreement, and defined the following as abnormal: WO% > 27% and late HMR ≤ 1.8.[29]

The effective radiation dose for the patient, resulting from the administration of 111 MBq/3mCi of ^{123}I-MIBG was estimated as approximately 4.8 mSv, comparable to one of the phases of myocardial perfusion studies with ^{99m}Tc-isonitrile.[30]

Statistical analysis

For this analysis, a sample of 76 patients was calculated to detect a 12% variation in the early or late ^{123}I-MIBG uptake (HMR), with 5% alpha error and 80% power (CI = 95%) for three groups of patients.

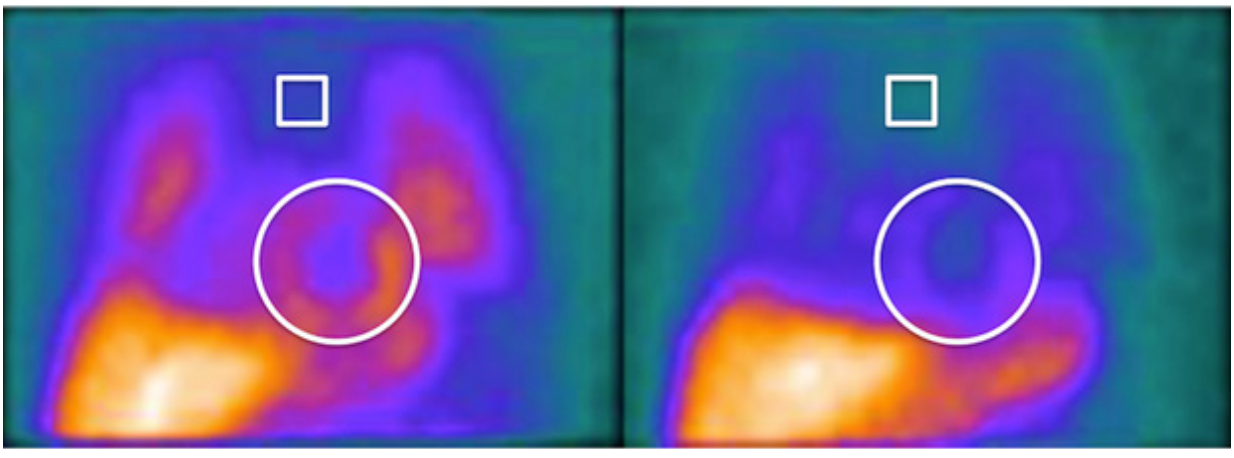

Figure 1 – *Early (15-minute) and late (180-minute) anterior planar imaging of the chest by ^{123}I-MIBG scintigraphy, with regions of interest (ROI) positioned on the superior mediastinum between the pulmonary fields and heart.*

To characterize the sample, descriptive analysis of the following variables was initially performed: sex, age, heart rate (HR), LVEF, early HMR, late HMR and WO% of ^{123}I-MIBG expressed according to the distribution of frequency or measures of central tendency and variability. This analysis was stratified per group (CCC, non-CCC and HT). When comparing the three groups, for the categorical variables (sex, HR, NYHA functional class, use of beta-blockers), Pearson chi-square test was performed; for the continuous variables (age, LVEF, WO%), one-factor analysis of variance (ANOVA) was used; and for multiple comparisons, the least significant difference (LSD) test was used. In addition, ANOVA was used to assess the variables (early and late HMR), based, however, on repeated-measures analysis and LSD test for multiple comparisons.

It is worth noting that the assumptions to use ANOVA were verified and accepted, that is, normally distributed residuals (Kolmogorov-Smirnov test) and constant variances (Levene's test).

To analyze the correlation between the measures of early HMR, late HMR or WO% and LVEF, Pearson correlation test and its respective p value were used. In all analyses, a 5% significance level was considered, and the statistical software SPSS, version 17.0 (SPSS Inc., Illinois, USA), was used.[31,32]

Results

Table 1 shows the demographic, clinical and echocardiographic data of the patients studied. Those with HF on angiotensin-converting-enzyme inhibitors (ACEI) and beta-blockers maintained their medications. In approximately 70% of the HT patients, HR was maintained over 80 bpm (mean of 90.7 bpm), while in individuals with CCC and non-CCC, the mean HR values were 72.7 bpm and 75.6 bpm, respectively ($p = 0.03$). No patient was on tricyclic antidepressants.

The early and late HMR values were greater than those reported for HT patients ($p < 0.001$) (Table 2), but did not differ in CCC or non-CCC patients with HF, even when adjusted for age and age group (early HMR: $p = 0.251$; and late HMR: $p = 0.011$). The early HMR values of CCC patients were 8.6% higher than those found in non-CCC patients, and 39.7% higher than those found in HT patients. The late HMR values of CCC patients were 9.7% higher than those of non-CCC patients, and 31.7% higher than those of HT patients (Figure 2).

The WO% values showed no statistically significant difference between individuals with HF, and between individuals with HF and those submitted to HT ($p = 0.577$) (Figure 3).

A weak positive correlation was observed between late HMR values and LVEF in CCC patients ($r = 0.42$; $p = 0.045$). Regarding the non-CCC patients, a positive correlation was observed both between LVEF and early HMR ($r = 0.46$; $p = 0.023$) and between LVEF and late HMR ($r = 0.49$; $p = 0.015$). However, none of the groups showed a correlation between LVEF and WO% (Figures 4, 5 and 6).

Discussion

This study investigated the presence and magnitude of cardiac dysautonomia in patients with HF and LVEF ≤ 45% by use of ^{123}I-MIBG scintigraphy. Patients were divided into three groups, CCC, non-CCC and HT, the latter, by representing the denervated heart model, served as the abnormality pattern.[23]

There was scintigraphic evidence of sympathetic hyperactivity, based on the findings of low ^{123}I-MIBG uptake (early and late HMR) by the presynaptic endings in the three groups studied, which is aligned with the literature.[13,23,24,28] The low ^{123}I-MIBG uptake indicates dysfunction of the receptors and integrity loss of the presynaptic sympathetic fibers, reinforcing the theory of sympathetic hyperactivity in the pathogenesis of HF.[8-10,12,24]

Scintigraphy is the only noninvasive and safe method, sufficiently sensitive to assess the autonomic sympathetic nervous system,[12,24] that can provide parameters known for their accuracy and reproducibility to estimate the efficacy of clinical treatment[13] and the prognosis of patients with HF.[13,24,25,33] However, the lack of standardization in the scintigraphic imaging acquisition and processing hinders the incorporation of the method into clinical practice, because there is no well-defined reference value.[28,29] In a meta-analysis of seven studies with 96 healthy individuals, Patel and Iskandrian have reported HMR of 2.13 ± 0.3 and WO% of 20 ± 10% (ranging from 10 ± 6% to 37 ± 5%) for healthy individuals.[29]

Table 1 – Demographic, clinical and echocardiographic characteristics of the patients

	CCC	non-CCC	HT	p
Male sex†	68.0	56.0	77.0	0.281[a]
Age (years)*	53.3 ± 9.2	43.3 ± 12	47.3 ± 13.1	0.016[c]
HR > 80 bpm†	30.8	33.3	69.2	0.072[a]
NYHA II-IV†	62.5	92.0	0.0	< 0.001[a]
LVEF % (Echo)*	30.6 ± 7.8	25.9 ± 8.0	66.6 ± 8.3	< 0.001[c] CCC = non-CCC < HT
ACEI†	91.3	88	77.3	0.394[b]
Beta-blockers†	91.3	100	18.2	< 0.001[a] CCC = non-CCC > HT

CCC: chronic Chagas cardiomyopathy; non-CCC: cardiomyopathy other than Chagas disease; HT: heart transplant; HR: heart rate (bpm); NYHA: New York Heart Association (heart failure functional classification); ACEI: angiotensin-converting-enzyme inhibitors; Echo: echocardiography; (*): expressed as mean and standard deviation; (†): expressed as percentage. Note: The probability of statistical significance for the comparison of the groups refers to (a) chi-square test, (b) Fisher exact test, and (c) analysis of variance.

Table 2 – Scintigraphic parameters of myocardial dysfunction (^{123}I-MIBG) in CCC, non-CCC and HT patients

^{123}I-MIBG	CCC	non-CCC	HT	p
Early HMR*	1.73 ± 0.24	1.62 ± 0.21	1.26 ± 0.10	< 0.001[a]
Late HMR*	1.58 ± 0.27	1.44 ± 0.16	1.20 ± 0.12	< 0.001[a]
WO%	41.60 ± 21.41	47.37 ± 14.19	43.29 ± 23.02	0.057[b]

CCC: chronic Chagas cardiomyopathy; non-CCC: cardiomyopathy other than Chagas disease; HT: heart transplant; early HMR: ratio of the heart/mediastinum radioactive counts estimated on 15-minute images (early uptake); late HMR: ratio of the heart/mediastinum radioactive counts estimated on 180-minute images (late uptake); WO%: cardiac washout of ^{123}I-MIBG, expressed as percentage; (*): values expressed as mean and standard deviation. Note: The probability of statistical significance refers to analysis of variance based on (a) repeated measures and (b) analysis of variance.

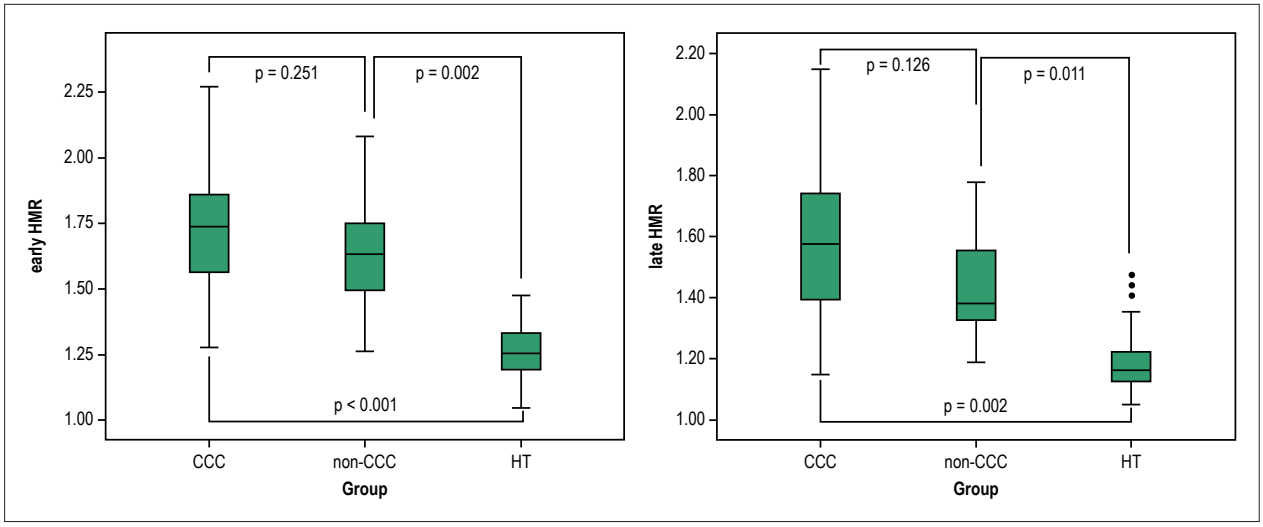

Figure 2 – *Early and late HMR of ^{123}I-MIBG in CCC or non-CCC or HT patients. Early HMR - ratio of the heart/mediastinum radioactive counts estimated on 15-minute images (early uptake); late HMR: ratio of the heart/mediastinum radioactive counts estimated on 180-minute images (late uptake). Groups: CCC: chronic Chagas cardiomyopathy; non-CCC: cardiomyopathy etiology other than Chagas disease; HT: heart transplant.*

The literature on HF has reported reduced late HMR values (1.80),[24] with a correlation between a reduction in uptake and worse prognosis, expressed as a higher number of cardiac events and higher mortality.[13,24,25] The late HMR values found for our patients with HF (Table 2) were lower than those adopted by different authors using cutoff point values < 1.75 (sensitivity of 84% and specificity of 60%),[13] < 1.68,[25] or even, more restrictive, < 1.60.[24]

The HMR values found for HT patients (1.20 ± 0.12) were lower than those found for individuals with HF, with statistical significance (p<0.001), which is aligned with that reported in the literature for patients within the first post-HT year, specially individuals with idiopathic heart disease.[23,29]

It is worth noting that the patients of this study were on regular use of beta-blockers and ACEI, which do not interfere directly in the uptake of noradrenaline. However, by improving the cardiac performance, and, thus, the sympathetic tone, they increment the ^{123}I-MIBG uptake.[34] Thus, supposedly, the HMR values of our patients are overestimated, reinforcing their sympathetic dysfunction degree.

Considering that the late HMR values of individuals with CCC (1.58 ± 0.27) are overestimated, and that Gadioli et al.[21] have reported a significant correlation between late HMR values of 1.68 ± 0.19 and severe ventricular arrhythmias, we assumed that the sympathetic dysfunction was more severe in the CCC group because of its arrhythmic findings as compared to the non-CCC group.[20,22] However, in our study, those values did not differ significantly from those of the non-CCC group (1.44 ± 0.16), even when statistically adjusted to age and LVEF (p = 0.111).

This fact might be explained by the advanced HF stage of our patients, when sustained autonomic sympathetic dysfunction represents a deleterious mechanism in the pathogenesis of HF itself,[8-10,13,24,25,33] independently of etiology, which is aligned with the report by other authors.[13]

On the other hand, the importance of sympathetic dysfunction in CCC has been questioned by different authors because of: variation in the intensity of denervation; absence of correlation between parasympathetic denervation and myocardial dysfunction extent;[19] presence of autonomic dysfunction in the early stages of Chagas disease;[4,15,19] correlation between persistence of the myocardial inflammatory process and those patients' morbidity and mortality,[35] despite the serum levels of catecholamines.

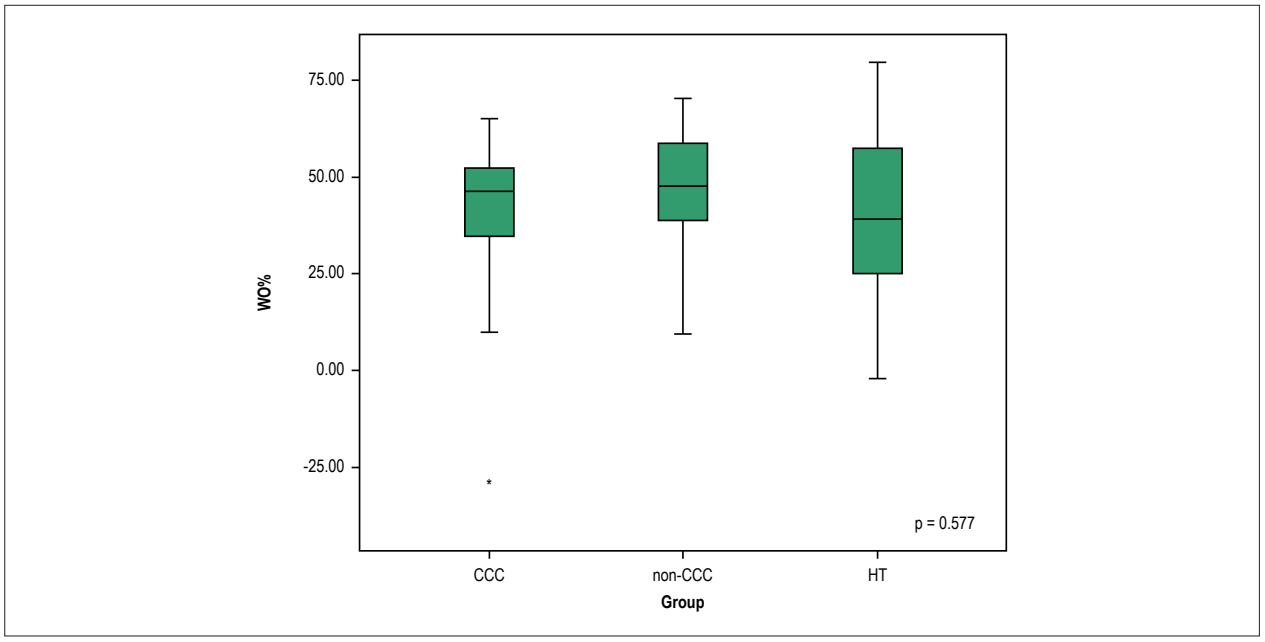

Figure 3 – *Cardiac washout (WO%) of ^{123}I-MIBG in CCC or non-CCC or HT patients. Groups: CCC: chronic Chagas cardiomyopathy; non-CCC: cardiomyopathy etiology other than Chagas disease; HT: heart transplant.*

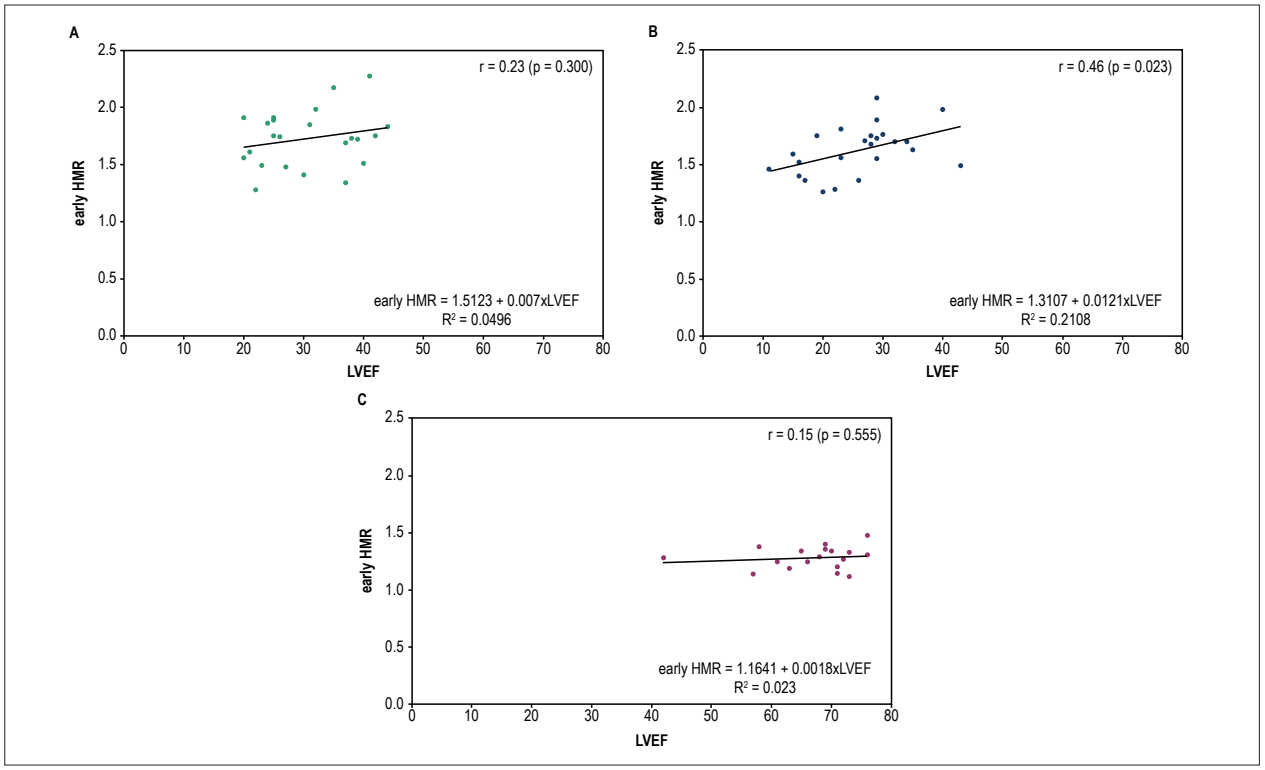

Figure 4 – *Dispersion graph to assess the correlation between LVEF and early HMR in CCC or non-CCC or HT patients. Early HMR: ratio of the heart/mediastinum radioactive counts estimated on 15-minute images (early uptake); LVEF: left ventricular ejection fraction. (A) CCC: chronic Chagas cardiomyopathy; (B) non-CCC: cardiomyopathy etiology other than Chagas disease; (C) HT: heart transplant.*

