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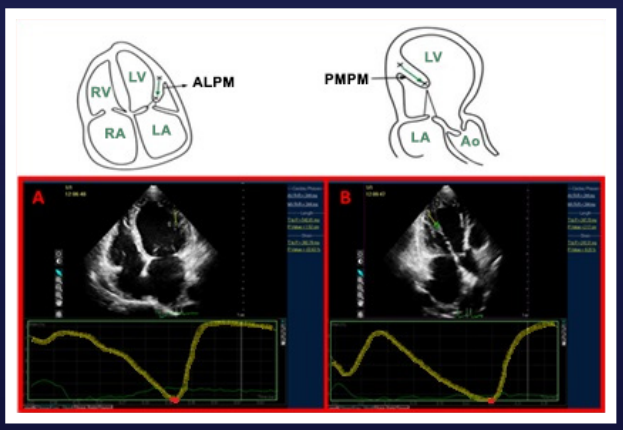

Figure 1 – *A) Free strain measurement of the APM from apical four chamber image in a patient with FMR. B) Free strain measurement of PPM from apical long axis image of the same patient. Page 341*

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SAMe-TT_2R_2 Score in the Outpatient Anticoagulation Clinic to Predict Time in Therapeutic Range and Adverse Events

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Abstract

Background: The SAMe-TT_2R_2 score was developed to predict which patients on oral anticoagulation with vitamin K antagonists (VKAs) will reach an adequate time in therapeutic range (TTR) (> 65%-70%). Studies have reported a relationship between this score and the occurrence of adverse events.

Objective: To describe the TTR according to the score, in addition to relating the score obtained with the occurrence of adverse events in patients with nonvalvular atrial fibrillation (AF) on oral anticoagulation with VKAs.

Methods: Retrospective cohort study including patients with nonvalvular AF attending an outpatient anticoagulation clinic of a tertiary hospital. Visits to the outpatient clinic and emergency, as well as hospital admissions to the institution, during 2014 were evaluated. The TTR was calculated through the Rosendaal´s method.

Results: We analyzed 263 patients (median TTR, 62.5%). The low-risk group (score 0-1) had a better median TTR as compared with the high-risk group (score ≥ 2): 69.2% vs. 56.3%, p = 0.002. Similarly, the percentage of patients with TTR ≥ 60%, 65% or 70% was higher in the low-risk group (p < 0.001, p = 0.001 and p = 0.003, respectively). The high-risk group had a higher percentage of adverse events (11.2% vs. 7.2%), although not significant (p = 0.369).

Conclusions: The SAMe-TT_2R_2 score proved to be effective to predict patients with a better TTR, but was not associated with adverse events. (Arq Bras Cardiol. 2017; 108(4):290-296)

Keywords: Atrial Fibrillation; Anticoagulants / adverse effects; Decision Support Techniques; Warfarin; Phenprocoumon; Vitamin K.

Introduction

Vitamin K antagonists (VKAs) reduce the risk for ischemic stroke in patients with atrial fibrillation (AF) by approximately 60%.[1] The efficacy of the treatment with VKAs is directly related to the time in therapeutic range (TTR), that is, percent time with prothrombin time/international normalized ratio (PT/INR) between 2.0 and 3.0.[2] A previous study[3] has suggested that the target TTR would be 58%-65%, below which there appears to be little benefit of oral anticoagulation with VKAs over dual antiplatelet therapy. Additional evidence has emphasized that stroke prevention with the use of VKAs is effective when individual mean TTR is high (> 70%).[4]

Predicting which patients are good candidates for anticoagulation therapy would be very useful. Scores are currently used to assess the risk for thromboembolic events ($CHADS_2$ and CHA_2DS_2-VASc),[5,6] as well as the risk for the major adverse effect from that therapy, bleeding (HAS-BLED).[7] Those scores allow us to assess the indication for that therapy and its risk; however, they provide no information on how the patient will respond to treatment, that is, whether the patient will maintain the target TTR. An easy prediction of which AF patients are likely to reach the target TTR by using VKAs could guide decision making in the strategy of anticoagulation with VKAs or new oral anticoagulants (NOACs).[8] Recently, Apostolakis et al.[9] have proposed and validated the SAMe-TT_2R_2 score. Those authors have reported the possibility of identifying AF patients on VKAs who reached the target TTR (score 0-1), as well as those who required additional interventions to reach the target TTR, achieving a low TTR with the use of VKAs (score ≥ 2), being thus likely candidates for the use of NOACs. Later studies have validated that score for the prediction of both TTR[8,10-17] and adverse events.[8,10-12,16,17] Others, however, have shown that the score cannot do that.[18-20]

In a previous study,[21] we have described our experience in an outpatient anticoagulation clinic of a Brazilian tertiary hospital, with a mean TTR of 64.8%. This study aimed at describing the TTR according to the SAMe-TT_2R_2 score, in addition to relating the score obtained with the occurrence of adverse events in patients with nonvalvular AF on anticoagulation with VKAs.

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Methods

This is a retrospective cohort including patients on oral anticoagulation with VKAs being followed up at the Outpatient Anticoagulation Clinic of the Internal Medicine Service of the Hospital de Clínicas de Porto Alegre (HCPA), a university-affiliated hospital for tertiary care in the Southern region of Brazil. Decisions regarding anticoagulation management were based on the protocol by Kim et al.[22] All patients attending consultations from January to March 2014 were screened, and those with nonvalvular AF were included in this study. Valvular AF was considered when moderate to severe mitral stenosis or prosthetic heart valve coexisted.[4]

The risk for ischemic stroke was estimated based on the CHADS$_2$ and CHA$_2$DS$_2$-VASc scores, while the risk for bleeding was estimated based on the HAS-BLED score.[5-7] To analyze the SAMe-TT$_2$R$_2$ score (0-8 points), the following variables were assessed: female sex (1 point), age < 60 years (1 point), presence of > 2 comorbidities (1 point), use of amiodarone to control heart rhythm (1 point), smoking within 2 years (2 points), and non-Caucasian race (2 points). The following were considered comorbidities: previous stroke, diabetes, peripheral artery disease, coronary artery disease, liver disease, lung disease, kidney disease, hypertension, and heart failure. Patients were categorized based on the SAMe-TT$_2$R$_2$ score into two groups: low risk (0-1 point) and high risk (≥ 2 points).[9]

Demographic and clinical data and results from complementary tests were obtained via retrospective assessment to electronic medical records, outpatient clinic consultations, visits to the emergency unit and admissions to the HCPA from January to December 2014. Patients lost to follow-up, those who died or whose anticoagulation with VKAs was suspended were also included in the analysis, and the TTR was analyzed up to the last available PT/INR test. Patients were assessed regarding anticoagulation control (via PT/INR tests) and occurrence of adverse events [major bleeding, stroke, transient ischemic attack (TIA), systemic embolism or death]. The TTR was estimated by use of the Rosendaal´s linear interpolation method.[23]

The laboratory tests, left ventricular ejection fraction (preferably assessed on echocardiogram) and number of drugs used were recorded based on the information available on the date closest to the beginning of follow-up. Anemia was considered when hemoglobin (Hb) < 13.0 g/dL for men or < 12 g/dL for women.[24] Uncontrolled hypertension was defined as systolic blood pressure > 160 mm Hg at the outpatient clinic visit closest to the beginning of follow-up.[7] Major bleeding was characterized as an event requiring hospitalization or transfusion of red blood cell concentrate, or Hb drop ≥ 2 g/dL.[7] Kidney disease was considered in the presence of kidney transplantation, chronic dialysis, or serum creatinine ≥ 2.26 mg/dL.[7] Liver disease was considered in the presence of chronic liver disease (ex.: cirrhosis) or biochemical evidence of significant liver damage (ex.: bilirubin > 2x the upper limit of normality, associated with aspartate aminotranferase, alanine aminotranferase or alkaline phosphatase levels > 3x the normal limit).[7]

Statistical analysis

Data were analyzed with the *Statistical Package for Social Sciences* (SPSS) software, version 21.0. Descriptive analysis was performed based on the distribution of absolute and relative frequency for qualitative variables, and based on mean ± standard deviation and median for quantitative variables with symmetrical and asymmetrical distribution, respectively. The median 25–75% percentiles were presented when deemed suitable. The groups were compared by using non-paired Student *t* test for symmetrical quantitative variables, Mann-Whitney U test for asymmetrical quantitative variables, and chi-square test for categorical variables. In low-frequency situations, Fisher exact test was used. The normality of the distribution of each variable was assessed by using Shapiro-Wilk test. Area under the *Receiver Operating Characteristic* (ROC) curve was calculated to assess the ability of the SAMe-TT$_2$R$_2$ score to predict the outcome 'TTR ≥ 65%' and the occurrence of adverse events, the best cutoff point of the score being considered that with the highest sensitivity x specificity product. Event-free survival was assessed by using Kaplan-Meier curves with the Log-Rank test. The significance level adopted for all tests was 5%. This study was submitted to the Committee on Ethics and Research from the HCPA, and approved.

Results

This study assessed 263 patients on oral anticoagulation with VKAs due to nonvalvular AF, corresponding to 38.5% of those being followed up at the Outpatient Anticoagulation Clinic of the HCPA. Of those, 205 patients (77.9%) completed the follow-up (Figure 1). Table 1 shows the demographic characteristics of the sample.

During follow-up, 2,754 PT/INR tests (median: 10 tests/patient) were performed, and 1,270 (46.1%) resulted between 2.0 and 3.0. Median TTR was 62.5% (P25-75 44.2%-79.5%). The median of subtherapeutic PT/INR time (< 2.0) was 18.9%, and that of supratherapeutic PT/INR time (> 3.0), 9.6%.

Regarding the SAMe-TT$_2$R$_2$ score, 138 patients (52.5%) had it 0-1 (low risk), while 125 (47.5%) had it ≥ 2 (high risk), the median being 1 (1-2). When assessing the SAMe-TT$_2$R$_2$ score criteria individually (Table 2), the criterion "medical history" (presence of > 2 comorbidities) was the most prevalent (57.0%). Low-risk (score 0-1) patients had a significantly higher median TTR as compared to high-risk (score ≥ 2) ones: 69.2% vs. 56.3% (p = 0.002). Likewise, the percentage of patients with TTR ≥ 60%, 65% or 70% was higher among low-risk patients for all cutoff points analyzed (Figure 2).

When assessing the ability of the SAMe-TT$_2$R$_2$ score to predict the outcome 'TTR ≥ 65%' by using the ROC curve (Figure 3), the cutoff point ≥ 2 showed the best combination of sensitivity and specificity (63.8% and 58.1%, respectively). The area under the curve was 0.612 (95%CI: 0.544 – 0.681; p = 0.002).

During follow-up, there were 24 (9.1%) adverse events, whose complete description is shown in Table 3. Neither TIA nor systemic embolism occurred during the period studied. High-risk patients (score ≥ 2) had more events, but with no

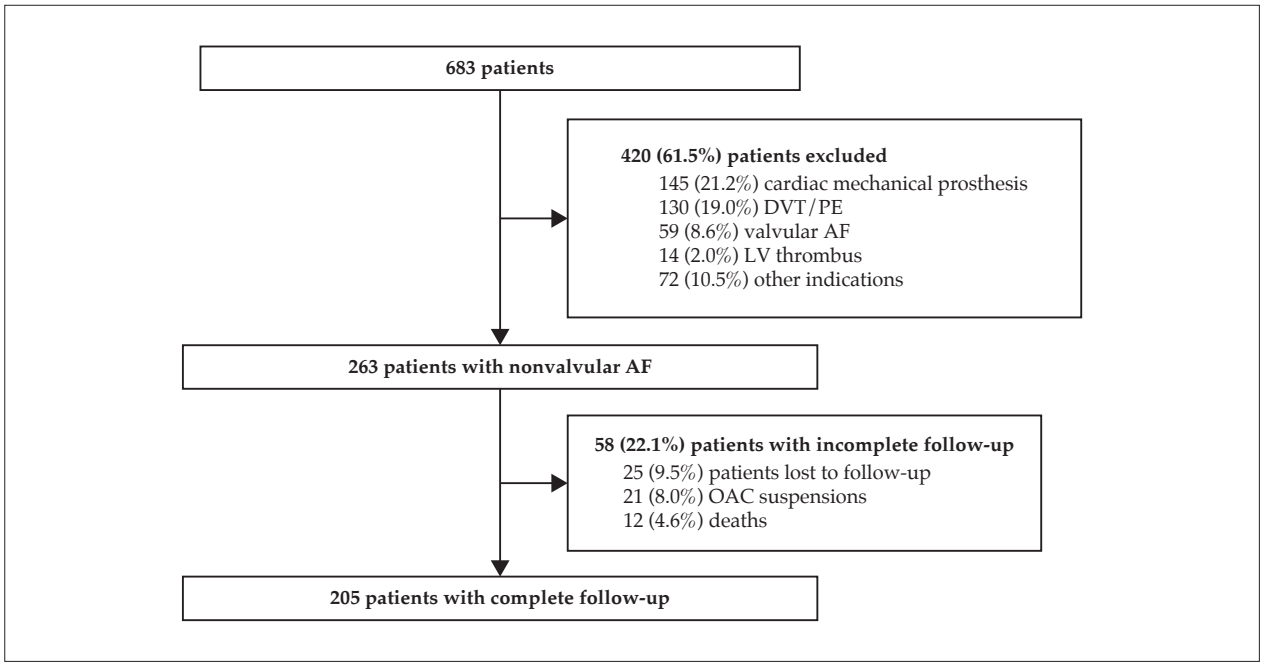

Figure 1 – *Study diagram. DVT: deep venous thrombosis; PTE: pulmonary embolism; AF: atrial fibrillation; LV: left ventricular; OAC: oral anticoagulation.*

Table 1 – Demographic characteristics of the sample

Variable	n = 263
Female sex	113 (43.0)
Age (years)	71.2 (64.1-78.5)
Use of warfarin	256 (97.3)
Labile PT/INR (TTR < 60%)	124 (47.1)
Hypertension	231 (87.8)
Uncontrolled hypertension	22 (8.4)
HF/LVEF < 40%	149 (56.7)
Diabetes	108 (41.1)
Previous stroke/TIA	96 (36.5)
Coronary artery disease	76 (28.9)
Use of antiplatelet drugs/NSAIDs	64 (24.3)
Anemia	67 (25.5)
Pulmonary disease	36 (13.7)
Previous major bleeding	24 (9.1)
Peripheral artery disease	25 (9.5)
Kidney disease	7 (2.7)
Liver disease	2 (0.8)
Number of medications	7 (6-9)
$CHADS_2$	3 (2-4)
CHA_2DS_2-VASc	4 (3-5)
HAS-BLED	2 (1-3)

PT/INR: prothrombin time / international normalized ratio; TTR: time in therapeutic range; HF: heart failure; LVEF: left ventricular ejection fraction; TIA: transient ischemic attack; NSAIDs: non-steroidal anti-inflammatory drugs. Categorical variables are shown as n (%), and continuous variables, as median (25%-75%).

Table 2 – Prevalence of the SAMe-TT$_2$R$_2$ score components

Score Component		n (%)
S	**S**ex (female)	113 (43.0)
A	**A**ge (< 60 years)	41 (15.6)
Me	**Me**dical history (> 2 comorbidities*)	150 (57.0)
T	**T**reatment (amiodarone)	26 (9.9)
T_2	**T**obacco use (within 2 years)	37 (14.1)
R_2	**R**ace (non-Caucasian)	22 (8.4)

**Previous stroke, diabetes, peripheral artery disease, coronary artery disease, liver disease, lung disease, kidney disease, hypertension, and heart failure.*

statistically significant difference (11.2% vs. 7.2%; p = 0.369). The area under the ROC curve of the score for the occurrence of adverse events was 0.566 (95%CI: 0.449 - 0.682; p = 0.289), ≥ 2 being again the best cutoff point, with sensitivity and specificity of 58.3% and 53.6%, respectively. Figure 4 shows the event-free survival curves.

Discussion

The use of anticoagulation in patients with AF to prevent thromboembolic events is known to be effective and TTR-dependent. Predicting which patients on VKAs are more likely to reach the target TTR is important, especially currently when new drugs that do not require PT/INR monitoring are available. In this study with a Brazilian sample, the SAMe-TT$_2$R$_2$ score proved to be a good predictor of TTR for nonvalvular AF patients on oral anticoagulation with VKAs. That score can be useful in the initial assessment of patients

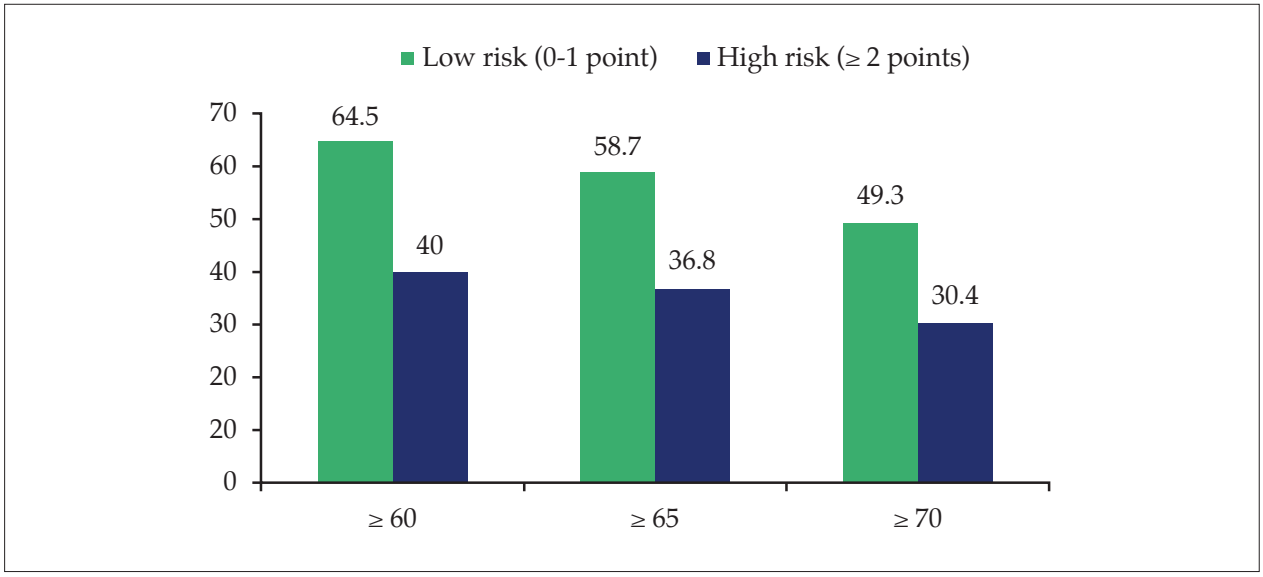

Figure 2 – *Percentage of patients with TTR ≥ 60%, 65% and 70% according to the points obtained in the SAMe-TT$_2$R$_2$ score (p < 0.001, 0.001 and 0.003, respectively).*

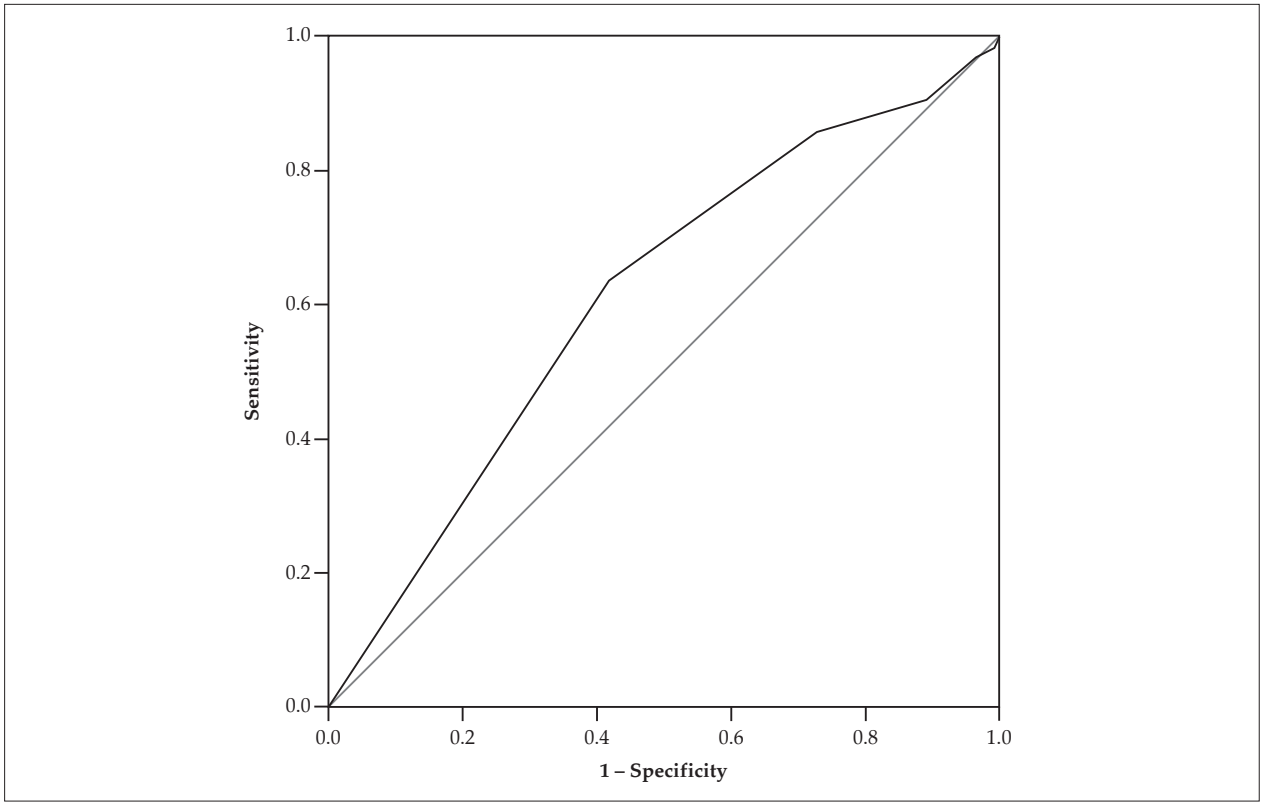

Figure 3 – *ROC curve for the outcome 'TTR ≥ 65%'.*

with indication for anticoagulation. Median TTR, as well as the percentage of patients with TTR ≥ 60%, 65% and 70%, were higher among patients with a low SAMe-TT$_2$R$_2$ score (0-1 point) as compared to the group whose score was ≥ 2.

The usefulness of that score in other populations and clinical settings has been reported. Ruiz-Ortiz et al.,[15] in a prospective analysis of Spanish cardiology outpatients, have reported a progressive decrease in mean TTR according to

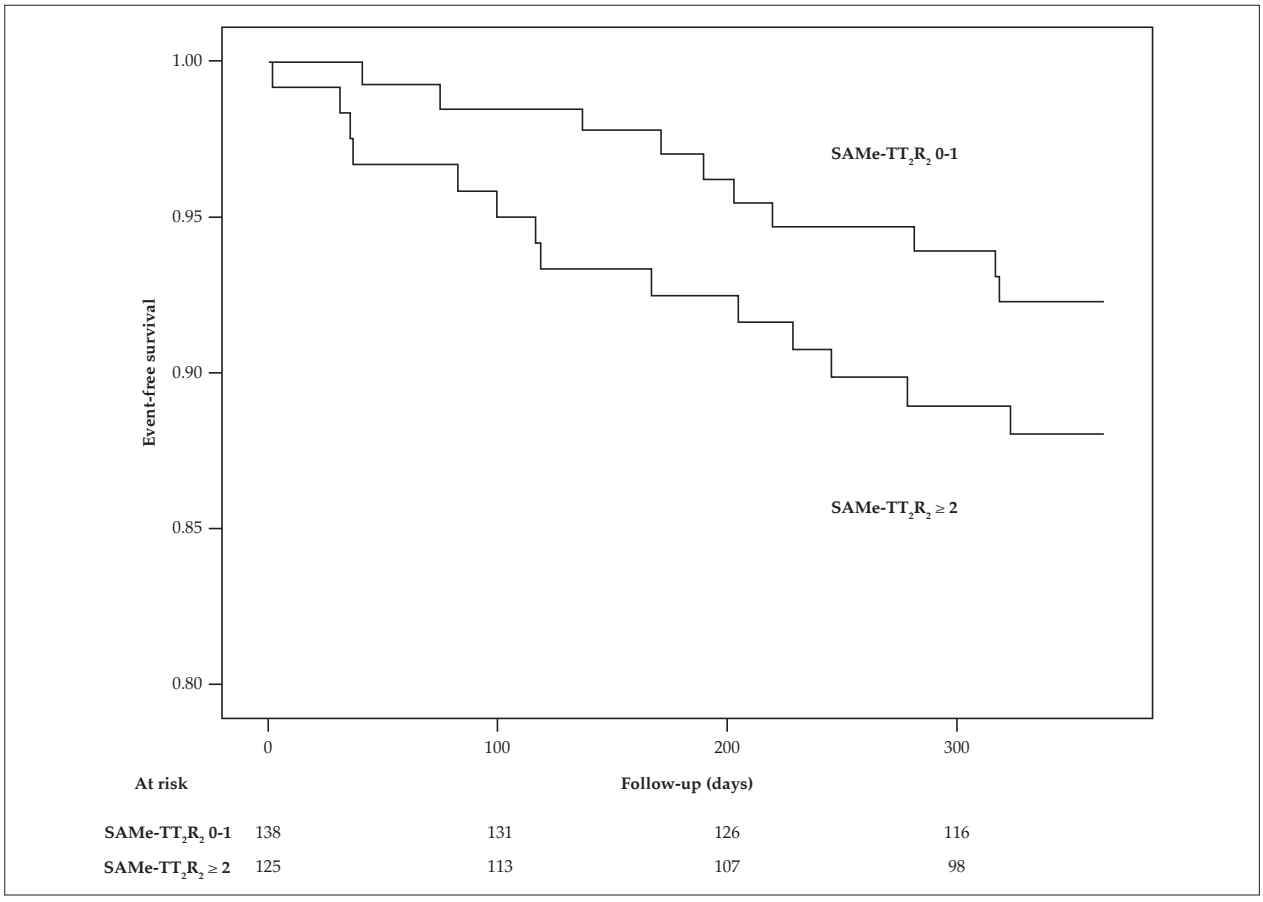

Figure 4 – *Event-free survival curve according to the points obtained in the SAMe-TT_2R_2 score (p = 0.224).*

Table 3 – Adverse events in total follow-up and according to the points obtained in the SAMe-TT_2R_2 score.

Adverse Events	n = 263	SAMe-TT_2R_2		p
		0-1 point	≥ 2 points	
Major bleeding	15 (5.7)	6 (4.3)	9 (7.2)	0.465
Stroke	4 (1.5)	1 (0.7)	3 (2.4)	0.349
Death	12 (4.6)	5 (3.6)	7 (5.6)	0.637
TOTAL	24 (9.1)	10 (7.2)	14 (11.2)	0.369

Data shown as n (%).

the score obtained. In their study, patients who scored 0 had a mean TTR of 67.5% ± 24.6%, while those who scored ≥ 4 had a mean TTR of 52.7% ± 28.7% (p < 0.01), with an area under the ROC curve for the outcome 'TTR ≥ 65%' of 0.57 (95%CI: 0.53 - 0.60; p < 0.0005). Roldán et al.,[14] assessing 459 patients of an outpatient anticoagulation clinic, have reported that those with a score of 0-1 had a mean TTR of 67% ± 18%, while those with a score ≥ 2 had a mean TTR of 61% ± 16% (p < 0.001). In their study, the odds ratio for reaching a TTR < 65% was 2.10 (95%CI: 1.44 - 3.06; p < 0.001) in patients with a score ≥ 2. In a retrospective study including 4,468 patients selected from a registry of primary care units in the United Kingdom, Martinez et al.[17] have reported that the proportion of patients with TTR ≥ 60% was 44.1% among those with a score of 0-1, and 37.1% among those with a score ≥ 2 (p < 0.01).

The association of the points obtained in the score with the occurrence of anticoagulation adverse events (major bleeding, stroke, systemic embolism and/or death) has been described in other studies[8,10-12,16,17] after the original study,[9] always relating the quality of anticoagulation, assessed via TTR, with the occurrence of those outcomes. Only the study

by Poli et al.[13] has not observed that relationship. In a retrospective study including 4,468 AF patients on VKAs with a 3-year follow-up, Martinez et al.[17] have reported a higher risk for stroke in patients with score ≥ 2 as compared to those with score of 0-1 (log rank $p < 0.01$). Lip et al.,[12] in a retrospective study with 8,120 patients (mean follow-up, 1,016 ± 1,108 days), have reported that the SAMe-TT$_2$R$_2$ score predicted stroke/thromboembolism, severe bleeding and death, reflecting a suboptimum TTR in patients with score ≥ 2. In the present study, the lack of association between the score and the occurrence of adverse events, specifically stroke, can be attributed to the low incidence of that complication.

Several studies have proposed the inclusion of the SAMe-TT$_2$R$_2$ score in the flowchart for strategic decision-making about which anticoagulant should be used for patients recently diagnosed with AF.[14,25-28] Based on the score obtained, for patients with ≥ 2 points, the use of NOACs should begin immediately, while those with a score of 0-1 should begin their treatment with VKAs, which should be changed to NOACs if target TTR (> 70%) was not achieved during follow-up. Current guidelines for AF management, however, have not included that strategy.[4,29,30]

Our study has some limitations. Its retrospective design has inherent limitations, which can affect the quality of the data analyzed. Nevertheless, we believe that there was no great loss of data necessary for this study, because at our institution patients undergo systematic care, by use of protocols and structured outpatient clinic visits. Thus, most data necessary for the study was systematically collected during outpatient visits. Another limitation is that the medical record review identified only in-hospital adverse events or events reported by patients during their visits to the outpatient clinic, and some events, especially the adverse ones, might have been missed. Finally, the single-center characteristic of this study ensures the uniform follow-up of the patients described in this cohort, but might have decreased its external validity.

Conclusion

Based on our findings, the SAMe-TT$_2$R$_2$ score proved to be effective to predict TTR for AF patients on anticoagulation with VKAs. Thus, the association of that score with the scores to assess the indication of anticoagulation (CHADS$_2$ and/or CHA$_2$DS$_2$-VASc), as well as the risk for bleeding (HAS-BLED), will provide a high-quality assessment of the treatment. For patients with a high SAMe-TT$_2$R$_2$ score (≥ 2), anticoagulation with VKAs is more likely to be less effective, and, thus, the use of NOACs should be considered. Low-risk patients (score 0-1), however, respond better to VKAs. Therefore, an intervention based on patients' risk allows the use of new technologies (in our case, NOACs), usually more expensive and less available, to be directed to a group of patients with a more specific indication.

Author contributions

Conception and design of the research, Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content: Pivatto Júnior F, Scheffel RS, Amon LC, Biolo A; Acquisition of data: Ries L, Wolkind RR, Marobin R, Barkan SS; Statistical analysis: Pivatto Júnior F, Scheffel RS; Writing of the manuscript: Pivatto Júnior F, Scheffel RS, Ries L, Wolkind RR, Marobin R, Barkan SS, Amon LC, Biolo A.

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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Comparison of Two Central Venous Pressure Control Strategies to Prevent Atrial Fibrillation After Coronary Artery Bypass Grafting

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Abstract

Background: Atrial fibrillation (AF) takes place in 10-40% of patients undergoing coronary artery bypass grafting (CABG), and increases cardiovascular mortality. Enlargement of atrial chambers is associated with increased AF incidence, so patients with higher central venous pressure (CVP) are expected to have larger atrial distension, which increases AF incidence.

Objective: To compare post-CABG AF incidence, following two CVP control strategies.

Methods: Interventional, randomized, controlled clinical study. The sample comprised 140 patients undergoing CABG between 2011 and 2015. They were randomized into two groups, G15 and G20, with CVP maintained ≤ 15 cmH_2O and ≤ 20 cmH_2O, respectively.

Results: 70 patients were included in each group. The AF incidence in G15 was 8.57%, and in G20, 22.86%, with absolute risk reduction of 14.28%, and number needed to treat (NNT) of 7 (p = 0.03). Mortality (G15 = 5.71%; G20 = 11.42%; p = 0.07), hospital length of stay (G15 = 7.14 days; G20 = 8.21 days; p = 0.36), number of grafts (median: G15 = 3, G2 = 2; p = 0.22) and cardiopulmonary bypass use (G15 = 67.10%; G20 = 55.70%; p = 0.22) were statistically similar. Age (p = 0.04) and hospital length of stay (p = 0.001) were significantly higher in patients who developed AF in both groups.

Conclusion: Keeping CVP low in the first 72 post-CABG hours reduces the relative risk of AF, and may be useful to prevent AF after CABG. (Arq Bras Cardiol. 2017; 108(4):297-303)

Keywords: Central Venous Pressure; Atrial Fibrillation/prevention; Myocardial Revascularization; Postoperative Care.