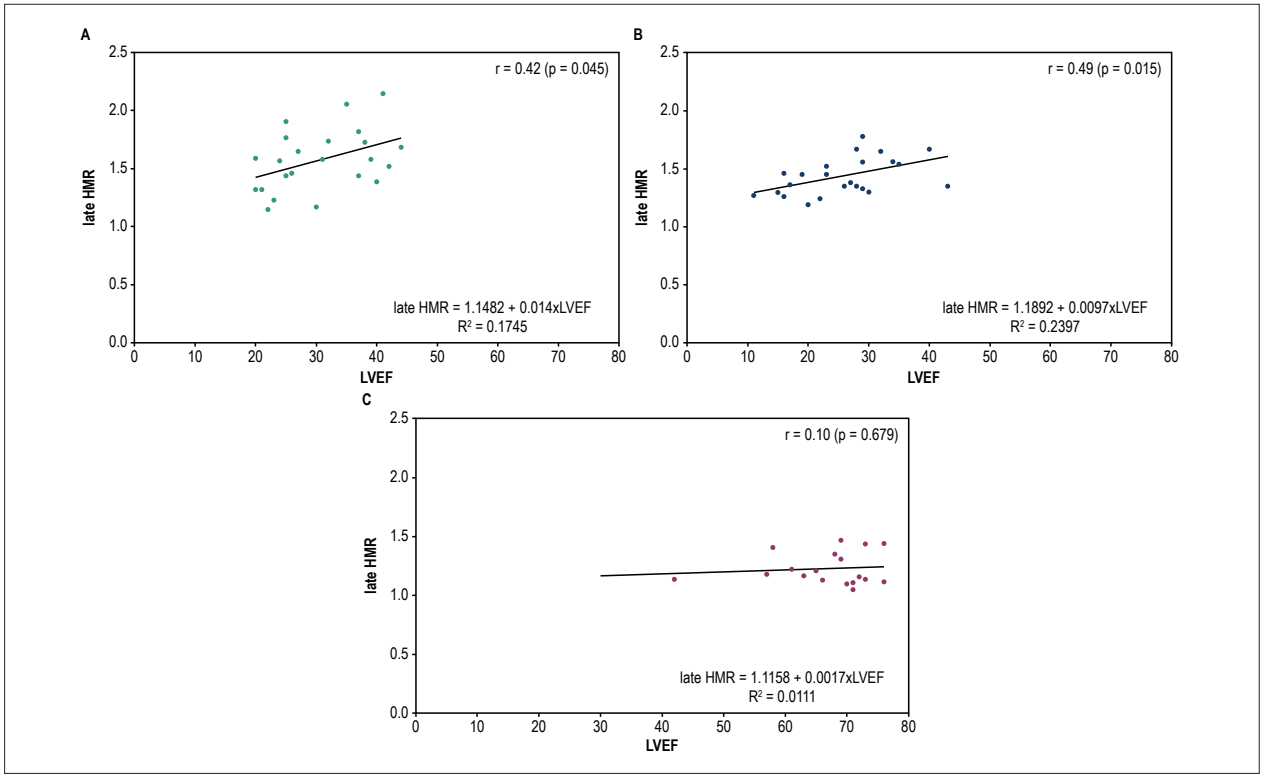

Figure 5 – *Dispersion graph to assess the correlation between LVEF and late HMR in CCC or non-CCC or HT patients. Late HMR: ratio of the heart/mediastinum radioactive counts estimated on 180-minute images (late uptake); LVEF: left ventricular ejection fraction. (A) CCC: chronic Chagas cardiomyopathy; (B) non-CCC: cardiomyopathy etiology other than Chagas disease; (C) HT: heart transplant.*

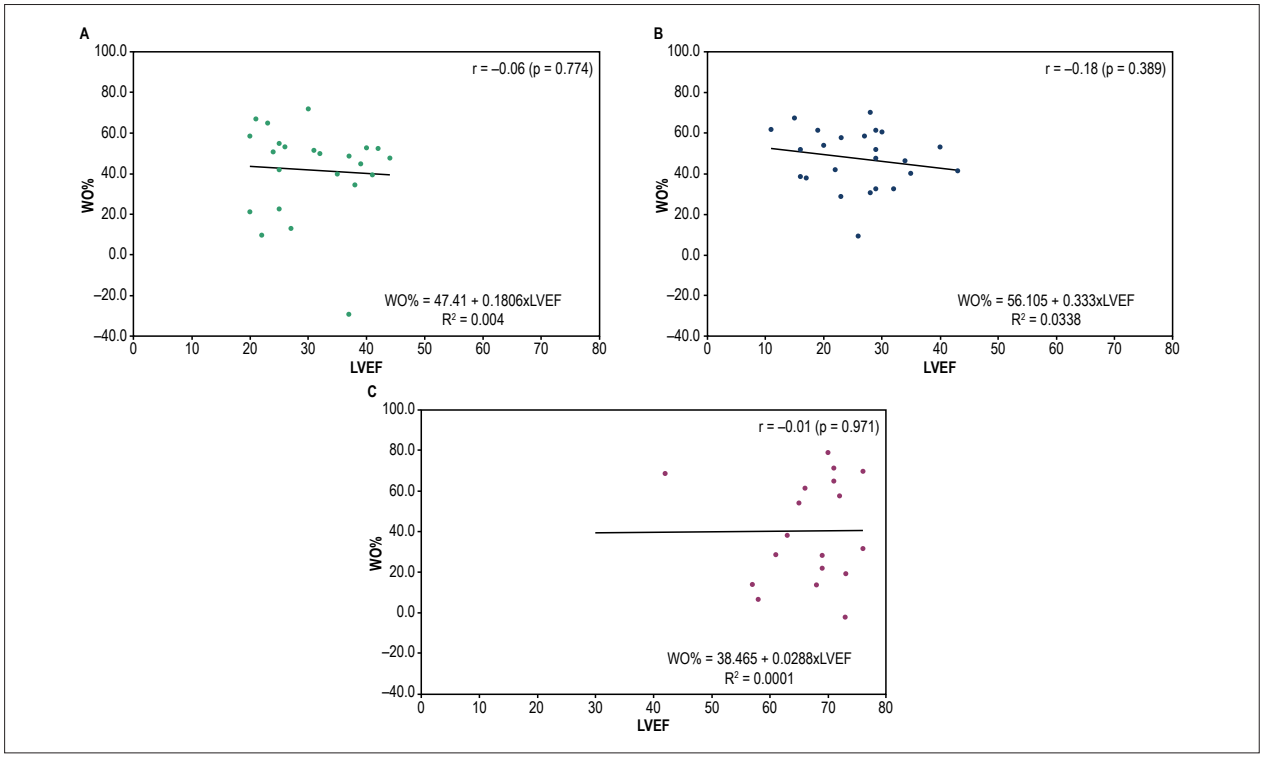

Figure 6 – *Dispersion graph to assess the correlation between LVEF and WO% of ^{123}I-MIBG in CCC or non-CCC or HT patients. CCC: chronic Chagas cardiomyopathy; LVEF: left ventricular ejection fraction. (A) CCC: chronic Chagas cardiomyopathy; (B) non-CCC: cardiomyopathy etiology other than Chagas disease; (C) HT: heart transplant.*

The estimated WO% values were elevated and abnormal in the three groups studied, being compatible with sympathetic dysfunction in patients with HF and not different from those of HT (p = 0.577). The sympathetic tone, translated by WO%,[13,28] might be altered earlier and more markedly than late HMR, and, thus, could be a more sensitive parameter for prognostic assessment, as described.[33]

Finally, a positive, although weak, but statistically significant correlation between HMR and LVEF was observed in individuals with HF. The LVEF is routinely used for the prognostic assessment of HF.[22,36] Thus, that weak correlation can indicate that this scintigraphic parameter is more accurate and earlier altered, as reported by Ogita et al., suggesting it is a better predictor of prognosis than LVEF.[33] In addition, it is worth noting that the correlation of early and late HMR with LVEF in CCC patients identified in this study has not been reported in the literature.

Study limitations

This is a cross-sectional nonrandomized study of patients with advanced HF (NYHA functional class II-IV), thus its findings cannot be generalized to all individuals with CCC. There are no strictly established reference values to quantify the scintigraphic parameters because different methodologies have been used (decay factor, correction of septal penetration of iodine).[28,29] The maintenance of the medication by the patients (ACEI and beta-blockers) might have led to overestimation of the HMR values and influenced our results, although the number of patients on medications did not differ between the CCC and non-CCC groups, and most studies in the literature have been performed considering the severity of HF.[24,25,33]

Conclusion

This study evidenced the presence of cardiac sympathetic autonomic dysfunction on myocardial ^{123}I-MIBG scintigraphy, regardless of the HF etiology, and its magnitude was equal in individuals with CCC and non-CCC as compared to HT patients.

Author contributions

Conception and design of the research: Marino VSP, Dumont SM, Mota LG, Moreira MCV; Acquisition of data: Marino VSP, Mota LG, Braga DS, Moreira MCV; Analysis and interpretation of the data: Marino VSP, Dumont SM, Moreira MCV; Statistical analysis: Marino VSP, Moreira MCV; Obtaining financing: Moreira MCV; Writing of the manuscript: Marino VSP, Dumont SM, Freitas SS, Moreira MCV; Critical revision of the manuscript for intellectual content: Marino VSP, Dumont SM, Mota LG, Braga DS, Freitas SS, Moreira MCV.

Potential Conflict of Interest

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

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Study Association

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais - Comitê de ética em Pesquisa (COEP) under the protocol number ETIC 0116.0.203.000-11. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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Cardiac Sympathetic Activity and the Neuro-Humoral Theory on Heart Failure with Reduced Ejection Fraction: Have We Learned Enough?

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Short Editorial regarding the article: Sympathetic Dysautonomia in Heart Failure by ^{123}I-MIBG: comparison between Chagasic, non-Chagasic and heart transplant patients

Since the 1970s,[1,2] in observational trials, and more recently in placebo-controlled randomized trials,[3,4] antagonizing beta-1 adrenergic receptors in the myocardium has been shown to mitigate the burden of heart failure with reduced ejection fraction (HFrEF). Mortality has been reduced by 34-65% according to these studies. However, inhibiting the action of the peripheral sympathetic nervous system (SNS) by blocking alpha1 or stimulating alpha-2 central receptors has shown negative results or even an increased mortality, despite reducing norepinephrine plasma levels.[5-7] This indicates that SNS effects on myocardial receptors, more than in peripheral receptors, play a pivotal role in HFrEF pathophysiology.

Hyper-activation of the SNS and the renin-angiotensin-aldosterone axis, combined with an increase in load-dependent peptides and inflammatory cascades constitute the neuro-humoral theory on HFrEF progression. While the neuro-humoral response counteracts and compensates for an initial myocardial insult, in the long-term it contributes to the progression of the disease to the point that cardiovascular homeostasis eventually succumbs if not treated properly. Therapies targeting all these neuro-humoral responses have dramatically changed the natural history of HFrEF.

Even though neuro-humoral theory has scaffolded for treatment of heart failure, the same has not occurred for HFrEF diagnosis. Except for the (still) scant use of brain natriuretic peptide (BNP), diagnosis and follow-up of such patients has been largely based on the estimation of ejection fraction (EF). This parameter is obviously of great importance, but in addition to being highly variable (inter-observer variability can be as high as 13%),[8] EF reduction occurs late in disease progression[9] when intervention is often less efficacious. Thus, new methods that comprise early detection of myocardium at risk and allow response to treatment assessment are desirable.

Assessment of cardiac sympathetic activity can, in theory, fulfill these criteria. Iodine^{123}– metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy is a well-known method to assess SNS cardiac activity and, though not widely applied, it can provide valuable information regarding early myocardial damage[10] and response to beta1- adrenergic receptors blockade.[11] In this issue of *Arquivos Brasileiros de Cardiologia*, Marino et al.[12] present a study with an instigating design and shed light on how cardiac sympathetic dysfunction occurs in HFrEF patients. In the study, treated patients with Chagas' cardiomyopathy appear to have similar sympathetic cardiac dysfunction compared to other treated HFrEF patients. These results could highlight the fact that treatment efficacy does not vary across HFrEF groups. Along with the current published scientific literature it is possible to speculate that cardiac Chagas disease begins with sympathetic denervation, evolves to perfusion disturbances and terminates in motility impairment.[13,14] Intervention with optimized medical treatment could, then, delay the progression to terminal disease. Also, the manuscript shows that sympathetic function in HFrEF treated patients is still below normal thresholds described in previous studies, thus suggesting a possible residual risk related to SNS hyper-activation.

The study by Marino et al.[12] is timely because it reminds clinicians and researchers that in order to maintain progress in treatment improvement and disease prevention it is of utmost necessity to keep track of its pathophysiology. Hyper-activation of SNS, renin-angiotensin-aldosterone axis and inflammatory cascades, among others, are cornerstones of the disease. Novel biomarkers to evaluate early myocardium at risk are highly needed, and some interesting ones seem to be in the pipeline. Late gadolinium enhancement, extracellular volume fraction and myocyte size quantification, assessed by cardiac magnetic resonance, as well as myocardial strain imaging by echocardiography and ^{123}I-MIBG global and regional cardiac scintigraphy by nuclear imaging are promising methods, but these novel methods require validation in larger cohorts and in controlled clinical trials. Established and novel methods can be then integrated to provide a thorough evaluation of the HFrEF patient and perhaps reduce even more the burden of such ominous disease.

Keywords

Heart Failure; Primary Dysautonomies; Chagas Cardiomyopathy; Myocardial/radionuclide imaging; ^{123}I-MIBC.

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The Role of Quantitative Aortographic Assessment of Aortic Regurgitation by Videodensitometry in the Guidance of Transcatheter Aortic Valve Implantation

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Abstract

Background: Balloon post-dilatation (BPD) is often needed for optimizing transcatheter heart valve (THV) implantation, since paravalvular leak (PVL) after transcatheter aortic valve implantation is associated with poor outcome and mortality. Quantitative assessment of PVL severity before and after BPD is mandatory to properly assess PVL, thus improving implantation results and outcomes.

Objective: To investigate a quantitative angiographic assessment of aortic regurgitation (AR) by videodensitometry before and after BPD.

Methods: Videodensitometric-AR assessments (VD-AR) before and after BPD were analysed in 61 cases.

Results: VD-AR decreased significantly from 24.0[18.0-30.5]% to 12.0[5.5-19.0]% ($p < 0.001$, a two-tailed $p < 0.05$ defined the statistical significance). The relative delta of VD-AR after BPD ranged from -100% (improvement) to +40% (deterioration) and its median value was -46.2%. The frequency of improvement, no change, and deterioration were 70% (n = 43), 25% (n = 15) and 5% (n = 3), respectively. Significant AR (VD-AR > 17%) was observed in 47 patients (77%) before and in 19 patients (31%) after BPD.

Conclusions: VD-AR after THV implantation provides a quantitative assessment of post-TAVI regurgitation and can help in the decision-making process on performing BPD and in determining its efficacy. (Arq Bras Cardiol. 2018; 111(2):193-202)

Keywords: Aortic Valve Insufficiency/diagnostic imaging; Angiography/evaluation; Heart Valve Prosthesis Implantation; Transcatheter Aortic Valve Replacement.

Introduction

Balloon post-dilatation (BPD) is often needed for optimizing transcatheter heart valve (THV) implantation, since paravalvular leak (PVL) after transcatheter aortic valve implantation (TAVI) is associated with long- term fatal prognosis.[1-5] The incidence of moderate or severe PVL following TAVI varies from 0% to 24% and that of mild PVL from 7% to 70%.[6] BPD is performed in 21% to 28% of cases with the first generation THVs.[7,8] Although newer generations of THVs have been designed to reduce the PVL, BPD is still performed in up to 17% of cases receiving the new generation of THVs.[7,9,10] Therefore, BPD remains one important technique to optimize implantation of the THV.

The Valve Academic Research Consortium-2 (VARC-2) consensus document recommends to perform quantitative and semi-quantitative hemodynamic assessments of PVL severity and other definitions for valve failure than mild PVL only.[11] TAVI under conscious sedation is increasingly adopted

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in clinical practice (i.e. the minimalist approach) restricting the usage of transesophageal echocardiography (TEE) as a guidance for TAVI and increasing the role of aortography as a screening tool to determine the severity of PVL during the procedure. We have previously reported the *in vitro* and *in vivo* validation of quantitative angiographic assessment of aortic regurgitation (AR) by videodensitometry technique after implantation of THV with an excellent reproducibility and accuracy.[12] This technique provides an accurate assessment of the severity of PVL and it has been shown that a Videodensitometric-AR (VD-AR) > 17% correlates with increased mortality and impaired reverse cardiac remodelling as determined by echocardiography after TAVI.[13,14] This prognostic cut-off value (VD-AR > 17%) could have the potential to guide operators in deciding the need for BPD. However, the change of VD-AR from before to after BPD has not been investigated. The aim of this study is to assess a quantitative aortographic approach of PVL by videodensitometry before and after BPD.

Methods

Study design

This is a report on patients enrolled in the the Brazilian TAVI registry including between January 2008 and January 2013. List of participating centers, inclusion and exclusion criteria and technical description of TAVI-procedure were previously reported.[15] The study protocol was approved by the ethics committee at each of the participating centers and all patients provided informed written consent. Three hundred ninety-nine patients were enrolled in the Brazilian TAVI registry in that period. VD-AR was performed and found to be analysable in 228 patients.[16] In this population, 102 patients underwent BPD, and in 17 cases, no angiography was available before BPD. Out of 85 cases with available aortograms before and after BPD, VD-AR was analysable at both time points in 61 cases (Figure 1). The reasons of non-analysable are discriminated in Figure 1.

Aortographic assessment of AR

Aortic root angiography was performed before and after BPD, using at least 20 ml of non-ionic contrast injected through a pigtail catheter positioned above the prosthetic valve stent (in case of a balloon-expandable device) or within the distal third of the prosthetic valve stent (in case of a self-expanding device). The decision on the total contrast volume and speed of injection, catheter size, and the projection were left to the discretion of the operators. Visual assessment of AR was performed by experienced observers based on Sellers' grade.[17] In a blinded fashion, assessment of post-BPD aortograms was performed by observers different from those who analyzed pre-BPD aortograms.

Quantification of AR using videodensitometric technology

VD-AR before and after BPD was analysed at an independent core laboratory (Cardialysis Clinical Trials Management and Core Laboratories, Rotterdam, the Netherlands) by experienced observers using a dedicated software (CAAS A-Valve 2.0.2; Pie Medical Imaging, Maastricht, The Netherlands). The details of this technique have been described elsewhere.[12-14,16,18] After drawing the contours of the aortic root (*i.e.* reference region) and the subaortic one third of the left ventricle (i.e. region of interest [ROI]), the contrast time-density curves were generated for both regions over at least three cardiac cycles after contrast injection. The areas under these curves (AUC) are automatically calculated and represent the time-density integral. VD-AR is automatically calculated as the ratio of the AUC of the

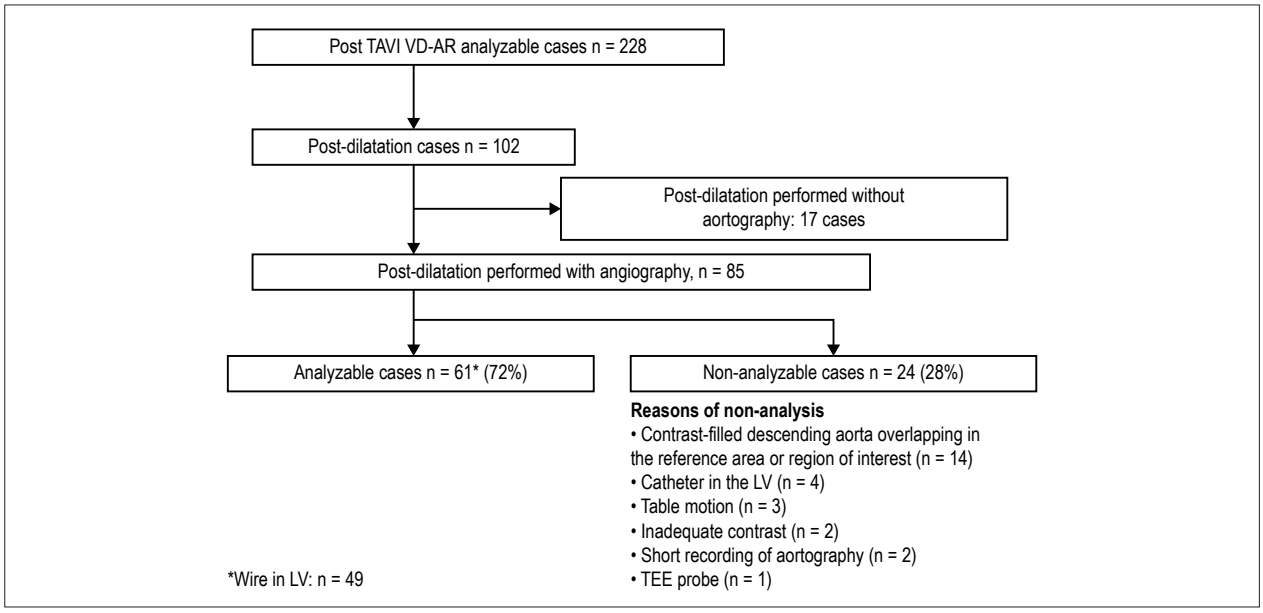


Figure 1 – *Flowchart of this study. TAVI: transcatheter aortic valve implantation; VD-RA: Videodensitometric of aortic regurgitation; TEE: transesophageal echocardiography.*

ROI to that of the reference region (Figure 2). Theoretically, the value of VD-AR ranges from 0.0% to 100%. The relative delta VD-AR was calculated as = (VD-AR after BPD - VD-AR before BPD)/VD-AR before BPD, where a negative value indicates an improvement of the severity of AR.