Introduction

Atrial fibrillation (AF) is an arrhythmia that results from abnormal depolarization, causing loss of the atrial contraction ability. It is related to increased risk for stroke and mortality.[1-3]

Atrial fibrillation in the postoperative period (PO) of coronary artery bypass grafting (CABG) occurs in 5-40% of patients, usually from the second to the fourth PO day, peaking on the second day.[3,4] Its pathophysiology is multifactorial and includes oxidative stress, systemic inflammatory response, excessive production of catecholamines, changes in autonomic tone and connexin expression. Such factors cause dispersion of atrial refractoriness, alter atrial electrical conduction and predispose to arrhythmia.[4,5]

The following risk factors are related to the higher incidence of post-CABG AF: advanced age, peripheral vascular disease, chronic obstructive pulmonary disease (COPD), diabetes mellitus, systemic arterial hypertension, valvular heart disease, left atrial enlargement, left ventricular dysfunction, history of previous AF or acute myocardial infarction (AMI), suspension of beta-blockers in the preoperative period, use of cardiopulmonary bypass (CPB), and increased PO sympathetic tone.[6,7]

Atrial fibrillation in the PO period of CABG worsens the patient's hemodynamic status, because of the increased risk for congestive heart failure and embolic events in the long run. Stroke is a major complication, observed in 2% of surgical patients. In addition, AF has been associated with higher in-hospital mortality and worse survival in the long run.[4-7] Such complications justify the need for prophylactic measures for post-CABG AF.

The rationale of this study is based on the following hypothesis: the increased volume of the atria is associated with increased AF incidence;[8] therefore, patients with higher central venous pressure (CVP) are expected to have greater atrial distension, and, thus, a higher AF incidence, in addition to being predisposed to pulmonary congestion, hypoxemia and atrial wall edema, factors that contribute to increase the incidence of that arrhythmia. Thus, CVP control could be useful to prevent post-CABG AF. Aiming at testing that hypothesis, the post-CABG AF incidence was assessed under two CVP control strategies.

Objective

This study aimed at assessing whether the incidence of AF 48 to 72 hours after CABG differs between two CVP control

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strategies (based on intent to treat), by comparing two groups: G15 (CVP ≤ 15 cmH_2O) and G20 (CVP ≤ 20 cmH_2O), AF being the primary outcome. The secondary outcome was to compare hospital length of stay after CABG, as well as mortality, between both groups.

Methods

Type of study

This is a clinical, randomized, controlled, interventional and prospective study performed at the Intensive Care Unit (ICU) of Santa Casa de Misericórdia of Ponta Grossa, Paraná State, in partnership with the State University of Ponta Grossa (UEPG). The analysis was performed with data prospectively collected. This study project was approved by the Ethics Committee in Research UEPG, abides by the 1975 Declaration of Helsinki, and all participants provided written informed consent before surgery.

Inclusion and exclusion criteria

This study sample comprised patients undergoing CABG at the Cardiac Surgery Service of the Santa Casa de Misericórdia of Ponta Grossa from January 2011 to December 2015.

Patients with the following characteristics were excluded: undergoing CABG in association with another procedure; history of preoperative AF; contraindication to maintain CVP below the established values (such as severe pulmonary hypertension); severe chronic renal failure, determined by a glomerular filtration rate < 30 mL/min; severe left ventricular dysfunction; use of high doses of vasoactive drugs, such as dopamine or dobutamine > 7mcg/kg/min or noradrenaline > 0.7mcg/kg/min; no use of beta-blocker or statin in the preoperative period, or no diet reintroduction, and after vasoactive drug suspension in the PO period; need for more than 20 ampoules of furosemide within 24 hours to maintain CVP levels.

Outcomes

Primary outcome: presence of AF 48 to 72 hours after CABG, on continuous electrocardiographic monitoring (cardioscope) and documented on 12-lead electrocardiogram (ECG).

Secondary outcomes: in-hospital mortality and hospital length of stay after CABG.

Definition of the groups and data collection

Patients were randomized into two groups by use of draw: G15, with a CVP goal of ≤ 15 cmH_2O; and G20, with a CVP goal of ≤ 20 cmH_2O. The strategy of CVP control consisted of measuring CVP every 2 hours for 72 hours after CABG, or until discharge from the ICU. The minimum ICU length of stay was 48 hours. Whenever CVP reached its cutoff point, a furosemide ampoule was intravenously administered, from the sixth hour on, because, in the first 6 post-CABG hours, hemodynamic instability is higher. Vasoactive or anti-hypertensive drugs were administered to maintain a mean arterial pressure (MAP) of 60-100 mm Hg. The AF incidence was compared between the groups, detected by use of continuous electrocardiographic monitoring (cardioscope) and confirmed on 12-lead ECG.

The following data were collected daily: CVP levels, need for furosemide, AF occurrence within the post-CABG period (48 to 72 hours), hospital length of stay and in-hospital death.[9] The following surgical data and comorbidities were collected from the patients' standard preoperative and PO forms: age, sex, previous AF, diabetes mellitus, COPD, chronic kidney disease, peripheral vascular disease, left ventricular function, recent AMI, use of CPB, and number of grafts.

Statistical analysis

The statistical analysis was performed in two steps. In the first, the following variables were compared between the G15 and G20 groups: age, sex, diabetes mellitus, COPD, peripheral arterial disease, recent AMI within three months from surgery, moderate left ventricular dysfunction (ejection fraction < 50% and > 35%), glomerular filtration rate, AF incidence, in-hospital death, post-CABG hospital length of stay (days), number of grafts, and use of CPB. The quantitative variables were expressed as medians for nonparametric data, or as means for parametric data, and coefficient of variation (CV). The qualitative variables were expressed as absolute numbers and percentages. In the second step, the patients were divided into two groups, one group of those who developed AF and the other group of those who did not developed AF, and the following variables were compared between the new groups: age, in-hospital death, post-CABG hospital length of stay (days), number of grafts, and use of CPB. Their statistical analysis was performed with the MedCalc software. The qualitative variables were assessed by using two-tailed Fisher exact test. The quantitative variables had their normality tested by use of Shapiro-Wilk test; because the data had nonparametric distribution, they were assessed by use of two-tailed Mann-Whitney test. To assess the effect size, absolute risk reduction (ARR) and relative risk reduction (RRR) were used, and, for qualitative variables, the number needed to treat (NNT). The statistical significance level adopted was $p < 0.05$.

Results

The sample comprised 140 patients randomized into two groups, with 70 patients each: G15, CVP maintained ≤ 15 cmH_2O; and G20, CVP maintained ≤ 20 cmH_2O. The comparative analysis of patients' age and sex is shown in Table 1. The mean ages of the groups were 60 years (CV = 0.17) in G15, and 63 years (CV = 0.15) in G20 ($p = 0.6$). The male sex predominated in both groups (G15=67.10%; and G20 = 81.43%; $p = 0.07$).

The analysis of comorbidities evidenced no statistical difference between the groups (Table 2).

In the 48-72 post-CABG hours, the incidence of AF differed statistically between the groups (Table 3). In G15, 8.56% of the patients developed AF, in contrast to 22.86% of those in G20 ($p = 0.03$). The ARR was 14.28% [95% confidence interval (95% CI): 2.14-26.28], and RRR were used of 62.50% (95% CI: 9.79-84.41) and NNT of 7. The sample power was 64.40%.

Table 1 – Patients' age and sex according to group (G15: CVP control ≤ 15 cmH_2O; and G20: CVP control ≤ 20 cmH_2O)

Variables	G15 (n = 70)	G20 (n = 70)	p
Age, mean (CV)	60 (0.17)	63 (0.15)	0.6 †
Male sex, n (%)	47 (67.1)	57 (81.43)	0.07*

(*) Fisher exact test (two-tailed); (†) Mann-Whitney test (two-tailed); CVP: central venous pressure; CV: coefficient of variation.

Table 2 – Assessed comorbidities according to group (G15: CVP control ≤ 15 cmH_2O; and G20: CVP control ≤ 20 cmH_2O)

Variables	G15 (n = 70)	G20 (n = 70)	p
Diabetes mellitus, n (%)	18 (25.71)	25 (35.71)	0.07*
COPD, n (%)	10 (14.28)	11 (15.71)	1.00*
Peripheral arterial disease, n (%)	7 (10.00)	12 (17.14)	0.27*
Previous recent AMI, n (%)	28 (40)	23 (32.85)	0.48*
LVD (EF < 50%), n (%)	8 (11.42)	16 (22.85)	0.11*
Renal function (GFR), mL/min (CV)	85.78 (0.37)	88.32 (0.49)	0.48 †

(*) Fisher exact test (two-tailed); (†) Mann-Whitney test (two-tailed); CVP: central venous pressure; COPD: chronic obstructive pulmonary disease; AMI: acute myocardial infarction; LVD: left ventricular dysfunction; EF: ejection fraction; CV: coefficient of variation; GFR: glomerular filtration rate.

Table 3 – Comparison of atrial fibrillation within 72 postoperative hours, in-hospital death, hospital length of stay after coronary artery bypass grafting, number of grafts and use of cardiopulmonary bypass between patients with CVP control ≤ 15 cmH_2O (G15) and CVP control ≤ 20 cmH_2O (G20)

Variables	G15 (n = 70)	G20 (n = 70)	p
Atrial fibrillation, n (%)	6 (8.57)	16 (22.86)	0.03*
In-hospital death, n (%)	4 (5.71)	8 (11.42)	0.07*
Hospital length of stay, mean, days (CV)	7.14 (0.70)	8.21 (0.68)	0.36†
Grafts, median	3	2	0.22 †
Cardiopulmonary bypass, n (%)	47 (67.10)	39 (55.70)	0.22*

(*) Fisher exact test (two-tailed); (†) Mann-Whitney test (two-tailed); CVP: central venous pressure; CV: coefficient of variation.

In G15, there were 4 in-hospital deaths, 2 due to pulmonary sepsis and 2 due to stroke. In G20, there were 8 in-hospital deaths, 5 due to stroke, 2 due to pulmonary sepsis and 1 due to urinary sepsis. Mortality showed no statistical difference (p = 0.07). In addition, there was no difference regarding the post-CABG hospital length of stay (days), number of grafts, and use of CPB.

In the group of patients who developed AF, the following variables were analyzed: mortality, hospital length of stay, number of grafts, and use of CPB (Table 4). Age differed statistically between the groups (p = 0.04), with mean of 65.68 years among patients who developed AF, and of 60.73 among those who did not develop AF. The hospital length of stay was significant (p = 0.0012) among patients who developed AF, with mean of 10.22 days (CV = 0.70). The other variables were statistically similar.

Discussion

This study compared the AF incidence in the first 72 post-CABG hours using two CVP control strategies, based on intention to treat, that is, the use of a diuretic was aimed at maintaining CVP below the cutoff points. Patients maintained with CVP ≤ 15 cmH_2O in that period had lower AF incidence as compared to patients whose CVP was maintained ≤ 20 cmH_2O (8.56% vs. 22.86%; p = 0.03). Measures of effect size were relevant: ARR of group G15 was 12.12%, equivalent to a NNT of 7, that is, 1 in every 7 patients benefited from CVP control ≤ 15 cmH_2O after CABG, suggesting that maintaining CVP under control can be effective in reducing AF incidence. The AF incidences in each group and in the whole sample (15.71%) are similar to those reported in the literature (5-40%).[4] The CVP was measured with a monitor in mm Hg and in water column; we chose to use the water column measure because some patients had only 24 hours of mmHg monitoring. For patients stable after 24 hours, it is routine procedure to end invasive blood pressure monitoring, CVP being controlled only by use of water column.

Atrial fibrillation in the PO period increases the risk for ischemic stroke, ventricular tachycardia, ventricular fibrillation, hypotension and heart failure.[3-7] Post-CABG AF is associated with increased hospital length of stay after surgery and

Table 4 – Comparison of age, mortality, hospital length of stay, number of grafts and use of cardiopulmonary bypass between patients who developed atrial fibrillation (AF) and those who did not

Variables	With AF (n = 22; 15.17%)	Without AF (n = 118; 84.29%)	p
Age, mean (CV)	65.68 (0.15)	60.73 (0.16)	0.04
In-hospital death, n (%)	2 (5.71%)	10 (11.42%)	1.00*
Hospital length of stay, mean, days (CV)	10.22 (0.70)	7.20 (0.67)	0.001†
Grafts, median	2	2	0.69†
Cardiopulmonary bypass, n (%)	12 (54.55)	74 (62.71)	0.48*

(*) Fisher exact test (two-tailed); (†) Mann-Whitney test (two-tailed); CV: coefficient of variation.

in-hospital mortality.[6] Sobral et al.[9] have reported a longer hospital length of stay of patients with AF (mean of 16.4 days; p = 0.004); however, they have not established whether AF was the cause of prolonged hospitalization or an indicator of the severity of more critically-ill patients. In addition, they have reported a peak incidence of 2.6 days (median, 2 days). Da Silva et al.[10] have shown a mean hospital length of stay of patients who developed AF after cardiac surgery of 16.9 days (p = 0.001). In their sample, the hospital length of stay was significantly longer among patients with AF (p = 0.0012). They have not assessed the peak incidence in relation to that time, but the period studied (72 hours) is in accordance with the predicted time in the literature for higher AF incidence.[5,9,10] The mortality rate reported by Sobral et al.[9] 1 year after surgery was 4.7% (n = 109; p = 0.001) for patients who developed AF, with a 30-day rehospitalization rate of 7.6% (n = 168; p = 0.004) and an 1-year rehospitalization rate of 18.7% (n = 417; p = 0.004). In our sample, in-hospital mortality and hospital length of stay were assessed, and were slightly higher in G20, but with no statistical difference.

Knowing the risk factors for post-CABG AF is highly important. It enables the use of prophylactic measures, aimed at reducing the incidence of AF, as well as of its complications.[11,12]

Some risk factors for AF have been demonstrated. Age over 65 years[9,10] has been reported as one of the most important risk factors.[7-12] Age is associated with myocardial structural changes due to degenerative processes (fibrosis and dilatation), which lead to lack of an effective refractory period, dispersion of atrial refractoriness and abnormal conduction and automaticity.[4-7] In addition, advanced age is related to increased in-hospital mortality.[9] In our study, mean age was 60 years in G15, and 63 years in G20, with no statistical difference (p = 0.07). Age showed significance when analyzing patients who developed AF as compared to those who did not (p = 0.04). The mean age of those who developed AF was 66 years, similarly to that reported in the literature.[9,10]

Diabetes mellitus leads to metabolic changes, such as increased oxidative stress, elevated levels of free fatty acids, and chronic tissue inflammation. Such alterations result in changes in atrial structure and electrical conduction, contributing to AF development in the PO period.[5,13] In our sample, the prevalence of diabetes mellitus was similar in both groups (p = 0.07).

Peripheral arterial occlusive disease associates with the severity of the patients' clinical profile and comorbidities that predispose to the appearance of AF after cardiac surgery.[9,14] El-Chami et al.[14] have identified peripheral arterial disease as a risk factor for AF, and considered it an independent predictor of mortality. In our study, the peripheral arterial occlusive disease prevalence did not statistically differ between the groups.

Other conditions associated with the development of post-CABG AF are COPD,[9,12,15] chronic renal failure,[9,12] previous AMI,[10,12] and left ventricular dysfunction.[16,17] The prevalence of those risk factors was similar in both groups.

The number of grafts performed during CABG and the use of CPB have been identified as risk factors,[7] but with some disagreement between different authors.[9,10,14-19] The CPB is an invasive technique related to atrial ischemia and inflammatory response in the PO period of cardiac surgeries.[7,20] The groups studied showed no statistical difference regarding those parameters.

Some strategies for post-CABG AF prevention have been developed, especially those related to pharmacological prophylaxis. Beta-blockers are the drugs of choice, because they significantly reduce the AF incidence, being related to lower PO morbidity and mortality.[4,6,7,21] They belong to the most studied and used drug class, especially because of the control they have on the increased sympathetic tone in patients submitted to cardiac surgery.[4,6] Beta-blockers are indicated for all patients undergoing CABG, except in the presence of contraindications.[4,7,21] Prophylaxis with amiodarone and intravenous magnesium is recommended when beta-blockers are contraindicated.[22] In older studies, the use of statins in the preoperative and PO periods was considered relevant to post-CABG AF prevention. Those drugs act by reducing the inflammation of patients with coronary arterial disease. A meta-analysis by Zheng et al.[23] has shown that statin therapy significantly reduced the AF incidence and hospital length of stay. Bockeria et al.[24] have shown that patients receiving statin before CABG had higher benefits in preventing early AF than those who did not. However, the literature is still controversial. In a recent meta-analysis,[25] the authors have concluded that PO statin therapy does not prevent AF in patients undergoing elective cardiac surgery. At our service, the use of beta-blockers and statins is maintained in the preoperative period and reintroduced with diet usually on the first PO day. If the patient is on dobutamine or noradrenaline, the beta-blocker is introduced after suspension of the vasoactive drug.

CVP is an important predictor of early mortality, independently of cardiac output and other variable clinical conditions, mainly in the first 6 PO hours, because of hemodynamic instability.[26] Strict CVP control is aimed at measuring the pressure to which the atria are submitted, taking the intravascular volume into consideration. The result of the intravascular volume overload is hypertension, atrial dilatation and contraction reduction, because of stretching of cardiac muscle fibers. However, that can be reversed with diuretics.[26-28] Kalus et al.[29] have shown that hemodynamic control based on the administration of large amounts of fluids accounted for the increase in atrial pressure, and could trigger AF in the PO period of cardiothoracic surgery. They have observed that cardiothoracic surgery patients who developed AF had received approximately 1 liter of fluids more than those who did not develop that arrhythmia, and that difference was more significant on the second PO day ($p = 0.04$). The limiting factor of that study was the lack of documentation of the increase in atrial pressure and volume after surgery.

Intravascular volume overload causes abnormal dispersion of atrial refractory period, because of the increase in atrial volume and pressure. Thus, the atrium becomes vulnerable to the development of AF.[11,15,30,31] Hwang et al.[31] have suggested that intravascular volume is an important parameter, as are arterial gas analysis, hemoglobin and serum potassium, which should be assessed when there is post-CABG AF, because they can clarify the reversible causes of that arrhythmia. In addition, Silva et al.[10] have shown that excessive fluid balance in the first 24 PO hours is a risk factor for post-CABG AF. Those authors have reported pulmonary congestion as a trigger for that arrhythmia. Koletsis et al.[15] have reported an association between positive fluid balance, reflecting an excessive intravascular volume, and occurrence of post-CABG AF. In addition, the positive fluid balance has been accounted for the increase in left atrial pressure and pulmonary congestion, leading to hypoxia.

The increased left atrial volume identified in the preoperative period predisposes to the development of AF after cardiac surgery. Wang et al.[32] have shown that the left atrial expansion index was associated with in-hospital mortality and post-CABG AF, being an independent risk factor. Osranek et al.[16] have reported that an increase over 32 mL/m^2 in left ventricular volume increases by 5 times the risk for AF, as a factor independent of age and other surgical parameters. Patel et al.[33] have found that for every 5-mm increase in the left atrium, on the echocardiogram, the risk for AF increases by 39%. Sanfilippo et al.[30] have concluded that maintaining sinus rhythm prevents the progression of left atrial hypertrophy and its adverse effects. Maceira et al.[34] have studied the right atrial dimensions by use of magnetic resonance imaging. The best independent indicators of increased atrial volume were area greater than 16 cm^2/m^2 and longitudinal diameter greater than 3.5 cm/m^2. The present study did not assess left atrial volume in the preoperative period, because not all patients underwent echocardiography before surgery.

In our study, CVP maintained ≤ 15 cmH_2O proved to be important to prevent post-CABG AF, as compared to CVP ≤ 20 cmH_2O. The statistical power of the sample was satisfactory (66.70%); however, further studies, with larger samples, are required to validate that approach as a prophylactic measure against AF in the PO period of cardiac surgery. Our data suggest that stricter CVP control is advantageous to prevent excessive volume overloads. Neither mortality after discharge, nor the appearance of AF after that have been assessed. Another study[35] has shown that strict CVP control can prevent post-CABG AF as compared to no CVP control. Our study strategy proved beneficial (NNT = 7) in this relatively small sample (140 patients) in a single-center study.

Multicenter studies with a larger sample that can assess not only AF incidence, but also mortality and the increase in costs with longer hospital length of stay, are required to better assess this type of treatment. It is worth noting that the use of diuretics caused no harm to the patients, there was increase in neither renal failure nor hemodynamic instability, in addition to being a very low cost strategy. Thus, CVP control can serve as a complementary method in post-CABG AF prophylaxis.

Conclusion

The CVP control maintained ≤ 15 cmH_2O, in the first 48-72 PO hours, can reduce the incidence of post-CABG AF. One in every seven patients benefits from that strategy.

Author contributions

Conception and design of the research: Costa MAC, Lirani W, Wippich AC, Schafranski MD; Acquisition of data, Analysis and interpretation of the data, Statistical analysis, Writing of the manuscript and Critical revision of the manuscript for intellectual contente: Costa MAC, Lirani W, Wippich AC, Lopes L, Tolentino ES, Zampar B, Schafranski MD; Obtaining financing: Costa MAC, Lirani W, Schafranski MD.

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

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A Multivariate Model for Prediction of Obstructive Coronary Disease in Patients with Acute Chest Pain: Development and Validation

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Abstract

Background: **Currently, there is no validated multivariate model to predict probability of obstructive coronary disease in patients with acute chest pain.**

Objective: **To develop and validate a multivariate model to predict coronary artery disease (CAD) based on variables assessed at admission to the coronary care unit (CCU) due to acute chest pain.**

Methods: **A total of 470 patients were studied, 370 utilized as the derivation sample and the subsequent 100 patients as the validation sample. As the reference standard, angiography was required to rule in CAD (stenosis ≥ 70%), while either angiography or a negative noninvasive test could be used to rule it out. As predictors, 13 baseline variables related to medical history, 14 characteristics of chest discomfort, and eight variables from physical examination or laboratory tests were tested.**

Results: **The prevalence of CAD was 48%. By logistic regression, six variables remained independent predictors of CAD: age, male gender, relief with nitrate, signs of heart failure, positive electrocardiogram, and troponin. The area under the curve (AUC) of this final model was 0.80 (95% confidence interval [95%CI] = 0.75 – 0.84) in the derivation sample and 0.86 (95%CI = 0.79 – 0.93) in the validation sample. Hosmer-Lemeshow's test indicated good calibration in both samples (p = 0.98 and p = 0.23, respectively). Compared with a basic model containing electrocardiogram and troponin, the full model provided an AUC increment of 0.07 in both derivation (p = 0.0002) and validation (p = 0.039) samples. Integrated discrimination improvement was 0.09 in both derivation (p < 0.001) and validation (p < 0.0015) samples.**

Conclusion: **A multivariate model was derived and validated as an accurate tool for estimating the pretest probability of CAD in patients with acute chest pain. (Arq Bras Cardiol. 2017; 108(4):304-314)**

Keywords: **CoronaryArtery Disease; Methods; Chest Pain; Models Statistical; Coronary Angiography; Troponin; Electrocardiography.**

Introduction

Acute chest pain is one of the most common reasons for emergency department visits. Since it may represent a clinical manifestation of cardiac ischemia, patient discharge is normally conditioned to a negative test for obstructive coronary artery disease (CAD).[1] However, the efficiency of this defensive strategy is challenged by a low yield of cardiac tests, since only a portion of patients ends up having obstructive CAD and a smaller part will need revascularization.[2] In addition, routine testing is not supported by evidence of beneficial effect[3] and may have unintentional consequences: overdiagnosis and overtreatment of coronary disease not causally related to symptoms, prolonged hospital stay, unnecessary invasive procedures due to false-positive test results, and increased medical expenses.[4]

Therefore, a more rational approach is to indicate additional tests on the basis of pretest probability. Traditionally, this pretest evaluation is restricted to electrocardiogram and necrosis markers. However, the use of a multivariate model has the potential to improve accuracy and provide a more continuous range of probabilities. In order to develop and validate a multivariate model to predict CAD based on variables assessed at admission to the coronary care unit, 370 consecutive patients were studied. Thirty-five variables were tested as candidate predictors of obstructive CAD in order to generate a final model that was further validated in a subsequent sample of 100 patients.

Methods

Sample selection

During a period of 30 consecutive months, all patients admitted to the coronary care unit of our hospital were included in the study. Admission took place whenever medical judgment recognized any chance of a coronary etiology, regardless of

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electrocardiogram or troponin. The only exclusion criterion was the patient's decline to participate. As defined *a priori,* the first 370 patients were utilized as the derivation sample and the next 100 patients as the validation sample. The study was approved by an institutional review committee, and all the subjects gave informed consent to participate.

Predictors of obstructive CAD

At baseline admission, three sets of variables were recorded as candidates for prediction of obstructive CAD. The first comprised 13 variables related to medical history, such as age, gender, previous history of CAD, risk factors for CAD, and comorbidities; the second set included 14 characteristics of chest discomfort; and the third set was composed of eight variables related to either physical examination or basic admission tests, including physical and radiologic signs of left heart failure, ischemic electrocardiographic changes (T wave inversion ≥ 1 mm or dynamic ST deviation ≥ 0.5 mm), positive troponin (> 99th percentile of the general population; Ortho-Clinical Diagnostics, Rochester, NY, USA), N-terminal pro–B-type natriuretic peptide (NT-proBNP, enzyme-linked fluorescent assay, Biomérieux, France), high-sensitivity C-reactive protein (CRP; nephelometry, Dade-Behring, USA), white cell count, plasma glucose, and hemoglobin. Laboratory tests were performed in plasma material collected at presentation to the emergency room. The medical history and chest pain characteristics were recorded by three investigators (M.C., A.M.C., and R.B.) trained to interview the patients in a systematic form, in order to decrease bias and improve reproducibility. Radiologic signs of ventricular failure and electrocardiogram were all interpreted by the same senior investigator (L.C.).

Outcomes definition

The primary outcome to be predicted by the model was a diagnosis of obstructive CAD, defined by subsequent tests performed during hospital stay. The outcome data were collected by three investigators (M.C., A.M.C., and R.B.) and adjudicated by a fourth investigator (L.C.). For diagnostic evaluation, the patients underwent invasive coronary angiography or a provocative noninvasive test (perfusion magnetic resonance imaging, nuclear single-photon emission computed tomography or stress-echocardiography with dobutamine), at the discretion of the assistant cardiologist. In the case of a positive noninvasive test, the patients had angiography for confirmation. Based on this diagnostic algorithm, obstructive CAD was defined as a ≥ 70% stenosis on angiography. A normal noninvasive test (ischemic defect size < 5% of the left ventricular myocardium) indicated the absence of obstructive CAD and no further test was required. Regardless of coronary tests, the patients were classified as presenting no obstructive CAD if one of the following dominant diagnoses was confirmed by image: pericarditis, pulmonary embolism, aortic dissection or pneumonia. Secondarily, the model was tested for the prediction of death within 30 days of admission.

Statistical analysis

The statistical analysis is depicted in Figure 1. The initial sample of 370 consecutive patients was utilized for the derivation of the model. First, univariate associations between obstructive CAD and baseline characteristics were tested by unpaired Student's *t* test for numeric variables and Pearson's chi-square test for categorical variables. Numeric variables not normally distributed were expressed as median and interquartile

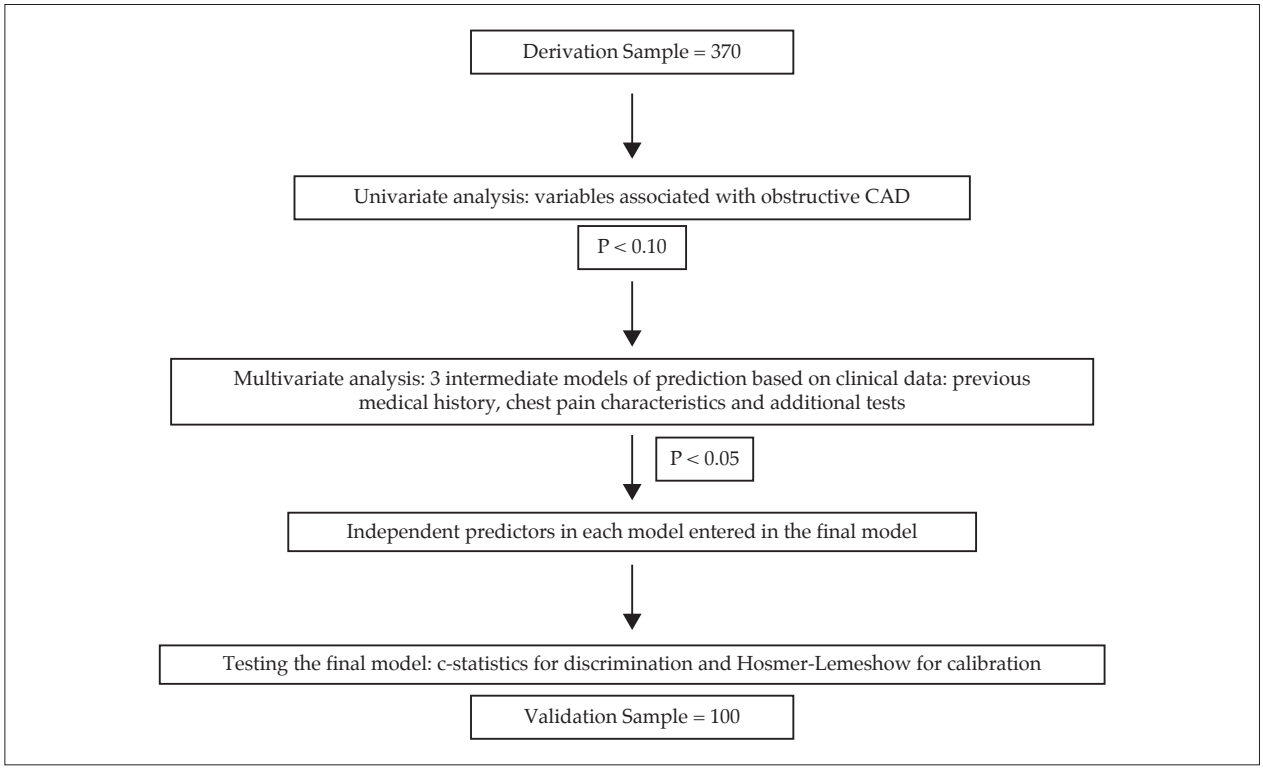

Figure 1 – *Flowchart of the statistical analysis.*

range and compared by nonparametric Mann-Whitney's test. Second, variables with a p value < 0.10 in the univariate analysis were included in the multivariate logistic regression analysis for prediction of obstructive CAD.

Multivariate models were developed by the stepwise method, forcing all selected variables into the regression and eliminating the least significant at each step, according to Wald's statistical test. Initially, three intermediate models were built, according to the type of predictive variables (medical history, chest pain characteristics or physical examination/laboratory tests). Independent predictors ($p < 0.05$) in each intermediate model were included as covariates in the final model. This final model was built hierarchically, with the order of variable imputation defined by clinical reasoning. The improvement of the model at each step was described by the decrease in -2Log likelihood.

Discrimination was evaluated by the area under the receiver operating characteristic (ROC) curve (AUC), while calibration was assessed by Hosmer-Lemeshow's test and correlation between predictive and observed prevalence of disease according to deciles of prediction. The incremental value of the full model in relation to the most basic model was evaluated by comparing the two AUCs by DeLong's test. In addition, *integrated discrimination improvement* by the full model was described according to Pencina's method.[5]

Subsequently, 100 consecutive patients served as the validation sample. In this sample, discrimination of CAD was tested by the AUC. Since calibration analysis by deciles would not be appropriate in a sample of 100 patients, observed CAD prevalence was compared among tertiles of CAD prediction. The incremental value of the full model in relation to the most basic model was evaluated by comparing the two AUC by DeLong's test. *Integrated discrimination improvement* by the full model was also described in this sample.

In a sensitivity analysis, the full sample of 470 patients was used to test whether the performance of the model changed according to the presence or absence of electrocardiographic or troponin changes. For this analysis, an interaction term was tested by logistic regression. The full sample was also used to test the prognostic value of the model. The AUC for 30-day mortality prediction was described and compared with the GRACE score[6] as a proxy of a model specifically created for a prognostic purpose. DeLong's test was used to compare the AUCs.

Statistical significance was defined as alpha < 0.05. For numerical variables with normal distribution, mean and standard deviation was used, while a non-normal distribution implied in the use of median and interquartile range. SPSS, version 21.0, was the software used for statistical analysis.

Acute chest pain score

In order to generate a score for CAD prediction, points were attributed to each positive variable, proportional to their regression coefficients in the final model. The prevalence of obstructive CAD was described according to score's deciles. Alternatively, the final regression formula was used to create a logistic calculator, provided as an Excel spreadsheet (electronic file) or application for smartphones (to be available in the near future).

Sample size determination

As described above, two consecutive samples of patients were selected: the derivation set and the validation set. For the derivation set, the sample size was planned to allow inclusion of at least 10 covariates in the logistic regression model. The calculation was based on the following assumptions: 30% prevalence of obstructive CAD and the need for 10 events for each covariate in the logistic regression model.[7] Therefore, a minimum of 300 patients would be required, and as a safety precaution, we planned to include a total of 370 individuals. The validation sample was set to test the discriminatory accuracy by the ROC curve analysis. Based on the assumption of an AUC of 0.70, to provide 90% power to reject the null hypothesis of an AUC equal 0.50, under the alpha of 5%, a minimum of 85 patients was required. Therefore, we planned to include 100 patients in the validation set.

Results

Sample population for model derivation

In total, 370 patients were studied, aged 60 ± 16 years, 57% males, 33% with a previous history of coronary disease. The median time elapsed between the onset of symptoms and first clinical evaluation in the hospital was 4 hours (interquartile range = 1.8 – 13 hours). At presentation, 52% of the patients had ischemic changes on the electrocardiogram, and 48% had positive troponin. Further investigation according to study protocol identified obstructive CAD in 176 patients, a prevalence of 48%. All cases had diagnostic confirmation by invasive coronary angiography. Regarding the 194 patients without CAD, 74 were classified by a negative angiography, 105 by a negative noninvasive test and 15 had another dominant diagnosis (four with pulmonary embolism, two with aortic dissection, seven with pericarditis, and two with pneumonia).

Predictors of obstructive CAD

Among the 13 variables related to medical history, only four were associated with obstructive CAD: older age, higher prevalence of male gender, previous history of CAD, and a trend towards more diabetes (Table 1). When these four variables were included in the logistic regression, age and male gender remained statistically significant (Intermediate Model 1) (Table 2).

Regarding chest pain characteristics, among 14 variables, only five had an association with CAD: relief with nitrates and similarity with previous myocardial infarction. On the other hand, worsening with manual compression, deep breath or arm movement were each more common in patients without CAD (Table 1). Of these, relief with nitrates, worsening with manual compression and with deep breath were the three independent predictors in the Intermediate Model 2 (Table 2).

Among the physical examination and laboratory tests, most variables were associated with CAD: ischemic electrocardiogram, positive troponin, and signs of left heart failure were more prevalent in patients with CAD. Also, four numeric variables had higher values in patients with CAD: NT-proBNP, CRP, white cell count, and hemoglobin (Table 1).

Table 1 – Comparison of medical history, chest pain characteristics, and laboratory tests between patients with and without obstructive coronary artery disease

	Obstructive Coronary Disease		p Value
	Yes (n = 176)	No (n = 194)	
Medical History			
Age (years)	63 ± 14	57 ± 16	< 0.001
Male gender	121 (69%)	90 (46%)	< 0.001
Body mass index (kg/m^2)	28 ± 4.8	28 ± 5.9	0.61
History of CAD	68 (39%)	55 (28%)	0.03
Diabetes	62 (36%)	51 (26%)	0.05
Hypertension	122 (70%)	138 (71%)	0.83
Current smoking	22 (13%)	18 (9.3%)	0.30
LDL cholesterol (mg/dL)	113 ± 64	116 ± 87	0.72
Family history of CAD	48 (28%)	42 (22%)	0.19
Chronic renal disease	9 (5.3%)	7 (3.6%)	0.45
Plasma creatinine (mg/dL)	0.95 (0.80 – 1.20)	0.80 (0.70 – 1.15)	0.10
Current statin therapy	85 (49%)	91 (47%)	0.71
Current aspirin therapy	75 (43%)	76 (39%)	0.44
Chest Pain Characteristics			
Left side location	137 (79%)	156 (81%)	0.70
Oppressive nature	97 (57%)	95 (49%)	0.14
Irradiation to neck	39 (23%)	51 (26%)	0.42
Irradiation to left arm	57 (33%)	53 (27%)	0.24
Vagal symptoms	61 (36%)	78 (40%)	0.35
Number of episodes	1 (1 – 2)	1 (1 – 3)	0.81
Duration (minutes)	40 (15 – 120)	40 (10 – 150)	0.82
Intensity (1 – 10 scale)	7.4 ± 2.5	7.1 ± 2.6	0.31
Relief with nitrate	84 (50%)	72 (37%)	0.02
Similar to previous infarction	70 (42%)	63 (33%)	0.08
Worsening with compression	7 (4.1%)	26 (13%)	0.002
Worsening with position	24 (14%)	36 (19%)	0.23
Worsening with arm movement	7 (4.0%)	16 (8.2%)	0.097
Worsening with deep breath	13 (7.5%)	36 (19%)	0.002
Laboratory Tests at Admission			
Ischemic changes on ECG	120 (68%)	73 (38%)	< 0.001
Positive troponin	116 (66%)	60 (31%)	< 0.001
X-ray and clinical signs of LVF	26 (15%)	5 (2.6%)	< 0.001
NT-proBNP (pg/mL)	363 (105 – 1850)	57 (20 – 235)	< 0.001
Plasma glucose (mg/dL)	120 (97 – 189)	112 (92 – 145)	0.22
C-reactive protein (mg/L)	7.3 (2.3 – 15)	5.7 (1.4 – 15)	0.09
White cell count	8.790 ± 4.300	7.701 ± 2.865	0.004
Hemoglobin (g/dL)	14.1 ± 1.9	13.7 ± 1.7	0.06

CAD: coronary artery disease; LVF: left ventricular failure. A family history of CAD implies in the presentation of the disease in a first-degree relative before the age of 55 years (females) or 45 years (males).

Table 2 – Intermediates logistic regression models of medical history (Model 1), chest pain characteristics (Model 2) and laboratory tests (Model 3)

Variables	Multivariate significance level
Model 1 (medical history)	
Male gender	< 0.001
Age (years)	< 0.001
Diabetes	0.10
HDL cholesterol	0.35
Previous CAD	0.84
Plasma creatinine (mg/dL)	0.95
Model 2 (pain characteristics)	
Sensible to manual compression	0.024
Sensible to deep breath	0.037
Relief with nitrate	0.045
Similar to a previous MI	0.17
Sensible to arm movement	0.57
Model 3 (laboratory tests)	
Ischemic changes on ECG	< 0.001
Positive troponin	< 0.001
X-ray or clinical signs of LVF	0.016
White cell count	0.29
Hemoglobin (g/dL)	0.67
NT-proBNP (pg/mL)	0.81
C-reactive protein (mg/L)	0.70

MI: myocardial infarction; CAD: coronary artery disease; LVF: left ventricular failure.

In the Intermediate Model 3, ischemic electrocardiogram, positive troponin, and signs of left heart failure were the independent predictors (Table 2).

Development of a model for CAD prediction

The eight variables independently associated with CAD in the Intermediate Models 1, 2, and 3 were candidates to the final model, which was built hierarchically in seven steps, defined by clinical reasoning: the first step comprised electrocardiogram and troponin together, followed by the second step that included left ventricular failure. These two first steps represented the severity of the clinical presentation. The third and fourth steps represented intrinsic characteristics of the patients, age, and gender. The fifth, sixth, and seventh steps were related to characteristics of chest pain, which were chosen to be last because of their subjectivity in clinical practice.

The first step of electrocardiogram and troponin had a -2Log likelihood of 437 (χ^2 = 69, p < 0.001), which sequentially improved by the inclusion of left ventricular failure (-2Log likelihood = 427, χ^2 = 9.8, p = 0.002), age (-2Log likelihood = 422, χ^2 = 4.9, p = 0.02), gender (-2Log likelihood = 401, χ^2 = 21, p < 0.001), and relief with nitrates (-2Log likelihood = 394, χ^2 = 6.8, p = 0.009). The inclusion of worsening with manual compression (-2 log likelihood = 391, χ^2 = 3.2, p = 0.07) and worsening with deep breath (-2 log likelihood = 389, χ^2 = 2.3, p = 0.13) did not promote further improvement in the model. Therefore, the first six variables constituted the final model.

The final model presented good discrimination, with an AUC of 0.80 (95%CI = 0.75 – 0.84) (Figure 2A). The Hosmer-Lemeshow's χ^2 of 1.95 indicated that the model was well calibrated (p = 0.98), as shown in the scatter plot of predictive probability *versus* observed prevalence of CAD by deciles (r = 0.99) (Figure 2B). The probability of CAD according to the final model ranged from a minimum of 3% to a maximum of 98%, with patients equally distributed across probabilities. Odds ratio, 95%CIs and regression coefficients, and p values of the final model are depicted in Table 3.

Incremental value of the full model

The AUC improved from 0.73 in the first model containing only electrocardiogram and troponin to 0.80 in the full model (95%CI of difference between the areas = 0.03 – 0.10, p = 0.0002). Discrimination progressively improved as variables were added: the AUC was 0.74 in the second model (adding left ventricular failure), 0.76 in the third model (adding age), and 0.79 in the fourth model (adding gender). The integrated discrimination improvement provided by the full model in relation to the first model was 0.09 (p < 0.001), a result of 0.05 of mean increase of probabilities in the group with events plus 0.04 of mean decrease of probabilities in the group free of events.

Validation by the independent sample

The validation sample consisted of 100 individuals, 62% males, aged 60 ± 13 years, with a 59% prevalence of obstructive CAD. In this group, the AUC was 0.86 (95%CI = 0.79 – 0.93) and Hosmer-Lemeshow's calibration χ^2 was 10.1 (p = 0.26) (Figure 3A). As the group was divided into tertiles of model's predicted probability (< 30%, 30 – 60%, > 60%), a progressive increase in disease prevalence was observed (24%, 59%, and 94%, respectively, p for trend < 0.001) (Figure 3B).

Compared with the basic model containing only electrocardiogram and troponin (AUC = 0.78), the increment provided by the full model was +0.07 (95%CI of difference between the areas = 0.004 – 0.14, p = 0.039). The integrated discrimination improvement provided by the full model in relation to the first model was 0.09 (p < 0.0015), a result of 0.02 of mean increase of probabilities in the group with events plus 0.07 of mean decrease of probabilities in the group free of events.

Sensitivity of the final model to electrocardiogram and troponin

The entire sample of 470 patients was utilized to test the model's sensitivity to electrocardiogram and troponin. There was no interaction between the model's prediction and presence (or absence) of electrocardiographic/troponin changes (p = 0.48), meaning that the performance of the model was not modified by these variables. The model's AUC

Table 3 – Final model of logistic regression defining the independent predictors of obstructive coronary artery disease

Variables	Beta	Odds Ratio (95%CI)	p Value
Age (each year)	0.025	1.03 (1.01 – 1.04)	0.003
Relief with nitrates	0.60	1.8 (1.1 – 3.0)	0.016
Ischemic ECG	1.10	3.0 (1.9 – 4.9)	< 0.001
Positive troponin	1.15	3.2 (1.9 – 5.1)	< 0.001
Male gender	1.16	3.2 (1.9 – 5.3)	< 0.001
Signs of LVF	1.55	4.7 (1.6 – 14)	0.004
Sensible to deep breath	----	----	0.06
Sensible to manual compression	----	----	0.18

LVF: left ventricular failure.

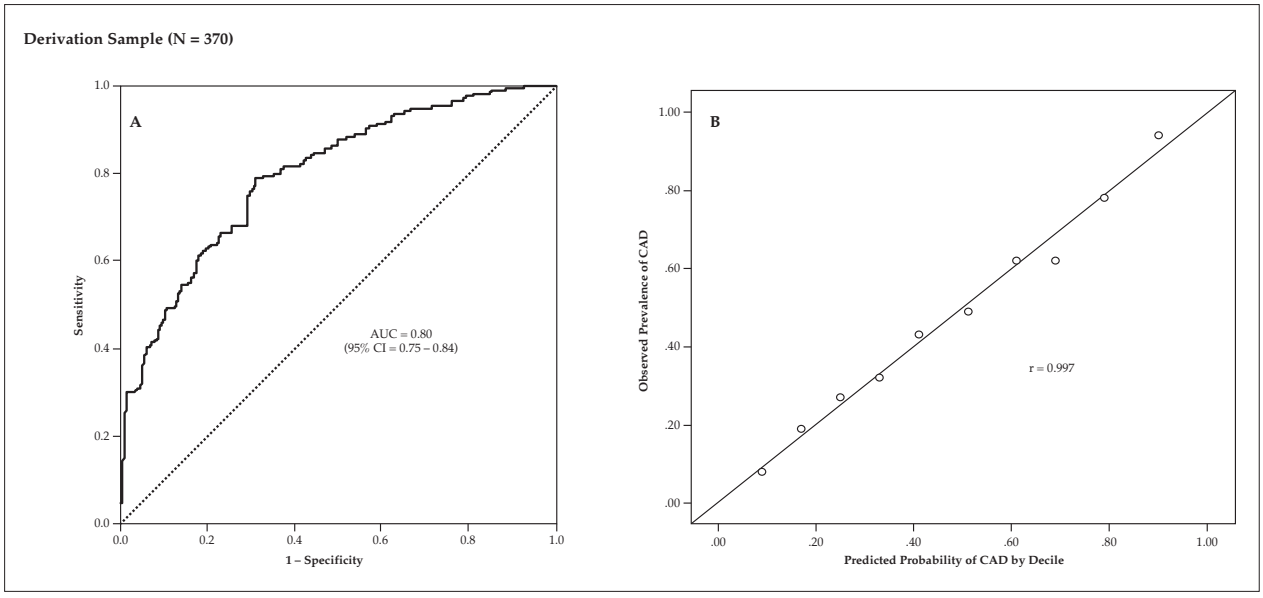

Figure 2 – *Analysis of the model's discrimination and calibration in the derivation sample of 370 patients. Panel A shows significant AUC of the probabilistic model for prediction of obstructive coronary artery disease. Panel B shows a significant correlation between predicted and observed probability of coronary artery disease (CAD). AUC denotes area under the receiver operating characteristic curve.*

of individuals with normal electrocardiogram and troponin (n = 147, 24% with CAD) was 0.74 (95%CI = 0.65 – 0.83), while individuals with either one abnormal (n = 323, 62% of CAD) had an AUC of 0.77 (95%CI = 0.71 – 0.82).

Prognostic value for 30-day mortality

In the entire sample of 470 patients, 10 patients (2.1%) died within the first 30 days from initial chest pain, eight during hospitalization and two after discharge. The ability of the model to predict death was shown by an AUC of 0.74 (95%CI = 0.61 – 0.87), similar to the GRACE score prognostic value of 0.72 (95%CI = 0.54 – 0.91, p = 0.83) (Figure 4A). There was no death in the first tertile of this entire sample (CAD probability < 30%), three deaths in the second tertile (30 – 62%), and seven deaths in the third tertile (> 62%, p for trend = 0.006) (Figure 4B).

Acute chest pain score

Points proportional to the regression coefficients were attributed to each positive variable: age (β = 0.025; 0.05 point for each year), relief with nitrates (β = 0.60; 1 point), male gender (β = 1.16; 2 points), ischemic electrocardiogram (β = 1.10; 2 points), positive troponin (β = 1.15; 2 points), and signs of left ventricular failure (β = 1.55; 3 points). The score presented the same AUC as the logistic model. There was a proportional increase in disease prevalence according to score deciles: 11%, 14%, 24%, 37%, 41%, 53%, 59%, 67%, 74%, and 95% (p for linear trend < 0.001) (Figure 5).

Discussion

The present study developed and validated a probabilistic model to predict obstructive CAD based on data from the initial presentation of acute chest pain. From a total of

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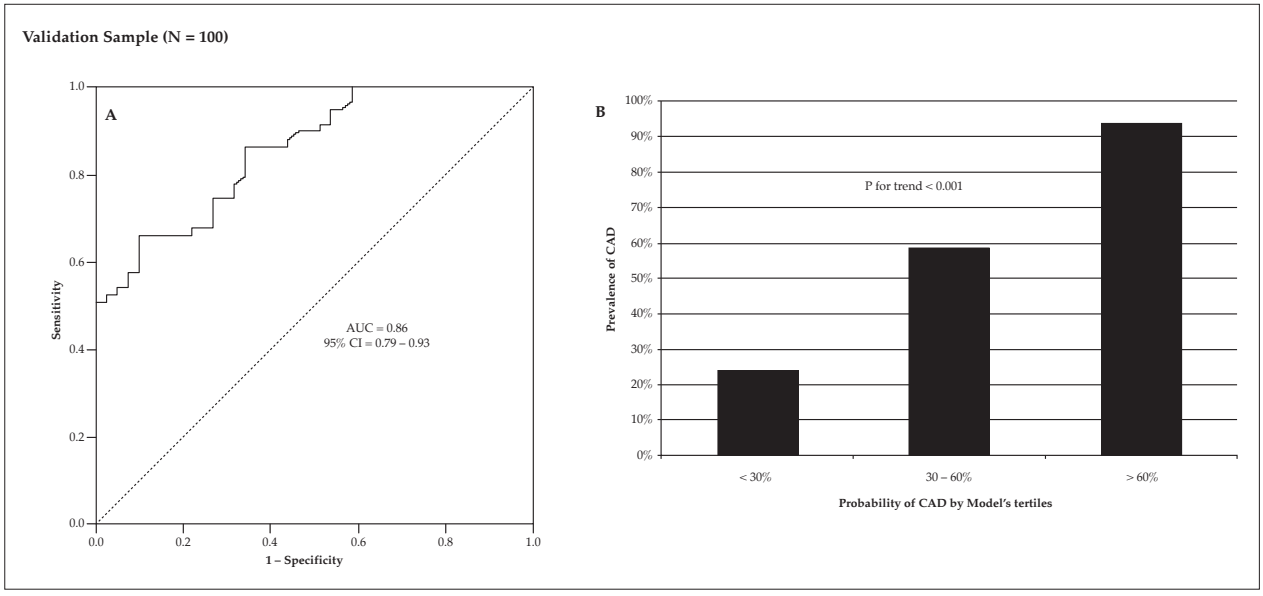

Figure 3 – *Analysis of the model's performance in the independent validation sample of 100 patients. Panel A shows a significant AUC of the probabilistic model for prediction of obstructive coronary artery disease (CAD). Panel B indicates a progressive increase in the prevalence of CAD according to tertiles of the model's prediction. AUC denotes area under the receiver operating characteristic curve.*

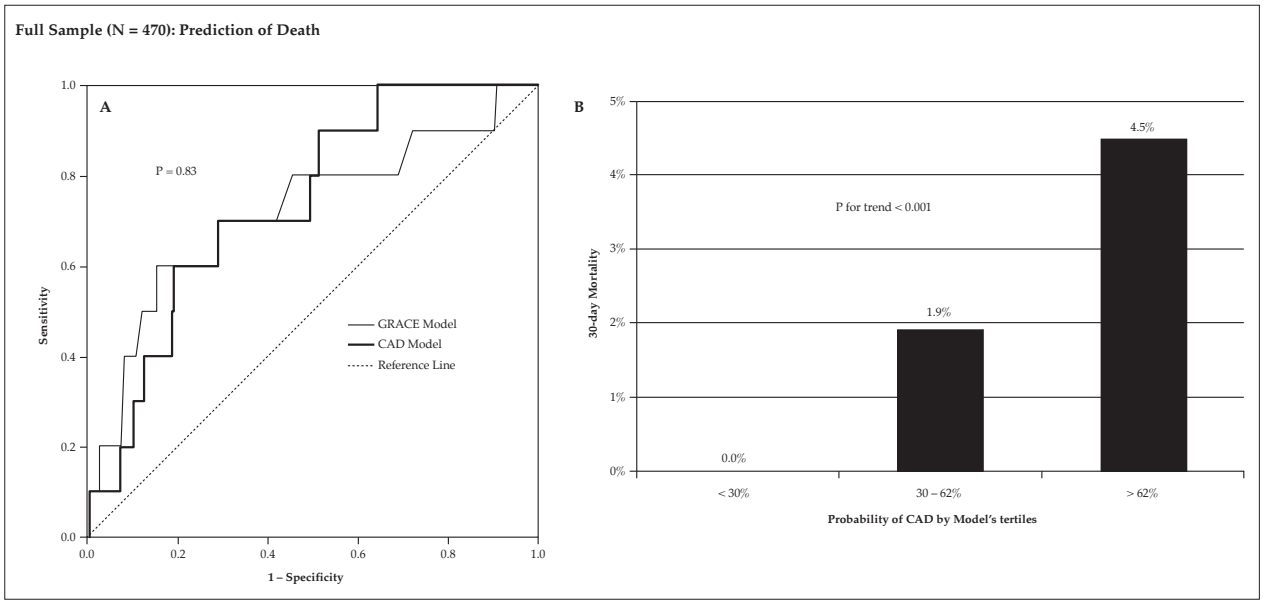

Figure 4 – *Mortality analysis in the full sample of 470 patients, showing a significant prognostic value of the model, which was originally derived for coronary artery disease (CAD) prediction. Panel A compares the C-index of the model versus GRACE score, indicating similar prediction. Panel B compares the incidence of CAD among tertiles of model's coronary disease prediction. AUC denotes area under the receiver operating characteristic curve.*

35 candidate variables, a final model of six independent predictors was generated, with good discrimination and calibration for assessing the pretest probability of the disease. Most importantly, the accuracy of the model proved to be superior to the traditional model that uses electrocardiogram and troponin.

The indication of diagnostic tests should take into account the pretest probability of the disease. However, in the selected setting of coronary care units, virtually all patients with undefined chest pain undergo testing for detecting obstructive CAD, regardless of pretest probability. Since the test will be negative in a significant proportion of patients,[2] this approach leads to unnecessarily prolonged hospital stay. Thus, eliminating the need for additional tests in patients with low probability of CAD will improve the efficiency of chest pain protocols. However, validated probabilistic models are not disseminated in

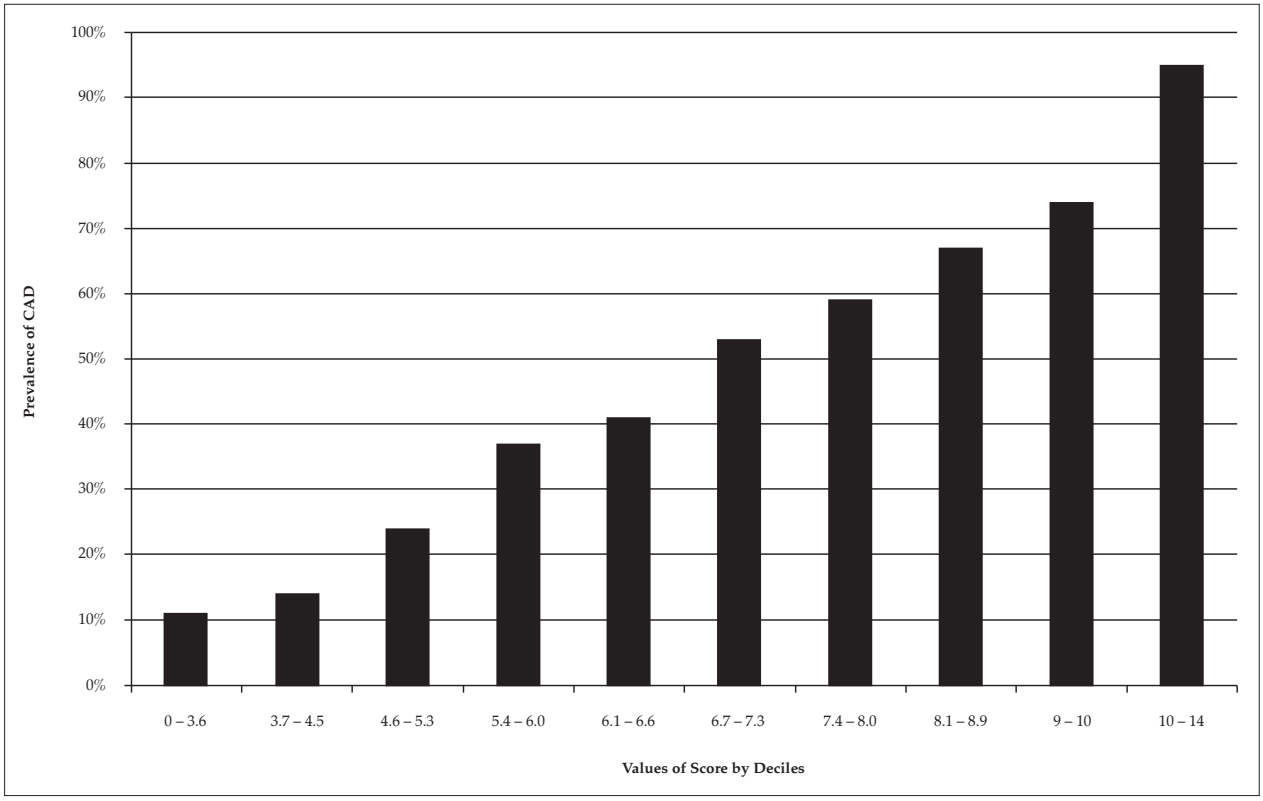

Figure 5 – *Prevalence of obstructive coronary artery disease (CAD) according to score's deciles.*

this clinical setting, making it hard for the emergency physician to tailor medical decision based on probability. At the most, the probability is evaluated in a binary form, based on whether the electrocardiogram or troponin is altered.

The use of such a probability model improves accuracy and offers a range of continuous probabilities, approximating medical thinking to the best form of dealing with uncertainty. As William Osler once said, "medicine is the science of uncertainty and the art of probability."

Our purpose to predict obstructive CAD should not be confused with previous studies that developed neural or logistic models for predicting the clinical diagnosis of myocardial infarction in patients with chest pain.[8-12] Such studies created models from clinical data, symptoms characteristics, and sometimes electrocardiogram, which were tested as predictors of a final diagnosis defined by a systematic analysis of the same variables in addition to markers of myocardial necrosis. Therefore, these mathematical models mainly serve as surrogates of medical thinking or, at the most, predictors of a final impression that will be obtained in a few hours of the initial presentation. In contrast, our model was built to predict the result of imaging tests before they are performed. Since noninvasive or invasive imaging tests aim the diagnosis of obstructive CAD, a model of this kind is clearly useful in efficiently selecting patients for these tests, based on the estimation of the pretest probability of the disease. In addition, the knowledge of a pretest probability permits the calculation of the post-test probability after a noninvasive imaging result is obtained.

Other scores focus on the risk of adverse events (HEART score,[13] TIMI score[14] or GRACE score[6]). Despite their prognostic value, they are not necessarily good predictors of obstructive CAD[15] and physicians are uncomfortable to discharge a patient with acute chest pain with no further testing. Thus, we believe that the calculation of the probability of obstructive CAD would encourage physicians to reduce overuse of imaging studies in patients with low probability, diminishing the phenomena of overdiagnosis and overtreatment. For example, patients with a normal electrocardiogram and negative troponin are known to have a good prognosis. In our study, 50% of these patients had a probability of significant CAD below 20%. Based on favorable prognostic and diagnostic probabilities, these patients could be discharged with no further testing. On the other hand, patients with normal electrocardiogram and troponin may have a significant probability of CAD that can be detected by the model. We should point out that future randomized clinical trials should validate the efficiency and safety of this approach.

Physicians normally rely on symptoms characteristics (typical or atypical) and traditional risk factors to estimate the chance of CAD in patients with acute chest pain. For example, a diabetic patient with typical chest pain is usually defined as having a high probability of CAD. However, in our study, no risk factors and chest pain characteristic (except for nitrate relief) independently predicted CAD. This is in agreement with previous studies, which indicate that the type of presentation has little influence on the diagnosis in the acute setting. In a comprehensive systematic review,

Swap and Nagurney et al.[16] showed low likelihood ratios for chest pain characteristics. Seemingly, a recent article by Khan et al.[17] demonstrated that most pain characteristics are not associated with coronary disease as the cause of the symptom. Therefore, our data reinforce that the approach to rely on risk factors and symptoms to stratify acute chest pain patients has low accuracy. The utilization of a probabilistic model prevents this type of cognitive error.

We purposed three easy forms of utilization of the probabilistic model. First, a score based on points attributed to each positive variable, accompanied by a chart relating summed results and probabilities (Figure 4). Considering the low number of variables, five of them of binary nature, the calculation is easily performed. Second, a logistic score within a spreadsheet with the regression formula, containing age as numeric variable and five "yes" or "no" answers. And, most friendly, an application for smartphones. We believe that by offering different forms of calculations, the clinicians will develop a greater interest in using probabilistic models.

Limitations of the present study should be recognized. The study was performed in a coronary care unit of a specific tertiary hospital, which limits external validity. The population of a chest pain unit is somewhat selected and tends to have a higher prevalence of disease than a general emergency room population. Thus, our model should be further validated for patients with a greater range of clinical presentation. On the other hand, the main purpose of the model is to estimate the pretest probability of hospitalized individuals, which also consist of a large subgroup of real world patients. In this sense, our external validity is not necessarily small; it is just more specific to the tested population.

We should recognize that our sample size is relatively small in comparison with examples of scores delivered from enormous databanks. We have three arguments in favor of our study of 470 patients: first, its novelty as the first successful attempt to develop such a score, which serves at least as a proof of concept that a multivariate model predicts the pretest probability of the disease. Second, in the absence of a multivariate probabilistic model, physicians use clinical judgment based on probabilistic intuition, which has been proved in different settings to be inferior to multivariate models. Thus, considering the remaining alternative of intuition, it might be a good idea to use such a score, not deterministically, but as a tool to avoid common cognitive biases related to intuition. Third, our sample size was based on *a priori* sample size calculation for the logistic regression and for testing the model with ROC curve. According to this calculation, our number of events was enough to provide the minimum power and precision required. Nevertheless, future reports should improve the precision of our estimates.

Finally, among patients who underwent noninvasive tests first, only those with positive results had confirmation by angiography. Nevertheless, predicting a negative noninvasive test (as opposed to no disease at all) is sufficient to prevent the patient from staying unnecessarily to undergo the test.

Conclusion

The present study developed and validated a novel model to predict obstructive CAD among patients who are admitted with acute chest pain in the coronary care unit. The utilization of such a model should have an impact in preventing overuse of tests, overdiagnosis, and overtreatment while improving the accuracy of pretest assessment of disease probability.

Author contributions

Conception and design of the research: Correia LCL, Cerqueira M, Carvalhal M, Ferreira F, Garcia G, Silva AB, Sá N, Lopes F, Barcelos AC, Noya-Rabelo M; Acquisition of data: Cerqueira M, Carvalhal M, Ferreira F, Silva AB, Sá N, Lopes F, Barcelos AC, Noya-Rabelo M; Analysis and interpretation of the data: Correia LCL, Cerqueira M, Garcia G, Silva AB, Sá N, Lopes F, Barcelos AC, Noya-Rabelo M; Statistical analysis: Correia LCL, Cerqueira M, Ferreira F, Garcia G, Noya-Rabelo M; Writing of the manuscript: Correia LCL, Cerqueira M, Carvalhal M, Ferreira F, Silva AB, Sá N, Lopes F, Barcelos AC, Noya-Rabelo M; Critical revision of the manuscript for intellectual contente: Correia LCL, Cerqueira M, Carvalhal M, Garcia G, Silva AB, Sá N, Lopes F, Barcelos AC, Noya-Rabelo M.

Potential Conflict of Interest

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

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Study Association

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

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Original Article

Effects of Continuous and Accumulated Exercise on Endothelial Function in Rat Aorta

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Abstract

Background: **The practice of exercise in short bouts repeated throughout the day may be an alternative strategy to lift people out of physical inactivity.**

Objective: **to evaluate if accumulated exercise, as occurs in continuous exercise training, improve endothelial function in rat aorta.**

Methods: **Wistar male rats were divided into three groups: continuous exercise (CEx, 1 hour on the treadmill) or accumulated exercise (AEx, 4 bouts of 15 minutes / day) for 5 days/week for 8 weeks, or sedentary (SED). During the training period, body weight gain and increase in exercise performance were recorded. On sacrifice day, aorta was dissected into rings (3-5 mm) and mounted on the organ bath.**

Results: **Fitness was significantly greater in CEx and AEx rats as compared with SED animals. In addition, compared with the SED group, CEx animals had a lower body mass gain, and the aorta obtained from these animals had reduced contractile response to norepinephrine and greater acetylcholine-induced relaxation. These results were not observed in ACEx animals.**

Conclusions: **Both CEx and AEx improved fitness, but only CEx led to reduced body weight gain and improved endothelial function. (Arq Bras Cardiol. 2017; 108(4):315-322)**

Keywords: **Rats; Exercise; Physical Fitness; Endothelium; Acetylcholine; Norepinephrine; Weight Loss.**

Introduction

Exercise has been considered an important instrument for the promotion of health and prevention of cardiovascular diseases. It is defined as any "physical activity that is planned, structured, and repetitive and [that] has as a final or intermediate objective the improvement or maintenance of physical fitness".[1,2] The pattern of regular exercise that brings better health benefits is still debated in the literature. Normally, it is recommended exercise of moderate intensity, at least 3 days a week.[3] Alternatively, exercise may be performed by bouts of at least 10 minutes of high intensity exercise interspersed with intervals of recovery, i.e., periods of mild exercises or simply rest.[4,5] On the other hand, current recommendations also suggest that short sessions of moderate-intensity physical activity accumulated throughout the day to attain a daily goal of 30 min of exercise - named accumulated exercise[2] - may be employed to improve the health or as adjuvant treatment of cardiovascular diseases.[6] Indeed, the practice of exercise in accumulated sessions can be an alternative to lift people out of physical inactivity.[7]

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Health benefits of the accumulated exercise have already been demonstrated: elevation of high-density lipoprotein levels,[8,9] reduction of postprandial triglycerides,[10] blood pressure levels,[11,12] skinfold thickness and waist circumference,[6] and improvement of fitness[13] and mood state.[6] However, there is no evidence of the influence of accumulated exercise on endothelial function.

The benefits of exercise on endothelial function occur mostly by the increment of shear stress on endothelial surface, thereby stimulating the expression of endothelial nitric oxide synthase (eNOS), cyclooxygenase-2 (COX-2) and superoxide dismutase-1 (SOD- 1).[14-17]However, it has been demonstrated in endothelial cell cultures that the exposure time to the shear stress influences the expression of these enzymes.[15,18] Particularly in relation to eNOS, it was demonstrated that the shear stress exposure time influences its degree of phosphorylation, thus regulating its activity.[19] Thus, it is reasonable to infer that the exposure to different exercise times may have different effects on the expression of endothelial enzymes. Thus, the aim of the present study was to verify whether training by accumulated exercise improves endothelial function in rat aorta such as it occurs in consequence of training by continuous exercise.