THV and post-dilatation balloon diameters / annulus diameter ratios

Multislice computed tomography (MSCT) was performed following the local radiological protocol. Cover index was calculated as "(prosthesis nominal diameter – annulus diameter) / (prosthesis nominal diameter) × 100". The post-dilatation balloon size / annulus diameter ratio was calculated as "(balloon nominal diameter – annulus diameter) / (balloon nominal diameter) × 100".

Statistics

When continuous variables were normally distributed, we summarized data as mean ± standard deviation.[19] If they were not normally distributed, median and inter-quartile range [IQR] were used. Mann-Whitney test was used to compare continuous variables between independent samples. Wilcoxon signed ranks test was performed to compare the serial changes between before and after BPD. All analyses were performed with SPSS 23 (IBM, Armonk, NY, USA). A two-tailed $p < 0.05$ defined the statistical significance.

Results

Baseline characteristics and echocardiographic data of this population (n = 61) are shown in Table 1. The mean age was 81.6 ± 7.6 years, and patients had a high Society of Thoracic Surgeons (STS)-Predicted Risk Of Mortality score, 8.8(4.6-16.3). Either CoreValve (Medtronic, Minneapolis, MN, USA) (72%) or SapienXT (Edwards Lifesciences, Irvine, CA, USA) (28%) have been implanted. In most cases, TAVI was performed with general anaesthesia (98%) and transfemoral approach (97%).

Influence of BPD on VD-AR

The change of VD-AR from before- to after- BPD is shown in Figure 3 and a representative case is displayed in Figure 2 and Movie 1. VD-AR decreased significantly from 24.0[18.0-30.5]% (before BPD) to 12.0[5.5-19.0]% (after BPD) ($p < 0.001$). The median value of absolute delta VD-AR was -10.0%, corresponding to a relative delta of - 46.2% (range: -100% to +40%). The frequencies of any improvement or deterioration of AR (as defined by VD-AR) were 82% (n = 50) and 18% (n = 11), respectively (Figure 4). The 25th percentile of the relative delta VD-AR was 20%, and this cut-point was arbitrarily used to define "a significant change" as follows: a relative delta < -20% defined as "a significant improvement", a relative delta of -20 to +20% as "no change", and a relative delta > +20% as "a significant deterioration". There were 43 patients (70%) with significant improvement, 15 patients (25%) with no change, and 3 patients (5%) with significant deterioration.

The THV cover index and the balloon size used in post-dilatation were both available in 38 out of 61 patients – 25 (66%) among those with significantly improvement of PVL after BPD, 11 (29%) with no change in VD-AR, and 2 (5%) with deterioration of AR. THV cover index was 11.5[4.1,15.9]

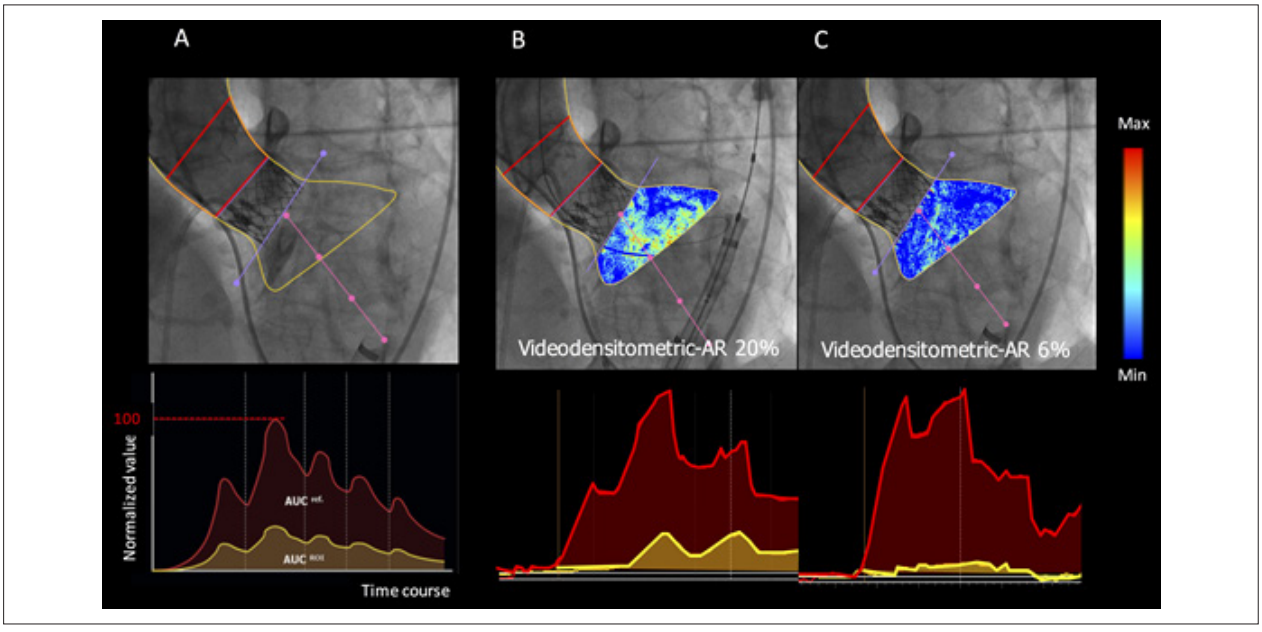
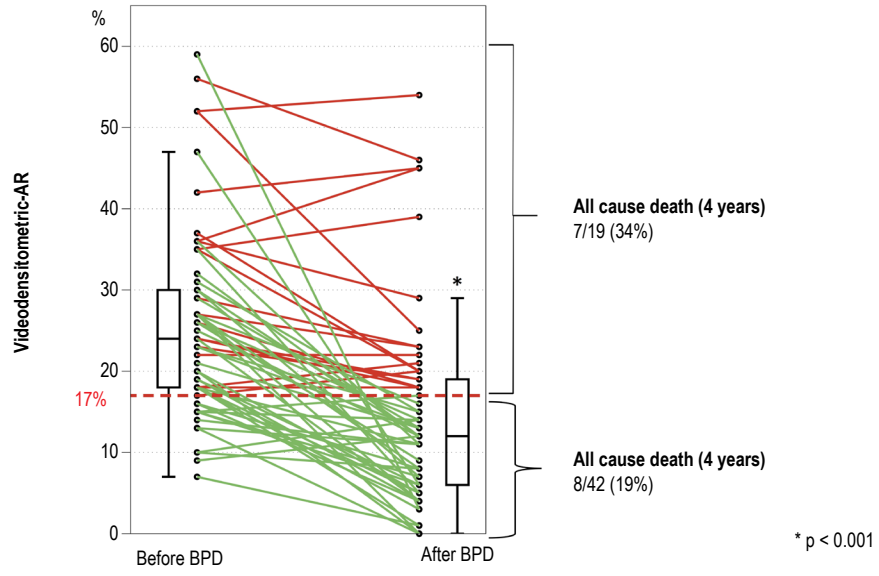


Figure 2 – *Videodensitometric assessment of aortic regurgitation. A) Delineation of the aortic root (reference region: red area in the aortography) and the subaortic one third of LV (ROI: yellow area in the aortography) are shown by the analyser. The time-density curves are provided for both ROI (yellow) and reference (red) regions, and the AUC is automatically computed by the software time-density integrals. VD-AR corresponds to the relative AUC, which is automatically calculated as the ratio of the relative AUC in the ROI (yellow) to that in the reference area (red). Theoretically, the value of VD-AR ranges from 0 to 1. B) An example of VD-AR measurement before BPD. C) An example of VD-AR measurement after BPD. Reproduce and adopted from Tateishi et al. EuroIntervention 2016[14]*

Table 1 – Baseline and echocardiographic characteristics of the study population (n = 61)

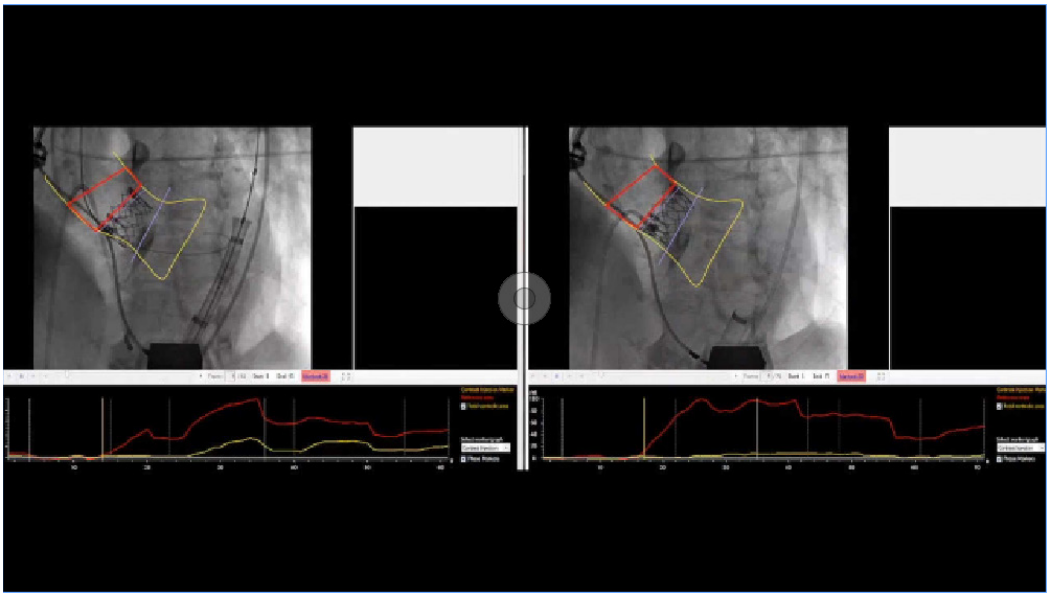
Variables	Median (IQR)/Frequency
Clinical characteristics	
Age, years (median[IQR])	81.6 ± 7.6
Male gender, n (%)	37(60.7)
BMI, kg/m^2	24.6 ± 3.9
NYHA II, n (%)	13(21.3)
NYHA III, n (%)	27(44.3)
NYHA IV, n (%)	21(34.4)
Hypertension, n (%)	47(77.0)
DM, n (%)	15(24.6)
Renal insufficiency*, n (%)	51(83.6)
CAD, n (%)	31(50.8)
PAD, n (%)	13(21.3)
COPD, n (%)	15(24.6)
PH**, n (%)	12(19.7)
Prior PCI, n (%)	15(24.6)
Prior CABG, n (%)	10(16.4)
Prior MI, n (%)	6(9.8)
Prior stroke, n (%)	6(9.8)
Prior BAV, n (%)	4(6.6)
Prior AVR, n (%)	1(1.6)
Prior PMI, n (%)	7(11.5)
Af/AFL, n (%)	9(15.0)
STS-PROM, %	8.8[4.6-16.3]
EuroSCORE, %	15.9[9.2-25.4]
Preprocedural echocardiographic parameters	
LVDd, mm	50.0[46.0-55.0]
LVEF,	61.0[45.0-68.0]
LVM index, %	136.9[114.2-162.9]
AVA, cm^2	0.6[0.5-0.8]
Peak PG, mmHg	75.0[64.0-92.5]
Mean PG, mmHg	47.0[41.0-61.0]
MR >mild, n (%)	16(26.2)
TEE guidance, n (%)	56(91.8)
General anesthesia, n (%)	60(98.4)
Transfemoral approach, n (%)	59(96.7)
Procedural characteristics	
CoreValve, n (%)	44(72)
CoreValve 26mm, n (%)	9(20.5)
CoreValve 29mm, n (%)	17(38.6)
CoreValve 31mm, n (%)	18(40.9)
Sapien-XT, n (%)	17(28)
Sapien-XT 23mm, n (%)	7(41.2)
Sapien-XT 26mm, n (%)	8(47.1)
Sapien-XT 29mm, n (%)	2(11.8)
Pre-dilatation performed, n (%)	18(29.5%)

*BMI: body mass index, NYHA: New York Heart Association, DM: diabetes mellitus, CAD: coronary artery disease, PAD: peripheral artery disease, COPD: chronic obstructive pulmonary disease, PH: pulmonary hypertension, PCI: percutaneous coronary intervention, CABG: Coronary artery bypass grafting, MI: myocardial infarction, BAV: balloon aortic valvuloplasty, AVR: aortic valve replacement, PMI: pacemaker implantation, AF: atrial fibrillation, AFL: atrial flutter, STS-PROM: the Society of Thoracic Surgeons - predicted risk of mortality, LVDd: left ventricular diastolic diameter, LVEF: left ventricular ejection fraction, LVM index: left ventricular mass index, AVA: aortic valve area, PG: pressure gradient, MR: mitral regurgitation, TEE: transesophageal echocardiography. * Defined as glomerular filtration rate < 60 mL/min, ** Defined as a systolic pulmonary artery pressure ≥ 60 mm Hg at rest*



	Before BPD (n = 61)	After BPD (n = 61)
Videodensitometric-AR (Median[IQR])	24.0[18.0-30.5]	12.0[5.5-19.0]
Videodensitometric-AR > 17%, n (%)	47(77%)	19(31%)
Videodensitometric-AR ≤ 17%, n (%)	14(23%)	42(69%)

Figure 3 – *Serial changes of the Videodensitometric-AR. Individual serial changes before and after balloon post-dilatation are shown in this figure. In patients with VD-AR > 17%, 7 deaths (34%) occurred, whereas in patients with VD-AR ≤ 17%, 8 deaths (19%) were observed.*



Video 1 – *Videodensitometric assessment of aortic regurgitation before and after balloon post-dilatation. Left panel shows VD-AR assessment before BPD (VD-AR = 20%). Right panel shows VD-AR assessment after BPD (VD-AR = 6%).*

and ranged from 0.0% to 22.8% in patients with a significant improvement of AR, and 13.8[3.3,16.5], ranging -29.0% to 19.3% in those with no change or a significant deterioration of AR. Post-dilatation balloon size / annulus diameter ratio was 0.0[-7.9,7.6] and ranged from -25.0% to 14.3% in patients with a significant improvement of AR, and 0.0[-5.6,13.4], ranging from -33.3% to 16.4% in patients with no change or with a significant deterioration of AR.

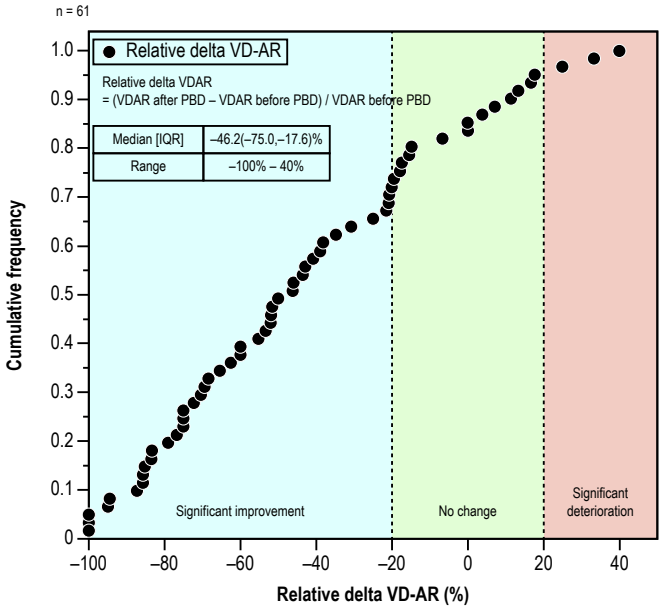


Figure 4 – *Cumulative frequency curve of the rate of improvement or deterioration of aortic regurgitation by balloon post-dilatation. The formula of the relative delta VD-AR was "(VD-AR after BPD - VD-AR before BPD)/VD-AR before BPD". Negative values indicate improvements of AR after BPD, whereas positive values stand for deterioration of AR after BPD. Using twenty-fifth percentile of the absolute delta VD-AR, arbitrarily we defined a relative delta of less than -20% as a significant improvement (blue), from -20 to 20% as no change (green), and 20% more as a significant deterioration (orange).*

Serial change of AR based on Sellers' grade

Before BPD, AR was visually classified as Sellers' III in 36 patients (59%), and as Sellers' II in 25 patients (41%). After post-dilatation, there were 3 (5%) cases with Seller' III, 19 (31%) cases with Sellers' II, 34 (56%) cases with Sellers' I and 5 (8%) cases with Sellers' 0. Out of 36 patients with Sellers' III before BPD, 34 patients had their Sellers' grade reduced (to Sellers' II in 16, Sellers' I in 17, and Sellers' 0 in one patient. Out of 25 patients with Sellers' II before BPD, PVL improved to Sellers' I in 17 patients and to Sellers' 0 in 4 patients, deteriorated to Sellers' III in one patient, and remained unchanged (Sellers' II) in three patients (Figure 5).

Efficacy of BPD

Before BPD, VD-AR > 17%, a value that has a prognostic significance in long-term follow-up, was observed in 47 patients (77%). Fourteen cases (23%) had a VD-AR ≤ 17%, eleven (11/14, 79%) were evaluated as Sellers' II before BPD and 3 (3/14, 21%) as Sellers' III. After BPD, VD-AR > 17% was observed in 19 patients (falling from 77% to 31% of subjects) – 3 patients (16%) in Seller's III, 10 patients (53%) in Sellers' II, and 6 patients in Sellers' I (32%) (Figure 6). In addition, in these patients with VD-AR > 17%, 7 deaths (34%) occurred during follow-up period, whereas among 42 patients with VD-AR ≤ 17%, 8 patients (19%) died.

Predilatation was performed in 18 patients and had no impact on the reduction of AR assessed by VD-AR. VD-AR was 25.5% (19.5%-36.0%) with predilatation and 23.0% (16.0%-29.0%) without predilatation (p = 0.159) before PBD, and 16.5% (9.5%-22.8%) with predilatation and 11.0% (5.0%-17.0%) without predilatation (p = 0.106) after PBD. Normalized delta VD-AR was -44.5 (-60.1 – -13.0) with predilatation and -50.0(-75.0 – -17.9) without predilatation (p = 0.569).

Discussion

This is the first study to report the value of VD-AR in assessing periprocedural changes in AR. In clinical practice, echocardiogram and aortography are the standard tools to define the device success. As mentioned in the Valve Academic Research Consortium-2 (VARC-2) consensus document, quantitative and semi-quantitative hemodynamic assessment are recommended to assess AR severity by echocardiogram and moderate-to-severe AR is defined as valve failure.[11, 20]

Nombela-Franco et al.[8] reported serial changes using semi-quantitative grading based on echocardiogram and showed a reduction of at least 1 degree of AR in 71% of patients. To make a decision whether BPD is needed or not, echocardiogram is an important tool to evaluate the severity of AR. However, we must consider that with the increasing minimalist TAVI approach, the usage of TEE as a guidance of TAVI is becoming unfeasible. Moreover, low inter-observer agreement for the PVL 4-class grading (kappa 0.481) and the 7-class grading (kappa 0.517) has been reported,[21] making a more reliable technique necessary.[18] These facts support the value of aortography with VD assessment as the most practical and objective screening tool to determine the severity of PVL during the procedure. The technique has a median time of execution of 3 minutes.