Methods

Animals

Thirty three male Wistar rats weighing 300-400 g were housed in plastic cages (50 x 40 x 20 cm), 5 animals per cage, with food

and water "ad libitum". The sample size (n) was established on the basis of studies that evaluated the effects of continuous exercise on endothelial function.[16,20] Notably, these studies were the basis for the present investigation that investigate the cardiovascular effects of accumulated exercise. During the exercise protocol, the animals were maintained in the training room under a 12 h light-dark cycle beginning at 7:00 h, at room temperature (25°C). This study was approved by the Ethics Committee on Animal Use of Marilia School of Medicine (protocol n° 627/13).

Exercise protocol

Rats were initially trained to walk on a treadmill (Movement Technology LX 170) then submitted to daily sessions of 10 minutes, from 0.3 up to 0.5 km/h, without slope, for 2 weeks. At the end of this period, the animals were submitted to the treadmill running test, consisted of graded treadmill exercise at increments of 0.3 km/h every 3 minutes, starting at 0.3 km/h and increased up to the maximal intensity attained for each rat. Based on the results in this test, the animals were randomly assigned to one of the following groups: sedentary (SED), trained by continuous exercise (CEx) or trained by accumulated exercise (AEx), with a similar mean maximal exercise capacity in each group. Subsequently, the animals of the CEx group were exposed to this exercise 5 days per week, 1 hour per day (starting at 09:00am) for 8 weeks. The exercise intensity was increased progressively by a combination of time and velocity, to a maximum of 2 hours per day at a velocity correspondent to 60% of maximal exercise capacity, which was attained by the third week. In parallel, the animals belonging to the AEx group were submitted to 4 short exercise sessions (15 minutes, at similar speed to the CEx group), regularly distributed throughout the day (starting at 07:30am, 10:25am, 01:05pm and 03:45pm), 5 days per week, for 8 weeks. Rats allocated to the SED group were also handled every day and put on a stopped treadmill. Body weight was measured weekly during the training period. Running capacity tests were performed on each rat at the beginning of the protocol and on week 6, for adjustment of exercise intensity and assessment of increase in performance.

Euthanasia and sample collection

At the end of the training period, the animals were sacrificed by inhalation of CO_2 and exsanguination by puncture of the vena cava. Blood samples were collected in heparinized syringe and centrifuged (3500 rpm/10 min/4°C) to obtain the plasma, which was stored at –80°C. Later, the aortas were removed and immediately immersed in cold Krebs-Henseleit solution, and the hearts were weighed.

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS levels were measured according to a method adapted from Yagi.[21] Briefly, the lipid peroxidation was determined by the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a pink chromogen that can be quantified by spectrophotometry (in 532 nm). The values of absorbance detected in the samples were interpolated to a tetramethoxypropane standard curve (0 to 100 μM).

Plasma antioxidant capacity (Ferric Reducing Ability of Plasma FRAP)

The method described by Benzie & Strain[22] is based on the ability of plasma to reduce Fe^{+++} to Fe^{++} ions in the presence of 2,4,6 tripyridyl-s-triazine (TPTZ) at low pH with the formation of Fe^{++}-tripyridyltriazin, a blue colored complex. Before the beginning of the experiments, three solutions were prepared: A (Acetate buffer: 300 mM, pH 3.6 and 40 mMHCl), B (TPTZ - 2,4,6-tri- [2-pyridyl]-s-triazine - 10 mm) and C ($FeCl_3.6H_2O$ - 20 mM). The working reagent was prepared by adding A + B + C in the ratio 10: 1: 1 (V/V). Later, the plasma samples (0,08 mL) were added to the mixture of deionized water (2,4 mL) and working reagent (0.25 ml). This solution was placed in microplates in parallel with the blank sample (only working reagent) and the standard curve samples ($FeSO_4$ 0-1000 mmol/L). These samples were read in spectrophotometer at 593 nm, and the concentrations (in uM/L) were calculated by interpolation in the standard curve.

Organ bath studies

In a Petri dish covered with paraffin containing Krebs-Henseleit solution, the aortas were carefully divided into rings (3-5 mm). These rings were, then, set in 2 mL organ baths, fixed to a lower stainless steel hook attached to a stationary support and to an upper one connected to an isometric force transducer. The organ bath contained Krebs-Henseleit solution of the following composition (mM): NaCl 130; KCl 4.7; $CaCl_2$ 1.6; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 15 and glucose 11.1. The Kreb-Henseleit solution was kept at 37°C, pH 7.4 and continuously bubbled with a mixture of 95% CO_2 and 5% O_2. Tension was continuously monitored and recorded using a Powerlab 8/30 data acquisition system (Australia ADInstruments). Prior to the addition of drugs, the rings were equilibrated for 60 minutes under a resting tension of 1.5 g.

All preparations were challenged with 10^{-4} mol/L acetylcholine (ACh), after precontraction induced by10^{-5} mol/L phenylephrine (Phe), to verify the endothelial integrity. Some preparations had their endothelium mechanically removed, which was confirmed by the absence of relaxation in response to ACh. Later, both intact and endothelium-denuded preparations were challenged with cumulative concentrations of norepinephrine (NE; 10^{-10} – 10^{-4} mol/L). Intact preparations were also challenged with cumulative concentrations of NE in presence of N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) 10^{-4} mol/L, a non-selective NOS inhibitor added 20 minutes before the challenging. In parallel, intact preparations were challenged with single concentrations of ACh (10^{-4} mol/L) after precontraction induced by10^{-5} mol/L Phe.

The evoked responses (in g) to the aforementioned vasoactive agents, cumulatively added into the organ bath, were plotted to obtain concentration-response curves. Non-linear regressions (variable slope) of these curves revealed the R_{max} (maximal response; highest point of each concentration-response curve) and the pEC_{50} (negative logarithm of the concentration that evoked 50% of the maximal response). The pEC_{50} is indicative of the sensitivity to the studied drug.

The following drugs were used: Acetylcholinechloride;L-(-)-norepinephrine(+)-bitartrate salt monohydrate, N_{ω}-Nitro-L-arginine methyl ester hydrochloride, and phenylephrine hydrochloride, all purchased from Sigma Chemical Co.

Statistical analysis

Data are reported as mean ± standard error of the mean (SEM). Data obtained from the CEx and AEx groups were compared independently with those obtained from SED group by unpaired Student "t" test. Before applying the Student "t" test, the Gaussian distribution of data was verified by the Shapiro-Wilk normality test. The statistical analysis was performed using the GraphPad Prism 6.0 software. P values less than 0.05 were considered statistically significant.

Results

Running capacity test

During the training period, a significant improvement in running performance was observed in the CEx and AEx groups (Figure 1A and 1B) as compared with the SED animals. In contrast, in the same period, a reduction in performance was observed in the SED group.

Body and heart weight

During the training period, body weight gain was significantly ($p < 0.05$) lower in CEx animals (11.69 ± 3.28%; n = 11) than in SED animals (21.38 ± 1.19%; n = 11). On the other hand, body weight gain in AEx animals (21.38 ± 1.19%; n = 11) was not statistically different in comparison with SED animals. Heart weight in CEx (1.30 ± 0.04 g; n = 11) or AEx (1.37 ± 0.05 g; n = 11) animals was not significantly different from that in SED animals (1.38 ± 0.05g; n = 11).

TBARS and FRAP

The TBARS values in CEx and AEx animals [17.85 ± 3.57 (n = 11) and 24.91 ± 5.18 (n = 11), respectively] were not significantly different in comparison with SED animals [20.88 ± 5.29 (n = 11)].

Both continuous and accumulated exercise had no effect on plasma antioxidant capacity, since FRAP values in CEx and AEx groups [1309.00 ± 74.04 (n = 11) and 1222.00 ± 55.98 (n = 11), respectively] were not significantly different than in SED animals [1215.00 ± 57.11 (n = 11)].

Vascular responses

The CEx reduced the magnitude of responses to NE in the aorta, with a significant reduction in R_{max} values compared to SED animals. However, no significant differences in pEC_{50} were observed between these groups (Figure 2A). This reduction in NE R_{max} was not observed in L-NAME pre-treated preparations (Figure 2C) or in preparations without endothelium (Figure 2E). On the other hand, the slight reduction of NE responses induced by ExA did not result in significant reduction of R_{max} or pEC_{50} (Figure 2B), and was suppressed by the presence of L-NAME (Figures 2C and D) or the endothelium removal (Figures 2E and F).

Moreover, the CEx also increased the 10^{-4} mol/L ACh-induced relaxation of intact aorta precontracted with 10^{-5} mol/L Phe (Figure 3A). This effect was not observed in AEx (Figure 3B).

Discussion

The practice of regular exercises has been proven effective in reducing the risk of cardiovascular diseases.[3] However, the concept that only intense and long-lasting sessions of exercises are beneficial to health may compromise the adherence to this practice.[23]Indeed, the flexibility of exercise plan, including intensity, duration and frequency, can lead to improved adherence.[6,24] The practice of short, but repeated exercise bouts throughout the day, may be an alternative way to get exercise benefits.[3]

Regarding cardiovascular diseases, further studies are needed to confirm the beneficial effects of exercise accumulated in several short bouts on vascular endothelium. In this context, we compared one continuous bout of exercise (1 hour/day) with the same amount of exercise distributed in short, repeated bouts (4 bouts/day), to assess the beneficial, cumulative effects of exercise. Although it was not the objective of this study to propose an exercise program that could be used in humans, which limits the extrapolation of our results to humans, the study raises the discussion about the usefulness of accumulated exercise in the clinical practice.

Interestingly, in the present study, not only continuous exercise, but also accumulated exercise increased the animals' running capacity on the treadmill. Despite the limitations of experimental models to reproduce exercise training protocols designed for humans, these findings suggest that the positive effects of accumulated exercise on fitness observed in animals, may also occur in humans.[11] For example, accumulated exercise can be an alternative approach to help individuals to get away from a sedentary lifestyle.

The improvements in running capacity on treadmill were not accompanied by changes in heart weight or in TBARS or FRAP values. These results indicate that, although the CEx and the AEx protocols were not able to increase the antioxidant defenses, no significant changes in plasma levels of free radicals were observed either. This may be explained by the intensity of exercise applied – 50-60% of maximum capacity – which is considered moderate.

In addition, accumulated aerobic exercise has been suggested as a strategy in weight control.[6,13]It has been proven effective in reducing blood pressure,[7,12]post prandial triglycerides,[10]skinfold thickness and waist circumference,[6] and in increasing high-density lipoprotein levels.[8,9] In the present study, however, as compared with SED group animals, body weight gain was significantly lower in the CEx group but not in the AEx group.

Exercise may also increase the laminar blood flow on endothelial surface, thereby increasing the shear stress on this surface.[25]An increased shear stress on the endothelium may induce the expression of several enzymes involved in the synthesis of substances that regulate vascular tone, local oxidative balance, coagulation process and endothelial inflammation.[26,27]Therefore, exercise may increase the

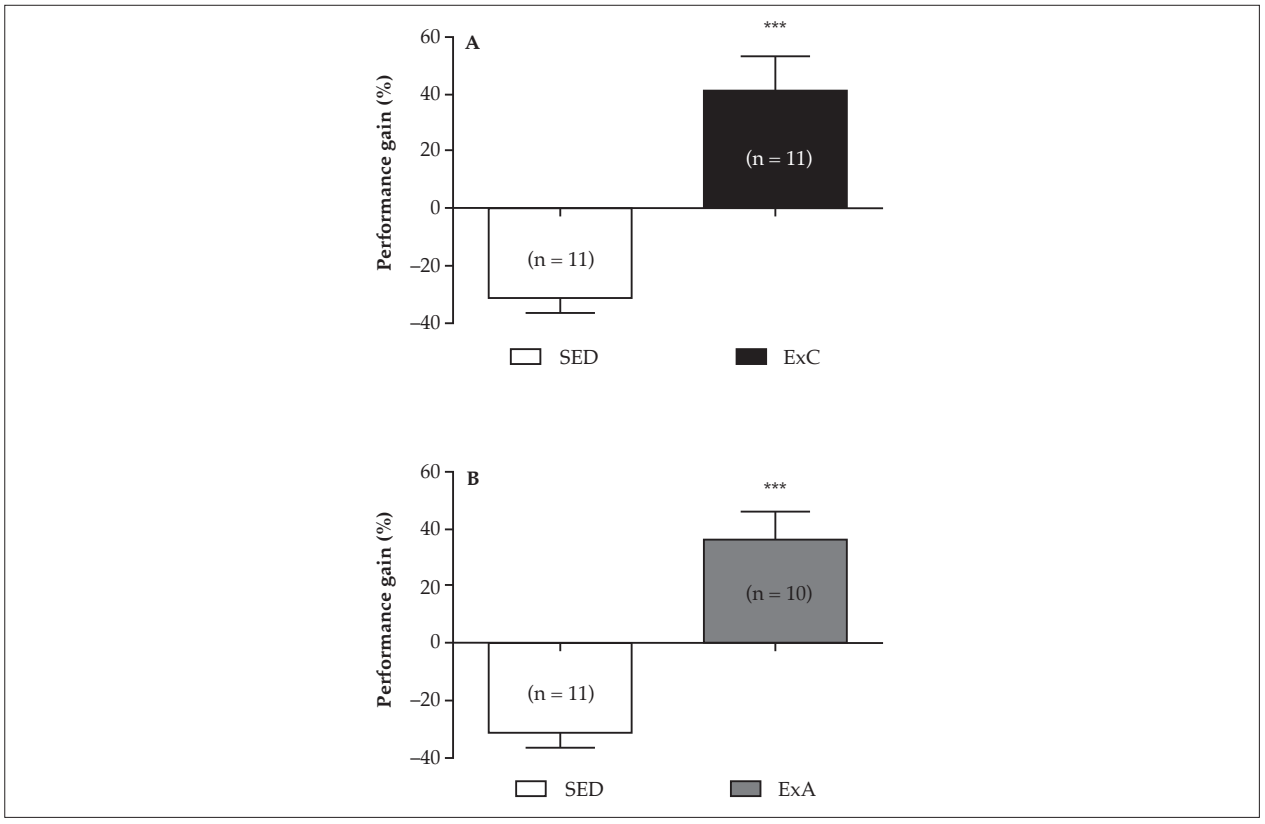

Figure 1 – *Performance gain (%) of animals submitted to continuous exercise training (CEx; A) or accumulated exercise (AEx; B) in comparison to sedentary animals (SED). Columns represent mean ± SEM; in parenthesis, number of independent determinations. *** p < 0.001 compared to SED animals (unpaired Student's t-test).*

endothelial production of vasodilator substances, and hence modulate the NE responses in several vascular beds, including rat aorta.[14,16]

The present study showed that the CEx decreased the responses to NE in aorta with a reduction of R_{max}, indicating an improvement in endothelial function. The main endothelium-derived relaxing factor is NO, a diffusible gas synthesized mainly by eNOS in the vascular endothelium. In our study, the reduction of R_{max} was suppressed by L-NAME, a non-selective NOS inhibitor, or reversed in preparations without endothelium. Since the eNOS expression may be increased by shear stress, thereby increasing the synthesis of NO,[18,20,27] the reduction of NE responses in rat aorta induced by the CEx may be due to a greater efficiency of endothelial NO-related mechanisms, resulting from an increased expression of endothelial eNOS. The involvement of endothelium-derived NO in the reduction of NE responses in aorta of animals exposed to CEx has been also described by other studies.[16,20,28]

The increased NE response, characterized by elevation of R_{max} and pEC_{50}, induced by CEx in aorta preparations without endothelium was unexpected, but also reinforces the pivotal role of the endothelium in the modulation of this response in these preparations. In addition, it has been proposed that NO is the principal mediator of the ACh-induced relaxation in rat aorta preparations.[29] In this manner, as previously described, the increased ACh-induced relaxation corroborates the involvement of NO-related mechanisms in the endothelium in these preparations.

Unfortunately, evidence of an exercise-induced enhancement in endothelial function has been demonstrated only by continuous or intermittent exercise studies. Evidences of a direct effect of AEx on the endothelium are scarce in the literature. Thus, once verified that CEx may improve the endothelial function in our experimental conditions, we began to investigate whether splitting the exercise in short bouts (with a total time corresponding to one continuous session) promoted similar effects on the endothelium. No significant increases in NE and ACh responses, induced by AEx were found in rat aorta preparations, suggesting that this type of exercise has no effect on endothelial function. It is worth mentioning that previous reports have shown that the effects of shear stress on the enzymes involved in the endothelial production of modulatory substances depends on time of exposure to exercise.[18,19,27] Our results suggest that the beneficial effects of exercise on endothelial function are achieved only if exercise are practiced for a long enough period.[30,31] However, the minimum time required for such effects remains to be determined.

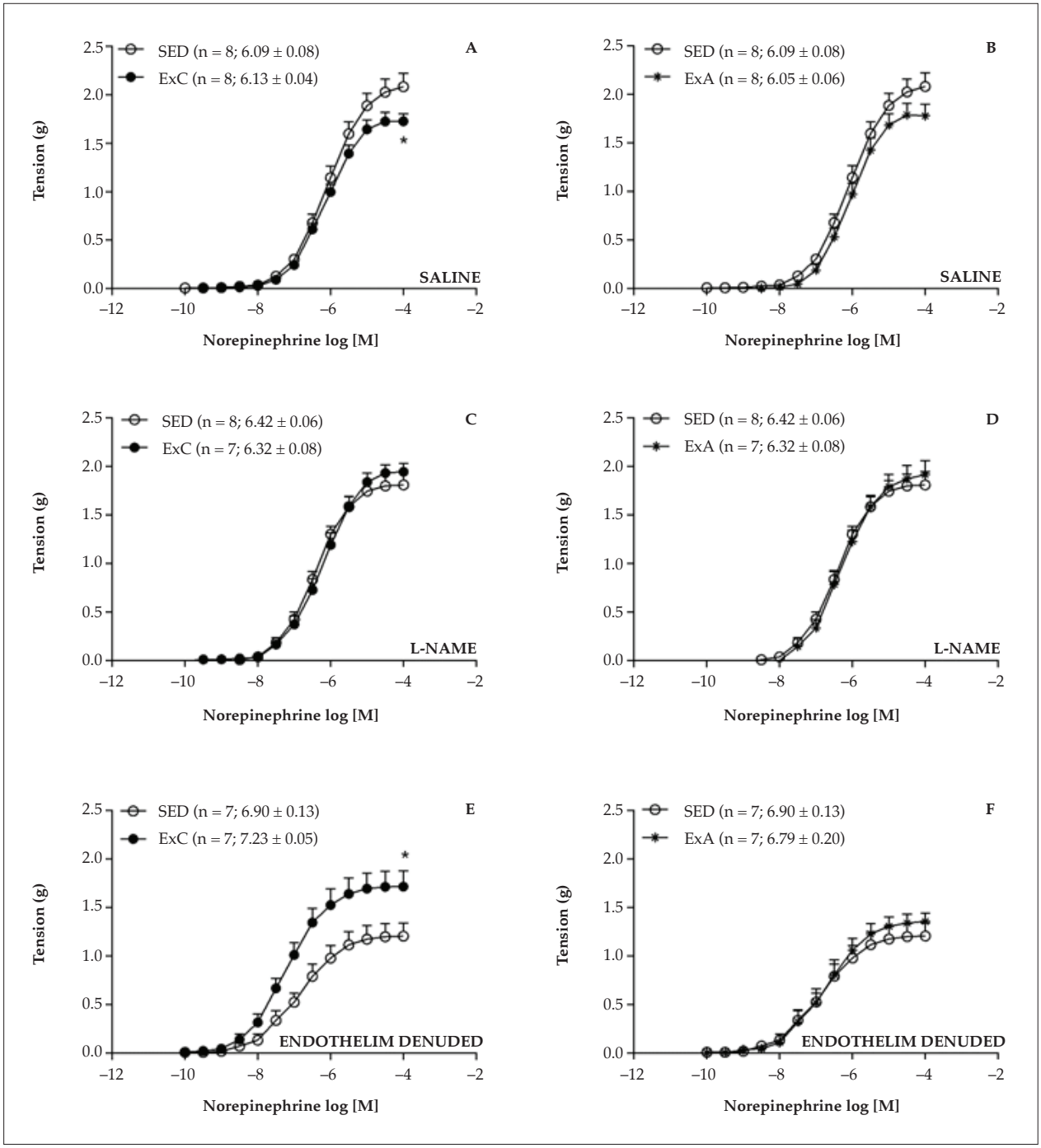

Figure 2 – *Concentration-response curves to norepinephrine determined in intact thoracic aorta preparations obtained from animals trained by continuous (ExA) or accumulated exercise (ExA), in comparison to sedentary animals (SED), not treated (A and B) or treated with 10^{-4} mol/L L-NAME (C and D) as well as in not treated endothelium denuded thoracic aorta preparations (E and F). In parentheses, the number of independent determinations (n) followed by pEC50 values. Data in mean ± SEM. * p < 0.05 compared with the SED group ("t" test of Student).*

The findings on the effect of AEx on vascular endothelium are not conclusive and may not be extrapolated to the clinical setting, since, to our knowledge, this is the first study investigating a direct effect of AEx on vascular endothelium in experimental conditions. Yet, in the present study, the concentration-response curves to NE had a descending trend. Thus, the improvement in endothelial function induced by AEx may occur in animals with endothelial dysfunction caused by aging, hypertension, atherosclerosis or diabetes and those chronically exposed to alcohol and/or smoke.[32]

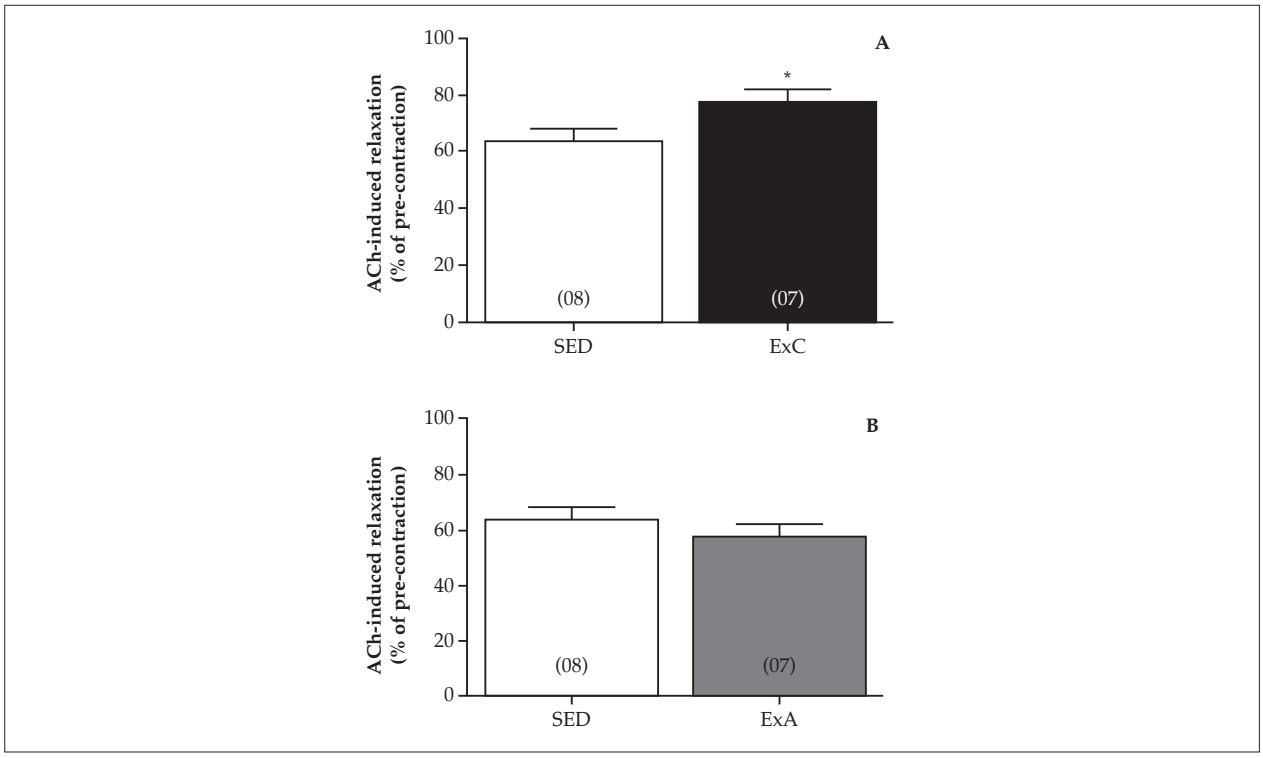

Figure 3 – *Relaxation induced by acetylcholine (ACh; 10^{-4} mol/L), in % of phenylephrine-induced pre-contraction (Phe; 10^{-5} mol/L), in animals submitted to continuous exercise (ExC; A) or accumulated exercise (ExA; B) training, in comparison with sedentary animals (SED). Columns represent mean ± SEM; in parenthesis, number of independent determinations. *p < 0.05 compared to the SED animals (unpaired Student's t-test).*

In fact, one of the few studies on the direct effect of AEx on the endothelial function was performed in adolescent boys submitted to an ingestion of a high-fat breakfast and lunch. This diet has induced endothelial dysfunction in these boys, which was reversed by short bouts of exercise that were repeated throughout a day.[33]Also, it was demonstrated that 30 min walk divided into sessions of 10 min (with intervals of 50 minutes to rest) was effective in reducing systolic blood pressure in pre-hypertensive individuals.[34] Such reduction in blood pressure may be involved at least partially in the exercised-induced improvement of endothelial function. Thus, further studies on endothelial dysfunction models are required to better understand the therapeutic potential of AEx.

Conclusion

In conclusion, the continuous and accumulated exercise protocols employed in this study increased the fitness of the animals, which suggests the usefulness of the AEx as a strategy to introduce people to physical training programs. However, as compared with CEx, AEx was not as effective in preventing body weight gain or improving the endothelial function of aorta of these animals.

Author contributions

Conception and design of the research, Statistical analysis, Obtaining financing and Writing of the manuscript: Martinez JE, Taipeiro EF; Acquisition of data, Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content: Martinez JE, Chies AB, Taipeiro EF.

Potential Conflict of Interest

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

Sources of Funding

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Study Association

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Kinetics of Hypotension during 50 Sessions of Resistance and Aerobic Training in Hypertensive Patients: a Randomized Clinical Trial

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Abstract

Background: Resistance and aerobic training are recommended as an adjunctive treatment for hypertension. However, the number of sessions required until the hypotensive effect of the exercise has stabilized has not been clearly established.

Objective: To establish the adaptive kinetics of the blood pressure (BP) responses as a function of time and type of training in hypertensive patients.

Methods: We recruited 69 patients with a mean age of 63.4 ± 2.1 years, randomized into one group of resistance training (n = 32) and another of aerobic training (n = 32). Anthropometric measurements were obtained, and one repetition maximum (1RM) testing was performed. BP was measured before each training session with a digital BP arm monitor. The 50 training sessions were categorized into quintiles. To compare the effect of BP reduction with both training methods, we used two-way analysis of covariance (ANCOVA) adjusted for the BP values obtained before the interventions. The differences between the moments were established by one-way analysis of variance (ANOVA).

Results: The reductions in systolic (SBP) and diastolic BP (DBP) were 6.9 mmHg and 5.3 mmHg, respectively, with resistance training and 16.5 mmHg and 11.6 mmHg, respectively, with aerobic training. The kinetics of the hypotensive response of the SBP showed significant reductions until the 20th session in both groups. Stabilization of the DBP occurred in the 20th session of resistance training and in the 10th session of aerobic training.

Conclusion: A total of 20 sessions of resistance or aerobic training are required to achieve the maximum benefits of BP reduction. The methods investigated yielded distinct adaptive kinetic patterns along the 50 sessions. (Arq Bras Cardiol. 2017; 108(4):323-330)

Keywords: Hypertension; Kinetics; Exercise; Exercise Movement Techniques; Clinical Trial.

Introduction

The practice of physical exercise is the most used strategy for nonpharmacological treatment of hypertension.[1,2] Aerobic stimuli between 40–60% of the maximum oxygen consumption (VO_{2max}) are recommended two to three times a week during sessions of 30 to 60 minutes, performed in association with resistance training using multiarticular exercises with at least one series of 8–12 repetitions for 30 to 60 minutes.[3]

Reductions of 6.9 mmHg in the systolic BP (SBP) and 4.9 mmHg in the diastolic BP (DBP) during rest have been reported as a result of adaptations enabled by aerobic training.[4] Although aerobic training is the most established strategy among the methods of physical training for hypertensive individuals, other methods have been shown to be effective in reducing BP levels, such as resistance dynamic,[5] isometric,[6] combined (aerobic and resistance),[7] and high-intensity interval training.[8]

Studies with resistance training as the only nonpharmacological strategy to treat hypertension have demonstrated BP reductions between 2 and 12 mmHg.[9,10] Even after interruption, the effects of training persist for up to 4 weeks.[11]

To the best of our knowledge, available studies directly comparing different training methods, such as aerobic *versus* resistance training,[12,13] have not identified the number of sessions required until stabilization of the hypotensive effect of the exercise in hypertensive patients. More precisely, it is important to clarify how many sessions are necessary to ensure that the training programs provide the maximum possible benefits. This outcome has not been investigated with priority, and the results regarding the number of sessions are still inconclusive in the literature (between 12 to 48 sessions),[14] hindering the interpretation of the adjustments provided by different methods of training and the consequent decision for the best treatment strategy.[15]

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Thus, the objective of this study was to establish the adaptive kinetics of the BP responses as a function of time and type of training (resistance or aerobic) in individuals classified with stage 1 hypertension.

METHOD

Experimental design

Clinical trial with two parallel groups conducted according to the CONSORT recommendations, but without registration. Eligible subjects were randomized into two independent training groups: resistance and aerobic. On the first visit, the subjects received instructions regarding the procedures of the study, had their questions answered, and signed a free and informed consent form (ICF). On the second visit, anthropometric and BP measurements were obtained. On the third visit, one repetition maximum (1RM) testing was performed in the resistance group, and recommendations regarding the prescription of training were delivered in the aerobic group. On the fourth visit, adaptations of the participants to their respective training methods were made. From the fifth visit onwards, the training protocols were carried out in both groups.

Subject

We recruited for the study 20 men and 49 women, whose characteristics are described in Table 1. All subjects participated voluntarily after being contacted through invitations and reports on the practice of physical activity for hypertensive patients, distributed on the campus of the *Universidade Federal de Pernambuco*. All participants used medication for BP control (Table 2). The research was approved by the Ethics Committee at *Centro de Ciências da Saúde* at *Universidade Federal de Pernambuco* (case 321/11).

As the inclusion criteria, the subjects should have stage 1 hypertension, use controlled medications, and be older than 60 years. On the first visit, we measured the participants' BP at rest, which was considered as the initial reference (moment 0) and was used to classify the subjects regarding their hypertension level.[16]

We excluded subjects using beta-blockers, since this type of medication changes the individual's cardiovascular responses, hindering the interpretation of the data and the use of the heart rate to prescribe training.[17] We also excluded participants who had any other disease affecting cardiovascular responses to physical exercise, or with joint limitations resulting in functional limitations. Figure 1 shows the flowchart of the subjects throughout the study.

For randomization, we used a digital tool available at www.randomizer.org. The eligible subjects were listed numerically in order of arrival by one of the researchers without access to any of the evaluations. A second researcher was blindly responsible for the allocation of the participants to each group.

Table 1 – General characteristics of the investigated subjects before training

Variables	Resistance Group	Aerobic Group
Age (years)	62.8 ± 1.22	63.9 ± 2.3
Weight (kg)	69.2 ± 13.7	70.6 ± 11.5
SBP	147.0 ± 9.4	151.8 ± 11.5
DBP	95.8 ± 7.9	93.9 ± 10.8
BMI (kg.m^{-2})	30.3 ± 30.1	29.2 ± 4.7
WHR	0.95 ± 0.21	0.90 ± 0.76
CI	1.55 ± 0.11	1.56 ± 0.23
WC (cm)	98.2 ± 6.0	97.9 ± 13.1
AC (cm)	102.0 ± 9.4	99.2 ± 12.3

SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; WHR: waist-hip ratio; CI: conicity index; WC: waist circumference; AC: abdomen circumference.

Procedures

Anthropometric assessments and weight indices

We measured the participants' body mass (kg), height (cm), and waist and hip circunferences (cm). Body mass was measured using a portable scale accurate to 0.1 kg (PL 200, Filizola S.A., São Paulo, Brazil). The height was measured with a stadiometer accurate to 0.1 cm (Professional Stadiometer Sanny, São Paulo, Brazil). The waist circumference was measured at the narrowest level between the rib margin and the iliac crest using a non-flexible anthropometric tape precise to 0.1 mm (SN-4010, Sanny, São Paulo, Brazil). The hip circumference was measured at the level of the pubic symphysis using the same tape. We then calculated the subjects' body mass index (BMI = body weight ÷ height^2), their waist/hip ratio (WHR = waist circumference ÷ hip circumference), and their conicity index [CI = (circumference of the abdomen ÷ 0.169) x √(body weight ÷ height)].[18]

Blood pressure measurement

The BP was measured at rest in the left superior limb according to recommendation by the American Heart Association, using a digital BP monitor (Digital Omron BP Monitor, Model 11 EM403c, Tokyo, Japan). Considered as the primary outcome in the present study, the BP was monitored before each training session, and the last measurement was performed 48 h after the 50th session. The subjects were instructed to not drink alcohol and/or caffeine for 24 h before the measurements. For each measurement, the subjects rested for 15 min in the sitting position with their feet supported and kept their arm at the heart level.

One repetition maximum testing

The 1RM test was performed according to the protocol of the American College of Sports Medicine.[3] For that, the subjects performed warm-up exercises with 10 repetitions

Table 2 – Frequency and percentage of medications used by the participants

Antihypertensive drugs	Resistance Group	Aerobic Group	Total Frequency
	(n = 28)	(n = 27)	(n = 55)
Angiotensin converting enzyme inhibitors	5 (55%)	4 (45%)	9 (16%)
Diuretics	5 (45%)	6 (55%)	11 (20%)
Angiotensin receptor II antagonists	15 (50%)	15 (50%)	30 (55%)
Calcium channel antagonists	3 (60%)	2 (40%)	5 (9%)

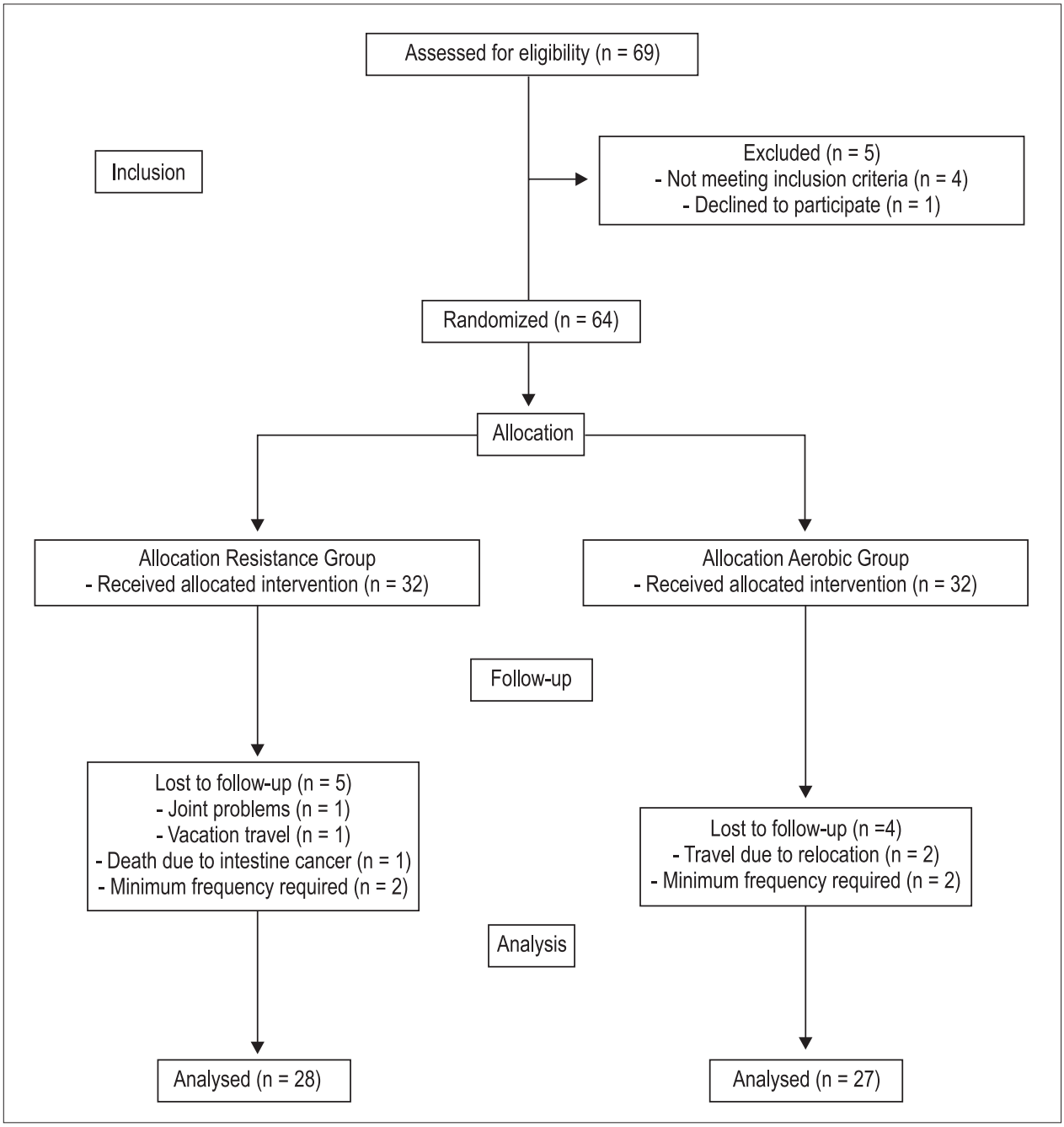

Figure 1 – *Flow diagram of the randomization of the subjects.*

with a light load. After 5 min, the 1RM load testing was carried out, in which each subject performed at the most five attempts of each exercise with an interval of 5 min between each one, in which the largest lifted load was the load selected.

Resistance training protocol

The resistance training sessions were carried out on exercise equipment (Technogym, Cesena, Italy). The subjects performed a program of resistance training alternated by segment, with two types of series (A and B), alternated by session (48 h). The order of the exercises was: A series - vertical bench press, seated leg curl, triceps cable curl, seated leg abduction, shoulder lift, plantar flexion, and upper abdominal; B series - frontal cable pull, leg press, shoulder abduction, leg extension, biceps curl, seated leg adduction, and lower abdominal. The training program was performed three times a week, with three sets of 12 repetitions at 50–70% of the maximum load and adjusted throughout the program for the achievement of a perceived exertion (Borg) classified as moderate. A 1 min recovery between each series and exercises was administered.

Aerobic training protocol

The sessions of aerobic training consisted of walking on track three times a week for 30 min, maintaining the heart rate between 40–60% of the predicted maximum rate for age.[19] The intensity was adjusted over the course of the sessions based on the participant's subjective perception of effort, aiming to reach a moderate intensity. All training sessions were supervised.

Statistical analysis

Quantitative variables are presented as mean ± standard deviation. Categorical variables are presented by their absolute and relative frequencies. The 50 training sessions were divided into quintiles, yielding five comparative moments (sessions 1–10, 11–20, 21–30, 31–40, 41–50). The BP result at each quintile represents the average of 10 sessions grouped for each variable investigated (SBP and DBP) measured before each training session. The pretreatment measurement of the dependent variables was used as a covariate to control the initial differences between the groups. Given the possibility of sampling mortality, the analyses conducted were not based on an "intention to treat". After verifying the conceptual assumptions, to compare the effect of the methods of resistance and aerobic training on the SBP and DBP measurements, we used two-way analysis of covariance (ANCOVA; training method x moment) with repeated measures for the second factor.

The identification of the differences between the investigated moments for each training method was established with one-way analysis of variance (ANOVA) with repeated measures. For both analyses, we used the *post hoc* Bonferroni test, when necessary. The analyses were performed using GraphPad Prism, v. 5.0 (GraphPad Software, San Diego, USA), with a significance level set at $p < 0.05$.

Results

We performed preliminary verifications to ensure that there was no violation of the assumptions of normality, linearity, variance homogeneity, regression slope homogeneity, and reliable covariate measurement. Figure 2 shows a comparison of the BP along the 50 sessions of resistance and aerobic training, and Table 3 highlights the differences (Δ) observed and their respective confidence intervals. ANCOVA indicated a significant interaction between the training methods in regards to the SBP ($F [4, 29] = 3.431$, $p = 0.021$), with a small *eta squared* effect size ($\eta^2 = 0.321$). The analysis of the main effects showed no significant differences between the training methods in terms of SBP ($p = 0.690$); however, the results suggested that the SBP responded with different reductions in both groups.

The interaction between the training methods in regards to the DBP showed an absence of statistically significant results ($F [4, 29] = 1.835$, $p = 0.149$), with a small effect size ($\eta^2 = 0.202$). In the analysis of the main effects in the DBP ($p = 0.091$), the groups responded in a similar manner.

The identification of the moments of BP stabilization as a result of the training strategies is presented in Table 4 for the SBP and in Table 5 for the DBP. The stabilization of the reductions in the SBP was observed in the 20th session for both methods. For the DBP, the reductions were significant until the 20th session of resistance training and up to the 10th session of aerobic training.

Discussion

The present study demonstrated that resistance training was able to reduce the SBP in 6.9 ± 2.8 mmHg and the DBP in 5.3 ± 1.9 mm Hg, while aerobic training showed reductions of 16.5 ± 3.4 mmHg in SBP and 11.6 ± 3.6 mmHg in DBP. The interaction between the methods investigated indicates apparently higher hypotensive effects with aerobic training when compared with resistance training. However, the comparison of the mean standardized reductions between the methods by the analysis of the η^2 showed a small magnitude for both strategies. In the temporal analysis of the training methods, we observed that the kinetics of the hypotensive response of the SBP showed significant reductions until the 20th session in both groups. After that, there was a plateau in the adaptations yielded by resistance training. This is a novel information that should be considered in therapeutic decisions using exercise as an adjuvant in BP treatment.

Even though a statistically significant difference occurred after the 40th session, a regression of the SBP to mean values close to those of the 10th session seems to have occurred. The mechanisms underlying such adaptation could not be identified. Future studies should investigate the hypothesis of the increased arterial stiffness generated by resistance training, as suggested by Okamoto et al.[20] In addition, aerobic training maintained nonsignificant reductions until the 50th session, which may clinically represent some treatment benefit, especially in patients within the classification limit of a given category (borderline), since

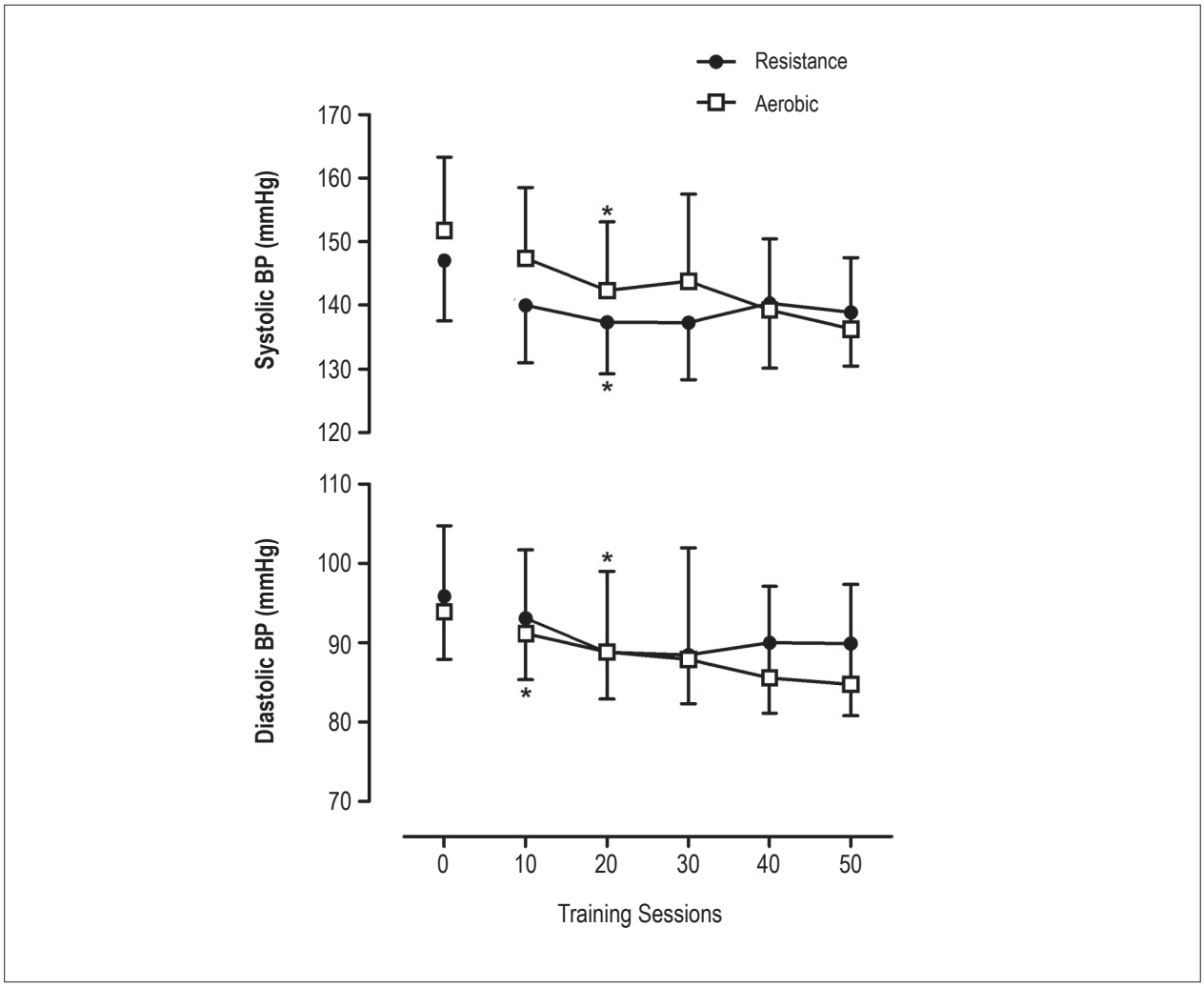

Figure 2 – *Responses in systolic and diastolic blood pressure at rest obtained before the exercise sessions in the resistance and aerobic groups. BP: Blood Pressure.*

an SBP reduction of 10 mmHg reduces the mortality risk by 13%.[21]

In a similar way, we observed that resistance training yielded a significant DBP reduction until the 20th session, while with aerobic training the stabilization occurred after the 10th session. Together, these results provide a better understanding of the adaptive behavior of the SBP and DBP as a result of the investigated training methods, since they provided different kinetic responses.

The physiological mechanisms explaining the BP reductions after physical exercise are, on the one hand, due to a decrease in cardiac output following a reduction in the systolic volume and heart rate and a decrease in the sympathetic tone[22] and, on the other hand, due to an increase in the baroreflex sensitivity and control, associated with a peripheral local action, mediated mainly by nitric oxide released in the endothelium as a result of stress generated by physical exercise (shear stress).[23] Together, these mechanisms trigger adaptations such as arterial vasodilation, generating a reduction in peripheral resistance and, consequently, in BP after physical exercise.[24] For example, Santana et al.[25] subjected hypertensive elderly women to aerobic exercise with one session at moderate intensity for 20 min and another session at high intensity for 20 min. Nitric oxide levels after the activity increased by 30% and 33%, respectively, and there was a significant reduction in BP with both interventions.

In a recent meta-analysis that investigated the effect of different exercise methods on the magnitude of the effect in reducing the BP, Cornelissen and Smart[26] did not find differences in effect size between aerobic and resistance training, concluding that both training methods provide BP reductions of similar magnitude. Furthermore, the results reported by the authors presented larger reductions with aerobic training. Both aspects were similar to those found in the present study. In addition, the results of the present study add information to these findings, setting the kinetic standard of BP responses yielded by the two investigated training methods. Future studies should investigate other training strategies.

Table 3 – Difference (Δ), standard deviation, and confidence intervals of the hypotensive responses of the systolic blood pressure (SBP) and diastolic blood pressure (DBP) at five different moments in the resistance and aerobic groups

Blood Pressure	Resistance Group		Aerobic Group	
	Mean ± SD	95%CI	Mean ± SD	95%CI
Systolic				
Δ 10-0	-7 ± 0.4	-7.2; -6.8	-4.4 ± 0.34	-4.6; -4.2
Δ 20-0	-9.7 ± 8.7	-14.0; -5.4	-9.5 ± 6.1	-13.0; -6.4
Δ 30-0	-9.7 ± 6.1	-13.0; -6.7	-8.0 ± 9.2	-13.0; -3.3
Δ 40-0	-6.7 ± 7.2	-10.0; -3.1	-13.0 ± 9.2	-17.0; -7.8
Δ 50-0	-8.2 ± 8.4	-12.0; -4.0	-16.0 ± 9.2	-20.0; -11.0
Diastolic				
Δ 10-0	-2.8 ± 0.2	-2.9; -2.7	-2.7 ± 0.3	-2.9; -2.6
Δ 20-0	-7.1 ± 5.6	-9.9; -4.3	-5.1 ± 7.0	-8.7; -1.5
Δ 30-0	-7.4 ± 6.1	-10.0; -4.4	-6.0 ± 9.2	-11.0; -1.3
Δ 40-0	-5.9 ± 8.4	-10.0; -1.7	-8.3 ± 7.7	-12.0; -4.4
Δ 50-0	-6.0 ± 8.0	-10.0; -2.0	-9.2 ± 8.6	-14.0; -4.7

Δ - Difference between the moments 10, 20, 30, 40, and 50 in regard to moment 0. CI: Confidence interval; SD: Standard deviation.

Table 4 – Indicator matrix of statistical significance of one-way analysis of variance (ANOVA) (within) with post hoc Bonferroni for systolic blood pressure comparisons at different moments

Moment	Resistance Group					Aerobic Group				
	10	20	30	40	50	10	20	30	40	50
0	NS	< 0.001	< 0.001	NS	< 0.01	NS	< 0.001	< 0.01	< 0.001	< 0.001
10	---	NS	NS	NS	NS	---	NS	NS	< 0.05	< 0.001
20	---	---	NS	NS	NS	---	---	NS	NS	NS
30	---	---	---	NS	NS	---	---	---	NS	NS
40	---	---	---	---	NS	---	---	---	---	NS

Table 5 – Indicator matrix of statistical significance of one-way analysis of variance (ANOVA) (within) with *post hoc* Bonferroni for diastolic blood pressure (DBP) comparison at different moments

Moment	Resistance Group					Aerobic Group				
	10	20	30	40	50	10	20	30	40	50
0	NS	< 0.01	< 0.001	< 0.05	< 0.05	< 0.05	< 0.001	< 0.001	< 0.001	< 0.001
10	---	NS	NS	NS	NS	---	NS	NS	NS	NS
20	---	---	NS	NS	NS	---	---	NS	NS	NS
30	---	---	---	NS	NS	---	---	---	NS	NS
40	---	---	---	---	NS	---	---	---	---	NS

About the kinetics of BP stabilization, we identified only one study using resistance training,[27] in which the SBP stabilized at the 6th training session, while in our study we observed significant reductions until the 20th training session. For the DBP, the same study found that the stabilization occurred in the 30th session, while in our study it occurred in the 20th session. It is possible that the differences encountered are the result of the difference in data sampling, since the present

study considered the training sessions grouped into quintiles. It is noteworthy that the protocols of resistance training in both studies were similar and were performed with moderate loads (between 50–70% of the 1RM load), with three sets of 12 repetitions.

Regarding aerobic training, Kokkinos et al.[28] compared the BP responses after 48 and 96 training sessions to the initial BP values, observing a nonsignificant decrease of 1.0 ± 4.0 mmHg ($p = 0.150$), but with a substantial reduction in the use of medications. On the other hand, Seals and Reiling[29] found BP reductions in elderly individuals after 72 sessions of aerobic training. Later, when 72 additional sessions of aerobic training were performed, there was an additional SBP reduction of 4.0 ± 4.0 mmHg ($p < 0.05$), but no DBP reductions. Jennings et al.[30] found a BP decrease at the 30th session of aerobic training, which corresponded to 75% of the hypotensive effect at the 60th session. This same proportion was found in the present study. Together, this evidence shows that the results of physical exercises on BP treatment in the long term seem to bring benefits only in the maintenance of the initial reductions and do not result in additional gains.

Although resistance training generates smaller reductions when compared with aerobic training,[26] its recommendation is supported by the reduction in BP responses in daily life activities, since the improvement in resistance promotes a relative reduction in the intensity in which daily tasks are performed, with consequent mitigation of BP responses. Considering that, resistance training seems to be a relevant strategy for BP control and maintenance of functional aspects. One should also consider that, in the light of the available knowledge, the clinical effects of BP reduction by resistance training are similar to those observed with aerobic training.

Some limitations of the present study need to be highlighted. The study did not take into account the doses of the medications used by each subject, which may have influenced the responses observed. However, this approach presents greater external validity considering that the individuals exercising in centers of physical activity and exercise clinics do not interrupt the use of their medications to practice their physical activities. In addition, physical exercise is considered an adjuvant treatment and should be performed along with the use of medications, which should be frequently evaluated for possible adjustments. Another limitation was the lack of use of ambulatory BP monitoring, which enables a more reliable measurement by evaluating the BP levels for a longer period of time. And finally, the absence of a control group limits the conclusion that it was only the exercise that determined the BP decrease. However, prior evidence has established with certainty the benefits of an exercise group (aerobic and resistance) in relation to a control group,[24, 28] which would characterize as ethically questionable the decision to deprive a group of individuals from exercise treatment.

Conclusions

We observed that 20 sessions of resistance or aerobic training are necessary to achieve BP reductions resulting from physical exercise, and that the BP reductions respond differently over the course of 50 sessions. A mean reduction per session of 0.5 mmHg in the SBP for both training methods, and 0.2 to 0.3 mmHg in the DBP for resistance and aerobic training, respectively, can be expected up to the 20th training session. The addition of more training sessions seems to provide smaller BP reductions, but without statistical significance. Our results support the recommendation of the use of resistance training with benefits close to those of aerobic training in reducing the BP.

Author contributions

Conception and design of the research and Acquisition of data: Damorim IR, Barros GWP, Carvalho PRC; Analysis and interpretation of the data and Statistical analysis: Damorim IR, Santos TM; Obtaining financing: Damorim IR, Carvalho PRC; Writing of the manuscript: Damorim IR, Santos TM; Critical revision of the manuscript for intellectual content: Damorim IR, Santos TM, Barros GWP, Carvalho PRC.

Potential Conflict of Interest

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

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Study Association

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

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MCP-1 Levels are Associated with Cardiac Remodeling but not with Resistant Hypertension

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Abstract

Background: Hypertension is a chronic, low-grade inflammation process associated with the release of cytokines and development of target organ damage. Deregulated monocyte chemoattractant protein-1 (MCP-1) levels have been associated with high blood pressure and cardiovascular complications; however, the mechanisms involved are complex and not fully understood.

Objective: This study aimed to compare the levels of MCP-1 in patients with resistant (RH) versus mild-to-moderate (HTN) hypertension and their association with the presence or absence of left ventricular hypertrophy (LVH) in all hypertensive subjects.

Methods: We enrolled 256 hypertensive subjects: 120 RH and 136 HTN, investigating the relationship between circulating MCP-1 levels and blood pressure, biochemical data, hematologic profile, and cardiac damage within the RH and HTN groups. Plasma MCP-1 levels were measured by ELISA and LVH was assessed by echocardiography.

Results: We found no difference in MCP-1 levels between RH and HTN subjects. On the other hand, we encountered lower MCP-1 levels in patients with LVH (105 pg/mL [100 – 260 pg/mL] versus 136 pg/mL (100 – 200 pg/mL), p = 0.005, respectively] compared with those without LVH. A logistic regression model adjusted for body mass index (BMI), age, race, aldosterone levels, and presence of diabetes and RH demonstrated that median levels of MCP-1 (2.55 pg/mL [1.22 – 5.2 pg/mL], p = 0.01) were independently associated with LVH in the entire hypertensive population.

Conclusion: Since MCP-1 levels were similar in both RH and HTN subjects and decreased in hypertensive patients with existing LVH, our study suggests a possible downregulation in MCP-1 levels in hypertensive individuals with LVH, regardless of hypertension strata. (Arq Bras Cardiol. 2017; 108(4):331-338)

Keywords: Refractory Hypertension; Cytokines; Monocyte Chemoattractant Proteins; Left Ventricular Hypertrophy.

Introduction

Resistant hypertension (RH) is defined as a condition in which patients present either (i) uncontrolled blood pressure (BP) (≥ 140/90 mmHg) despite the use of maximal recommended or tolerated doses of three or more antihypertensive drugs, or (ii) controlled BP with the use of at least four medications.[1,2] The high prevalence of target organ damage (TOD), such as left ventricular hypertrophy (LVH), is higher in patients with RH compared with those with mild/moderate hypertension (HTN)[1,3] and is considered a predictor of future cardiovascular events in this specific RH population.[4]

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Many lines of evidence have established that hypertension is a chronic low-grade inflammation process that plays a role in the development and maintenance of TOD.[5,6] Several inflammatory mediators are enhanced in hypertensive subjects,[7] including monocyte chemoattractant protein-1 (MCP-1).[8] MCP-1, also known as CCL2, can be produced by different cells and is responsible for migration of monocytes and macrophages cells to the tissue,[9] exacerbating the local damage.

Experimental models of hypertension have shown that infiltration of inflammatory cells (macrophages) in the vascular walls is strongly related to increased BP[10] and cardiovascular alterations.[11,12] A clinical study has suggested that MCP-1 levels may vary according to the degree of hypertension,[8] indicating a stage-dependent biomarker of the disease.

Although some authors have shown that increased levels of MCP-1/CCL2 and macrophages in the heart contribute to cardiac damage,[13,14] others have pointed out that macrophages have cardioprotective effects.[15] In fact, one study showed that depletion of macrophages accelerates the development of cardiomyopathy in hypertensive rats.[15] This effect could be explained by an ability to maintain cardiac homeostasis during some cardiac diseases.[14]

Despite these findings, the relationship of MCP-1 with RH and cardiac damage in the clinical setting has not been established yet. Therefore, this study was designed to assess the levels of MCP-1 in RH compared with HTN subjects and its association with LVH in all hypertensive groups.

Methods

Study subjects

A convenience sample of 256 hypertensive patients from the Resistant Hypertension Outpatient Clinic at University of Campinas (Campinas, Brazil) were enrolled in this cross-sectional study.

Patients were diagnosed with RH after a 6-month protocol to exclude pseudoresistance – white-coat hypertension and poor medication adherence – with ambulatory BP monitoring (ABPM), the Morisky questionnaire, and pill count. Secondary hypertension (renal artery stenosis, pheochromocytoma, and primary hyperaldosteronism) was also excluded. These subjects were enrolled in the RH group. Also, patients with controlled BP using three or less antihypertensive drugs, or not yet controlled using two or less of these medications were classified as having HTN and also enrolled in the study.

The patients were classified into two groups: RH (n = 120) and HTN (n = 136). In addition, we combined both RH and HTN groups together and assessed the MCP-1 levels according to (1) the presence or absence of LVH (115 g/m^2 for men and 95 g/m^2 for women)[16] and (2) LVH severity, considering patients without LVH as level 0; patients with LVH and left ventricular (LV) mass index (LVMI) < median (121 g/m^2) as level 1; and patients with LVH and LVMI ≥ median (121 g/m^2) as level 2.

All ethical requirements for experiments conducted in human subjects were strictly followed. The study was approved by the Research Ethics Committee at Faculty of Medical Sciences, University of Campinas (São Paulo, Brazil) (approval no. 1.112.881/2015) and was conducted in accordance with the Declaration of Helsinki. All participants signed a written informed consent form before study enrollment.

Office blood pressure measurements

A trained health professional measured the office BP at least three times using a certified digital sphygmomanometer (HEM-907 XL OMRON Healthcare Inc., Bannockburn, IL, USA) in accordance with the 2013 European Society of Hypertension (ESH) guidelines.[17] The average of two or three consecutive measurements was used if a difference between the measurements was below 5 mmHg.

Ambulatory blood pressure monitoring

24-hour ABPM was carried out using an automatic oscillometric device (Spacelabs 90207, Spacelabs Inc.). The measurements were obtained every 20 minutes throughout the 24 hour period. The subjects were instructed to maintain their normal daily activities, avoid excessive physical activity, and take note of their sleep period in a personal diary. The mean BP was calculated during waking and sleep.

Echocardiography

Experienced specialists blinded to the patients' clinical data measured echocardiographic parameters (Siemens Acuson CV70, Munich, Bavaria, Germany) using two-dimensional targeted M-mode echocardiography. Diastolic and systolic LV diameters and the interventricular septal and posterior wall thicknesses were measured according to the QRS wave of the electrocardiogram. The LV mass was calculated by the American Society of Echocardiography (ASE) recommended formula[18] and the LVMI was calculated by dividing the LV mass by the body surface. An LVMI greater than 115 g/m^2 for men and 95 g/m^2 for women characterized the presence of LVH.[16]

Serum collection and laboratory assessments

Blood samples were withdrawn from the antecubital vein, with atraumatic venipuncture, after 8 hours of overnight fasting. Plasma levels of MCP-1 were measured using enzyme-linked immunosorbent assay (ELISA; R&D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer's instructions. Radioimmunoassay (Immunotech SAS, Marseille, France) was used to measure the plasma level of aldosterone according to the manufacturer's instructions. The neutrophil/lymphocyte ratio (NLR) was calculated by absolute neutrophil count divided by absolute lymphocyte count. In addition, serum total cholesterol, low- and high-density lipoprotein cholesterol, triglycerides, glucose, and creatinine were measured. Creatinine clearance (mL/min/1.73 m^2) was measured in a urine sample collected during 24 hours.

Statistical analysis

Descriptive data are shown as mean ± standard deviation (SD) for parametric data or median (interquartile range [IQR]) for nonparametric data. The distribution of the data was assessed by the Shapiro-Wilk test. Non-paired Student's *t* test or Mann-Whitney test was performed to compare two groups, while Kruskal-Wallis or analysis of variance (ANOVA) test, followed by Dunn's or Bonferroni post-test, respectively, were used for groups of three, according to data distribution. Categorical variables are presented in frequencies and/or percentages and were compared by Fisher's test. Spearman's correlation tested the association of nonparametric data. Also, we performed multiple logistic regression for the presence of LVH adjusted for age, aldosterone levels, body mass index (BMI), race, presence of diabetes, presence of RH, and MCP-1 median levels (categorized according to the median value of < 125 pg/mL) in hypertensive subjects. The level of statistical significance taken into account was < 0.05.

The analyses were performed using the software SigmaPlot (Systat Software, Inc, v.12, Chicago, IL, USA).

Results

Table 1 shows the general characteristics, biochemical data, and hematologic profile of the 256 hypertensive subjects. We found a higher percentage of diabetic individuals and black race in the RH compared with the HTN group. Moreover, RH patients showed higher office systolic BP (SBP) and aldosterone

Table 1 – General characteristics of the subjects with resistant and mild-to-moderate hypertension

	Hypertensive (n = 256)		
	HTN (n = 136)	RH (n = 120)	p value
Clinical Data			
Age (years)	65 ± 10	60 ± 11	< 0.001
Women (%)	62	67	0.50
Black race (%)	13	44	< 0.001
Diabetes (%)	38	51	0.05
Office SBP (mmHg)	139 (131 – 148)	147 (134 – 160)	< 0.001
Office DBP (mmHg)	81 (76 – 86)	83 (78 – 92)	0.09
ABPM SBP (mmHg)	127 (118 – 135)	130 (117 – 143)	0.18
ABPM DBP (mmHg)	76 (70 – 81)	77 (70 – 83)	0.34
LVMI (g/m^2)	100 (87 – 119)	113 (95 – 142)	< 0.001
Biochemical Data			
C-reactive protein (mg/dL)	0.3 (0.2 – 0.6)	0.3 (0.2 – 0.6)	0.72
Cholesterol (mg/dL)	165 (140 – 185)	181 (151 – 209)	0.003
HDL (mg/dL)	49 (42 – 57)	46 (38 – 54)	0.02
LDL (mg/dL)	87 (67 – 107)	98 (79 – 127)	0.002
Triglycerides (mg/dL)	108 (80 – 151)	129 (93 – 185)	0.019
HbA1c (%)	6.0 (5.8 – 6.5)	6.3 (6.0 – 7.8)	0.007
Glucose (mg/dL)	97 (90 – 107)	101 (89 – 132)	0.12
Creatinine (mg/dL)	0.94 (0.8 – 1.1)	0.97 (0.8 – 1.2)	0.15
Creatinine clearance (mL/min/1.73m^2)	65 (28 – 93)	81 (62 – 98)	0.05
Aldosterone (ng/dL)	68 (43 – 115)	98 (60 – 179)	< 0.001
Hematologic Profile			
Leukocytes (mm^3)	6.6 (6 – 8)	7.4 (6 – 8)	0.03
Monocytes %	8 (7 – 9)	8 (6 – 9)	0.79
Lymphocytes %	30 ± 7	30 ± 8	0.85
Basophils %	0.4 (0.2 – 0.5)	0.4 (0.3 – 0.6)	0.41
Eosinophils %	3 (2 – 4)	2 (1 – 3)	0.43
Neutrophils %	59 ± 7	58 ± 10	0.60
NLR	2 (1.8 – 2.3)	2 (1.4 – 2.6)	0.80

HTN: mild–to-moderate hypertensive subjects; RH: resistant hypertensive subjects; SBP: systolic blood pressure; DBP: diastolic blood pressure; ABPM: ambulatory blood pressure measurement; LVMI: left ventricular mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HbA1c: glycated hemoglobin; NLR: neutrophil/lymphocyte ratio; Continuous variables are presented as mean ± standard deviation (SD) for parametric data or median (1st, 3rd quartiles) for nonparametric data. Categorical variables are presented as percentages. Student's t test or Mann-Whitney test was performed according to data distribution, and Fisher's test was used to compare categorical variables.

levels, a higher incidence of LVH, and imbalance of lipid and glucose profiles compared with HTN subjects. On the other hand, we found no difference in hematologic parameters between the groups.