We have previously shown that a VD-AR > 17% correlates with increased mortality and with impaired cardiac reverse remodelling as determined by echocardiography after TAVI with excellent reproducibility.[13,14] This value (VD-AR > 17%)

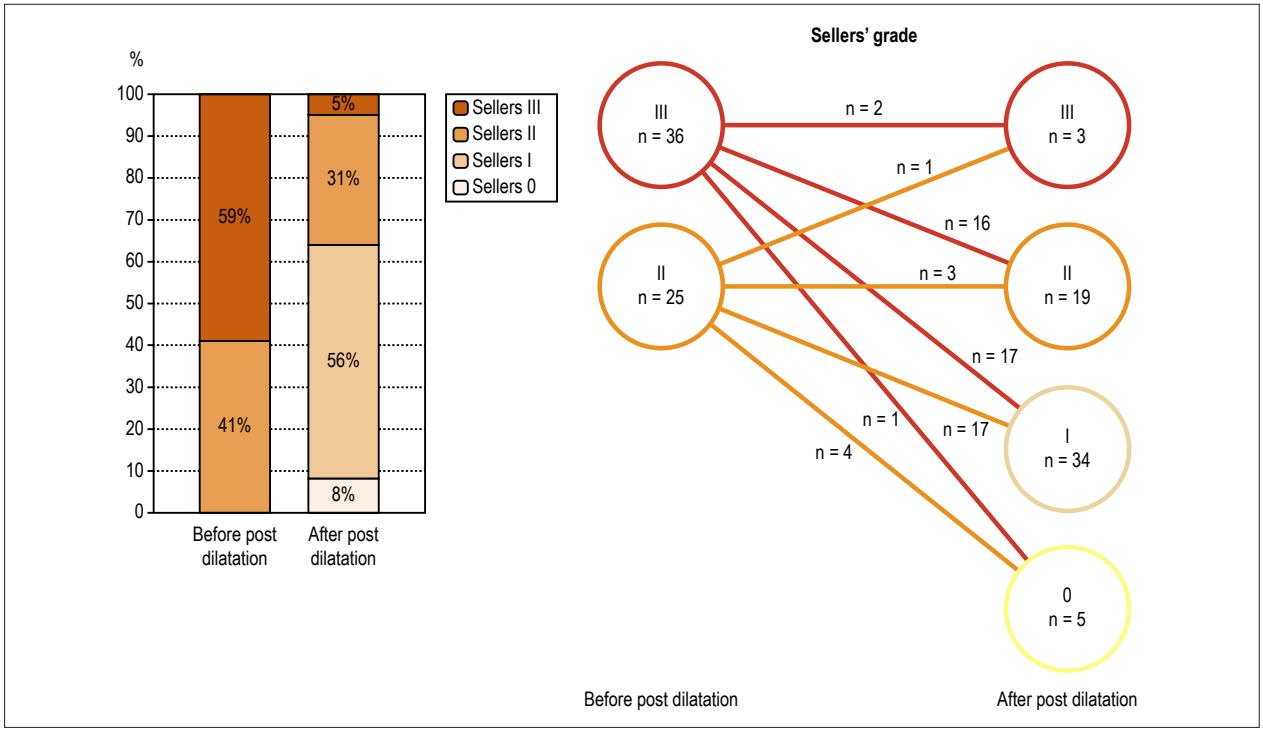


Figure 5 – *Serial changes of visual aortographic assessment.*

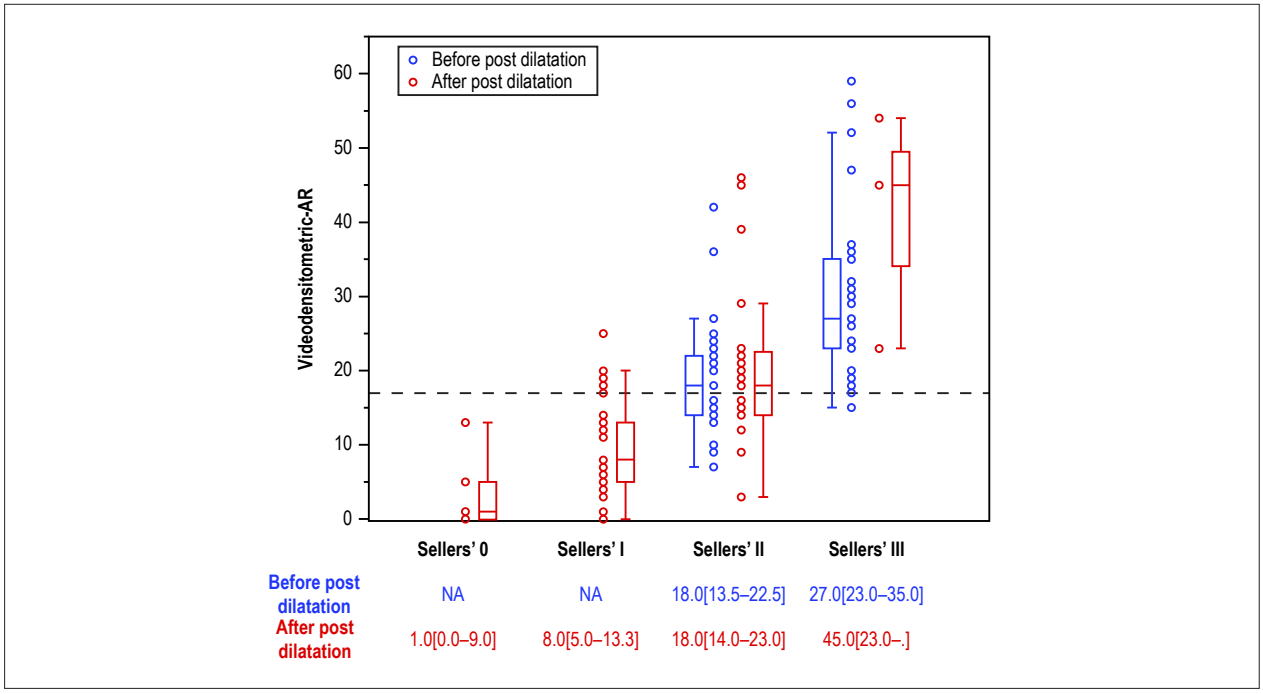


Figure 6 – *Videodensitometric- assessment of aortic regurgitation and Sellers' grade before and after balloon post-dilatation.*

could be decisive in helping the operator to make a decision as whether BPD should be performed during the procedure. When BPD was performed, we showed that before BPD, 77% of patients had a VD-AR > 17%, and the other patients (VD-AR ≤ 17%) (23%) would not require BPD. This finding is important, since BPD is associated with higher rate of cerebrovascular events compared to the patients without BPD.[8,22] Avoiding unnecessary BPD would possibly reduce the risk of cerebrovascular events as well as procedural costs. Moreover, most cases of VD-AR ≤ 17% before BPD were found in Sellers' II, suggesting that the visual assessment of the Sellers' classification could lead to unnecessary PBD.

After BPD, VD-AR > 17% was still seen in 31% of patients. Based on current available data, for patients with residual AR (VD-AR > 17%), additional measures should be taken. We found higher mortality in patients with VD-AR > 17% compared to patients with VD-AR ≤ 17% during the follow-up (34% vs. 19%). Although the difference in mortality was not significant (log rank p = 0.273) in this small population with BPD, a tendency for high mortality was previously reported in patients with VD-AR > 17% in a large population.[14,16]

VD-AR deteriorated numerically in 11 patients, and this deterioration was significant in 3. This comes in agreement with previous studies which also reported AR deterioration in a small proportion of patients after BPD,[8] and could be due to prosthetic overexpansion with secondary leaflet maladaptation and transvalvular regurgitation.[23]

Serial changes of VD-AR showed predominantly improvement of AR. A reduction of the regurgitation by BPD was reported in 68%-91% in the literature.[8,24] The mechanisms of regurgitation after implantation of THV are multifactorial as, for example, calcification of the native aortic annulus and left ventricular outflow tract (LVOT) and cover index are well known predicting factors of regurgitation after implantation of a THV.[19,26-34]

To make a decision whether BPD is needed or not and to judge its efficiency, repeated injections of large doses of contrast medium would be needed. Contrast medium volume used in this population was 150[131-209] ml/procedure. In the setting of TAVI, peri-procedural acute kidney injury (AKI) develops in 12% to 57% of cases and portends a significant increase in early and late mortality.[34,35] The mechanisms of AKI following TAVI are multifactorial, and the role of the contrast medium volume is controversial.[36] However, there is some evidence suggesting that a larger contrast volume is related to an increased risk of AKI after TAVI.[34,37] Taking into account the important role of aortography in the minimalist TAVI era, repeated aortograms cannot be avoided. However, the possibility of reducing contrast medium is reported using a diastolic phase-synchronized injection of only 8 ml of contrast medium in an *in-vitro* setting.[12] This technique could enable the reduction of the total amount of contrast medium during the procedure.

Limitations

After implantation of the THV, the guidewire is frequently left in the left ventricle and may produce artificial transvalvular regurgitation.[38] However, the effect of the guidewire on AR during TAVI is variable according to the weight of the wire. Most operators decide whether to perform BPD with or without a guidewire in LV by using echocardiography and aortography. Indeed, in the present study, VD-AR before BPD was analysed either with (n = 49) or without (n = 12) the guidewire being left in the left ventricle.

One limitation of our study is the absence of data on aortic regurgitation index, thus lacking the possibility of comparing this to our method. Limitations of VD-AR assessment are its feasibility. The current report is a retrospective study so that the acquisition of aortography was not dedicated for VD-AR assessment. In order to perform videodensitometric assessment appropriately, the acquisition of aortography should be done without overlapping ROI with contrast filled ascending/descending aorta. Recently, Teng et al.[39] reported how to plan an overlap free projection for VD-AR assessment.[39] A dedicated acquisition protocol would achieve a high feasibility of assessment. We tried to overcome this limitation by choosing the cases that did had an adequate acquisition of images, lowering our sample size. However, a prospective clinical study is needed to confirm this hypothetical assumption. So far, CAAS-A-valve software is available as an offline system. Currently, attempts are being made to allow online assessment.[40] In the near future, online system will probably foster the VD-AR as guidance for TAVI.

In this registry, no echocardiographic parameters recorded were reported after THV deployment but before BPD. The information of calcification of the native aortic valve, annulus and LVOT from computed tomography were not available.

Conclusion

VD-AR after THV implantation enables the operator to assess quantitatively regurgitation, to rationalise BPD and to assess its efficacy.

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Author contributions

Conception and design of the research: Miyazaki Y, Modolo R, Abdelghani M, Tateishi H, Cavalcante R, Collet C, Asano T, Tenekecioglu E, Mangione JA, Abizaid A, Soliman OII, Onuma Y, Serruys PW, Lemos PA, Brito Jr. FS; Acquisition of data: Miyazaki Y, Modolo R, Abdelghani M, Tateishi H, Cavalcante R, Katagiri Y, Sarmento-Leite R, Mangione JA, Abizaid A, Soliman OII, Onuma Y, Serruys PW, Lemos PA, Brito Jr. FS; Analysis and interpretation of the data: Miyazaki Y, Modolo R, Abdelghani M, Tateishi H, Cavalcante R, Collet C, Asano T, Katagiri Y, Tenekecioglu E, Mangione JA, Abizaid A, Soliman OII, Onuma Y, Serruys PW; Statistical analysis: Miyazaki Y, Modolo R, Abdelghani M, Cavalcante R, Collet C, Asano T, Katagiri Y, Mangione JA, Abizaid A, Soliman OII, Serruys PW, Brito Jr. FS; Writing of the manuscript: Miyazaki Y, Modolo R, Abdelghani M, Asano T, Mangione JA, Abizaid A, Serruys PW; Critical revision of the manuscript for intellectual content: Miyazaki Y, Modolo R, Abdelghani M, Tateishi H, Cavalcante R, Collet C, Asano T, Katagiri Y, Tenekecioglu E, Sarmento-Leite R, Mangione JA, Soliman OII, Onuma Y, Serruys PW, Lemos PA, Brito Jr. FS.

Potential Conflict of Interest

Rogério Sarmento-Leite, José A. Mangione, and Fabio S. de Brito Jr are proctors for Medtronic and Edwards Lifesciences. Pedro A. Lemos is a proctor for Edwards Lifesciences and Boston Scientific. All other authors have no relevant conflicts of interest to declare.

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Study Association

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Ethics approval and consent to participate

This study used data from the Brazilian TAVI Registry. This Registry was approved by the Ethics Committe of all the Institutions participating, and all patients included prospectively provided written informed consent. All procedures were in accordance with the Decalration of Helsinki.

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New Method Improves the Assessment of Aortic Regurgitation Grade during TAVR by Aortography

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Transcatheter aortic valve replacement (TAVR) is a rapidly expanding alternative to surgical aortic valve replacement for patients deemed inoperable or at high or intermediate operative risk. Yet, residual aortic regurgitation (AR) secondary to paravalvular leaks (PVL) remains a procedural limitation.[1] Although residual AR after TAVR is frequent, affecting up to approximately 70% of the treated patients,[2-4] it is moderate to severe in approximately 12% of these[4] and steadily below 5% with current-generation devices, which come with specific sealing features.[1] Noteworthy, moderate/severe AR has a detrimental clinical impact after TAVR, with a 3-fold increase in 30-day mortality and a 2.3-fold increase in 1-year mortality.[4] Thus, its accurate assessment and quantification with a multimodality approach is key for appropriate utilization of additional procedures to reduce PVL, such as balloon post-dilatation (BPD), valve-in-valve, or leak closure.[1,5]

While Doppler echocardiography has been the most common method to assess AR following TAVR, its accurate quantification is challenging since AR jets are often multiple and eccentric.[3,5-7] Therefore, other methods for proper AR assessment have been evaluated in recent years, such as 3D echocardiography, hemodynamic AR index, aortography and even cardiovascular magnetic resonance, each one with its specific advantages and disadvantages.[1,5,7]

In the current issue of the journal, Miyazaki et al.[8] investigate a quantitative angiographic assessment of AR by videodensitometry (VD-AR) before and after BPD was performed. VD-AR was shown to decrease significantly from 24.0 [18.0-30.5] % to 12.0 [5.5-19.0] % ($p < 0.001$) after BPD, with some degree of AR grade improvement for up to 70% of patients treated. Of note, significant AR (VD-AR > 17%) was observed in 47 patients (77%) before and in 19 patients (31%) after BPD; moreover, in up to a quarter of these, pre-BPD VD-AR was below 17%, indicating that this additional maneuver could have been avoided. The study has its inherent limitations, e.g., the cohort was relatively small, with retrospective patient selection and imaging acquisition, and the decision whether or not to perform BPD was left to the discretion of the operators. Accordingly, the study only comprises cases where BPD was deemed necessary, and only aortograms with good quality imaging were selected.

Notably, the technique used to quantify VD-AR is a novel method that can accurately determine the regurgitation fraction in aortograms performed during TAVR; it uses dedicated software and showed excellent reproducibility and accuracy.[9,10] This technique provides an accurate assessment of the severity of PVL, and a VD-AR index greater than 17% correlated with increased mortality and with impaired cardiac reverse remodeling after TAVR.[11,12] And while VD-AR measurements are performed offline only, real-time online assessment is underway so as to enable this method to help guiding TAVR in the near future. After all, BPD is currently performed in about 10% to 20% of patients following TAVR, and it reduces the severity of PVL by at least one grade in more than two thirds of patients.[13,14] Nevertheless, BPD may be associated with an increased risk of cerebrovascular events and annular trauma, therefore judicious utilization of this procedure is recommended.[13,14]

In conclusion, since PVL has a negative impact on clinical outcomes after TAVR, its proper assessment through a multimodality, multiparametric, integrative approach is fundamental. Priority should be given to PVL prevention through accurate sizing of aortic annulus by 3D imaging techniques, THV devices with improved sealing features, and optimal THV sizing and positioning. Still, if PVL does occur after TAVR, the interventional cardiologist can consider corrective procedures such as BPD, valve-in-valve, or leak closure. The novel VD-AR after TAVR also allows quantitatively assessing post-TAVR regurgitation and may assist decision making on whether or not to perform BPD, as well as determining its efficacy. Future prospective studies are warranted to further confirm the present results.

Keywords

Transcatheter Aortic Valve Replacement/methods; Aortic Valve Insufficiency/mortality; Aortic Valve Insufficiency/diagnostic imaging; Echocardiography, Three-Dimensional/methods; Aortography/methods.

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Antiplatelet Therapy in Breast Cancer Patients Using Hormonal Therapy: Myths, Evidence and Potentialities – Systematic Review

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Abstract

Breast cancer is the most frequently diagnosed tumor in women worldwide, with a significant impact on morbidity and mortality. Chemotherapy and hormone therapy have significantly reduced mortality; however, the adverse effects are significant. Aspirin has been incorporated into clinical practice for over 100 years at a low cost, making it particularly attractive as a potential agent in breast cancer prevention and as an adjunct treatment to endocrine therapy in the prophylaxis of cardiovascular complications. The objective of this study was to evaluate the role of aspirin in reducing the incidence of breast cancer and to evaluate the impact of its use on morbidity and mortality and reduction of cardiovascular events as adjuvant therapy during breast cancer treatment with selective estrogen receptor modulators. A systematic review was performed using the PRISMA methodology and PICO criteria, based on the MEDLINE, EMBASE and LILACS databases. The original articles of clinical trials, cohort, case-control studies and meta-analyses published from January 1998 to June 2017, were considered. Most studies showed an association between the use of selective estrogen receptor modulators and the increase in thromboembolic events. The studies suggest a protective effect of aspirin for cardiovascular events during its concomitant use with selective estrogen receptor modulators and in the prevention of breast cancer. This systematic review suggests that aspirin therapy combines the benefit of protection against cardiovascular events with the potential reduction in breast cancer risk, and that the evaluation of the benefits of the interaction of endocrine therapy with aspirin should be further investigated.

Introduction

Breast cancer is the most frequently diagnosed tumor in women worldwide, with a significant impact on morbidity and mortality. According to the World Health Organization, it is estimated that more than 1.5 million new cases of breast cancer are annually diagnosed worldwide. Despite advances in treatment, breast cancer mortality is still high, with 570,000 deaths in 2015. The disease, recurrent or metastatic, remains incurable in most cases.[1]

Chemotherapy and hormone therapy have significantly reduced mortality, but their adverse effects are considerable. Endocrine therapy has revolutionized the treatment of breast cancer patients with positive Estrogen Receptor (ER), although there are cases that develop resistance to this therapy. An appropriate strategy would be the combination of Selective Estrogen Receptor Modulators (SERMs) or another hormonal class with other therapeutic agents, aiming at attaining a synergistic antitumor effect. The use of non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin, has been associated with reduced risk of breast cancer.[2,3] This therapy could also antagonize thrombogenic effects in women treated with tamoxifen.

The increasing number of breast cancer survivors is confronted with the shortage of information among clinicians on the subject.

The aim of the present study is to evaluate the role of aspirin in reducing the incidence of breast cancer and to evaluate the impact of its use in reducing cardiovascular events as an adjuvant therapy during the treatment of breast cancer with SERMs.

Methods

This systematic review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology.[4] The study included original articles of clinical trials, cohort, case-control studies and meta-analyses published from January 1998 to June 2017, with full-texts in English, Spanish and Portuguese, obtained from the MEDLINE, EMBASE and LILACS databases. The research was performed using the following descriptors: (selective estrogen receptor modulators OR tamoxifen OR raloxifene hydrochloride OR toremifene) AND (platelet aggregation inhibitors OR aspirin) AND (cardiovascular disease) AND (breast CA).

This study was based on the PICO (acronym for Population, Intervention, Control and Outcome) criteria. The objective was to evaluate whether aspirin use implies in the reduction of events, especially cardiovascular events, in women with breast cancer using SERMs. The studies were selected according to the following criteria: use of SERMs in women with breast cancer; regular aspirin use; and evaluation of mortality, metastases, and adverse effects using SERMs and/or aspirin. Case reports, articles with other types of endocrine therapy, and animal experimental models were excluded.