We observed that RH individuals used a greater number of antihypertensive drugs and a higher proportion of almost all antihypertensive classes, except for angiotensin II receptor antagonists (ARAs) (Table 2). Furthermore, the number of individuals using statins was greater in the HTN compared with the RH group.

Regarding MCP-1 levels, we found no differences between RH and HTN subjects (153 ± 93 pg/mL *versus* 178 ± 120 pg/mL, respectively, p = 0.47) (Figure 1). However, when we combined both RH and HTN groups together and assessed the MCP-1 levels according to the presence or absence of LVH, we found lower MCP-1 levels in patients with LVH compared with those without LVH (105 pg/mL [100 – 260 pg/mL] *versus* 136 pg/mL [100 – 200 pg/mL], respectively, p = 0.005) (Figure 2A). Also, when we stratified the LVMI levels into three degrees

Table 2 – Medication use in resistant and mild-to-moderate hypertensive subjects

	Hypertensive (n = 256)		
	HTN (n = 136)	RH (n = 120)	p value
Antihypertensive drugs			
Number of classes	2 (2 – 3)	4 (4 – 5)	< 0.001
Diuretics (%)	66	91	< 0.001
Spironolactone (%)	2	40	< 0.001
ACEIs (%)	16	37	< 0.001
ARBs (%)	74	55	0.003
CCBs (%)	46	84	< 0.001
Beta-blockers (%)	14	69	< 0.001
Others drugs			
Hypoglycemic agents (%)	38	51	0.05
Statin (%)	75	57	0.003

HTN: mild–to-moderate hypertensive subjects; RH: resistant hypertensive subjects; ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; CCBs: calcium channel blockers. Categorical variables are presented as numbers and percentages. Fisher's test was performed to compare categorical variables.

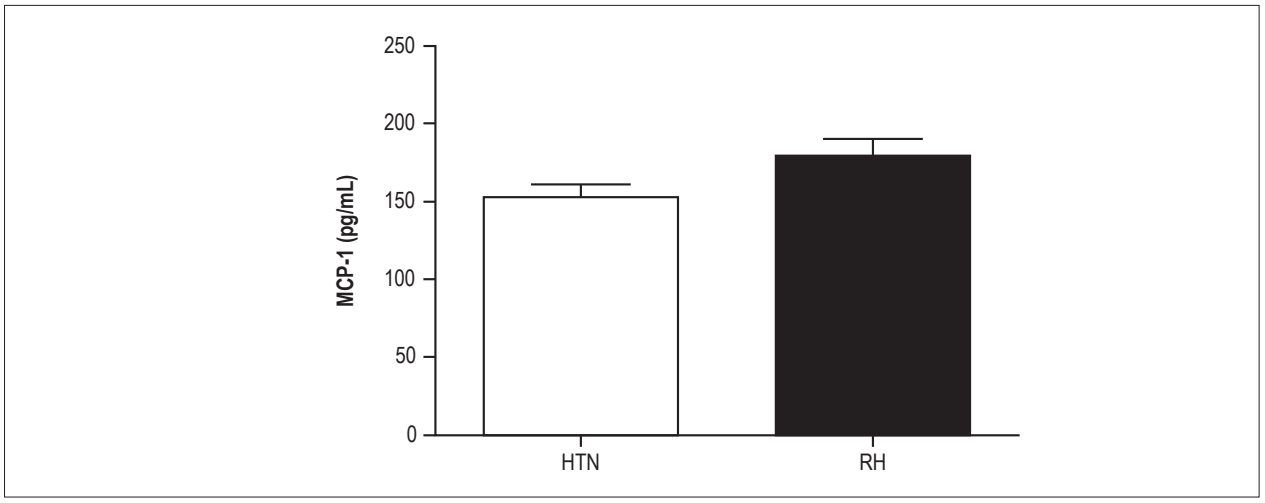

Figure 1 – *Plasma MCP-1 levels in subjects with resistant hypertension (RH, n = 119, 153 ± 93 pg/mL) and mild-to-moderate hypertension (HTN, n=114, 178 ± 120 pg/mL, p = 0.47). Values are expressed as mean ± standard deviation (SD).*

of LVH severity, we found that patients with the highest degree of hypertrophy (LVMI > 125 g/m^2 - level 2) showed lower MCP-1 levels compared with those with the lowest degree (levels 0 and 1) (Figure 2B). Also, the subjects at the lowest (level 0) and intermediate levels (level 1) of LVH demonstrated similar MCP-1 levels.

Finally, the logistic regression model demonstrated that MCP-1 levels were inversely associated with the presence of LVH after adjustment for BMI, age, race, aldosterone level, and the presence of diabetes and RH (Table 3).

Discussion

The main finding of this study was the association between MCP-1 levels and presence of LVH in hypertensive subjects, especially in those with advanced LVH, regardless of resistance to antihypertensive treatment.

Strong evidence supports the role of the inflammatory process in hypertension.[19] Both RH and HTN patients present higher levels of inflammatory cytokines and their levels are related to TOD.[6] MCP-1 is a proinflammatory cytokine with potent chemoattractant activity for monocytes and macrophages.[5] The recruitment and activation of monocytes in rat models appear to be involved with hypertension and TOD process[20] by increasing oxidative stress in the vascular wall.[10] Moreover, mice lacking MCP-1 receptor present no cardiac fibrosis or fibroblast accumulation in the heart after angiotensin infusion,[21] suggesting that MCP-1 and its receptor may have an important role in cardiac damage.

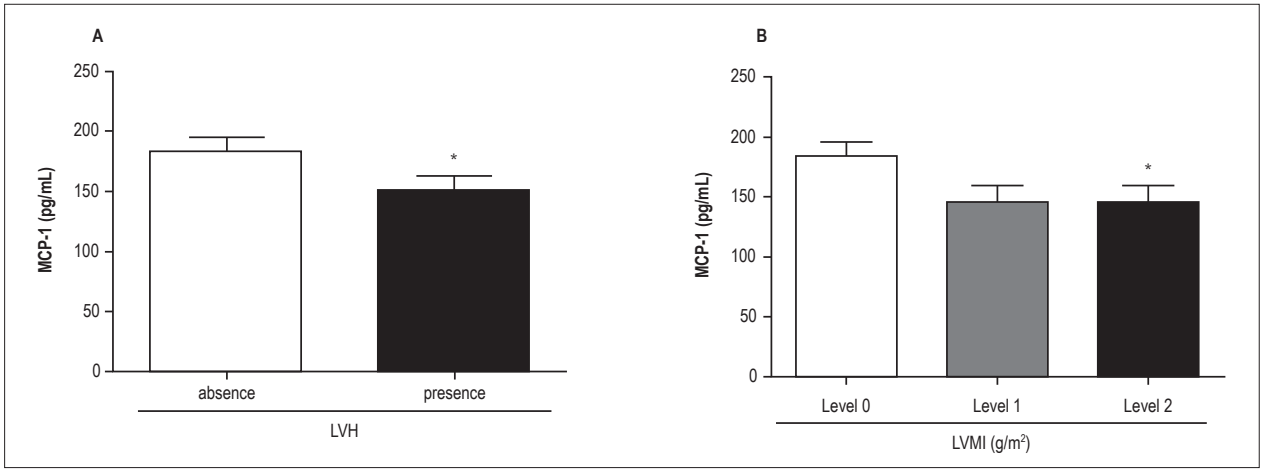

Figure 2 – *Plasma MCP-1 levels according to (A) presence (n = 96) or absence (n = 94) of left ventricular hypertrophy (LVH; cut-off value of 115 g/m² for men and 95 g/m² for women) and (B) level of LVH in all hypertensive subjects (with resistant hypertension and mild-to-moderate hypertension): level 0 = patients without LVH (left ventricular mass index [LVMI] < 115 g/m² in men and < 95 g/m² in women); level 1 = patients with LVH and LVMI levels < 121 g/m²; and level 2 = patients with LVMI levels ≥ 121 g/m². Values are expressed as mean ± standard deviation (SD). (A) *p = 0.005 compared with the absence of LVH and (B) *p = 0.01 compared with level 0.*

Table 3 – Multiple logistic regression for the presence of left ventricular hypertrophy (LVH) in all hypertensive subjects (resistant hypertension and mild-to-moderate hypertension)

Variable	Odds ratio (95% confidence interval)	p value
MCP-1 < 125 pg/mL	2.55 (1.2 – 5.2)	0.01
Presence of diabetes	0.64 (0.3 – 1.1)	0.21
Resistant hypertension	3.7 (1.5 – 8.6)	0.003
Aldosterone (ng/dL)	1.0 (1 – 1)	0.97

MCP-1 was categorized by median levels.

A recent study demonstrated that RH individuals have higher levels of MCP-1 compared with normotensive subjects.[22] However, we found no differences in MCP-1 levels between RH and HTN subjects, suggesting no association between MCP-1 and resistance to antihypertensive drugs.

Regarding MCP-1 levels in hypertensive patients, the data are limited and conflicting.[11,23] One study has shown higher levels of MCP-1 in patients with untreated hypertension compared with controls and patients with isolated systolic hypertension.[23] Mirhafez et al.[8] proposed that cytokines are dependent on the hypertension stage. Indeed, these authors found similar MCP-1 levels among normotensive, pre-hypertensive, and stage 2 hypertensive subjects, but higher MCP-1 levels in stage 1 hypertensive ones compared with their controls.[24] On the other hand, we found similar MCP-1 levels between RH and HTN subjects despite SBP differences between the groups. Also, a multiple logistic regression analysis showed no influence of BP levels in circulating MCP-1 after adjustment for potential confounders (data not shown).

It is well described that RH subjects represent a group with unfavorable phenotype compared with HTN ones. Therefore, the RH subgroup was expected to have high aldosterone levels,[1] presence of LVH,[25] and a greater number of individuals of black race, since these are characteristics closely related to the presence of RH. However, there are no data in the literature showing influence of these parameters on MCP-1 levels.

Equally to the similar MCP-1 levels in our groups, we found no difference in C-reactive protein levels and the number of monocytes between RH and HTN subjects, showing that the inflammatory state may be similar in both groups, corroborating other studies that found no difference in some inflammatory mediators between these groups.[6,26,28] The similar findings between RH and HTN may indicate a BP-independent inflammatory process.

Cardiac damage is an adaptive response to chronic BP overload resulting in hypertrophic growth of cardiomyocytes.[29] To date, the underlying mechanism involved in this TOD remains unknown, although evidence supports the fact that specialized inflammation cells – including monocytes – contribute to tissue lesion through cell-cell interaction performed by chemokines such as MCP-1.[30]

Recently, the idea that the innate immune system plays an important role in the initial and chronic phases of cardiac injury has been brought to knowledge. An experimental study in the early inflammatory phase of infarct healing has shown a marked upregulation of MCP-1 levels resulting in

intense monocyte infiltration into the myocardium, while an opposite situation was observed in the chronic phase – a downregulation of MCP-1.[31]

Additionally, Weinberger et al.[12] have shown that macrophages in the myocardium undergo dynamic changes in the course of life and the CCL-2 – receptor for MCP-1 – helps especially to identify macrophages that recently migrated from the circulation. Taken both studies together, we speculate that MCP-1 might also vary during TOD development in hypertension, where MCP-1 is downregulated in patients with LVH and with long-term hypertension. This may contribute to support our findings that MCP-1 may be differently regulated according to the degree of organ damage.

It is important to highlight that antihypertensive drugs have some antiinflammatory properties and may exert influence on chemokines.[32] Consistent with these reports, a decrease in MCP-1 levels after administration of angiotensin-converting enzyme inhibitors (ACEIs)[33] has been encountered. On the other hand, the use of losartan did not change MCP-1 levels.[33] The authors suggested that only ACEIs could shift the MCP-1 levels by increasing oxide nitric and prostaglandin synthesis. [33] However, the precise mechanism still deserves further investigation.

Since RH subjects took a greater number of antihypertensive drugs compared with HTN ones, we assessed the potential influence of these medications on MCP-1 levels. A multiple linear regression analysis, adjusted for age, presence of LVH, and RH, revealed that only beta-blockers were independently associated with MCP-1 levels (beta coefficient = 55, standard error [SE] = 20, $p < 0.01$). Nevertheless, this possible interference might not have affected the outcome of our study, since RH subjects had similar MCP-1 levels as HTN ones, despite the use of a greater proportion of beta-blocker agents.

Since MCP-1 levels do not necessarily reflect their tissue concentration, this would be appointed as the main limitation of our study. We may also cite as limitations the lack of a normotensive control group and the possible interference of antihypertensive drugs in MCP-1 levels. However, due to ethical reasons, washout of these drugs in RH patients cannot be performed. Hence, as this is an observational study, we cannot infer a causal relationship between progression of the cardiac remodeling and changes in MCP-1 levels.

Conclusion

The similar levels of cytokine betwen RH and HTN subjects and the lower MCP-1 levels in LVH patients suggest (i) a possible downregulation of MCP-1 levels in hypertensive patients with advanced stage of cardiac damage, and (ii) high activation of monocyte migration by MCP-1 in hypertensive patients developing cardiac structural changes. The modulation of chemokines represents an interesting therapeutic approach; therefore, further large clinical studies are required to define the potential involvement of the courses of hypertension and cardiac remodeling and changes in MCP-1 levels.

Author contributions

Conception and design of the research and Writing of the manuscript: Ritter AMV; Acquisition of data: Ritter AMV, Faria APC, Sabbatini A, Corrêa NB, Brunelli V; Analysis and interpretation of the data: Ritter AMV, Faria APC, Sabbatini A; Statistical analysis: Ritter AMV, Faria APC; Obtaining financing: Moreno H; Critical revision of the manuscript for intellectual content: Ritter AMV, Faria APC, Sabbatini A, Corrêa NB, Brunelli V, Modolo R, Moreno H.

Potential Conflict of Interest

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

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Study Association

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

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Original Article

Papillary Muscle Free Strain in Patients with Severe Degenerative and Functional Mitral Regurgitation

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Abstract

Fundamento: The role of papillary muscle function in severe mitral regurgitation with preserved and reduced left ventricular ejection fraction and the method of choice to evaluate PM have still been the subjects of controversy.

Objectives: To evaluate and compare papillary muscle function in and between patients with severe degenerative and functional mitral regurgitation by using the free strain method.

Methods: 64 patients with severe mitral regurgitation - 39 patients with degenerative mitral regurgitation (DMR group) and 25 patients with severe functional mitral regurgitation (FMR group) - and 30 control subjects (control group) were included in the study. Papillary muscle function was evaluated through the free strain method from apical four chamber images of the anterolateral papillary muscle (APM) and from apical three chamber images of the posteromedial papillary muscle (PPM). Global left ventricular longitudinal and circumferential strains were evaluated by applying 2D speckle tracking imaging.

Results: Global left ventricular longitudinal strain (DMR group, –17 [-14.2/-20]; FMR group, –9 [-7/-10.7]; control group, –20 [-18/-21] $p < 0.001$), global left ventricular circumferential strain (DMR group, –20 [-14.5/-22.7]; FMR group, –10 [-7/-12]; control group, –23 [-21/-27.5] $p < 0.001$) and papillary musle strains (PPMS; DMR group, –30.5 [-24/-46.7]; FMR group, –18 [-12/-30]; control group; –43 [-34.5/-39.5] $p < 0.001$; APMS; DMR group, (–35 [-23.5/-43]; FMR group, –20 [-13.5/-26]; control group, –40 [-32.5/-48] $p < 0.001$) were significantly different among all groups. APMS and PPMS were highly correlated with LVEF ($p < 0.001$, $p < 0.001$; respectively), GLS ($p < 0.001$, $p < 0.001$; respectively) and GCS ($p < 0.001$, $p < 0.00$; respectively) of LV among all groups. No correlation was found between papillary muscle strains and effective orifice area (EOA) in both groups of severe mitral regurgitation.

Conclusions: Measuring papillary muscle longitudinal strain by the free strain method is practical and applicable. Papillary muscle dysfunction plays a small role in severe MR due to degenerative or functional causes and papillary muscle functions in general seems to follow left ventricular function. PPM is the most affected PM in severe mitral regurgitation in both groups of DMR and FMR (Arq Bras Cardiol. 2017; 108(4):339-346).

Keywords: Mitral Valve Insufficiency / diagnostic; Mitral Valve Insufficiency / physiopathology; Papillary Muscles / physiopathology; Diagnostic Imaging; Echocardiography / methods; Ventricular Function; Ventricular Remodeling.

Introduction

Mitral regurgitation (MR) is one of the most common valve diseases in developed countries. The main etiologies of MR are classified as degenerative, dilatative and ischemic.[1] Severe MR may compromise left ventricular function and worsen patients' prognosis.[2] The mitral subvalvular apparatus contributes significantly to left ventricular function and occurrence of mitral regurgitation. The impairment of the subvalvular apparatus is detrimental to the left ventricular systolic function and mitral regurgitation.[3,4] Papillary muscle dysfunction has previously been shown as a mechanical cause of mitral regurgitation in patients with functional mitral regurgitation (FMR) in some studies, but, in some others, no correlation was found between mitral regurgitation and papillary muscle dysfunction and even an attenuating effect of papillary muscle dysfunction was reported in most studies.[2,3,5-8] In experimental studies, under the range of normal loading and inotropic conditions, papillary muscle contraction normally follows the general characteristics of left ventricular contraction,[4] but ischemia or stunning may disrupt this course. It is reported that in ischemic mitral regurgitation, diminished papillary muscle shortening, which is termed as papillary muscle dysfunction, paradoxically decreases the degree of MR.[5,7] In patients with normal LV function and MR, fractional shortening has been shown to be normal, similarly to patients with mild or more severe MR.[3]

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In patients with degenerative mitral regurgitation (DMR), there is no sufficient knowledge about the role of papillary muscle (PM) dysfunction. The role of papillary muscle function in severe MR and the method of choice to evaluate PM are still controversial.[3,7] The objective of this study is to evaluate and compare papillary muscle functions in and between patients with severe DMR and severe FMR by using the free strain method.

Methods

Study Population

This is a prospective study and included 64 patients with severe MR who were referred for echocardiographic examination at the Kartal Kosuyolu Heart Education and Research Hospital between January 2014 and April 2015. A total of 39 patients had degenerative severe MR (DMR group) and 25 patients had functional severe MR (FMR group). The control group consisted of 30 subjects with no MR and normal ejection fraction. Patients with DMR (mitral valve prolapse, chordae tendinea rupture) and normal ejection fraction (> 60%), and patients with ischemic or non-ischemic FMR with ejection fraction < 40% were enrolled in the study prospectively. In the DMR group, 6 patients had anterior leaflet prolapsus, 26 patients had posterior prolapsus (18 patients with P2 scallop prolapsus, 4 patients with P1 scallop prolapsus and 4 patients with P3 scallop prolapsus) and 6 patients with Barlow's disease. In the FMR group, 21 patients had ischemic heart disease that did not require revascularization, and 4 patients had non-ischemic dilated heart disease. Patients with organic MR caused by other reasons, including rheumatic or senile degenerative heart valve disease, mitral annular calcification, infective endocarditis, and patients with reduced ejection fraction were excluded from the study. Only patients with appropriate echocardiographic images were included in the study. The Local Ethics Committee approved this study.

Standard Echocardiography

Standard echocardiographic evaluations were performed using a 1 to 5 MHz X5-1 transducer (iE33, Philips Healthcare Inc., Andover, MA). Patients were examined in the left lateral position. Measurements were averaged over 3 consecutive heart cycles. All standard 2D transthoracic echocardographic images from parasternal long axis, short axis, apical four, three and two chamber views, color Doppler and Tissue Doppler images were stored in cine loop format triggered to the QRS complex. Left ventricular diastolic and systolic diameters were measured using M-mode or 2-dimensional echocardiography. Left ventricular ejection fraction (LVEF) was calculated according to Simpson's formula employing a two-dimensional image of the LV chamber during systole and diastole in the four- and two-chamber apical views.

The quantification of MR was assessed as recommended.[9] The proximal isovelocity surface area (PISA) was visualized from apical four-chamber view. The radius of the PISA was measured at mid-systole using the first aliasing. Regurgitant volume (RV) and effective orifice area (EOA) were obtained using the standard formula. For DMR; RV > 60 mL/beat or EOA > 0.4 cm^2, and for FMR; RV > 30 mL and EOA > 0.2 cm^2 were considered as severe MR.[10] The configuration of mitral leaflets was assessed from the parasternal long axis and apical views. In addition to 2D transthoracic echocardiographic views, all patients with severe DMR underwent 2D and 3D transesophageal echocardiographic (TEE), which provided precise information on type and extent of anatomical lesions, mechanism of regurgitation, etiology and reparability of the valve. The mitral annular diameter seen from the bi-commissural view (MAbic) was measured by conventional 2D TEE at 60-75 degrees and anterior-posterior diameter (MAap) was measured at 120 degrees in the parasternal long-axis view. Anterior and posterior leaflet lengths were measured in diastole at 120º.

Speckle Tracking Echocardiography (STE): Left ventricular strain (circumferential and longitudinal) was evaluated using 2D speckle-tracking imaging. Global circumferential strain (GCS) was assessed from parasternal short axis views of the left ventricle at three levels (base-mid-apical). Global longitudinal strain (GLS) was assessed from apical four, three and two chamber views.

Longitudinal myocardial strain of PMs was evaluated using the free strain method from apical four chamber view for anterolateral PM (APM) and apical long axis view for posteromedial PM (PPM). Patients in whom PM views were visually clear in both systole and diastole were considered eligible for the assessment. Of 110 patients, 15% were excluded from the study because of inadequate image quality.

Free strain is an application of the commercially available software program of Philips (CMQ Q-app). This method enables the measurement of user defined custom local velocities, displacement and deformation using unlimited directional chords display technic. This workflow measures strain within the myocardial region, free of restraints on the location or direction of the measurements, which can be radial, longitudinal, and circumferential. Free strain is thought to be an easy, quick and practical method of measuring myocardial deformation. This method may be particularly preferable in measuring the deformation of PMs since these structures are relatively separate from the LV myocardium and are not included in the commercially available LV strain models.

In order to measure the longitudinal strain by using the free strain method, a region of interest should be selected by clicking two points manually. The first point was selected from the base of the PM at its attachment zone to the LV wall. The second point was selected from the tip of the PM with special attention to keep a 3-5 mm distance from the chordae in order to avoid artifacts.

All STE acquisitions were performed at frame rates between 50-70 Hz frames per second. The average value of strain was taken from the three consecutive beats. The peak systolic values were recorded for GCS, GLS and longitudinal S of APM and PPM.

The details of the longitudinal strain measurement with the "free strain" method for both PMs are presented in Figure 1.

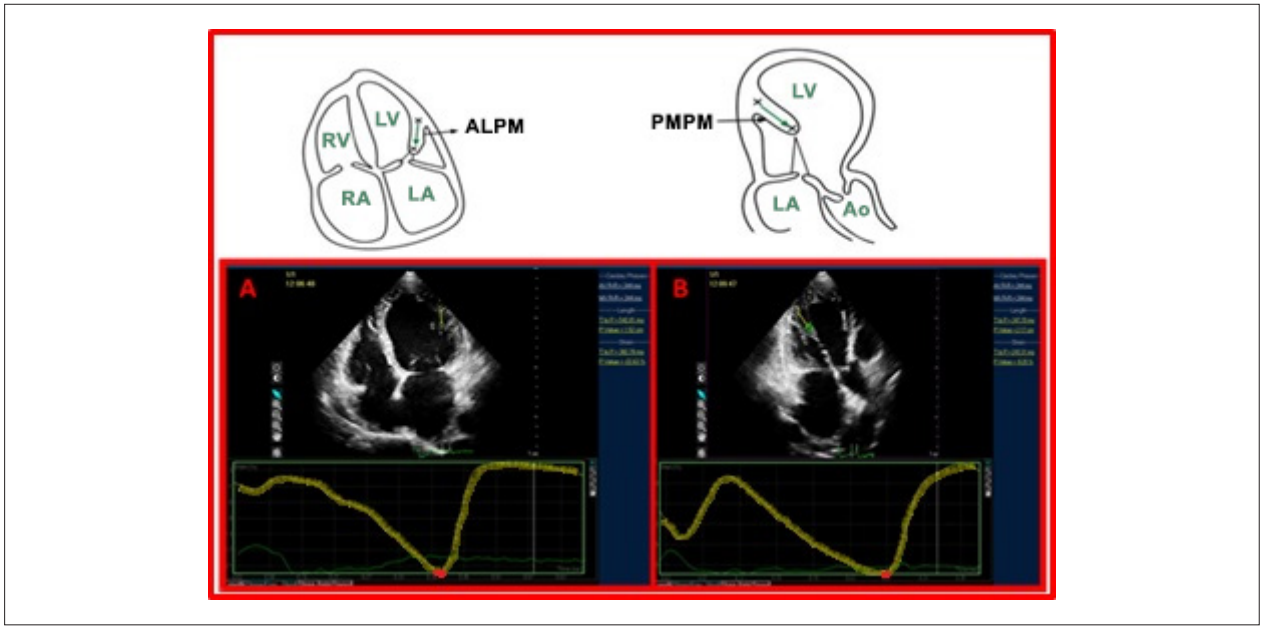

Figure 1 – *A) Free strain measurement of the APM from apical four chamber image in a patient with FMR. B) Free strain measurement of PPM from apical long axis image of the same patient.*

Statistical analysis:

Data management and analysis were performed using IBM SPSS Statistics 16.0 (SPSS, Chicago, IL) software. Continuous variables are expressed as mean (SD) or median (25th to 75th interquartile range [IR]) depending upon variable distribution. Normal distribution was analyzed using the Kolmogorov-Smirnov test. Categorical variables are presented by absolute and percentage numbers and compared using Chi-Square or Fisher's Exact test as appropriate. One-way ANOVA with Tukey post hoc was used to compare continuous variables among groups; when homogeneity of variance was not present, the Kruskal-Wallis test was used for nonparametric independent samples. Mann-Whitney test for nonparametric independent samples for inter-group comparisons were performed to confirm significance. Correlations were tested by Pearson or Spearman's correlation tests, as appropriate.

A p value < 0.05 was considered statistically significant.

Results

Demographic characteristics of the study population are presented in Table 1. Age and gender were similar in all groups. Standard echocardiographic and STE characteristics are presented in Tables 2 and 3. LA and LV diameters were statistically different among all groups. Atrial fibrillation ratio was statistically different between DMR and FMR groups but this did not seem to significantly affect the results of the study.

Global left ventricular longitudinal strain and PM longitudinal strains were significantly different among all groups. Posteromedial PM strain (PPMS) of the control group was better than PPMS of the DMR and FMR groups. There was no significant difference in anterolateral PM strain (APMS) between the DMR and control groups, and both strains of the FMR group were significantly lower than PM longitudinal strains of the DMR and control groups. PPMS had the lowest values in both MR groups. Global left ventricular longitudinal and circumferential strains of all three groups followed the same order as PPMS, and were better in the control group than in the DMR group, and the DMR group was better than the FMR group (Figure 2).

APMS and PPMS were highly correlated to LVEF (both $p < 0.001$), GLS (both $p < 0.001$) and GCS (both $p < 0.001$) of the LV among all groups.

No correlation was found between PM strains and EOA in either group with severe MR.

In the DMR group, there was no statistical correlation between PM longitudinal strains and EOA. Any scallop prolapse in the anterior leaflet versus posterior leaflet was correlated to APMS ($p = 0.04$). Moreover, there was a moderate correlation between the left ventricular end diastolic diameter (LVEDD) and EOA ($r = 0.38$, $p = 0.02$). APMS and PPMS were not correlated with LVEF ($p = 0.55$, $p = 0.13$; respectively), GLS ($p = 0.62$, $p = 0.54$; respectively) and GCS ($p = 0.77$, $p = 0.38$; respectively).

In the FMR group, there was also no correlation between EOA and PM longitudinal strains. MAbic was negatively correlated with APMS ($r = -0.76$, $p = 0.03$). Posterior leaflet length was correlated with PPMS ($r = 0.88$, $p = 0.01$). APMS and PPMS were not correlated with LVEF ($p = 0.18$, $p = 0.09$; respectively), GLS ($p = 0.33$, $p = 0.33$; respectively) and GCS ($p = 0.83$, $p = 0.93$; respectively).

Also, the in the control group, APMS and PPMS were not correlated to LVEF ($p = 0.80$, $p = 0.65$; respectively), GLS ($p = 0.25$, $p = 0.43$; respectively) and GCS ($p = 0.63$, $p = 0.85$; respectively).

Table 1 – Baseline Clinical Characteristics of Study Population

Variable	DMR (n: 39)	FMR (n: 25)	Control (n: 30)	p value
Age, years	52.5 ± 15	57 ± 15	52.7 ± 9.4	0.40
Gender, male	29 (%74)	20 (%80)	20 (%67)	0.58
NYHA class 3-4	10 (%26)	6 (%24)	0 (% 0)	0.011
Creatinine, mg/dL	0.88 ± 0.26	1.2 ± 0.78	0.81 ± 0.15	0.06
DM	4 (%10)	5 (%20)	3 (%10)	0.45
SBP (mmHg)	128.8 ± 6.8	113.4 ± 8	127.6 ± 9.1	< 0.001
DBP (mmHg)	78.2 ± 5.3	71±5.2	80.8 ± 6.1	< 0.001
Chronic AF	2 (5.1%)	11 (44%)	0	< 0.001

DM: Diabetes Mellitus; SBP: systolic blood pressure; DBP: diastolic blood pressure; AF: atrial fibrillation.

Table 2 – Baseline characteristics of mean and median values of echocardiographic parameters

Groups	DMR Group (n: 39)	FMR Group (n: 25)	Control Group (n: 30)	p value (all groups)	Pα	Pβ	Pγ
LA (cm)	4.18 ± 0.73	4.72 ± 0.79	3.31 ± 0.37	< 0.001	0.008	< 0.001	< 0.001
LVESD (cm)	3.56 ± 0.67	5.14 ± 0.73	2.89 ± 0.40	< 0.001	0.01	< 0.001	< 0.001
LVEDD (cm)	5.80 ± 0.74	6.51 ± 0.81	4.71 ± 0.41	< 0.001	< 0.001	< 0.001	< 0.001
LVEF (%)	64.5 ± 2.02	33.4 ± 9.06	65.1 ± 1.94	< 0.001	< 0.001	0.22	< 0.001
EOA (cm²)	68.75 ± 27.23	33.43 ± 11.03		< 0.001			
RV (ml)	95.97 ± 30.6	50.3 ± 13.9		< 0.001			
AL LENGHT (mm)	27.8 ± 6.41	25.8 ± 3.6		0.465			
PL LENGHT (mm)	17.5 ± 4.15	14 ± 1.41		0.049			
MAbic (mm)	46.6 ± 7.13	33.5 ± 14.8		0.001			
MAap (mm)	41.0 ± 5.62	34.6 ± 1.86		0.009			

LA: left atrium; LVESD: left ventricular end systolic diameter; LVEDD: left ventricular end diastolic diameter; LVEF: left ventricular ejection fraction; PISA: proximal velosity surface area; RV: regurgitant volume; AL LENGHT: anterior leaflet length; PL LENGHT: posterior leaflet length; MAbic: bi-commissural mitral annulus diameter; MAap: A2P2 mitral annulus diameter; Pα: p value of comparing groups DMR-FMR; Pβ:p value of comparing groups DMR-control; Pγ: p value of comparing groups FMR-control.

Table 3 – Strain values of study population

Groups	DMR (n: 39)	FMR (n: 25)	Control (n: 30)	P value (all groups)	Pα	Pβ	Pγ
GCS (%)	-20 (-14.5/ -22.7)	-10 (-7/-12)	-23 (-21/-27.5)	<0.001	<0.001	0.002	<0.001
GLST (%)	-17 (-14.2/-20)	-9 (-7/-10.7)	-20 (-18/-21)	<0.001	<0.001	0.005	<0.001
APMS (%)	-35 (-23.5/-43)	-20 (-13.5/-26)	-40 (-32.5/-48)	<0.001	<0.001	0.102	<0.001
PPMS (%)	-30.5 (-24/-46.7)	-18 (-12/-30)	-43 (-34.5/-39.5)	<0.001	<0.001	0.012	<0.001

GCS: global circumferential left ventricular strain; GLS: global longitudinal left ventricular strain; APMS: anterolateral papillary muscle strain from apical four chamber; PPMS: posteromedial papillary muscle strain from apical long axis; Pα: p value of comparing groups DMR-FMR; Pβ: p value of comparing groups DMR-control; Pγ: p value of comparing groups FMR-control.

Discussion

Our study demonstrated that PM functions acts in a manner similar to the left ventricle, and is diminished in severe degenerative and functional MR similarly to global ventricular strain. PPMS of the control group was better than in the DMR group and the PPMS of the DMR group was better than in the FMR group. APMS of the control group was similar to the DMR group and better than the FMR group. Although patients had normal ejection fraction in the DMR group, PM longitudinal strain values ordinarily followed GLS and GCS, which were diminished when compared to control group, reflecting a latent systolic dysfunction in the DMR group. Also, in the FMR

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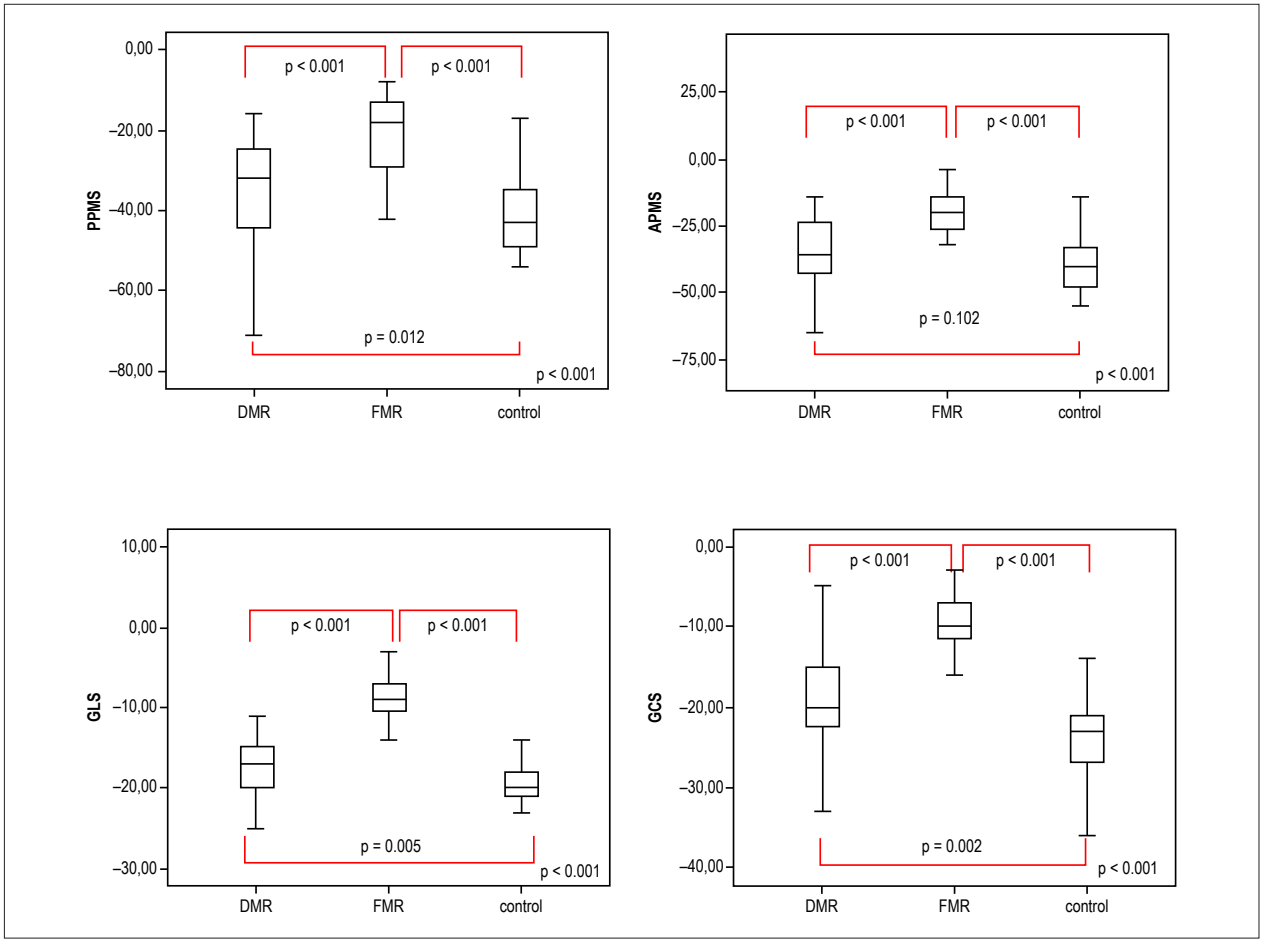

Figure 2 – *Box-plot values of median (IQR) for APMS, PPMS, GCS, GLS values according to the groups DMR, FMR and controls.*

group, PM longitudinal strain values were well-matched to the diminished global longitudinal and circumferential strain of the impaired LV. No correlation was found between PM free strains and EOA in either group with severe MR. Kisanuki et al. showed that the occurrence of moderate to severe MR was significantly more frequent in patients with combined anterior and posterior PM dysfunction than in those with isolated PM dysfunction or normal PM function. However, they supposed that isolated or combined PM dysfunction was not the only cause for MR unless it was together with left ventricular wall motion abnormalities.[11] It has been shown in experimental studies that selective paresis of the PMs does not affect the competence of the mitral valve and does not cause MR in a normally contracting ventricle.[11,12] The main mechanism of MR in FMR is increased tethering forces and reduced coaptation of the mitral valve leaflets by medial/ lateral and apical displacement of the PMs.[13,14] Tigen et al. [6] demonstrated that PM desynchrony was the independent predictor of moderate or moderate-to-severe MR in patients with non-ischemic dilated cardiomyopathy. But in another study that included patients with ischemic and non-ischemic cardiomyopathy, the circumferential strain of PM was evaluated and a straight relationship between PM desynchrony and MR degree was not found; conversely, an inverse relationship between PM longitudinal strain and the degree of MR was found in patients with basal inferior LV remodeling.[2] There are several studies supporting the paradoxical decrease in ischemic MR by PM dysfunction.[5,7] This is attributed to a decreased shortening of PMs resulting in the reduction of tethering and MR by PM dysfunction. Although some studies show acute improvement of MR with cardiac resynchronization therapy, the main mechanism is ambiguous and it is considered that if there is PM desynchrony with left ventricular desynchrony, improved coordination of PM contraction can cause acute improvement of MR.[15,16] Also, in patients with normal ejection fraction, it is reported that PM dysfunction had no significant role in the occurrence of MR.[3]

Our study demonstrated that in the DMR group, APMS was similar to the control group and better than PPMS. In degenerative mitral valve disease, perivalvular ventricular fibrosis and PM fibrosis have been shown in some pathological and MRI studies.[17] Foci of necrosis are also common in patients with recent onset severe valvular regurgitation, and, in our study, most of the patients had chordal rupture with prolapse. Necrosis or fibrosis may be either focal or diffuse and can involve only one PM or both. The APM is slightly larger and has a richer blood supply

than the PPM. Thus, if only one PM contains foci of fibrosis, it is almost always the PPM.[18] Moreover, combined PM dysfunction is frequently seen in patients with FMR, in contrast to patients with apparent mitral valve prolapse in which combined PM dysfunction was noted in a small number of patients.[8]

In addition, in our study, any scallop prolapse on anterior leaflet was associated to a decreased APMS value when compared to posterior leaflet prolapse. This may be because the anterior leaflet is larger, longer and usually thicker than the posterior leaflet. The posterior leaflet is crescent-shaped with a short radial length and a long circumferential base.[19,20] Thus, severe MR may cause less shortening of PM in systole caused by redundant anterior leaflet movement towards the left atrium by the driving force of the mitral regurgitant jet. In addition, the mitral annulus is a nonplanar saddle-shaped structure. The anterior portion of the mitral annulus is continuous with the rigid aortic annulus and is elevated towards the atrium as a 'horn'. However, the posterior mitral annulus is more flexible, allowing a systolic apical bending along a commissural axis. This helps to reduce tissue stress.[19,21] Anterior leaflet prolapse may be more associated with increased tissue stress than with posterior prolapse.

In the FMR group, an increase in MAbic is associated with decreased APMS. When the LV dilates, the mitral annulus also dilates and flattens, loses its saddle shape and systolic annular contraction. This causes malcoaptation of mitral leaflets,[22,23] and an increase in tethering forces resulting in less shortening of PMs. In addition, we found that posterior leaflet length was associated with PPMS. In FMR, tethering of the mitral leaflets is often on the posterior leaflet and particularly on the posteromedial scallop.[24] According to this finding, as the posterior leaflet length increases, tethering of the mitral leaflet diminishes and PM function improves.

As far as we know, ours is the only study that compares PM function in degenerative and functional severe MR patients by using the free strain method. In previous studies, longitudinal and circumferential strain methods were used to evaluate PM function. We used the free strain method to measure the longitudinal strain of two points on the PMs, which seems easier and more practical in clinical use, although there is no standard guideline on free strain in PM function evaluation so far.

Some studies with animals have shown that under the range of normal loading and inotropic conditions, PM dynamics closely follow the dynamics of the LV as a whole. They shorten during ejection like the rest of LV, and their lengths change only very slightly during the isovolumic periods. During isovolumic contraction they shorten slightly and during isovolumic relaxation they lenghten slightly. Under ischemic conditions, the dynamic behaviour of PMs reverse during isovolumic contraction and isovolumic relaxation.[4,25,26] In the study by Kisanuki et al., fractional shortening of PMs was calculated by using end diastolic and end systolic length of PMs on 2D TTE.[11] In our study, the values of longitudinal strain of PMs, using free strain method, were correlated with their values of fractional shortening of PMs.

Limitations

Only patients with severe MR were included in the study. Patients with mild or moderate MR were excluded. We evaluated PM function in severe MR comparing and associating with EOA in DMR and FMR patients. The behavior of free strain patterns in patients with mild or moderate MR is unknown. We used the free strain method to evaluate PM function, but there is no standard usage or values about this method. Since this is study with a small population, the present results should be confirmed in further studies with a larger number of patients.

Conclusion

Our study, in accordance with previous studies, has demonstrated that PM dysfunction plays a small role in severe MR due to degenerative or functional causes, and PM function in general seems to follow LV function. PPM is the most affected PM and has the lowest longitudinal strain values in both severe MR groups. Free strain is a practical and applicable method of choice to measure PM longitudinal strain.

Author contributions

Conception and design of the research: Kılıcgedik A, Kahveci G; Acquisition of data: Kılıcgedik A, Gurbuz AS, Karabay CY, Guler A, Efe SC, Aung SM, Arslantas U, Demir S; Analysis and interpretation of the data: Kılıcgedik A, Kahveci G, Gurbuz AS, Karabay CY, Guler A, Efe SC, Aung SM, Arslantas U, Demir S, Izgi IA, Kirma C; Statistical analysis: Kılıcgedik A, Kahveci G, Gurbuz AS, Karabay CY, Efe SC, Kirma C; Obtaining financing: Kılıcgedik A; Writing of the manuscript: Kılıcgedik A, Kahveci G, Aung SM, Arslantas U, Demir S, Izgi IA, Kirma C; Critical revision of the manuscript for intellectual contente: Kılıcgedik A, Kahveci G, Guler A, Izgi IA, Kirma C.

Potential Conflict of Interest

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

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Original Article

Quality of Life Score as a Predictor of Death in Dogs with Degenerative Mitral Valve Disease

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Abstract

Background: **The knowledge of the variables predicting mortality is important in clinical practice and for therapeutic monitoring in mitral valve disease.**

Objectives: **To determine whether a quality of life score evaluated with the Functional Evaluation of Cardiac Health questionnaire would predict mortality in dogs with degenerative mitral valve disease (DMVD).**

Methods: **Thirty-six client-owned dogs with mitral valve disease underwent clinical, laboratory, and echocardiographic evaluations at baseline and were monitored for 6 months. Cardiovascular death was the primary outcome.**

Results: **The 36 dogs were classified as survivors or nonsurvivors. Higher values of the following variables were obtained at baseline in the nonsurviving group (12 dogs): amino-terminal pro-B-type natriuretic peptide (NT-proBNP) levels, plasma norepinephrine, heart rate, quality of life score, diastolic left ventricular internal dimension to aortic root ratio, systolic left ventricular internal dimension to aortic root ratio, and left atrium to aortic root ratio. NT-proBNP levels and quality life score were independently associated with death in the multivariable analysis.**

Conclusion: **The quality life score was an independent variable for cardiac death in dogs with DMVD. This result is encouraging, as this score is easy to apply and does not require any technology, only a veterinarian and an observant owner. (Arq Bras Cardiol. 2017; 108(4):347-353)**

Keywords: **Dogs; Quality of Life; Mortality; Heart Valve Diseases; Mitral Valve / abnormalities.**

Introduction

Degenerative mitral valve disease (DMVD) is the most commonly diagnosed disease in routine veterinary cardiology in dogs. Therefore, the knowledge of the variables that can predict mortality in DMVD is important for the clinical practice and for therapeutic monitoring of these patients.[1]

Diagnostic tests, such as electrocardiography, echocardiography, chest radiography, and blood pressure measurement, are routinely used to evaluate these patients and the effectiveness of their treatments.[2] Other tests have been identified as useful in monitoring the progression of this valvular heart disease. For example, exacerbated activation of the sympathetic nervous system developed during heart failure associated with mitral valve disease can be monitored by measuring the plasma concentration of norepinephrine (NE), which is associated with severe symptoms and a higher risk of death.[3,4] The importance of the amino-terminal pro-B-type natriuretic peptide (NT-proBNP), an inactive amino-terminal fragment of the prohormone brain natriuretic peptide, has been recognized in recent years. In veterinary medicine, studies in dogs have suggested that NT-proBNP is a marker of the presence and severity of cardiac disease. Cutoff values for the concentrations of this peptide have been established and used to estimate the risk of the onset of congestive heart failure and to predict mortality in dogs with mitral valve disease.[5-7]

In addition to this biochemical marker, the echocardiographic variables left ventricular end-diastolic diameter, left atrial (LA) to aortic root (Ao) ratio (LA/Ao), and E wave transmitral peak velocity are predictors of all-cause mortality in dogs with DMVD.[8] These diagnostic variables may be used to predict mortality in therapeutic management. Nevertheless, several clinical variables, such as respiratory signs, difficulties with mobility, etc., could together be an important tool to predict death and be very useful in veterinary clinics where technology is unavailable. The aim of this study was to investigate whether a score obtained with the Functional Evaluation of Cardiac Health, a quality of life questionnaire, could be used as a predictor of death in dogs with DMVD.

Methods

Animals

The dogs included in this prospective study were referred from a private veterinary ambulatory clinic at the time of their

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first presentation of signs or symptoms of congestive heart failure. The inclusion criteria for participation in the study were dogs with mitral regurgitation (MR) and LA enlargement (LA/Ao > 1.2) normal laboratory renal (creatinine < 2.1 mg/dL) and liver function results, and no other associated diseases. All dogs underwent a clinical evaluation consisting of physical examination, electrocardiography, blood pressure measurement, thoracic radiography, blood cell count, plasma and serum biochemical analysis, and two-dimensional, M-mode spectral-pulsed Doppler echocardiography. Therapeutic adjustments were only made when the dogs had undergone all diagnostic tests and the quality of life questionnaire had been applied, which occurred after the selection of the animals.

The definitive diagnosis of DMVD was obtained during an echocardiographic examination performed by a veterinary specialist who was blinded to the quality of life questionnaire and laboratory results. The dogs were classified as having grade I to IV DMVD according to the New York Heart Association functional class scoring system modified for veterinary use.[9] Briefly, functional class I was defined as a heart murmur of mitral origin with no signs of heart enlargement and no limitation to physical activity, class II included slight limitation to physical activity with varying degrees of heart enlargement without clinical signs, class III included marked limitation of physical activity with radiologic signs of congestive heart failure, and class IV comprised severe limitation of physical activity with radiologic signs of congestive heart failure.

Before enrollment in the study, 17 animals were already being treated with diuretics, inotropic agents, and/or angiotensin converting enzyme inhibitors. The drugs administered at the beginning of the study were adjusted according to the severity of the disease and included angiotensin converting enzyme inhibitors, inotropic agents, diuretics, and beta-blockers (when well tolerated). During the 6-month follow-up period, the treatment was adjusted whenever necessary. The owners of the dogs were asked to inform the researcher in case a cardiac-related death occurred outside of the hospital. None of the dogs were euthanized.

An informed written consent was obtained from each dog owner, and the study was approved by the Ethics Committee Heart Institute (InCor), University of São Paulo Medical School (number 072/05).

The variables of the survivors and nonsurvivors at baseline were compared before any therapeutic adjustment was made. The significance of the variables that were clinically relevant in predicting death was analyzed after 6 months of follow-up.

Assessment of quality of life

A total of 36 client-owned dogs were chosen by convenience for our study. As previously described, the Functional Evaluation of Cardiac Health quality of life questionnaire was developed based on widely accepted clinical signs of cardiac disease in dogs.[10] The questionnaire consists of 17 questions answered by the dog owner, who grades the severity of symptoms on a scale from 0 to 5, in which 0 = few symptoms and 5 = several symptoms , with higher scores indicating a poorer health-related quality of life. The questions are mainly related to respiratory signs, difficulties with mobility (such as walking and climbing stairs), physical activity, irritability, appetite, sleepiness, and frequency of urination and vomiting. The score was established using information obtained from the owner by a veterinarian during the anamnesis.

Laboratory measurements

Blood samples were obtained early in the morning for measurement of plasma concentrations of NE, NT-proBNP, and other biochemical variables. An appropriately sized heparinized catheter was inserted into the saphenous vein of each dog. The dog was then placed in lateral recumbency on a table with minimal restraint for 20 minutes.[11] The first mL of blood collected from the catheter was discarded. The subsequent 3 to 5 mL of blood were collected and immediately transferred to ice-chilled tubes containing a mixture of ethylene glycol tetraacetic acid - glutathione (20 μL of anticoagulant/mL of blood) for NE analysis. Other samples were collected from the same catheter and transferred to an EDTA tube for NT-proBNP measurement and into a plain tube for other biochemical analyses. Within 1 hour of blood collection, the plasma and serum were separated and immediately frozen at –70°C. NE levels were determined by high-performance liquid chromatography with an electrochemical detector,[12] (Model 515, Waters Corp, Milford, MA, USA) and sodium (Na) levels were analyzed with a selective electrode (Dimension RXL, Dade Behring, Newark, DE, USA). Specific kits for automated equipment were used to measure urea and creatinine levels (Dimension RXL). The concentrations of plasma NT-proBNP were measured in duplicate using a commercial ELISA kit specific for canine NT-proBNP (Vet Sign Canine CardioSCREEN NT- Pro-BNP kit, Guildhay, UK).

Echocardiographic and electrocardiographic evaluation

The arterial blood pressure was measured indirectly by vascular Doppler (Medmega DV-610, Medmega, São Paulo, Brazil) while the dogs were in lateral recumbency. The cuff width was approximately 40% of the limb circumference. Each systolic and diastolic arterial blood pressure value was calculated as the mean of three to four measurements.

The heart rate (HR) and cardiac rhythm were evaluated using a short-term electrocardiographic recorder (Ecafix model E.C.G.-6, Ecafix , São Paulo, Brazil).[13,14] The echocardiographic examination was performed using an ultrasound system with a 5-MHz microconvex transducer (Aloka SSD 650 Ultrasound System, Aloka Inc., Tokyo, Japan).

The M-mode echocardiographic variables studied were the diastolic interventricular septal thickness (IVSd), diastolic left ventricular wall thickness (LVWd), diastolic ventricular internal dimension (LVIDd), systolic ventricular internal dimension (LVIDs), fractional shortening (FS), Ao, and LA dimension. The left ventricular dimensions and the LA were indexed to the Ao. FS values were calculated using the equation FS = [(LVIDd - LVIDs) / LVIDd] X 100. The intraobserver variability of the M-mode echocardiographic variables was calculated using 15 measurements of each variable (obtained from three recordings measured five times each) in five dogs (the coefficients of variation ranged from 2.6% to 6.5%).[15]

The severity of the MR was estimated with spectral-pulsed Doppler ultrasonography based on the percentage of the LA occupied by the regurgitant jet (mild < 20%, moderate 20 to 50%, severe > 50%).[16,17]

Statistical analysis

Data with normal distribution are expressed as mean ± standard deviation (SD), while those with non-normal distribution are shown as median and interquartile range (IQR). The Kolmogorov-Smirnov normality test was used to test for the normality of the data. When the data were normally distributed, the parametric Student's *t* test for independent samples was used, as displayed in Table 1. When the data were not normally distributed, the nonparametric Mann-Whitney *U* test for independent samples (Table 2) and Kruskal-Wallis test (NT-proBNP) were used. In addition, the chi-square test and Fishers' exact test were used when the groups were evaluated in relation to their proportions. The Spearman test was used to measure the statistical association between two variables.

We performed a multivariable logistic analysis in a forward stepwise approach considering death at 6 months as the dependent variable. The independent variables were functional classification, LA/Ao, creatinine, quality of life score, ranked NT-proBNP, and dichotomized HR as ≤ 130 bpm or > 130 bpm. NT-proBNP values were ranked in units of 1,000 pmol/L, in order to make it easier to interpret the results.[18] Only variables with $p < 0.1$ were included in the multivariable regression model.

Receiver operating characteristic (ROC) analyses were performed to determine the optimal cutoff values for selected variable.[19] Odds ratios (OR) were calculated as part of the logistic regression analysis. The significance level adopted for the statistical tests was 5%. Statistical analyses were performed using the Statistical Analysis System (SAS) software program for Windows, version 9.2 (SAS Institute Inc., 1989-1996, Cary, NC, USA).

Results

The following breeds of dogs were enrolled in the study: 23 Poodles, five mixed-breed dogs, one Basset hound, one Beagle, one Cocker Spaniel, one Dachshund, one Lhasa Apso, and three Pinschers. The baseline characteristics of the 36 DMVD dogs are presented in Tables 1 and 2. The dogs were classified as having mild (n = 4), moderate (n = 18), or severe (n = 14) MR.

We investigated the correlation between laboratory, electrocardiographic, echocardiographic, and clinical variables obtained at baseline. A positive correlation was identified between quality of life scores and the following variables: functional classification of the dog ($r = 0.729$, $p < 0.0001$), LA/Ao ($r = 0.591$, $p = 0.0001$), and plasma NE ($r = 0.430$, $p = 0.009$).

NT-proBNP concentrations correlated positively with LA/Ao ($r = 0.615$, $p < 0.001$), LVIDd/Ao ($r = 0.502$, $p = 0.0018$), and LVIDs/Ao ($r = 0.622$, $p = 0.0001$) and negatively with FS ($r = -0.386$, $p = 0.020$). The only clinical and biochemical variables that correlated positively with NT-proBNP levels were the quality life score ($r = 0.537$, $p = 0.001$) and the plasma NE levels ($r = 0.383$, $p = 0.021$).

Dogs with mild (n = 4), moderate (n = 18), and severe (n = 14) MR had NT-proBNP values of 751 pmol/L (IQR 539 – 1017 pmol/L), 1183 pmol/L (IQR 701 – 1850 pmol/L), and 2070 pmol/L (IQR 878 – 5461 pmol/L), respectively (Kruskal-Wallis test, $p = 0.0849$).

The 36 DMVD dogs were further classified as survivors and nonsurvivors. We compared the clinical, laboratory, and echocardiographic variables of the dogs in both groups to identify factors predictive of death (Tables 1 and 2). The following variables were significantly higher among the animals that did not survive when compared with those that survived: NT-proBNP, NE, HR, quality of life

Table 1 – Baseline characteristics of 36 dogs with degenerative mitral valve disease (DDMV) categorized as survivors or nonsurvivors. Variables with normal distribution, described as mean and standard deviation (SD)

	All	Mortality		
Variables	**DMVD dogs (n = 36)**	**Surviving dogs (n = 24)**	**Nonsurviving dogs (n = 12)**	**p**
Age (SD) yrs	10.7 (2.0)	10.5 (2.2)	11.0 (1.5)	0.478
Male, n (%)	23 (63.9)	12 (50.0)	11 (91.7)	0.025
FC III-IV, n (%)	15 (41.7)	6 (25.0)	9 (75.0)	0.004
FETCH (SD)	14.9 (10.5)	10.5 (7.9)	23.7 (10.0)	< 0.001
Na (SD) mEq/L	147.0 (4.00)	147.2 (4.19)	146.4 (3.60)	0.550
HR (ECG),(SD),bpm	144.3 (33.8)	137.3 (36.6)	158.3 (22.5)	0.041
SBP (SD) mmHg	135.5 (24.8)	134.8 (26.2)	136.7 (23.0)	0.831
DBP (SD) mmHg	80.1 (16.9)	79.3 (15.3)	81.9 (21.1)	0.689
LVIDd/Ao (SD)	2.23 (0.44)	2.07 (0.39)	2.54 (0.36)	0.0014
LVIDs/Ao (SD)	1.17 (0.28)	1.07 (0.23)	1.36 (0.28)	0.0025
FS (%)	47.7 (6.7)	48.5 (7.0)	45.9 (6.0)	0.277

FC: functional classification; FETCH: Functional Evaluation of Cardiac Health; Na: sodium; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; LVIDd/Ao: diastolic left ventricular internal dimension/aortic root ratio; LVIDs/Ao: systolic left ventricular internal dimension/aortic root ratio; FS: fractional shortening.

Table 2 – Baseline characteristics of 36 dogs with degenerative mitral valve disease (DDMV) categorized as survivors or nonsurvivors. Variables without normal distribution, described as median and interquartile range (IQR)

	All	Mortality		
Variables	**DMVD dogs (n = 36)**	**Surviving dogs (n = 24)**	**Nonsurviving dogs (n = 12)**	**p**
Weight (IQR) kg	6.2 (4.5-9.9)	6.2 (4.6-9.9)	6.0 (4.3-10.0)	0.920
NT-proBNP (IQR) pmol/L	1282 (699-2477)	859 (619-1345)	4055 (2070-6452)	< 0.001
NE (IQR) pg/mL	386 (250-574)	293 (214-430)	574 (357-998)	0.017
Creatinine (IQR) mg/dL	0.85 (0.70-1.00)	0.80 (0.70-1.00)	0.90 (0.80-1.10)	0.119
IVSd (IQR) cm	0.60 (0.50-0.70)	0.60 (0.50-0.70)	0.60 (0.50-0.70)	0.890
LA/Ao (IQR)	1.56 (1.38-2.00)	1.44 (1.30-1.65)	2.09 (1.70-2.28)	< 0.001

NT-proBNP: amino-terminal pro-B-type natriuretic peptide; NE: norepinephrine; IVSd: diastolic interventricular septal thickness; LA/Ao: left atrium dimension/aortic root ratio.

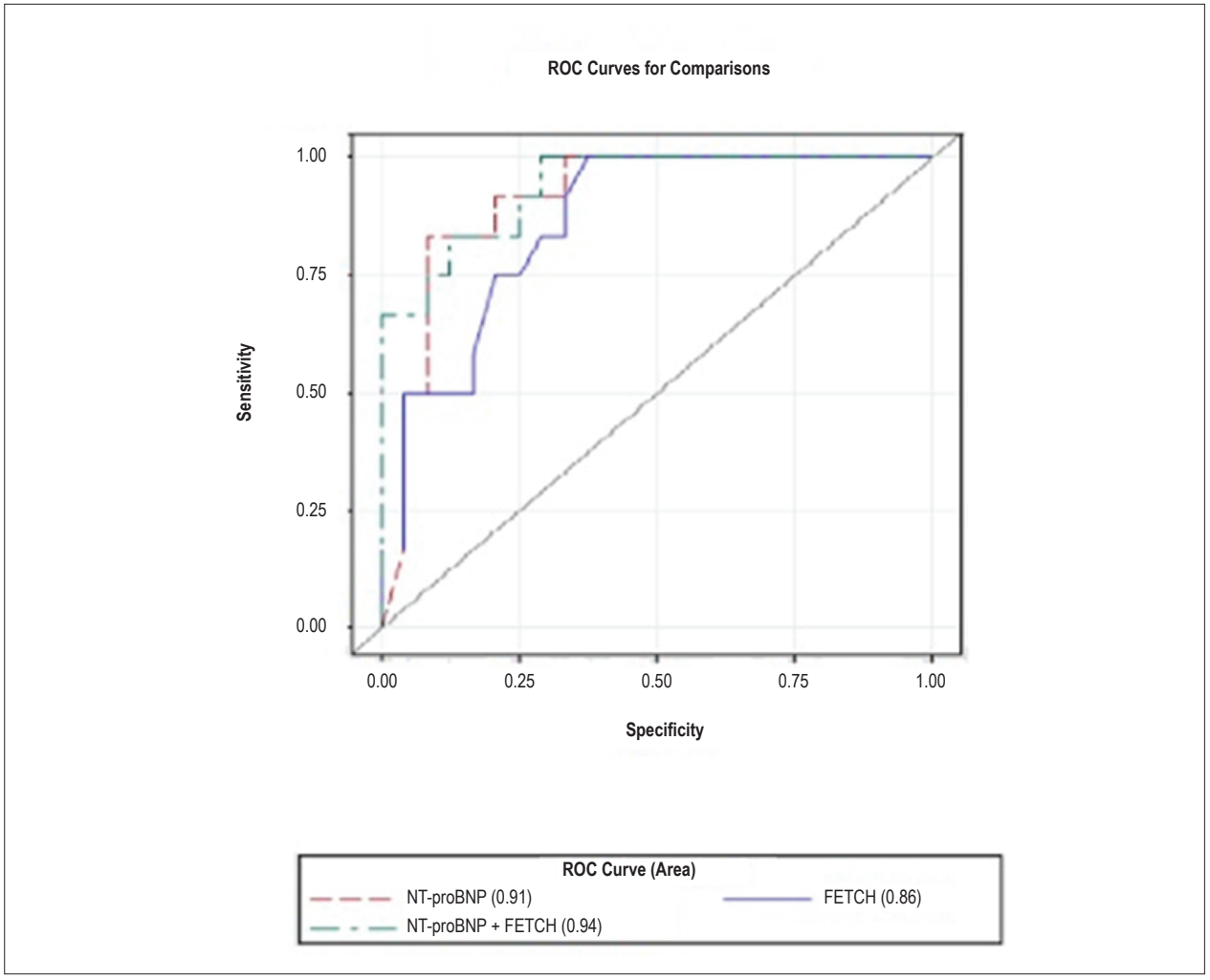

Figure 1 – *Receiver operating characteristic (ROC) curves for the comparison of the Functional Evaluation of Cardiac Health (FETCH) score and NT-proBNP levels.*

score, LVIDd/Ao, LVIDs/Ao, and LA/Ao. Additionally, most nonsurvival dogs were male (91.7%) and had functional classes III-IV (75.0%).

On multivariable logistic analysis, the variables independently associated with death were NT-proBNP (OR = 2.29, 95% confidence interval [95%CI] 1.24 – 4.2, p = 0.008) and quality of life score (OR = 1.22, 95%CI 1.02 – 1.45, p = 0.027).

The area under the curve, sensitivity, and specificity (obtained from ROC curves) of the univariate models associating NT-proBNP (cutoff = 1850 pmol/L) and the quality of life score (cutoff = 17) with death were 0.91 (95%CI 0.77 – 0.98, standard error [SE] = 0.05, p < 0.0001), 0.83 and 0.88, respectively, and 0.86 (95%CI 0.70 – 0.95, SE = 0.06, p < 0.0001), 0.75 and 0.79, respectively.

Finally, ROC curves were developed for the multivariable model with NT-proBNP and the quality of life score as predictors (Figure 1).

Discussion

The dogs enrolled in this study had mainly MR, LA enlargement, and preserved renal function. According to our results, the quality of life scores correlated with the functional classification and NE concentrations, while NT-proBNP values correlated with the quality of life score, NE concentrations, and certain echocardiographic findings. On multivariable regression analysis, NT-proBNP concentrations and quality of life score emerged as independent predictors of death after a follow-up period of 6 months. We were also able to calculate the NT-proBNP levels and the quality of life score cutoff values that best predicted mortality.

The association between quality of life scores and NE values with the severity of mitral valve disease has been previously described in the veterinary literature.[3,10] The positive correlation between these variables suggests that dogs with mitral valve disease that develop heart failure and experience increased sympathetic activity have a decreased quality of life.

The correlation between natriuretic peptide levels and the echocardiographic variables LVIDd/Ao, LVIDs/Ao, LA/Ao, and FS observed in this study have been previously reported by other investigators,[20-22] confirming that this peptide is a marker of cardiac remodeling and left ventricular dysfunction in dogs with mitral valve disease.

Furthermore, animals with higher concentrations of NT-proBNP or a higher quality of life score had a higher risk of death. The prognostic value of NT-proBNP has been discussed by other investigators. Chetboul et al.[20] demonstrated the ability of NT-proBNP to predict the transition from asymptomatic mitral insufficiency to a symptomatic disease in dogs. In a prospective study of dogs with symptomatic mitral valve disease over a 6-month follow-up period, Serres et al.[21] demonstrated that NT-proBNP was a good predictor of survival.

Questionnaires assessing the health-related quality of life of dogs have been validated for a variety of diseases, including cardiac disease, diabetes, neuropathic pain, and skin diseases.[10,23-25] The questionnaire used in the present study has been already validated in dogs with heart failure.[10] All studies recommend using the owner-perceived quality of life score for disease management.

In the multivariable regression model, both NT-proBNP concentrations and quality of life score were equally significant and independent predictors of mortality. Still, our most interesting finding was the quality of life score as a predictor of risk of mortality. This result is encouraging, as this questionnaire is easy to apply and does not require any technology, only a veterinarian and an observant owner.

One limitation of our study was the small sample size, which may limit the validity of the results. Another limitation was that the dogs were at different stages of the disease, as shown by their different functional classification. Finally, it is possible that the owner-reported data may have introduced subjectivity into the evaluation.

Conclusion

The quality of life score was an independent predictor of cardiac death in dogs with DMVD.