Keywords

Breast Neoplasms/drug therapy; Indicators of Morbidity and Mortality; Aspirin; Tamoxifen; Raloxilene Hydrochloride; Cardiovascular Diseases/prevention & control; Selective Estrogen Receptor Modulators.

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A total of 221 abstracts met the search criteria and other 15 were manually retrieved. A total of 159 duplicated articles were eliminated and 77 abstracts were evaluated. Of these, 57 were selected for the review. We excluded 25 because they did not meet the previously established criteria, resulting in 32 full-text articles, which were evaluated in relation to their scientific quality. Five articles were excluded according to the inclusion/exclusion criteria. A total of 27 articles were analyzed, according to figure 1.

Selective estrogen receptor modulators and reduction of morbidity and mortality in breast cancer

Most breast cancers have positive ER and three main drugs are being used for their treatment and/or prevention, namely: tamoxifen, raloxifene and toremifene. All of these agents are competitive inhibitors of estrogen binding to its receptors, and have mixed agonist and antagonist activity, depending on the target tissue.[5] Tamoxifen is the most well-studied SERM and often the drug of choice for breast cancer treatment. Its mechanism of action involves tumor cell growth inhibition through competitive ER inhibition.[6]

The benefits of tamoxifen have been consolidated through the US Financial Service Task Force (USPSTF) meta-analysis.[7] In comparison with placebo, the use of tamoxifen resulted in: reduced risk of invasive breast cancer (Relative Risk – RR = 0.70; 95% Confidence Interval – 95%CI: 0.59-0.82); reduction in the incidence of non-vertebral fractures (RR = 0.66, 95%CI: 0.45-0.98); and no difference in mortality from breast cancer or from all causes. On the other hand, a pro-coagulant effect is described when tamoxifen is added to chemotherapy – especially an increase in thromboembolic events.[8,9]

Raloxifene differs from tamoxifen because it does not stimulate endometrial tissue, although it exerts the same beneficial effects of tamoxifen on breast tissue. In preclinical studies, raloxifene has been shown to prevent the onset of new breast cancers, as well as prevent the growth of preexisting cancers.[10] In the STAR (Study of Tamoxifen and Raloxifene) study,[11] 19,747 women were randomized to receive 20 mg of tamoxifen or 60 mg/day of raloxifene for 5 years. The results showed that raloxifene had the same efficacy as tamoxifen in the prevention of breast cancer *in situ*, both with a 50% risk reduction (RR of 1.02, 95%CI: 0.82-1.28). However, raloxifene did not show protection against invasive types of breast cancer, whereas tamoxifen reduced its incidence by around 50%. It was observed that the group treated with raloxifene had an almost 30% reduction in thromboembolic events such as Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE) (RR = 0.70, 95%CI: 0.54-0.91). Both groups had the same incidence of cerebrovascular accident, myocardial infarction and fractures.

The MORE (Multiple Outcomes of Raloxifene Evaluation)[12] study randomized 7,705 postmenopausal patients who had osteoporosis and had no history of breast or endometrial cancer for the use of placebo or 60 mg/day or 120 mg/day of raloxifene. After 4 years of follow-up, a 72% reduction of breast cancer risk was observed.[13] In the CORE (Continuing Outcomes relevant to Evista) study,[14] the patients were randomized to either raloxifene 60 mg/day or placebo. A 59% reduction (RR = 0.41, 95%CI: 0.24-0.71) was observed in the incidence of breast cancer and a decrease of 66% (RR = 0.34, 95%CI: 0.18-0.66) of ER-positive invasive breast cancer, when compared with the placebo group. When analyzing both studies together, the incidence of invasive breast cancer was reduced by 66% (RR = 0.34, 95% CI: 0.22-0.50) and, for ER-positive cases, 76% (RR = 0.24, 95%CI: 0.15-0.40), relative to the placebo group. No protection was observed against non-invasive cancers.

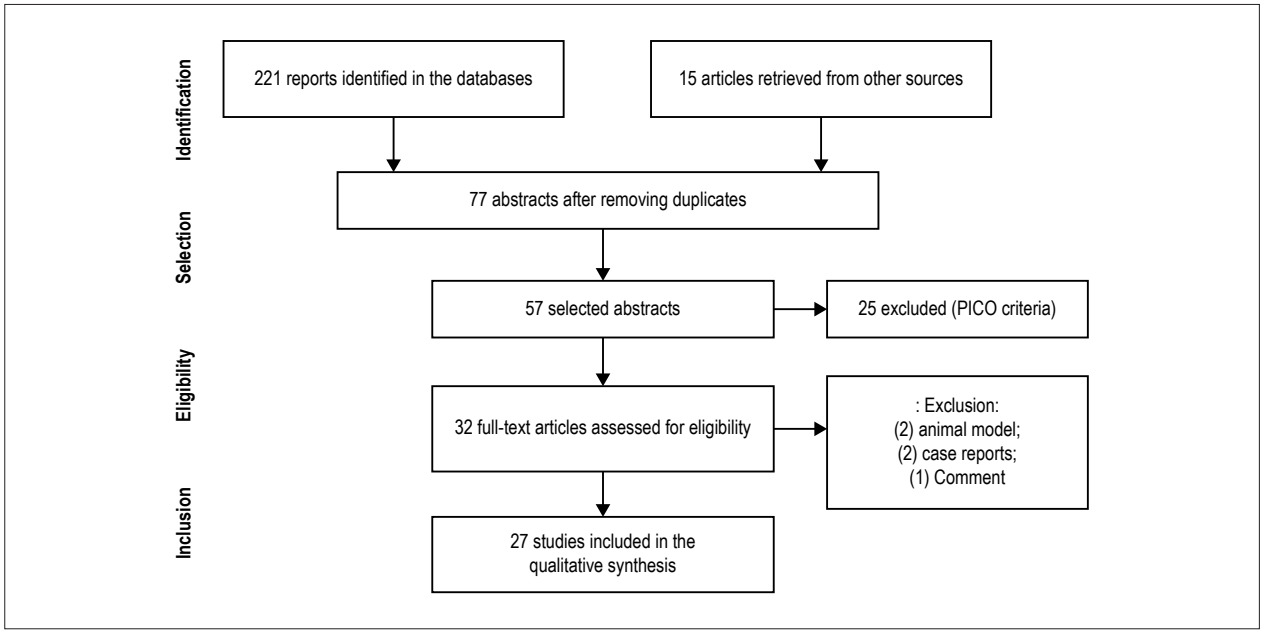

Figure 1 – *Flowchart of the evaluated studies.*

A significant reduction in the amount of microvessels in breast cancer was observed after treatment with raloxifene 60 mg/day for 28 days in postmenopausal women without previous endocrine treatment.[15] There is evidence that the benefits of treatment with SERMs were seen not only during the 5 years of active treatment, as well as 5 years after the end of treatment, indicating a long-term effect on the prevention of breast cancer. Adverse effects, notably the thromboembolic events and endometrial cancer, should be considered when assessing the risk-benefit ratio for each patient.[16]

Selective estrogen receptor modulators and thromboembolic events

A number of studies have demonstrated that the use of tamoxifen is associated with an increased rate of venous thromboembolic events (VTE) and that there is an additional procoagulant effect when tamoxifen is added to chemotherapy.[6,8,9] Raloxifene is also associated with a higher risk of VTE, but with a lower incidence than tamoxifen. The NSABP (National Surgical Adjuvant Breast and Bowel Project) Tamoxifen Prevention Trial[9] allocated 13,388 women at high risk of breast cancer to receive tamoxifen or placebo. The incidence of PE and DVT increased in women who received tamoxifen, especially in patients older than 50 years (RR for PE = 3.0, 95%CI: 1.1-11.2, RR for DVT = 1.6; 95%CI: 0.9-2.9). The IBIS-1 (International Breast Cancer Intervention Study)[8] allocated 7,154 women at risk for breast cancer to receive tamoxifen or placebo. The use of tamoxifen was associated with an increased risk of developing VTE (Odds Ratio – OR = 2.1, 95%CI: 1.1-4.1). The risk of developing PTE or PE was significantly higher during the 5 years of active treatment with tamoxifen (RR of 2.3; 95%CI 1.4-3.9) but did not persist after its cessation.

A meta-analysis of seven trials and 30,023 patients, which compared outcomes in women with breast cancer assigned to treatment with tamoxifen or an aromatase inhibitor, found a higher rate of VTE in those receiving tamoxifen (2.8% vs. 1.6%).[16] An analysis of 13 trials of the NSABP,[17] which evaluated the risk of contralateral breast cancer in 20,878 women who received tamoxifen after primary treatment for this disease, found an increased risk of VTE with tamoxifen. The risks of PE, DVT and superficial phlebitis increased two to three-fold in patients treated with tamoxifen, and 11 to 15-fold in patients treated with tamoxifen plus chemotherapy.[18] The STAR (Study of Tamoxifen and Raloxifene)[11] study suggested a lower incidence of DVT and PE in women receiving raloxifene vs. those treated with tamoxifen. This study randomized 19,747 women at risk for breast cancer to raloxifene and tamoxifen use for 5 years.

Selective estrogen receptor modulators and cerebrovascular accident

In the EBCTCG (Early Breast Cancer Trialists' Collaborative Group)[6] meta-analysis, which compared 21,457 women to receive tamoxifen or placebo, there was an increase in cerebrovascular accident (CVA) rates, but without statistical significance. In a case-control study of 11,045 women with breast cancer, the risk of CVA was not increased by the use of tamoxifen.[19] In a meta-analysis that evaluated the use of tamoxifen in primary or secondary prevention in 39,601 breast cancer patients, the frequency of ischemic CVA was higher in those who received tamoxifen than in the controls.[20] Tamoxifen was associated with an increased risk of CVA, but with a low absolute risk.

In the RUTH (Raloxifene Use for The Heart) study,[21] raloxifene was associated with an increased risk of fatal CVA when compared with placebo. The IBIS-1 study[22] did not show statistical significance between the treatment groups (tamoxifen vs. placebo) regarding cerebrovascular or cardiovascular events. A sub-analysis of the MORE[12] study suggested that in women at high risk for arterial events, raloxifene reduced the incidence of coronary events and CVA. However, after 8 years of treatment, the incidence of cardiovascular, coronary, or cerebrovascular events did not significantly differ between the raloxifene and placebo groups. In the STAR study,[11] the risk of CVA was similar in the raloxifene and tamoxifen groups.

Selective estrogen receptor modulators and lipid profile

There is evidence of changes in the lipid profile with the use of SERMs. The reduction of serum total cholesterol and low-density lipoprotein cholesterol (LDL-c) levels is a consensus. However, an increase in serum triglyceride levels has also been reported. Sawada and Sato[23] reported that tamoxifen reduced total and LDL cholesterol levels, as well as significantly increased triglycerides. Atalay et al.[24] did not find a significant effect of tamoxifen on total cholesterol or high-density lipoprotein-cholesterol (HDL-c) but reported a borderline increase in triglycerides. Taken together, these studies suggest that although tamoxifen consistently lowers LDL-c levels, the effects on HDL-c are mild, and tamoxifen use increases serum triglyceride levels. Changes in the lipid profile associated with the use of tamoxifen are summarized in Table 1.

Selective estrogen receptor modulators and coronary artery disease

Even after consolidation of the clinical use of tamoxifen, there is no definitive evidence of its effect on coronary

Table 1 – Tamoxifen and lipid profile

Changes	Total cholesterol	LDL-c	HDL-c	Triglycerides
Tamoxifen	Reduction	Reduction	Mild alteration	Increase

LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol.

artery disease (CAD). Evidence suggests a modest protective effect of tamoxifen against death from CAD. There is a controversy over the effects of SERMs on atherosclerosis and its complications (Table 2). The publication of the RUTH (Raloxifene Use for The Heart) study[21] confirmed a neutral effect of raloxifene. Evidence available in the world literature suggests neutral effects or discrete benefits of SERM use in overall cardiovascular risk.[25]

In the NSABP study,[26] 13,388 women at increased risk of breast cancer were assigned to receive tamoxifen 20 mg/day or placebo. Cardiovascular follow-up was available for 13,194 women, of which 1,048 had clinically manifest CAD. The rates of cardiovascular events were not significantly different between women receiving tamoxifen and those receiving placebo, regardless of the preexisting disease. A case-control study of women diagnosed with breast cancer found that the use of tamoxifen was not associated with a reduced risk of myocardial infarction for the observed 137 cases of myocardial infarction.[27] Another case-control study demonstrated that women with breast cancer who received tamoxifen had a reduced risk of angina pectoris or myocardial infarction (OR = 0.4, 95%CI: 0.2-0.7) compared to patients who did not receive it.[28]

Nordenskjold et al.[29] reported a significant reduction in mortality due to CAD in women who received 5 years vs. 2 years of tamoxifen, with a higher dose of 40 mg/day. The study carried out by the Early Breast Cancer Trialists' Collaborative Group[30] reported a reduction in mortality from CAD in more than 15,000 women randomized to receive approximately 5 years of tamoxifen vs. placebo, although there was no statistical significance (120 vs. 132 deaths, p = 0.06).

Selective estrogen receptor modulators and aspirin

Cancer can lead to a state of hypercoagulability, platelet abnormalities and thromboembolic events. Platelets can contribute to the metastasis process by promoting angiogenesis and by releasing the Vascular Endothelial Growth Factor (VEGF).[31,32] the platelets and coagulation cascade components involve tumor cells, which prevents lysis by natural killer cells, allowing the spread of metastases.

Tamoxifen rapidly increases free calcium in human platelets.[33,34] Jhonson et al.[35] demonstrated that tamoxifen and its metabolite 4-hydroxytamoxifen altered the platelet function, with a reduction in the angiogenic and metastatic potential. Angiogenic proteins are released during the platelet activation process, and platelet deposition is observed at the tumor site.[36] The alpha and beta forms of ER were found in the platelet membrane.[37,38] Some studies have suggested that estradiol, as well as the tamoxifen metabolites, can increase platelet aggregation, suggesting that ER function may influence the release of intraplatelet proteins, such as VEGF and endostatin, when platelets are stimulated in the tumor environment.[39]

Holmes et al.[40] carried out a study that evaluated the concentrations of VEGF and endostatin before and after tamoxifen or aromatase inhibitors in 30 women with breast cancer. Tamoxifen therapy resulted in increased VEGF concentrations in platelets, but no change in plasma VEGF levels. The use of aspirin attenuated the increase in the VEGF levels associated with tamoxifen and reduced serum levels of VEGF. The data from this study suggest that antiplatelet therapy may interfere with angiogenic protein levels in women treated with endocrine therapy.

Women with breast cancer who used tamoxifen and 45 days of aspirin had reduced intraplatelet VEGF levels, as well as increased serum and intraplatelet levels of the antiangiogenic factor thrombospondin-1.[41] These changes were reversed with the aspirin discontinuation. In this study, a dose of 325 mg/day was used. Aspirin decreased the pro-angiogenic effects of tamoxifen, suggesting that antiplatelet therapy may improve tamoxifen efficacy.

Table 2 – Events associated with the use of selective estrogen receptor modulators (SERMs)

Study, year	Type of study	Patients (n)	Assessed/ compared SERMs	Breast cancer	VTE	CVA	CAD
STAR, Vogel et al.[11] 2006	Clinical trial	19,747 postmenopausal women	Tamoxifen and raloxifene	Risk reduction of 50% (*in situ* - tamoxifen and raloxifene and invasive - tamoxifen)	Increase, raloxifene < tamoxifen of 30%	Reduction (tamoxifen and raloxifene)	Increase (tamoxifen and raloxifene)
MORE, Cauley et al.[13] 2001	Clinical trial	7,705 postmenopausal women	Raloxifene and placebo	Risk reduction of 72% after 4 years	Increase	Neutral	Neutral
CORE / Martino et al.[14] / 2004	Clinical trial	5,213 postmenopausal women	Raloxifene and placebo	Risk reduction of 59%	Increase		
NSABP / Fisher et al.[9] / 1998	Clinical trial	13,388 at risk for breast cancer	Tamoxifen and placebo	Risk reduction of 49%	Increase	Increase	Neutral
IBIS-1 / Cuzick et al.[8] / 2002	Clinical trial	7,152 at risk of breast cancer	Tamoxifen and placebo	Risk reduction of 32%	Increase	Neutral	Neutral
RUTH / Barret-Connor et al.[21] / 2006	Clinical trial	10,101 postmenopausal women	Raloxifene and placebo	Invasive cancer risk reduction of 55%	Increase of 44%	Increase of 49%	Neutral

VTE: venous thromboembolism; CAD: coronary artery disease.

Cheng et al.[42] carried out a study that showed that aspirin not only inhibits the growth of the MCF-7 RE-positive breast cancer cell line, but also has a potential function to overcome resistance to tamoxifen in MCF-7/TAM cell lines. The concomitant action of aspirin makes cells more sensitive to tamoxifen, indicating that aspirin can regulate proteins to overcome tamoxifen resistance.

The RUTH[21] study evaluated the effects of antiplatelet therapy concomitant with the use of raloxifene regarding the risk of VTE. The increased risk of VTE with raloxifene when compared to placebo was not different between the women who used antiplatelet agents and those who did not use it.[43] The key findings of the abovementioned studies are summarized in Table 3.

Aspirin and Cancer Prevention

A prospective observational study of 4,164 women with breast cancer showed that, among women who were alive at least 1 year after the breast cancer diagnosis, the use of aspirin was associated with a reduction in the risk of recurrence and death from breast cancer.[44] Contrarily, another study with 27,426 women with breast cancer showed that there was no association between aspirin use and death from breast cancer.[45]

A retrospective cohort study was carried out in Taiwan with 148,739 diabetic women, of which 27,378 used aspirin at a dose ranging from 75 mg to 165 mg/day, which were compared to women who did not use aspirin. Overall, aspirin use reduced the risk of breast cancer by 18% (Hazard Ratio – HR= 0.82, 95%CI: 0.71-0.94). Specifically, a cumulative dose of aspirin > 88,900mg was observed to reduce the risk of breast cancer by 47% .[46] A cohort in Scotland identified 4,627 women with breast cancer throughout 11 years. The use of aspirin after the diagnosis was identified in 1,035 women (22.4%). Most of them used a 75 mg dose/day. It was concluded that low-dose aspirin was associated with reduced risk of death from all causes and breast cancer.[47] Another cohort, with 27,616 postmenopausal women, identified 938 cases of breast cancer in 6 years of follow-up, meaning a RR of 0.71 (95% CI: 0.58-0.87) for those who took aspirin at least six times a week, when compared with those who did not use the medication.[48] Evidence from case-control and cohort studies suggest an approximately 10% reduction in the risk of breast cancer for aspirin use.[49,50] Similar results were found with other NSAIDs and Cycloxygenase-2 inhibitors (COX-2).[51]

Rothwell et al.[52] analyzed seven randomized trials for the regular use of aspirin with a minimum duration of 4 years to determine the effect of aspirin on the risk of death from cancer. Daily aspirin reduced death rates from several types of cancer during and after the studies. The benefit increased with treatment duration and was consistent in all the different studied populations.

Harris et al.[53] found 393 cases of breast cancer in 32,505 patients after 5 years of follow-up. This study reported a 50% reduction in the incidence of breast cancer using ibuprofen ($p < 0.01$) and 40% with regular aspirin use ($p < 0.05$), suggesting that other NSAIDs may also be effective in breast cancer prophylaxis.

Aspirin emerged as the most likely NSAID for use in chemoprevention due to its benefits also in preventing cardiovascular events. Other NSAIDs have also been studied as adjuvants in the chemoprevention of several types of cancer, especially colorectal, breast and stomach neoplasms, although these drugs do not offer cardioprotection.[54] Mortality reduction is more evident in colon cancer, probably in prostate and possibly also in breast neoplasms.[55,56]

Discussion

The clinical trials mentioned in this review report an increase in VTE with the use of SERMs and, regarding cerebrovascular and coronary events, the results were discordant. The currently used treatment, consisting of chemotherapy and hormone therapy, has reduced breast cancer mortality, but morbidity

Table 3 – Aspirin and selective estrogen receptor modulators (SERMs)

Author, year	Type of study	Patients (n)	SERMs and/or aspirin	Main Conclusions
Holmes et al.,[40] 2008	Clinical trial	30	Tamoxifen or aromatase inhibitor + ASA	ASA attenuated the increase in VEGF associated with tamoxifen
Holmes et al.,[44] 2010	Prospective Cohort	4,164	ASA	Reduction in the recurrence and death from breast cancer
Holmes et al.,[41] 2013	Clinical trial	12	Tamoxifen + ASA	Reduction in VEGF and increase in TSP-1
Holmes et al.,[45] 2014	Case-control	27,426	ASA	There is no benefit during end-stage illness
Yang et al.,[46] 2017	Retrospective Cohort	148,739	ASA	Reduction in breast cancer risk in diabetics
Fraser et al.,[47] 2014	Cohort	4,627	ASA	Reduction in death risk from all causes
Jhonson et al.,[48] 2002	Prospective Cohort	27,616	ASA	Reduction in breast cancer risk
Harris et al.,[53] 1999	Prospective Cohort	32,505	ASA/ibuprofen	Reduction in breast cancer risk
Duvernoy et al.,[43] 2010 (RUTH Trial)	Clinical trial	10,101	Raloxifene + ASA	Did not change the risk of VTE

ASA: acetylsalicylic acid; VEGF: vascular endothelial growth factor; TSP-1: thrombospondin 1; VTE: venous thromboembolism.

and mortality are still high, with considerable side effects and high financial costs. There are great expectations regarding new treatments, with low toxicity and cost reduction.

Aspirin has been incorporated into clinical practice for over 100 years at a low cost, making it attractive as a potential adjunct treatment. The observational studies included in this review suggest a reduction in the risk of breast cancer in patients regularly taking aspirin. Randomized clinical trials are required to assess the impact of aspirin use on breast cancer prevention, whether associated with endocrine therapy or disease-free survival in breast cancer patients.

Aspirin use is a consensus for the secondary prevention of myocardial infarction and ischemic CVA in patients with pre-existing cardiovascular disease and for primary prevention in high-risk groups. Current indications for the prophylactic use of aspirin are based on cardiovascular risk, considering the side effects, especially gastrointestinal bleeding, of which incidence increases with age. Other potential benefits of using aspirin need to be proven in the context of cancer.

Conclusion

Breast cancer is the most frequently diagnosed tumor in women worldwide, with a significant impact on morbidity and mortality. Although there are controversies in the analyzed studies, considering the possible benefits regarding breast cancer prevention and reduction in cardiovascular events, this systematic review suggests that therapy with selective estrogen receptor modulators and aspirin should be better investigated, and emphasizes the need for randomized trials. Future studies should address issues such as dose, age at the start, duration, efficacy, and safety of a clearly defined treatment regimen.

Author contributions

Conception and design of the research and Acquisition of data: Leite AM, Martins WA; Analysis and interpretation of the data: Leite AM, Macedo AVS, Jorge AJL, Martins WA; Writing of the manuscript: Leite AM; Critical revision of the manuscript for intellectual content: Macedo AVS, Jorge AJL, Martins WA.

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Downstream Change of the Primary Endpoint in the ISCHEMIA Trial: the Elephant in the Room

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The ongoing ISCHEMIA trial (International Study of Comparative Health Effectiveness with Medical and Invasive Approaches, NCT01471522),[1,2] aims to overcome the limitations of previous trials to identify the best management strategy for patients with stable ischemic heart disease (SIHD). Percutaneous coronary intervention (PCI), the most common form of coronary revascularization, and coronary artery bypass grafting (CABG) have been used successfully to improve angina symptoms and quality of life in patients with severe coronary obstructions.

The findings of recent trials such as COURAGE (Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation), BARI 2D (Bypass Angioplasty Revascularization Investigation 2 Diabetes), and FAME 2 (Fractional Flow Reserve versus Angiography for Multivessel Evaluation) fail to show any difference in mortality or myocardial infarction (MI) between patients with SIHD who were treated invasively compared with those who were treated with optimal medical therapy (OMT). However, these trials had a substantial proportion of patients with no significant myocardial ischemia, potentially underestimating the beneficial effect of revascularization. Therefore, the ISCHEMIA trial was set to resolve this issue by including only patients with moderate-to-severe ischemia inducible at stress imaging.

The primary aim of the ISCHEMIA[1,2] trial is to test the hypothesis that the use of a routine invasive strategy with cardiac catheterization followed by revascularization (PCI or CABG) plus OMT is superior to a conservative strategy of OMT, with cardiac catheterization and revascularization reserved for those who fail OMT. ISCHEMIA is a major study funded by the National Institutes of Health and is the largest clinical trial comparing alternative treatment strategies in patients with SIHD. With an average follow-up period estimated to be about 3.5 years (minimum of 12 months), the trial was designed to provide at least 83% power (with a two-sided alpha = 0.05) to detect an 18% relative reduction in the composite primary endpoint (from 20% to 16.4%) in the invasive strategy compared with the conservative strategy. Enrollment began in mid-2012 and ended recently (January 31, 2018) with 5,179 participants randomized in 328 sites in 37 countries.[1,2]

Keywords

Myocardial Ischemia/physiopathology; Cardiac Catheterization; Myocardial Revascularization; Treatment Outcom; Evaluation Studies.

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Modification of the Trial Protocol

Recently we learned that the protocol of the ISCHEMIA trial published on ClinicalTrials.gov had a major modification in January 2018, only 11 months before the estimated completion of this 7-year trial. The primary endpoint was changed from the composite of cardiovascular death or nonfatal MI (2012 version) to a 5-component endpoint that also includes resuscitated cardiac arrest, hospitalization for unstable angina and hospitalization for heart failure (January 2018 version).[1,2]

Then, in February 2018, the full version of the 2012 ISCHEMIA protocol was published on ClinicalTrials.gov. This version of the protocol stated that if the incidence of the primary endpoint pooled across randomized groups is lower than expected, an independent advisory panel can decide to extend the follow-up period or change the primary endpoint.

Strict adherence to study protocol is a cornerstone of the trial methodology. Extending follow-up improves precision towards the true effect and conserves the primary hypothesis of the trial. By contrast, the current wisdom in randomized clinical trials is that once the primary endpoint is selected, the trial should proceed with no further changes. Although trialists recognize that there may be appropriate reasons for modifying endpoints after the trial has started,[3] evidence suggests that such changes often appear to favor the intervention group,[4] raising the risk that failure to adhere to predetermined endpoints can inflate type I errors.

A change in primary endpoint is considered appropriate and unbiased when the decision is based on external information, independent of the trial data, such as the results of other studies. On the other hand, modification based on data from the trial itself will have a detrimental effect on the validity of the trial's findings. A decision based on the pool incidence of events permits reasonable prediction of the main result and induces operational bias.[3]

The Elephant in the Room

The decision to modify the primary endpoint of ISCHEMIA discards the original hypothesis and misses the elephant in the room: the incidence of death or MI was lower than expected, suggesting that the prognosis of patients with stable coronary disease is quite satisfactory. Such a good

overall prognosis may reduce statistical power, but also makes futile the choice for an invasive procedure expected to protect patients from an unlikely outcome, at the expense of physical and mental stress, unintended consequences and monetary costs. In fact, the need for a higher than expected statistical power indicates the intention to detect an absolute risk reduction that may be clinically irrelevant.

The new components added to the composite primary endpoint also have implications for the trial's findings. The original outcomes of cardiovascular death or MI are unequivocal, whereas hospitalization for angina or heart failure is mediated by a physician's reaction to a clinical scenario. In an open study such as ISCHEMIA, it is possible that the knowledge that the patient was not revascularized could lower the physician's threshold for admitting patients due to symptoms.

Although unstable angina and MI belong to the same spectrum of pathophysiological processes collectively described as acute coronary syndromes, the diagnosis of unstable angina involves significant subjectivity on the part of the treating clinician, the investigator, and adjudication committees.[5] Additionally, the prognostic relevance of unstable angina is much lower than that of MI and, of course, cardiovascular death. Therefore, the inclusion of hospitalization for unstable angina in the composite primary endpoint is susceptible to ascertainment bias and may alter the results towards a benefit for the routine invasive strategy.

Heart failure is a heterogeneous syndrome related not only to atherosclerosis but also to hypertension, renal disease, and other causes that are generally not discernible from the records available to the study adjudicators. It is also often difficult to distinguish heart failure from other causes of acute dyspnea.

In conclusion, it is highly questionable whether improving statistical power at the cost of impairing validity and relevance justifies this protocol modification. Changing the primary endpoint of a trial often evokes cynicism from the medical community and a study that uses a less relevant end point may not provide answers to clinically important questions. Time will tell whether such a strategy was a wise decision.

Author contributions

Conception and design of the research, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Correia LCL, Rassi Junior A.

Potential Conflict of Interest

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

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Case 4 – A 59-Year-Old Woman with Rheumatic Mitral Valve Disease (Severe Stenosis and Regurgitation), Severe Dyspnea, Shock and Pulmonary Condensation

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A 59-year-old female patient with double mitral lesion was hospitalized with fever, cough and worsening dyspnea with shock.

At 58 years old, the patient reported onset of dyspnea in medium exertions for five months, associated with dry cough at night with dyspnea, which was relieved with orthostatism. Cardiac murmur was detected and the patient was referred to InCor, a heart specialist hospital, for treatment (17/Sept/2010).

There was no reference to rheumatic outbreaks in the past, and the patient had arterial hypertension and hypothyroidism.

Physical exam when the patient was first examined (17/Sep/2010) showed the patient weighed 73 Kg, was 1.55 m tall, body mass index was 30.6 kg/m^2, cardiac frequency was 88 bpm, arterial blood pressure 140 x 90 mmHg; pulmonary auscultation resulted normal; cardiac auscultation revealed hypophonic 1st heart sound, hypophonic pulmonary component of 2nd heart sound and mitral holosystolic murmur ++++/6+; abdomen exam resulted normal; there was no edema in the lower limbs and pulse palpation was normal.

The ECG (14/Sep/2010) showed sinus tachycardia, with 127 bpm frequency, PR interval 200 ms, dQRS 92 ms, SÂQRS + 150º reverse, QTc 459 ms, overload of the left atrium and indirect signs of overload of the right atrium (Peñaloza-Tranchesi signal), low-voltage front plane and overload of the right ventricle (Figure 1).

Laboratorial exams (14/Sep/2010) showed red blood cells 5.0 million/mm^3, hemoglobin 14.6 g/dL, hematocrit 45%, creatinine 1.08 mg/dL (FG = 55L/min/1.73 m^2), potassium 4.4 mEq/L and sodium 142 mEq/L.

The echocardiogram (25/Aug/2010) revealed aortic diameter 25 mm, left atrium 52 mm, right ventricle 44 mm, left ventricle 34/21 mm, ejection fraction 70%, septum thickness and posterior wall 11 mm; there was no alteration in segment contraction of the left ventricle; right ventricle's systolic function was normal; mitral valve presented thickened cusps with commissural fusion and reduced opening, compatible with a severely compromised rheumatic condition, and there was significant valve insufficiency. The maximum diastolic gradient between the left atrium and the ventricle was estimated at 30 mm Hg, and the medium, at 18 mm Hg; the aortic valve showed discrete signs of fibrocalcification without functional alterations; the tricuspid valve had severe insufficiency. Pulmonary artery systolic pressure was estimated at 140 mmHg.

Losartan 100 mg, furosemide 40 mg, digoxin 0,25 mg and acetylsalicylic acid 100 mg daily were prescribed.

Surgical treatment of the mitral valve was indicated.

In December 2010 the patient sought emergency medical attention due to tachycardia and dyspnea.

The ECG (16/Dec/2010) revealed nodal reentrant tachycardia, with 178 bpm frequency (Figure 2). The patient underwent chemical cardioversion with intravenous amiodarone.

At the outpatient ward (5/Apr/2011) the patient was asymptomatic, with controlled blood pressure (120/80 mmHg) and heart rate of 84 bpm, and the physical exam resulted normal, except for preexisting alterations in the cardiac auscultation. The patient used 200 mg of amiodarone, 40 mg of furosemide, 100 mg of losartan and 60 mg of diltiazem.

The patient continued waiting to be operated and on 16/Sep/2011 she sought emergency medical care, with dyspnea in small exertions and productive cough with purulent sputum, and no fever was reported.

The physical exam showed a sleepy patient, with cold extremities and a heart rate of 98 bpm, blood pressure 93 x 58 mmHg. Pulmonary auscultation revealed crackling rales in the lower third of both hemithorax; cardiac auscultation revealed rhythmic heart sounds, mitral regurgitation systolic murmur +++/6+ and diastolic arrhythmia ++/6+; the abdomen had no abnormalities and there was edema ++/4+ in the lower limbs.

The ECG (16/Sep/2011) showed sinus rhythm with 97 bpm frequency, PR 168 ms, dQRS 89 ms, SÂQRS + 150º reverse, QTc 513 ms, biatrial overload, giant P wave positive at V1 and right ventricular overload (Figure 3).

The echocardiogram (17/Sep/2011) revealed hypokinesia of the right and left ventricles, the latter with 55% ejection fraction, from moderate to strong mitral insufficiency, maximal mitral transvalve gradient at 22 mm Hg and medium, at 13 mmHg. Pulmonary artery pressure was estimated at 81 mmHg; however, the patient had systemic arterial hypotension, 53 mmHg medium pressure.

Keywords

Mitral Valve/complications; Heart Murmurs; Mitral Valve Insufficiency; Arrhythmias, Cardiac; Pulmonary Embolism.

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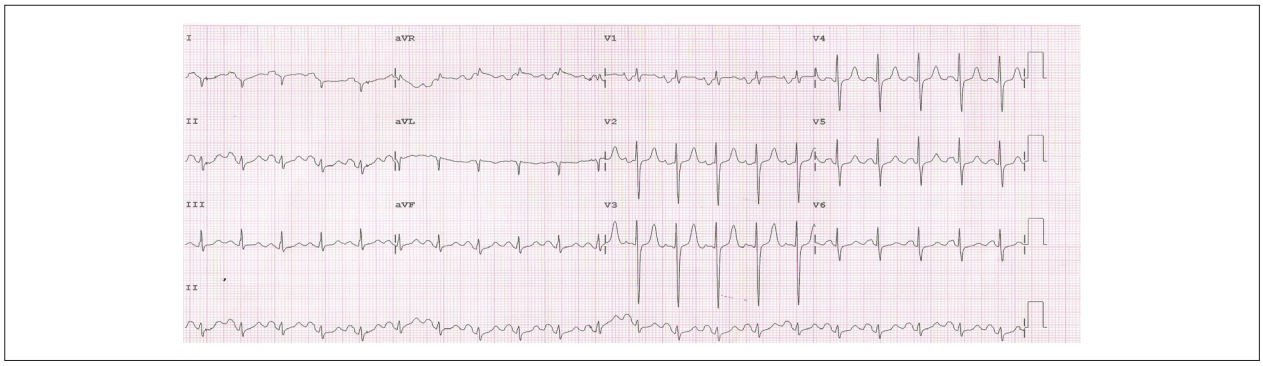

Figure 1 – *ECG. Sinus tachycardia. Overloaded left atrium and indirect signs of overloaded right atrium (Peñaloza-Tranchesi signal) and overloaded right ventricle.*

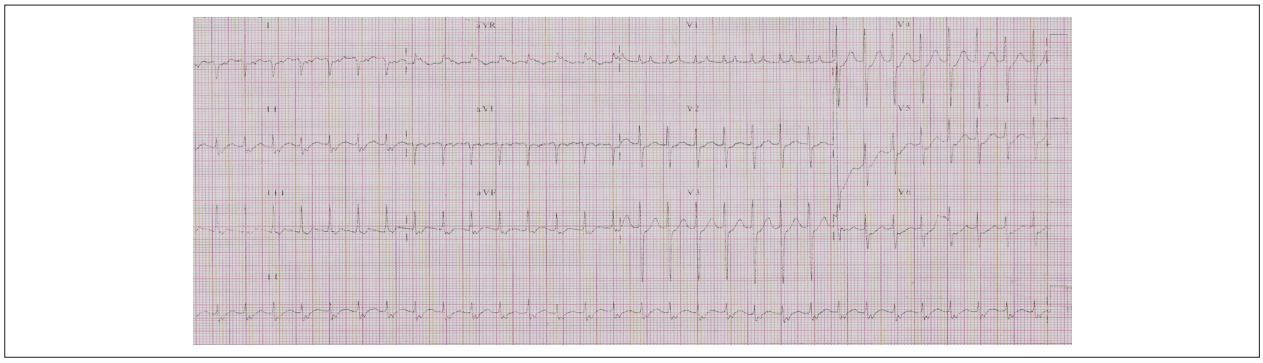

Figure 2 – *ECG. Nodal reentrant tachycardia, retrograde P wave (negative II, III and aVF) after QRS with short PR interval (< 70 ms)*

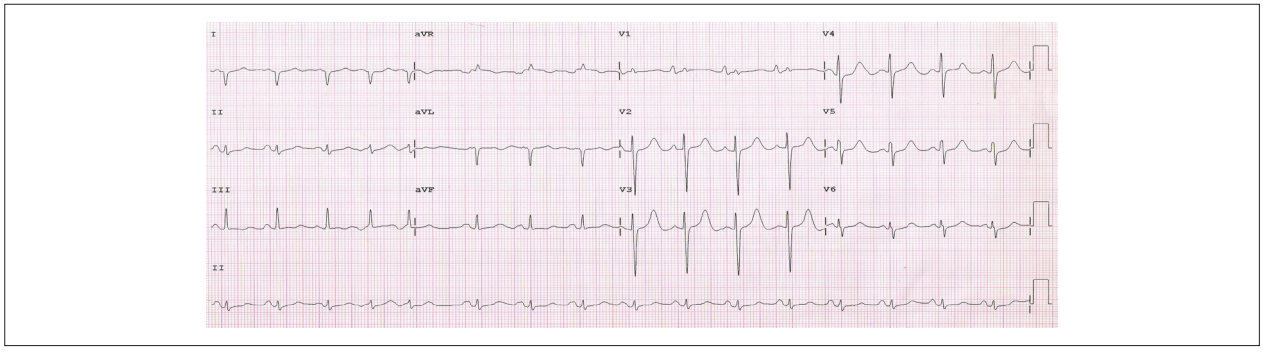

Figure 3 – *ECG. Sinus rhythm; Both atria overloaded with giant P wave positive at V1 and overloaded right ventricle.*

Antibiotics were prescribed (ceftriaxone and clarithromycin), which later were changed for the piperacillin/tazobactam and vancomycin association, in addition to vasoactive amines, oxygen mask, and then orotracheal intubation for respiratory support.

Laboratorial exams (17/Sep/2011) showed: red blood cells 4.2 million/mm^3, hemoglobin 12 g/dL, hematocrit 39%, VCM 93 fL, RDW-CV 17.9%, leukocytes 13840/mm^3 (90% neutrophils, 7% lymphocytes and 3% monocytes), platelets 161000/mm^3, urea 63m/dL, creatinine 1.44 m/dL (FG = 40 mL/min/1.73 m^2), magnesium 1.3 mEq/L, sodium 137 mEq/L, potassium 3.9 mEq/L, prothrombin time (INR) 1.7 and APTT rel 1.26.

Thorax radiography (18/Sep/2011) revealed pulmonary congestion, opacification at the right base and increased cardiac area (presence of double contour and bulging unfolding of the mid aortic arch) (Figure 4).

The coronary angiography (20/Sep/2011) did not reveal coronary obstructions; and there was severe calcification of the mitral valve.

Laboratorial exams (20/Sep/2011) revealed hemoglobin 11 g/dL, hematocrit 36%, VCM 92 fL, RDW-CV 17.8%, leukocytes 13440/mm^3 (90% neutrophils, 7% lymphocytes, 3% monocytes), platelets 142000/mm^3, urea 74 mg/dL, creatinine 1.97 mg/dL (FG = 28 mL/min/1.73 m^2), AST 34 U/L, ALT 34 U/L, calcium 4 mEq/L, magnesium 1.3 mEq/L, arterial lactate 155 m dL.

Exams of 21/Sep/2011 showed hemoglobin 10.5 g/dL, hematocrit 37%, VCM 102 fL, RDW-CV 16.8%, leukocytes

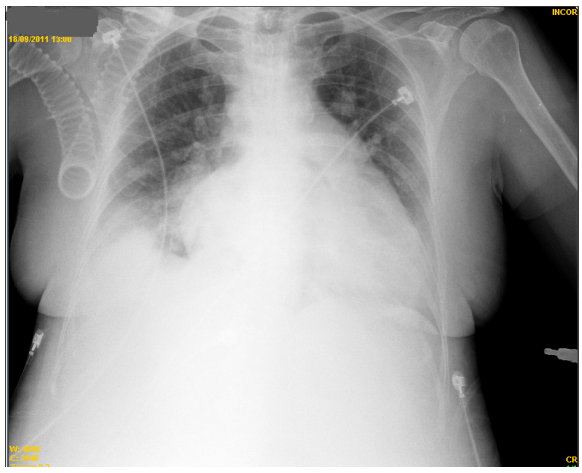

Figure 4 – *Chest X-ray. Pulmonary congestion, opacification at the right base, and cardiomegaly with double contour (enlargement of the left atrium), and abnormal enlargement or bulging at mid-arch of the aorta (enlargement of the right ventricle).*

17840 mm/mm^3 (93% neutrophils, 5% lymphocytes, 2% monocytes), platelets 148000/mm^3, urea 104 mg/dL, creatinine 2.88 mg/dl (FG = 18 mL/min/1.73 m^2), sodium 145 mEq/L, potassium 4.5 mEq/L, AST 1863 U/L, ALT 426 U/L, gammaGT 87 U/L, alkaline phosphatase 152 U/L, magnesium 1.9 mE L, total bilirubin 4.05 mg/dL, direct bilirubin 3.5 mg/dL, arterial lactate 173 mg/dL; arterial gasometry: pH 7, 16, pCO_2 32.7 mm Hg, pO_2 160 mm Hg, O_2 Saturation 99%, bicarbonate 11mEq/L, base excess (-) 16.2 mEq/L; TP (INR) 3.2; TTPA rel times 3.13.

Thorax radiography (21/Sep/2011) showed hypotransparency in the right pulmonary base and an increase in the cardiac area (presence of double contour and abnormal enlargement or bulging at mid-arch of the aorta) (Figure 5).

During hospitalization the patient developed hemodynamic instability, consumption coagulopathy, and presented cardiac arrest in pulseless electrical activity, with no response to resuscitation maneuvers, and she passed away (16h and 55min; 21/Sep/2011).

Clinical aspects

This is about a 59-year-old woman with double mitral lesions with pulmonary hypertension who, while awaiting surgery, had arterial hypotension, respiratory failure with hypotransparency in the right lung field. It evolved without improvement with vasoactive drugs, orotracheal intubation and antibiotic therapy, and she passed away in electrical activity without pulse.

The etiology of this patient's valve disease should be attributed to rheumatic fever, although there is no description of an acute outbreak of that disease in the patient's medical history. That is not unusual in a rheumatic disease scenario once using echocardiogram raises its frequency from 5 to 10 times when compared to a clinical diagnosis.[1-4]

That difference in frequency between clinical diagnosis and echocardiography may be due to the autoimmune response triggered by molecular mimicry,[5] which may go ahead with predominance of humoral response mediated by Th2 lymphocytes, causing more symptoms and leading more easily to the use of secondary prophylaxis. Others show a predominance of cellular response, mediated by Th1 lymphocytes with milder forms of clinical manifestations, but those where cardiac involvement predominates. Thus, patients who would benefit the most from the use of that prophylaxis fail to do it and are subject to relapses that aggravate valve lesions.[6]

Those subtypes of CD4 + (Th1 and Th2) lymphocytes produce different cytokines, those of Th1-type produce interleukin-2 and interferon-gamma cytokines, and those of Th2 subtype secrete Interleukins 4, 5 and 10.[7-9]

The guidelines of the World Health Organization and the US National Institute of Health (NIH) have defined the diagnosis of rheumatic heart disease by the presence of cardiac murmur consistent with mitral or aortic insufficiency, and echocardiographic evidence of rheumatic valve damage, or history of acute rheumatic fever without echocardiogram done in the acute outbreak.[10]

The predominant clinical onset of the heart rheumatic disease is dyspnea, which occurs between the third and fourth decade in life, mostly in women.[11]

In this case, clinical manifestations happened later, but like in the rheumatic disease, they presented alterations in the mitral valve, the valve most frequently affected in the disease. Mitral regurgitation usually occurs earlier than stenosis, attributed to persistent or recurrent valvulitis.[12]

Despite its earlier onset, mitral regurgitation generally has a longer asymptomatic period due to increased atrial compliance due to its progressive increase, maintenance of cardiac output by dilatation of the left ventricle, and decreased regurgitation fraction in exertions to decrease peripheral resistance in the exertion.

Still on this case, the initial clinical scenario of de-compensation due to the presence of tachycardia, once a shorter diastole is more detrimental to mitral stenosis, suggests

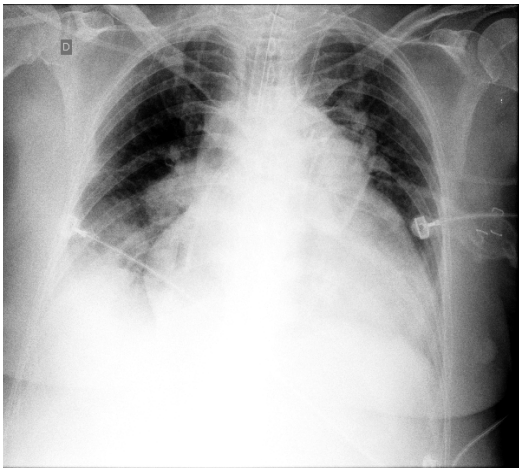

Figure 5 – *Chest X-ray. More penetrated than the previous one. Presence of endotracheal cannula. Pulmonary congestion, opacification at the right base and cardiomegaly with double contour (enlargement of the left atrium) and abnormal enlargement or bulging at mid-arch of the aorta (right ventricle enlargement).*

its predominance. Additionally, the first echocardiogram (2010) was more compatible with the predominance of mitral stenosis with major hemodynamic repercussion, once the dimensions of the left ventricle were normal and there was an increase in the left atrium and in the pulmonary systolic pressure, as well as dilation of the right ventricle.

Echocardiographic criteria for severe stenosis were present, although the valve area was not calculated, which would be less than 1.0 cm^2/m^2 or < 1.5 cm^2, there was fusion of the cusps, in addition to the pressure gradient between the left atrium and the upper ventricle at 10 mmHg, and pulmonary arterial hypertension above 50 mmHg.[13,14]

The indication for percutaneous and/or surgical treatment is based on the presence of symptoms or atrial fibrillation, or systemic embolism, in patients using anticoagulant and moderate or severe mitral stenosis, and in asymptomatic patients with pulmonary systolic pressure ≥ 80 mmHg. Percutaneous valvuloplasty still requires the presence of favorable morphology - mobile thin cusps, free from calcification.

In the second echocardiogram, one year after the accompaniment had started, the predominance of mitral insufficiency was not discarded because there was malfunction of the left ventricle.

The right time of an indication for surgery in patients with severe mitral incompetence has always been controversial due to the symptoms' late onset. Surgical treatment is indicated for the onset of symptoms, of dilatation (systolic diameter > 4.5 cm), or left ventricular dysfunction (ejection fraction < 60%). The guidelines define severe mitral regurgitation based on several parameters: valve morphology - ring dilation ≥ 3.5 cm; characteristics of the regurgitation jet - a rotating jet (multicolor) that reaches the posterior atrial wall, or lateral jet that fills at least 40% of the atrial surface; the *vena contracta* - width of the jet next to the valve ≥ 0.7 cm; effective regurgitation orifice ≥ 0.4 cm^2; regurgitation volume ≥ 60 mL; regurgitation fraction ≥ 50%, ventricular filling pattern – ratio of the mitral valve's velocity-time integral and the aortic valve above 1.3; wave velocity E ≥ 150 cm/s; pulmonary artery systolic pressure ≥ 50 mm Hg; indexed atrial volume 60 mL/m^2; and left ventricle systolic diameter > 4.5 cm.[3,15]

Thus, whichever was the predominance of valvular dysfunction, the patient already had indication for surgical treatment of the mitral valve because there was already severe hemodynamic repercussion and, as it usually happens in valvopathy with a rheumatic origin, there was a double lesion.

Other ways in which the disease may appear are atrial arrhythmias, the most common being atrial fibrillation, embolic events, acute heart failure, or infective endocarditis.

In this case there was de-compensation of heart failure due to nodal reentrant tachycardia, arrhythmia is usually not associated with mitral valvopathy. In mitral stenosis there is usually atrial fibrillation due to dilation and atrial fibrosis, in addition to an inflammatory process in the acute phase (Aschoff nodules).

Atrial phenomena in mitral insufficiency are similar to those of stenosis as interstitial fribrosis and inflammation; however, there is no hypertrophy, myolysis and necrosis of atrial myocytes.[16]

As to the patient's final condition, there are three possible causes: infectious endocarditis, pulmonary infection or pulmonary thromboembolism.

For the diagnosis of endocarditis there would be only fever and de-compensation of heart failure, lacking worsened murmur and vegetation in the valves.

In other words, the involvement of the endocardium in systemic infection has not been proven. Blood culture is not a diagnostic criterion. The clinical criteria of strong suspicion are: new valve injury (insufficiency), embolic events of unknown origin, sepsis of unknown origin, hematuria and fever in a patient who has a prosthesis, previous valvopathy and dental or endoscopic manipulation of the colons, new conduction disorders (atrioventricular blockage due to perivalvular abscess), first episode of cardiac de-compensation, positive

blood cultures, skin complications (Osler spots, Janeway) or ophthalmic complications (Roth), peripheral abscesses (kidneys, spleen).[17]

According to their frequency, the most common complications - heart failure > systemic embolization > stroke > intracardiac abscess.[18]

However, endocarditis cannot be ruled out because in rheumatic valvular disease it can be difficult to diagnose the vegetation.

Infection at any place can be responsible for the de-compensation of heart failure in patients with severe valvopathy. In this case, pneumonia was suspected due to the presence of suggestive image at the right base (Figure 4) and leukocytosis, and antibiotic therapy was introduced; however, there was no change in the clinical situation compatible with the presence of pneumonia.

As the last and most probable cause of the onset of the patient's final hemodynamic status there is pulmonary thromboembolism.

The clinical situation is very non-specific and may be mixed up with acute coronary syndrome or pneumonia. Favoring it there is the image at the base of the right lung and dysfunction of the right ventricle.

In the "International Cooperative Pulmonary Embolism Registry (ICOPER)" pulmonary thromboembolism is associated with the presence of heart failure (hazard ratio 2.4), right ventricle hypokinesia (2.0), systolic arterial hypotension < 90 mmHg (2.9), age > 70 years (1.6), cancer (2.3), chronic obstructive pulmonary disease (1.8). In the same sense, pulmonary thromboembolism with right ventricle hypokinesia doubled the mortality within 3 months.[19]

The exam deemed "gold standard" in the diagnosis of pulmonary thromboembolism is angiotomography, but failing that, or due to the patients' hemodynamic instability, the finding of dilation and right ventricle dysfunction on the echocardiogram can be diagnostic alternatives.

And as to hepatic alterations – elevation of transaminases and disorders in coagulation (elevation of TAP-INR- and relation of APTT times) – they are compatible with ischemic hepatitis with extensive liver necrosis due to low cardiac output in patients with high ventricular diastolic pressures. Its onset takes on average one week after the episode of low hepatic flow leading to centrilobular necrosis.[20] **(Dr. Desiderio Favarato)**

Diagnostic hypotheses: Rheumatic mitral valvopathy (double lesion), pulmonary thromboembolism, cardiogenic shock, multiple organ failure. **(Dr. Desiderio Favarato)**

Necropsy

The exam of the heart showed increased weight (470 g) as well as moderate to significant increase in volume of both atria. The mitral valve showed a lesion characterized by fusion of commissures and marked calcification of the cusps, compatible with rheumatic disease sequelae (Figures 6 and 7). The other valves suffered no significant morphological alterations. The left ventricle had its volume unchanged. There was hemothorax on the left (about 1000 ml). The lungs showed macroscopically thromboembolic vessels in hilar vessels, in addition to purplish-red areas with a firmer consistency in the lower lobes (Figure 8). Histological exam showed recent pulmonary infarctions, organized thrombi in pulmonary arteries, and signs of chronic passive congestion (Figure 9). The wall of the pulmonary veins had thickened, with intimal fibrosis (Figure 10) and hypertrophy of the tunica media of the arteries and arterioles (Figure 11).

In the other organs there were signs compatible with shock, such as, for instance, recent centrilobular hepatic necrosis, acute renal tubular necrosis, and small sub-endocardial infarcts on ventricular walls.

Anatomopathological diagnoses: rheumatic heart disease with mitral valve sequelae (calcified mitral stenosis); chronic passive pulmonary congestion with signs of passive pulmonary hypertension; hemothorax on the left without a defined causal factor.

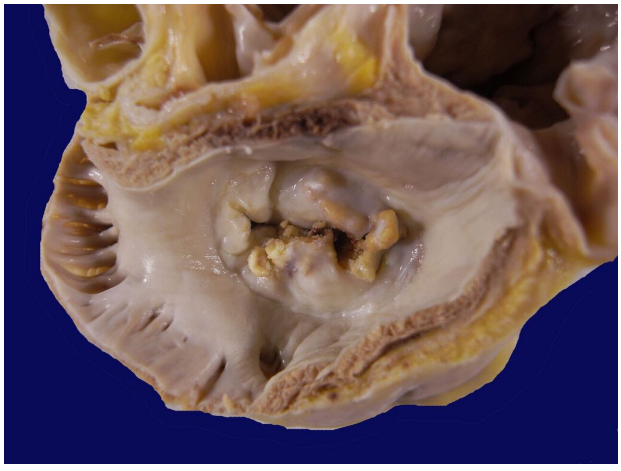

Figure 6 – *Right atrium open showing the mitral valve with fusion of commissures and multiple foci of calcification.*

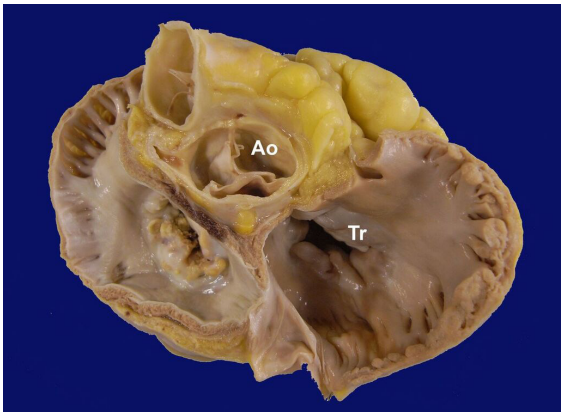

Figure 7 – *Base of the heart from where the atria were removed. Observe that the aortic valve (Ao) is preserved and the tricuspid valve (Tr) shows insufficiency secondary to the ring dilatation.*

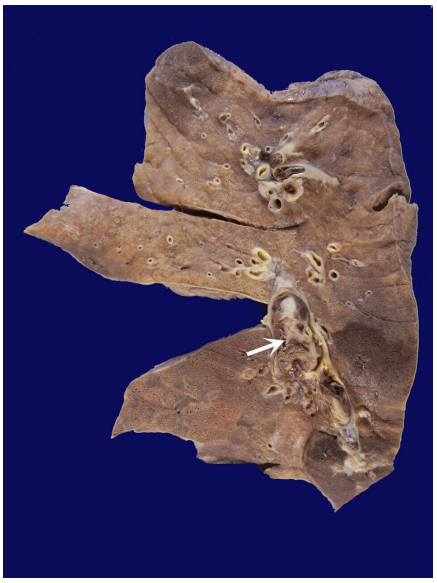

Figure 8 – *Surface of a lung cut with thrombus-plunger in hilar artery (arrow) and purplish-red areas at the base.*

Cause of death: Pulmonary thromboembolism **(Prof. Dr. Vera Demarchi Aiello)**

Comments

The involvement of the mitral valve in this case is typical of rheumatic disease sequelae, and the heart showed signs of de-compensation, such as marked dilation of the atria. Signals of terminal shock were found in the various organs.

The involvement of only the mitral valve is common in rheumatic disease, and it can be found in over 50% of the cases of chronic rheumatic diseases as double dysfunction (stenosis and insufficiency) or just insufficiency.[21]

No lesions were found in arterial or venous thoracic vessels able to explain hemothorax.

The pulmonary situation included recent aspects, such as thromboembolism of hilar vessels and pulmonary infarcts, with other chronic ones, characterized by long-term chronic passive congestion. Passive congestion ends up by causing passive pulmonary hypertension, which starts in the venous territory. The case under discussion presented lesions in venules characteristic of this type of impairment. In the last classification of pulmonary hypertension, this group (of hypertension secondary to lesions of the left heart) is known as pulmonary hypertension group 2.[22] In addition to the lesions in the cardiac valves on the left side, myocardial diseases can also evolve chronically with secondary pulmonary hypertensive involvement. Individuals thus affected can have a troubled evolution in the postoperative period of valve surgery, or that of a cardiac transplantation, when this is a therapeutic option. A recent study by our laboratory revealed that venous lesions of pulmonary hypertension group 2 are frequent and that the appearance of phosphodiesterase 5 in pulmonary vessels of those patients is greater than in the normal ones.[23] **(Prof. Dr. Vera Demarchi Aiello)**

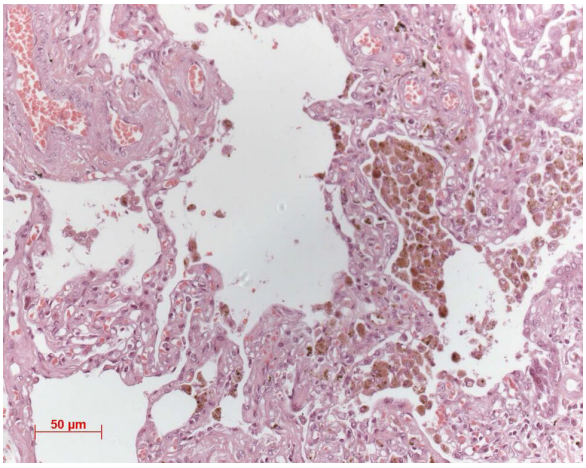

Figure 9 – *Lung photomicrograph showing histiocytes with hemosiderotic pigment in alveolar lumen (cells of the cardiac defect). Hematoxylin-eosin staining, lens with increase = 5X.*

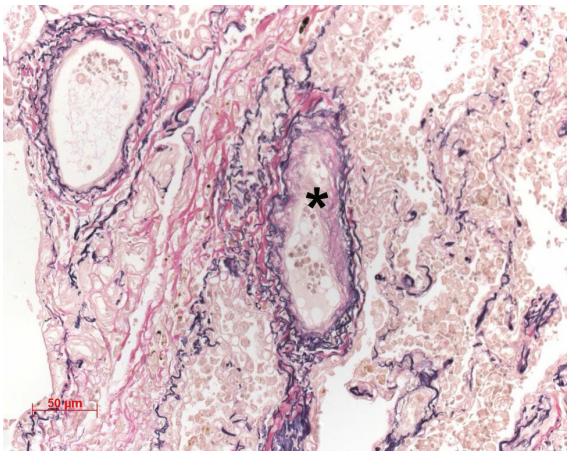

Figure 10 – *Photomicrography of pulmonary venules with fibrotic lesions in the tunica intima (asterisk). Miller elastin staining, 20X macro lens.*

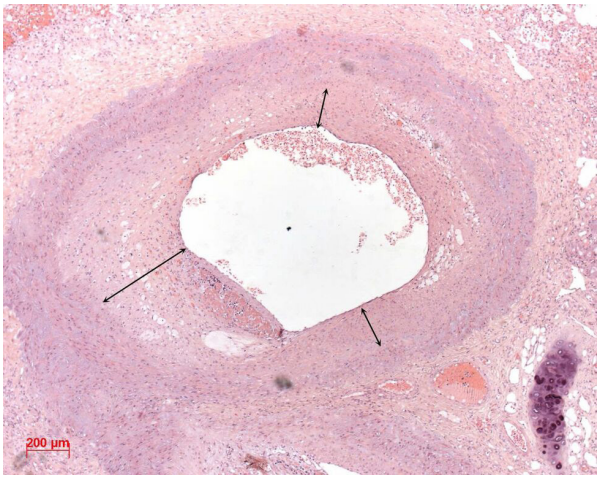

Figure 11 – *Photomicrograph of a muscular pulmonary artery showing tunica media hypertrophy and proliferative lesion of the tunica intima, concentric (double arrows). Hematoxylin-eosin staining, 5X macro lens.*

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Transcatheter Closure of a Traumatic VSD with an ASD Occluder

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Introduction

Traumatic ventricular septal defects (VSD) are exceptionally rare. They can be a consequence of either blunt or penetrating trauma. It is believed that most patients die before reaching the hospital, which makes this condition even more challenging.

Percutaneous closure of traumatic VSD has been presented as an alternative to open conventional surgery.[1] Transcatheter intervention may have some benefits. The objective of this case report is to present a situation where the defect was closed using an Amplatzer septal occluder.

Case Report

A 23-year-old man was admitted in the emergency department after a frontal car collision. He had suffered severe blunt trauma, which included cervical subcutaneous emphysema, bilateral pulmonary contusion, left hemothorax, pneumomediastinum and complex fractures of both femurs. He was in hemorrhagic shock and was immediately taken to the operatory room. After external fixation of both femurs and reaching hemodynamic stability, he was transferred to the Intensive Care Unit. The following morning the presence of a loud holosystolic murmur was noted. The 12-lead electrocardiogram showed only sinus tachycardia. A transthoracic and later a transesophageal echocardiogram (TEE) were performed and both demonstrated a large muscular ventricular septal defect, located in the mid anteroseptal segment with signs of dissection through the basal septum (Figure 1). It measured 19 mm on the left ventricular (LV) side and 7 mm on the right ventricular (RV) side. The peak left to right shunt gradient was estimated in 84 mmHg and the Qp/Qs ratio was estimated in 1.8/1.0. Cardiac catheterization showed limited hemodynamic repercussion (systolic pulmonary artery pressure of 35 mmHg and a Qp/Qs ratio of 1.9/1.0) and the patient remained clinically stable, so a conservative strategy was decided at that time to allow the edges to heal and create a more delimited defect.

He was released after recovering from orthopedic surgery.

Keywords

Heart Septal Defects, Ventricular / complications; Myocardial Contusions; Hemolysis; Heart Septal Defects, Ventricular / surgery.

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Three months later the patient was reevaluated and remained asymptomatic. He repeated cardiac catheterization, which showed a Qp/Qs ratio of 2.95/1.0. Because the shunt increased significantly, it was decided to close the defect percutaneously.

The procedure was done under general anesthesia and guided by transesophageal echocardiography. Cardiac catheterization was performed using the right femoral artery (6-Fr sheath) and vein (7-Fr sheath) and unfractionated heparin was administered. Angiogram of the LV confirmed a VSD with an oblique entry from the LV into the right ventricular outflow tract. The VSD was crossed using a retrograde arterial approach with a floppy guidewire, which was advanced into the pulmonary artery. The guidewire then snared and brought out the femoral venous sheath. This created an arteriovenous loop to allow the delivery of the closure device. A NuMed sizing balloon catheter was subsequently utilized to measure the defect, but it was not possible to maintain it steady. Therefore, the echocardiographic calculations were used to choose the device size. An 8-mm Amplatzer septal occluder was first selected and loaded into the sheath. The device was advanced across the VSD, but prolapsed back to the RV when it was being released. After this failed attempt, a slightly different approach was used. The VSD was crossed once more using the guidewire, this time in the opposite direction into the right subclavian artery. Once again, it was snared to make an arteriovenous loop, but on this occasion pulled out through the femoral arterial sheath. For this second attempt, it was decided to employ a 10-mm Amplatzer septal occluder. The device was advanced through the venous sheath and this time was successfully placed (Figure 2). LV angiogram after the procedure revealed a mild residual shunt and the Qp/Qs ratio reduced to 1.53/1.0.

A transesophageal echocardiography was repeated a month after the procedure, which showed the device well adapted to the defect. Nevertheless, a residual shunt remained in the superior border of the device with a peak gradient estimated in 90 mmHg (Figure 2).

Another complication of this procedure was the appearance of transient self-limited hemolysis. Initial blood analysis showed a LDH value > 2000 UI/L and haptoglobin < 6 mg/dL. The condition remained stable and resolved without the need of blood transfusions.

The patient continued to be asymptomatic and has returned to his previous professional life.

Discussion

There are a few possible mechanisms that explain the development of traumatic VSDs. In this case, cardiac contusion after compression between the sternum and the spine or due to high intrathoracic pressures at impact seems to be the most probable explanation.[1]

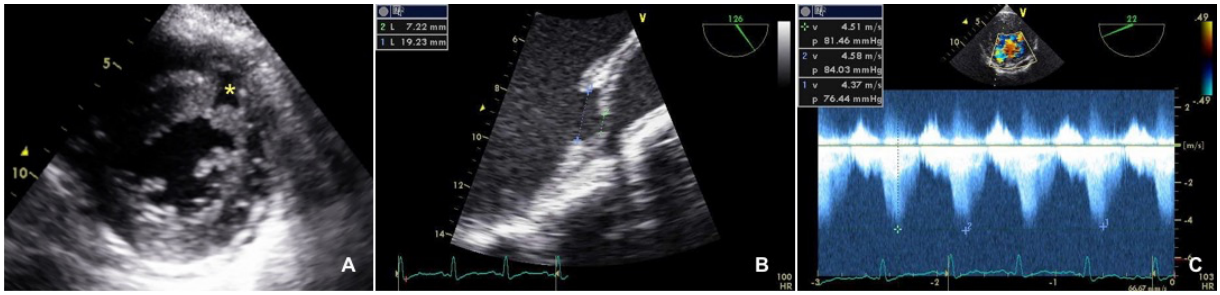

Figure 1 – *Echocardiographic images of the VSD. Panel A: VSD located in the mid anteroseptal segment. Panel B: VSD measuring 19 mm on the LV side and 7 mm on the RV side. Panel C: continuous wave Doppler estimating peak gradient at 84 mmHg.*

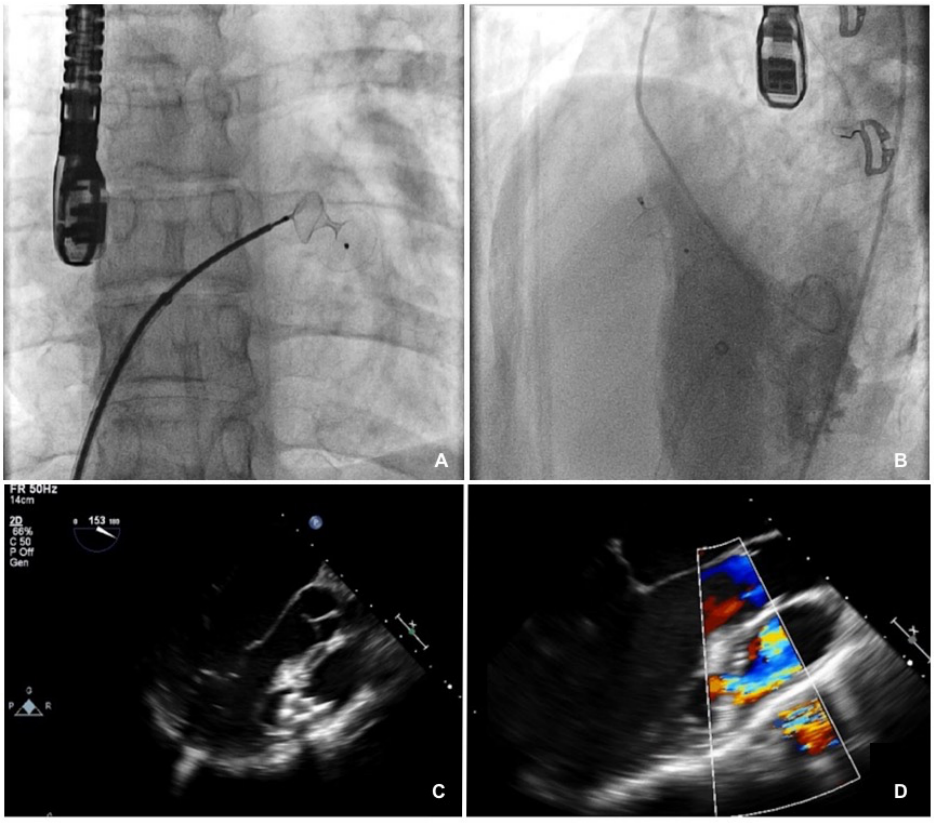

Figure 2 – *10 mm ASD Occluder deployed in the VSD. Panel A: position of the device in the VSD. Panel B: LV angiogram showing mild residual shunt. Panel C and D: TEE displaying the residual shunt through the superior edge of the device.*

During his stay our patient was clinically improving, which led to our decision to delay the intervention. Furthermore, it is known that in VSDs fibrotic tissue facilitates the device placement in elective closures.[2] However, the progressively increasing shunt, led to the decision of closing it. He was initially considered for surgery, but given the risks associated this procedure, the alternative approach was pondered. Transcatheter closure can be a successful substitute with some advantages. It removes cardiopulmonary bypass, avoids arrhythmogenic scar formation related with ventriculotomy and reduces hospital stay and recovery time.

Because these are rare cases with diverse features, it can be challenging to size accurately the defect. In this case, imprecise echocardiographic measurements and the difficulty in operating the sizing balloon catheter, led to the inappropriate choice of the first device.

A possible complication of selecting this type of devices is the appearance of hemolysis. The probable mechanism is the passage of high-velocity turbulent blood flow through

the device, which causes mechanical fragmentation of erythrocytes. Although there are reports of chronic hemolysis, it is usually self-resolving.[3] Like previous cases,[4,5] we encountered the same complication. Our patient remained asymptomatic and the hemolysis resolved without the need of blood transfusions.

Conclusion

Transcatheter devices can be selected as the first choice for closing traumatic VSD. We demonstrate that ASD Occluder can be successfully implanted and that acceptable clinical effectiveness can be achieved.

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Unexpected Mass in the Left Atrium

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A 60-year-old Caucasian female with a history of rheumatic mitral stenosis, permanent atrial fibrillation and chronic lymphocytic leukemia was admitted due to decompensated chronic heart failure. The transthoracic echocardiogram depicted a severe mitral stenosis (anatomic valve area of 0.9 cm^2), mild mitral regurgitation, aneurysmatic left atrium and mildly compromised left ventricular ejection fraction. Given the indication for mitral valve replacement, coronary angiography was performed, revealing an abnormal vascularized mass at the level of the left atrium beyond normal coronary arteries (Panel A). For better characterization an angio-CT was requested. A well-delimited, 7x4x3cm left atrial homogeneous, slight hyperdense mass was observed along the lateral portion of the atrial roof (Panel B and C). The patient underwent both surgical mass resection and mitral valve replacement with an uneventful recovery. The pathological analysis showed a multifocal left atrial wall and pericardial fat infiltration with CD20+, CD5 +, bcl-2+, cyclin D1+, CD10- and CD23- lymphoid cells, in addition to a left atrial adherent thrombus (Panels D-I). These findings were compatible with lymphocytic lymphoma/chronic lymphocytic leukemia and the patient remains clinically stable.

Secondary or metastatic tumors are much more common than primary tumors of the heart. A recent necropsy study revealed that cardiac metastases in patients with leukemia and lymphomas may be present in 25% of patients.[1] Despite being mostly clinically silent, cardiac imaging improvements and availability has led to increased incidental recognition and awareness.

Keywords

Heart Atria Heart Neoplasms/surgery; Leukemia,Lymphoid/physiopathology; Mitral Valve Stenosis; Echocardiography; Coronary Angiography.

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Writing of the manuscript: Guimarães TIO; Critical revision of the manuscript for intellectual content: Plácido R, Quadros AC, Costa JM, Pinto FJ.

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Study Association

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

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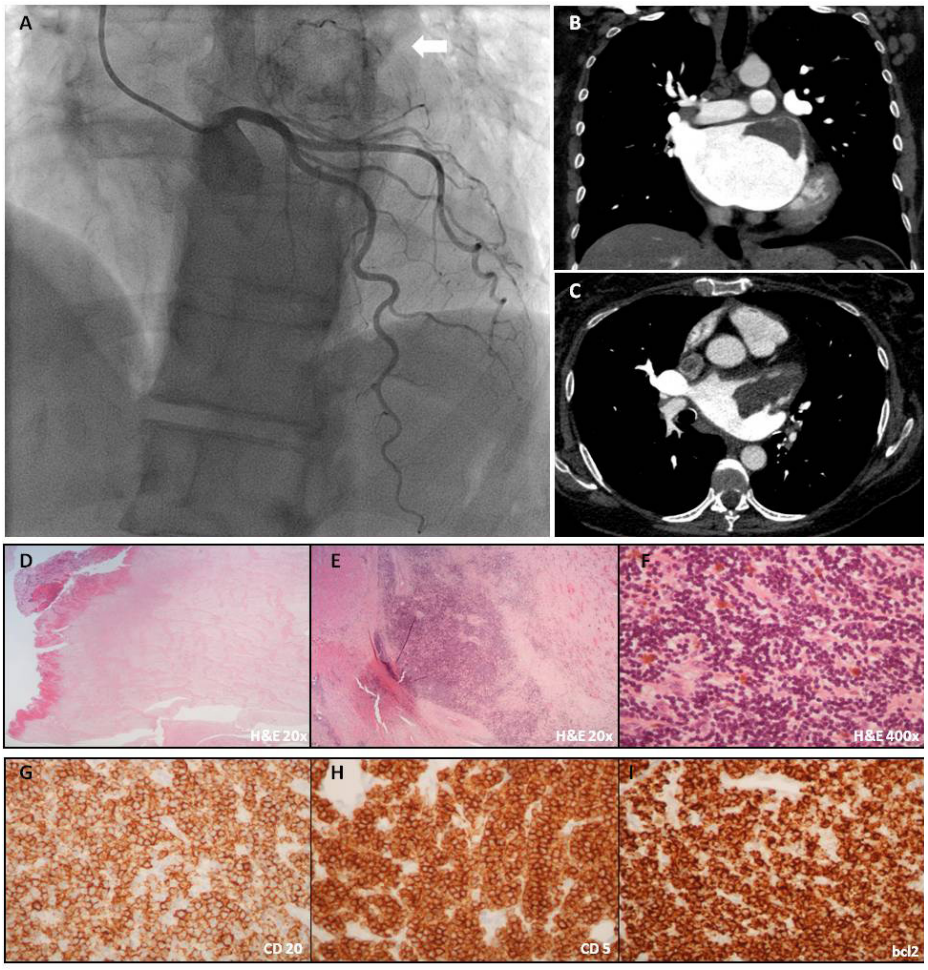

Figure 1 – *(Panel A) Selective left coronary angiogram (left anterior oblique 30° position) showing an abnormal vascularized mass (arrow) in the left atrium. (Panel B and C) Coronal and axial angio-CT planes in arterial phase, respectively, demonstrating a well-delimited, homogeneous and slight hyperdense mass, along the lateral portion of the atrial roof. (Panel D) Recent thrombus, partially in organization (H&E 20x). (E and F) Myocardium and adipose tissue infiltrated by small lymphoid cells, with scant cytoplasm and nuclei with peripherally clumped chromatin (H&E 20x and 400x). (Panel G-I) CD20, CD5 and bcl2 immunoreactivity (400x), respectively.*

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The Need for Sex Hormone Analysis in Addition to Long-Term Follow-Up of Phytosterol Supplementation

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Dear Editor,

I read with great interest the article entitled "Phytosterols in the Treatment of Hypercholesterolemia and Prevention of Cardiovascular Diseases", by Cabral and Klein, published in the Brazilian Archives of Cardiology.[1] The authors discuss phytosterol doses in the treatment of hypercholesterolemia, showing the current consensus of renowned guidelines and approval by global regulatory agencies.

As already mentioned, the main phytosterol mechanism of action occurs through the reduction (30% to 50%) in the intestinal absorption of cholesterol.[1] The authors, however, make it clear that long-term follow-up is essential to assess the association of phytosterol supplementation with the risk of cardiovascular diseases. Additionally, I emphasize another important investigation: analyses of serum sex hormones during randomized clinical trials based on phytosterol administration.

I recently showed that cholesterol intake may be related to increased total testosterone in men, whereas statin use may annul this potential.[2] Perhaps the use of phytosterols can also attenuate serum total testosterone levels in men (Figure 1).

In one test, the ingestion of 8.6 g/d of phytosterols reflected in the daily excretion of 28 mg cholesterol/g of fecal dry weight, resulting in an increase of 20 mg / g in comparison to the period prior to the test.[3] These proportions reflect in the daily excretion of approximately 230 mg of fecal cholesterol without the ingestion of phytosterols and 810 mg with the ingestion of phytosterols, since the average daily fecal excretion in humans is 128 g of wet weight, which corresponds to 29 g of dry weight.[4]

I point out that daily intake of 500 to 1,000 mg of cholesterol may result in an approximate increase of 130 ng/dL of total testosterone in men. In rats, the ingestion of phytosterols for 22 days reduced serum testosterone by 33%, in comparison to controls.[5] To the best of my knowledge, there are no studies that analyzed sex hormones in association with phytosterol administration in humans. Therefore, in addition to the aforementioned need to analyze the long-term administration of phytosterols, it is also important to consider sex hormone measurements in this context.

Keywords

Cardiovascular Diseases/prevention&control; Hypercholesterolemia; Phytosterois; Steroids

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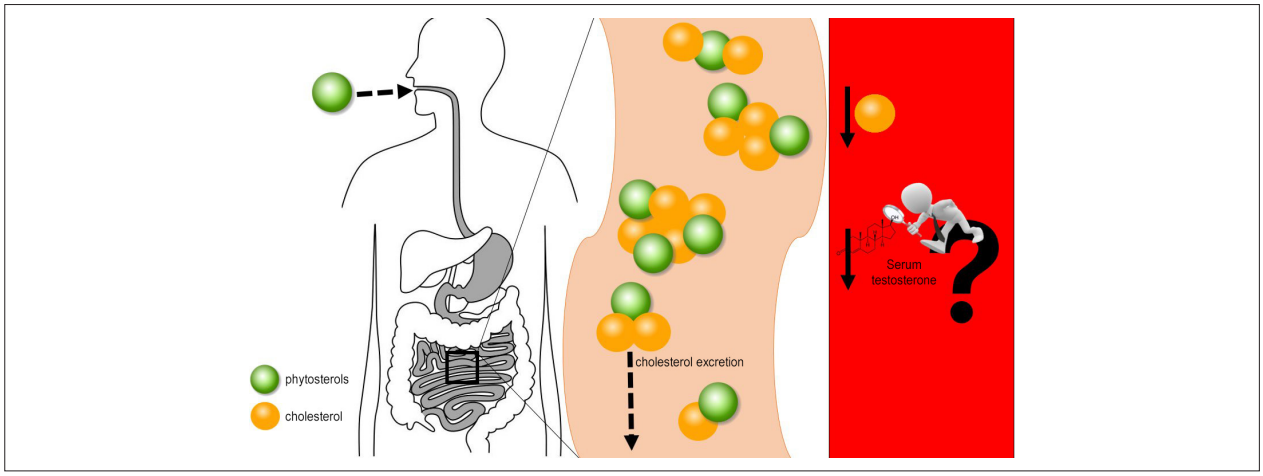

Figure 1 – *Proposal to analyze serum testosterone levels during phytosterol supplementation. Phytosterols increase the excretion of cholesterol, resulting in serum cholesterol decrease. Since cholesterol is important for the synthesis of sex hormones, the decrease in serum testosterone combined with phytosterol supplementation is a hypothesis. Therefore, the testing of this concept is a useful and, at first, an innovative concept. Dotted arrows indicate the conduction of phytosterols; continuous arrows indicate decreased levels of serum cholesterol and testosterone.*

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Reply

We thank you for your interest and comments related to our recent review article entitled "Phytosterols in the Treatment of Hypercholesterolemia and Prevention of Cardiovascular Diseases," [1] which aimed specifically to address the available evidence in the literature on the association between phytosterols and risk of cardiovascular diseases.

We agree that it is important to investigate the effects of phytosterols on serum levels of sex hormones, especially due to the potential risk of reducing plasma testosterone levels. We are not aware of studies carried out in humans that have performed such an evaluation. Experimental animal studies are scarce, and a reduction in plasma testosterone levels has been observed in some,[2,3] but not all of them.[4]

Sincerely,

Carlos Eduardo Cabral

Márcia Regina Simas Torres Klein

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