Acknowledgements

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Author contributions

Conception and design of the research:Strunz CMC; Acquisition of data:Marcondes-Santos M, Fragata FS; Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content:Strunz CMC, Marcondes-Santos M, Takada JY, Fragata FS, Mansur AP; Statistical analysis: Takada JY, Mansur AP; Obtaining financing:Strunz CMC, Marcondes-Santos M; Writing of the manuscript:Strunz CMC.

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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Myosin-binding Protein C Compound Heterozygous Variant Effect on the Phenotypic Expression of Hypertrophic Cardiomyopathy

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Abstract

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant genetic disease caused by mutations in genes encoding sarcomere proteins. It is the major cause of sudden cardiac death in young high-level athletes. Studies have demonstrated a poorer prognosis when associated with specific mutations. The association between HCM genotype and phenotype has been the subject of several studies since the discovery of the genetic nature of the disease.

This study shows the effect of a *MYBPC3* compound variant on the phenotypic HCM expression.

A family in which a young man had a clinical diagnosis of HCM underwent clinical and genetic investigations. The coding regions of the *MYH7*, *MYBPC3* and *TNNT2* genes were sequenced and analyzed.

The proband present a malignant manifestation of the disease, and is the only one to express HCM in his family. The genetic analysis through direct sequencing of the three main genes related to this disease identified a compound heterozygous variant (p.E542Q and p.D610H) in *MYBPC3*. A family analysis indicated that the p.E542Q and p.D610H alleles have paternal and maternal origin, respectively. No family member carrier of one of the variant alleles manifested clinical signs of HCM.

We suggest that the *MYBPC3*-biallelic heterozygous expression of p.E542Q and p.D610H may cause the severe disease phenotype seen in the proband.

Introduction

Hypertrophic cardiomyopathy (HCM) is a genetic myocardial disorder characterized by ventricular hypertrophy (VH), which is frequently asymmetrical in the interventricular septum and can lead to a dynamic obstruction of the left ventricle (LV) outflow tract.[1] It is the main cause of sudden cardiac death (SCD) in young people, with a 2-4% annual mortality rate in adults and 6% in adolescents and children.[2] A benign outcome of HCM may also occur, such as late onset, mild hypertrophy, and a history of non-malignant events.[3] Modifier genes, environmental influences, genetic variant diversity and the effect of multiple variants could explain the great clinical heterogeneity between individuals of the same family or from different families.[4]

HCM is a relatively common (0.2%) Mendelian disorder, caused mainly by mutations in sarcomere protein genes, most commonly those encoding β-myosin heavy chain (*MYH7*), myosin-binding protein C (*MYBPC3*) and troponin T (*TNNT2*).[5] Recent studies suggest that this prevalence is even higher, around 1:200, in the general population,[6] and around 5% of those who have HCM carry more than one disease-causing gene variant.[7-9] The hypothesis of gene dosage effects in patients with multiple variants is supported by some authors who have reported a more severe clinical feature, with greater risk of SCD, major LV hypertrophy, and earlier onset of HCM.[7,10]

In this context, we present a case herein in which a compound heterozygous variant led to a HCM manifestation with disease phenotype magnification.

Methods

Subjects

The proband with clinical HCM diagnosis was referred to genetic analysis at the National Cardiology Institute (Instituto Nacional de Cardiologia - INC) in Rio de Janeiro. A genealogical tree, including the highest possible number of generations, was built based on his family history. Family members were submitted to clinical assessments and genetic investigations. The local ethics committee approved this study. Written informed consent was obtained for every analyzed family member.

Clinical assessment

The proband underwent clinical and cardiovascular examination, including a 12-lead electrocardiogram (ECG), transthoracic echocardiography (TTE) and 24-hour Holter monitoring. Diagnosis of HCM was based on TTE: major echo diagnostic criteria were defined by a maximal LV end-diastolic wall thickness ≥ 15 mm. The same clinical examination was performed for the phenotypic analyses of all family members, and cardiac magnetic resonance imaging (CMR) was requested as a complementary exam.

A risk score proposed by the European Cardiac Society (ESC) was used to predict the risk for SCD in five years for patients with HCM.[11]

Keywords

Hypertrophic cardiomyopathy, sarcomere genes, compound variant, MYBPC3 gene

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Genetic analysis

Sanger sequencing

The genetic analysis of the proband was performed through direct sequencing of the three sarcomere genes: *MYH7*, *MYBPC3* and *TNNT2*. Genomic DNA obtained from leukocytes according to Miller et al.[12] was submitted to a polymerase chain reaction (PCR) of all coding exons, using previously described primers and others designed by us (Tables 1, 2 and 3), and the same amplification program. PCR products were cleaned-up with EXOSAP-IT (Affymetrix, Santa Clara, CA), subjected to the sequencing reaction using the BigDye® Terminator v3.1 reagent (Thermo Fisher Scientific, Waltham, MA) and subsequently analyzed on a ABI 3500xL genetic analyzer (Thermo Fisher Scientific, Waltham, MA). Sequence analyses were performed using the Geneious® v.6.1.6 software package (Biomatters, Auckland, NZ). The family was submitted to a mutation-specific screening according to the HRS/EHRA expert consensus statement.[13]

Variant pathogenicity prediction

Effects of missense mutations were predicted by using the PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT/PROVEAN (http://SIFT.jcvi.org/) and PredictProtein (http://predictprotein.org/home) tools. A5YM48 and Q14896 were used as *MYBPC3* reference sequences (UniProtKB).

Results

A seventeen-year-old (y) male proband presenting with a clinical manifestation of HCM and syncope history was submitted to a cardioverter-defibrillator implantation for syncope primary prevention. The diagnosis was based on TTE and showed a reverse curve asymmetric septal hypertrophy, with 39-mm thickness with preserved LV systolic function and normal LV ejection fraction (Figure 1). Additionally, diastolic type II dysfunction, maximum gradient LV/Aorta of 25 mmHg, systolic anterior motion of the mitral valve, obstruction of the LV outflow tract, and enlarged left atrium (46 mm) were also present. The ECG showed LV and LA overload and 24-hour Holter monitoring failed to document the presence of ventricular tachycardia. The risk of SCD was considered high, at 7.69%. The genetic analysis identified a compound heterozygous missense variant, c.1624G>C (p.E542Q) and c.1828G>C (p.D610H) in *MYBPC3* (Figure 2). The variant p.E542Q (rs121909374) has been associated with HCM in ClinVar and in the Human Gene Mutation Database (HGMD). The *in silico* analysis performed by PolyPhen-2 predicts this variant as possibly harmful, while SIFT/PROVEAN and PredictProtein classify this mutation as tolerable. On the other hand, p.D610H (rs371564200) is classified as a variant of uncertain significance (VUS), although pathogenicity prediction tools rank p.D610H as probably deleterious/harmful. Both variants affect conserved residues in the polypeptide chain (Figure 2).

The proband is the only member that manifests the HCM phenotype in his family. His father was adopted, so only maternal ascendants are known. The constructed heredogram revealed 30 relatives, over five generations, in which only one unexplained death of a 30-year-old female with no HCM diagnosis was detected (Figure 2).[14]

Genotyping of maternal family members - grandmother (59y), aunt (29y), uncle (35y) and mother (39y) - detected the p.D610H variant. All family members were asymptomatic, with normal TTE and ECG, with no evidence of VH. On the other hand, the allele p.E542Q was detected in the father (40y) and a paternal sibling (8y), both with normal clinical assessment results (Table 4). CMR was performed in the mother, aunt, and father, and resulted in normal findings, specifically normal LV wall thickness and no signs of fibrosis (Figure 1).

Discussion

The present study reports on a young individual with severe HCM who carries a compound *trans*-heterozygous variant in the *MYBPC3* gene, with one allele - p.D610H - inherited from the mother and the other - p.E542Q - inherited from the father.

Individuals with a single variant did not show any HCM phenotype. The p.E542Q variant, found in the paternal relatives, is associated to HCM, with good prognosis and moderate wall hypertrophy, although only a few studies mentioning this mutation are available[10,15-17]. Pathogenicity prediction of p.E542Q is in agreement with literature data[18-21].

Moreover, the p.D610H variant, identified in the maternal relatives, also did not manifest any HCM phenotype, even in the oldest investigated familiar member (59y). The association between p.D610H and HCM remains uncertain, despite the fact that pathogenicity predicting tools classified this as probably pathogenic. Only a single study in the literature has identified this mutation, although it did not correlate it with the disease[22].

In general, a single HCM-heterozygous mutation is sufficient to affect myocardial function and lead to hypertrophy; however, early studies have associated variants in the *MYBPC3* gene with incomplete penetrance, mild VH, low SCD risk and benign clinical evolution[23-25].

In conclusion, it is suggested that, individually, the p.E542Q and p.D610H variants generate mild changes in protein structure/function, insufficient to cause a strong phenotype. However, the expression of these variants in *trans* may be responsible for early disease onset, a more severe clinical phenotype and increased risk of malignant events in the proband. In other words, double or compound variants by themselves are not decisive for a poorer HCM prognosis, but the allelic composition of these variants may be determinant in this regard.

Study limitations

The present study investigated the three major HCM-genes that account for approximately 60-70% of HCM cases[5,14]. However, several other genes have already been associated to this disease[5,14], which are yet to be investigated.

Author contributions

Conception and design of the research: Cruz Filho FES, Dias GM; Acquisition of data: Rafael JF, Gottlieb I, Cazelli JG, Siciliano AP, Dias GM; Analysis and interpretation of the data: Rafael JF, Cruz Filho FES, Gottlieb I, Dias GM; Obtaining funding: Dias GM; Writing of the manuscript: Rafael JF, Dias GM; Critical revision of the manuscript for intellectual content: Cruz Filho FES, Carvalho ACC, Dias GM.

Table 1 – Primers for MYH7 sequencing

Exon	Forward Primer 5'-3'	Reverse Primer 5'-3'	Amplicon†	A.T.‡
3	TCTTGACTCTTGAGCATGGTGCTA	TCTGTCCACCCAGGTGTACAGGTG	381 bp	62ºC
4	AGGAAGGAGGGAAAGCCCAGGCTG	TCTGCATGCACTCAATCTGAGTAA	380 bp	62ºC
5	ATCTTTCTCTAACTCCCAAAATCA	ACTCACGTGATCAGGATGGACTGG	398 bp	60ºC
6	TGTCACCGTCAACCCTTACAAGTG	GAGGCTGAGTCTATGCCTCGGGG	394 bp	62ºC
7	CTTGCTGGTCTCCAGTAGTATTGT	CTGCGGTACAGGACCTTGGAGGGC	198 bp	62ºC
8	GCCCTCCAAGGTCCTGTACCGCAG	GTCCAAGTCCCAAGGCCAAGGTCA	200 bp	62ºC
9	GACAACTCCTCCCGCTTCGTG	AACAGAGGGAGGGAGGGGAGAG	281 bp	62ºC
10	CCTTTTGCTTGCTACATTTATCAT	GCCACAAGCAGAGGGGACCAG	252 bp	60ºC
11	CTGCTTCCTCAGGCCATGTGCTGT	ACCAATGGCCAGAGTCTTAGCTCT	284 bp	62ºC
12	CACAGGGATTAAGGAGACAAGTTT	TTACAGCTGCCCCAAGAATC	273 bp	58ºC
13	AGTCATCTCTTTACCAACTTTGCTA	ATTATCATCTGAAGATGGACCCACC	186 bp	62ºC
14	CAAGTTCACTCTTCCCAACAACCCT	ATGTGGGAGCGAGTGAGTGATTGTT	258 bp	62ºC
15	ACTCACACCCACTTTCTGACTGCTC	GAATTCAGGTGGTAAGGCCAAAGAG	247 bp	62ºC
16	ATAACTGTACTCAGAGCTGAGCCTA	TCCATCCCACTGAGTCTGTAAACCT	578 bp	62ºC
17	GCAAATGCCAGCAAGGATGTAAAG	AGAGAAGGGAGATGGGAAGTAA	359 bp	58ºC
18	CATCTCTGTGACTTCTCGAATTCT	CACTGTGGTGGTAGGTAGGGAGAT	300 bp	60ºC
19	ACAAAGCCAGGATCAGAACCCAGA	GTCCAGAGTCACCCATGCTCTGCA	323 bp	62ºC
20	TGGGTATGAGGGTGCACCAGAGCT	GCATCAGAGGAGTCAATGGAAAAG	330 bp	62ºC
21	TAGGCTGTTACCCTTCCTAAGGTA	GCCTCTGACCCTGTGACTGCAGTG	374 bp	62ºC
22	GGACCTCAGGTAGGAAGGAGGCAG	TGTGCAGGGAGGTGCAGGGTTGTG	390 bp	62ºC
23	TCCTATTTGAGTGATGTGCCTCTC	ATGGTCTGAGAGTCCTGATGAGAC	390 bp	62ºC
24	AGATGGCACCAAGCTGGTGACCTT	TCTGGGCACAGATAGACATGGCAT	290 bp	62ºC
25	GGCAATCTCACAGTCCCCTAATAA	TTTTTGCCAGGGAGGACCATCTAA	508 bp	60ºC
26	ACTCTTTACCTGTATCATTACCAT	GCCTCCATGGACACATAATCAGTT	306 bp	60ºC
27a*	AGCCGAGAGCCTTTTAGAGCCG	GTCCCGCCGCATCTTCTGGA	274 bp	64ºC
27b*	TCCAGAAGATGCGGCGGGAC	AGGGGAGGTGGGAGGAGGAAGT	266 bp	64ºC
28	TCCCACTTCCCTTCCTCTGCCT	CAGCACTCCTCTCTATCCCCACCT	438 bp	56ºC
29	GGTGGGGATAGAGAGGAGTGCTGA	TGTGGCAGGGTTTGGGCTGT	315 bp	64ºC
30	GAGAAGGGCAAGGGTGGGGT	CCTGAGAGGAGAAGGAGGTGGG	422 bp	58ºC
31	TTGTCCCCATCCACACCCTCCA	GCTCCGACTGCGACTCCTCATACT	469 bp	56ºC
32	GCTGAAGAGTGAGCCTTGTCCC	TCCGCTGGAACCCAACTGCT	396 bp	56ºC
33	AGTATGAGGAGTCGCAGTCGGA	GGGGATGAGAACAGGGAGCCAA	500 bp	60ºC
34	CTGCCCTGTGCCCTGACTGT	CCAGCCTCGGTTCCCTTCACT	500 bp	64ºC
35	GTGAAGGGAACCGAGGCTGGC	GTTGGGCAGAGCAGGAAAAGCA	364 bp	62ºC
36	TCCGTGCCAACGACGACCTGAA	GTCCTCACACACTTGCTGCCCA	497 bp	60ºC
37	TGGGCAGCAAGTGTGTGAGGA	GGTTGTCACTGTGGCTATGGTGC	391 bp	62ºC
38 / 39	ACCTTCTATGACTGTGCCATCTTCAC	GTTTGAGGGTGCTCTGTCTGG	464 bp	62ºC
40	ATGCCCTGTCCCTGCCCAATAC	TTTCCACCTCCCCTATGCCAGACC	268 bp	60ºC

(*) Necessary more than one primer pair to cover the exon; (†) Size of the amplified fragment; (‡) Annealing temperature.

Table 2 – Primers for MYBPC3 sequencing

Exon	Forward Primer 5'-3'	Reverse Primer 5'-3'	Amplicon*	A.T.†
2	GACCTCAGCTCTCTGGAATTCATC	GCTCAGAGGCCACGTCCTCGTCAA	311 bp	62°C
3	GTGCACGCTCCAACCAG	CAGCAAAGGCAAGAAAGTGTG	429 bp	65°C
4	CTGGGACGGGGAGGAGAATGTG	GCTTTTGAGACCTGCCCTGGAC	385 bp	62°C
5	GGGCACCTGCGGTCCCAGCTAACT	ACGCGGGCTGAGAAGGTGATG	378 bp	62°C
6	CTACCCCTGGAGCCCCCGATGACC	TGCCTCCCAGATTCCCCACACC	449 bp	62°C
7	CTGGAGCTCCTGGTCTTATGTGAT	GGAGCCGTGACACCAAGATGATAA	528 bp	62°C
8	GCTTCTCAAACGGCCCCCTCTG	AGCTCCGCCCCGCAAATCATCC	213 bp	62°C
9	GGGCTGGGGATGATTTG	GGAGGGAGAAAGGGACACTA	226 bp	63°C
10	AATCTGGCTAGTGTCCCTTTCTCC	AGCCCTTTAACTCCTTCCACACTG	322 bp	62°C
11	TCGGCCCAACTGACTTA	CCCATGGGCCTTTACTT	389 bp	58°C
12	CGGCTCCCCACGGACAG	CCCAGGCCAGGCAGGACT	405 bp	67°C
13	TCCCCAGCCCCTCTTCA	GCCGGACTCCGCTCTTT	515 bp	62°C
14	GGCGGCACAGAGGGGATTG	ACCGGCAGGAGCAAAAGGATG	402 bp	62°C
15	ATCCGGCTGACCGTGGAACT	CAGTGCGCCCCGTGATAATC	375 bp	65°C
16	AACACTTCAACGGCCCCTTCTG	GCCCCCTCCTCCGATACTTCACAC	451 bp	62°C
17	CGGACGACGCAGCCTACCAGT	GTCAGCTCCACCCCGTCCTTCA	366 bp	62°C
18	GGAGGAGGGGGCGCAAGTCAAAT	GTCAAAGGCCCAAGGTCACAGAGG	400 bp	62°C
19	ACAGGCACACGTGTTTTCAC	CAGTCTCCACCTGTCCCATC	345 bp	61°C
20	AGAATACCAACAAGCCAGGACAAG	GCGGGAAAGTGAGCAGAACC	402 bp	62°C
21	TGCCTTTGCCCCCGTGCTACTTG	GCCCCAGGACCCCCACTTTTGAT	187 bp	62°C
22	TCCTCCTGGCTCTCCCGTTTCTCT	GCGCCCCTCTGCTGCTTCTTC	379 bp	62°C
23	GCTCCTCTGCTCCCTACTTCC	ATGGCCATCAGCACACTTCAC	310 bp	62°C
24	TCGGTGCCACAGAGATGATTTTGA	GGCTGCCCCTCTGTGTTCTCCA	367 bp	62°C
25	CCTGTGGCGGTTAGTTGG	CACCGGTAGCTCTTCTTCTTCTTG	350 bp	62°C
26	CCGAGGGAAGGTGGTGTGG	TCTGTAAAATGCGGCTGAGTATCC	404 bp	62°C
27	GGAAGTGCCCCCTATGT	TCGCACTGCTCAAAGAAG	457 bp	62°C
28	TCAGAGGAGTGGGCAGTGGGAGTG	CTGGGGTGTCAATGGCGGGTCTT	292 bp	62°C
29	GCCTGGAGTTGCTGTGTTAG	GGCTGCCCCTCTTTGGTC	467 bp	62°C
30	GCGGCCGGCCCTTGGAGT	TGGAAAATGTGAGCTGTGGGTTGG	356 bp	62°C
31	GCATTCAGGCACTTACCAGGTGACG	CACGGTGAGGACAGTGAAGGGTAGC	527 bp	60°C
32	GGCCGCAGCTACCCTTCAC	GGCCCCTCTCCCTGTTCC	392 bp	65°C
33	GGCCTCTCGGTACCAAGTCCTGTC	CAACGTCGGGGCCTGTGAGC	232 bp	65°C
34	GCAGGGCCATGGTACTCACTCTTG	CCGCCCGCTCTTCCCATCTC	404 bp	62°C
35	CACAGTGACATGGCCTCCTCTTCT	GCCCCTACAGCCTCCCATTTACT	159 bp	62°C

(*) Size of the amplified fragment; (†) Annealing temperature.

Potential Conflict of Interest

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

Sources of Funding

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Study Association

This article is part of the thesis of Master submitted by Julianny Freitas Rafael, from Instituto Nacional de Cardiologia.

Table 3 – Primers for TNNT2 sequencing

Exon	Forward Primer 5'-3'	Reverse Primer 5'-3'	Amplicon*	A.T.†
2	ACAGCTCATGAGGGGTGGAACTA	GTGCTCTGCCTGGGATCTACAACC	376 bp	65ºC
3 / 4	ATGAGAACGGCAGGCCAGGCTAGTG	GTTTGCCTCAAGACCCGAGCAACC	506 bp	65ºC
5	GTGGCGGGAGGTAGCCGACAGT	TGGGCAATCAATGGTTGAATCTTA	403 bp	65° C
6	TTGACCCAGCGCTTCTCTTGTGTC	ACTGGGTGCCACCAATGCAACTTC	449 bp	65° C
7	CCAGTGCCGGGAGGGACTCAC	CAGCCCGTGTCCACTGCACCATAC	262 bp	65° C
8	GGATCAGGGGCCCTGCCTGTCCTGACA	TCCTCCTCCTCTTTCTTCCTGTTCT	538 bp	62° C
9	GCCAGGCCCTGCCAGAGGTCTT	CCCTGGGGGAGGCCTGAAACAG	494 bp	70° C
10	ACGTCCGTGGAGCTGGTTGAAAGT	CCCGGCCAATATTGTCTCTTGACT	373 bp	62° C
11	TGGGAGCTACCCTCTCAGAA	CACAGCAGCTGGGAATCTCT	369 bp	60° C
12	GTAAACCCGGCTGACTACAG	AGCCAGCCCAATCTCTTCAC	258 bp	62° C
13	CAGGGGGTTTGGGGAGGGTTAG	GTGGGGCACCTGCTCAGTTCTCT	402 bp	60° C
14	GGAGGGCCCTTTCTTACTGGAC	CCGGACCCAGTGAACCAGGAGGAG	207 bp	68° C
15	GCCCCTCCTGACCCTTAACTATCC	CGGAGGAGCCAGAGAAGGAAACCT	353 bp	62° C
16	GGGGGTGAAATGTGGGGCGGAGAA	GTGTGGGGGCAGGCAGGAGTGGTG	383 bp	62° C

(*) Size of the amplified fragment; (†) Annealing temperature.

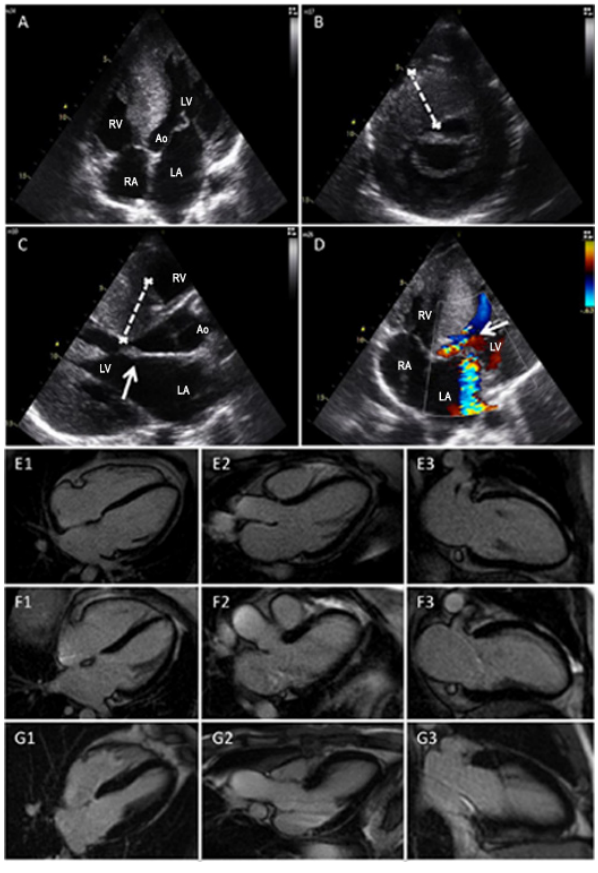

Figure 1 – *TTE of the proband and CMR of the family. A) TTE image of the four heart chambers and aorta revealing the reverse curve septal hypertrophy. B) Parasternal short-axis view showing the septal hypertrophy. C) Parasternal long-axis view displaying the LV and septal hypertrophy and the enlarged left atrium. The white arrow shows the systolic anterior motion of the mitral valve. D) TTE image showing the obstruction and the turbulence in the outflow tract of the left ventricle (white arrow). Mild mitral regurgitation in the left atrium is visible. CMR of the proband's father (E), aunt (F) and mother (G), showing no hypertrophy or fibrosis signs. CMR in the inversion-recovery sequence (delayed enhancement) in 4CH axes (E1, F1, G1), LVSV (E2, F2, G2) and 2CH (E3, F3, G3). RA: right atrium; RV: right ventricle; LA: left atrium; LV: left ventricle; Ao: aorta.*

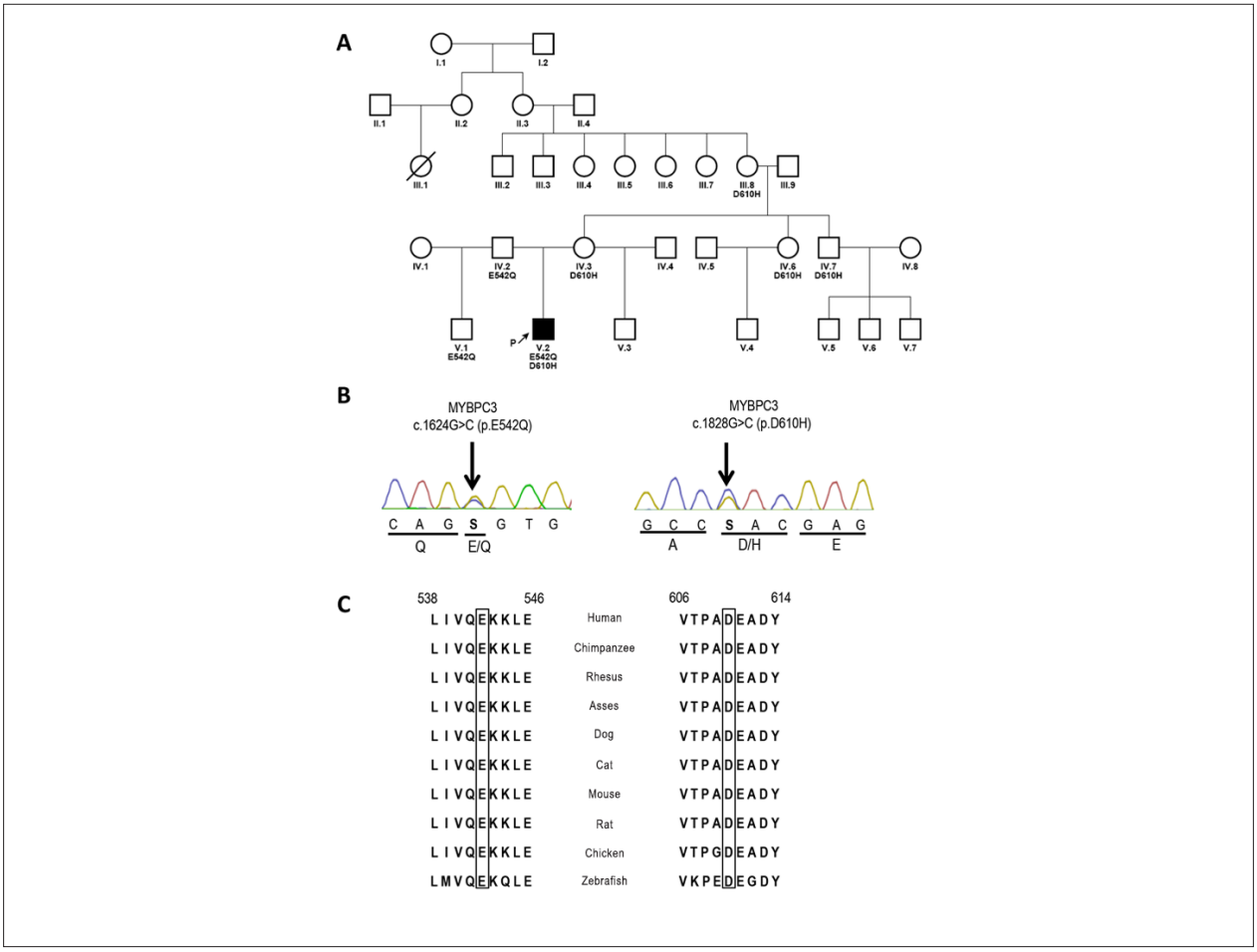

Figure 2 – *A) Pedigree showing five generations of the maternal family. The proband is the only HCM-affected member. The family variant allele carriers are indicated by E542Q+ and D610H+. B) Electropherograms of the compound missense variant regions of the MYBPC3 gene of the proband. C) Multiple species alignment of the myosin-binding protein C amino acid sequence for residues 538 to 546 and 606 to 614. The conserved residues, glutamic acid and aspartic acid, are indicated by a rectangle.*

Table 4 – Clinical assessment data of the individuals

	Epidemiology				ECG						TTE					
ID	Age (Y)	Sex	HCM	Variant	LAO	LVO	ABN T wave	LVH +	LVH type	Form	Max LVWT (mm)	LVOG mmHg	LVSD	LVDD	SAM	LA size (mm)
III.8	59	F	No	D610H	No	No	No	No	-	-	10	No	No	No	No	28
IV.2	40	M	No	E542Q	No	No	No	No	-	-	9	No	No	No	No	35
IV.3	39	F	No	D610H	No	No	No	No	-	-	9	No	No	No	No	37
IV.6	29	F	No	D610H	No	No	No	No	-	-	8	No	No	No	No	32
IV.7	35	M	No	D610H	No	No	No	No	-	-	8	No	No	No	No	36
V.1	8	M	No	E542Q	No	No	No	No	-	-	7	No	No	No	No	37
V.2	17	M	Yes	D610H E542Q	Yes	Yes	Yes	Yes	Septal	Reverse Curve	39	25	No	Type I	No	46

The identification numbering (ID) of individuals follows the standard adopted in the pedigree charts (Figure 2); ECG: electrocardiography; TTE: Transthoracic echocardiography; (Y): years; HCM: hypertrophic cardiomyopathy; LAO: left atrial overload; LVO: left ventricular overload; ABN T wave: abnormal T wave; LVH + : left ventricular hypertrophy showed by echo; LVH type: type of the left ventricular hypertrophy; Max LVWT: maximal thickness of the left ventricular wall; LVOG: left ventricular outflow gradient; LVSD: left ventricular systolic dysfunction; LVDD: left ventricular diastolic dysfunction; SAM: systolic anterior motion; LA size: left atrial size.

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Association between Obstructive Sleep Apnea and Myocardial Infarction: A Systematic Review

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Abstract

Obstructive sleep apnea (OSA) has been associated to cardiovascular risk factors. However, the association between OSA and cardiovascular disease is still controversial. The objective of the present study was to verify the association between OSA and myocardial infarction (MI). This is a systematic review of the literature performed through electronic data sources MEDLINE/PubMed, PubMed Central, Web of Science and BVS -*Biblioteca Virtual em Saúde* (Virtual Health Library). The descriptors used were: 'obstructive sleep apnea' AND 'polysomnography' AND 'myocardial infarction' AND 'adults NOT 'treatment.' The present work analysed three prospective studies, selected from 142 articles. The studies followed a total sample of 5,067 OSA patients, mostly composed by male participants. All patients underwent night polysomnography, and all studies found an association between OSA and fatal and non-fatal cardiovascular outcomes. Thus, we were able to observe that 644 (12.7%) of the 5,067 patients suffered MI or stroke, or required a revascularization procedure, and 25.6% of these cardiovascular events were fatal. MI was responsible for 29.5% of all 644 analysed outcomes. There is an association between OSA and MI, in male patients, and apnea and hypopnea index (AHI) are the most reliable markers.

Introduction

Studies have demonstrated the association between obstructive sleep apnea (OSA) and myocardial infarction (MI).[1-5] Up to 65% of patients who seek medical attention for a cardiovascular event are diagnosed with OSA.[1] There is a need to study OSA's ability to predict cardiovascular events; some cohort studies, following apneic patients, have identified a high number of fatal and non-fatal cardiovascular outcomes.[2-8] However, this association is still controversial.[1] Results suggest that intermittent hypoxia could work as a protection factor for ischemic events, a phenomenon that has been observed in apneic individuals who develop a cardiac lesion that is less severe than those of patients with no OSA after a MI.[5] Considering the prevalence of OSA, as well as the importance of cardiovascular diseases, the objective of the present systematic review of the literature was to verify the association between OSA and MI.

Keywords

Sleep Apnea, Obstructive; Myocardial Infarction; Review; Adults; Polysomnography / methods; Sleep Wake Disorders.

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Methods

Study design and research strategy

This is a systematic review of literature and thus approval by a Research and Ethics Committee was not required. The search was performed in the electronic data sources MEDLINE/PubMed, PubMed Central® (PMC), Web of Science and BVS, through a combination of descriptors, including the terms of Medical Subject Heading (MeSH) and Health Sciences Descriptors (DeCS). The descriptors chosen to be used together were: "obstructive sleep apnea" AND "polysomnography" AND "myocardial infarction" AND "adults" NOT "treatment".

The search for descriptors on MEDLINE/PubMed yielded: *((("obstructive sleep apnea"[All Fields]* OR *"sleep apnea, obstructive"[MeSH Terms]* OR *("sleep"[All Fields]* AND *"apnea"[All Fields]* AND *"obstructive"[All Fields]) OR "obstructive sleep apnea"[All Fields]* OR *("obstructive"[All Fields]* AND *"sleep"[All Fields]* AND *"apnea"[All Fields]))* AND *("polysomnography"[MeSH Terms] OR "polysomnography"[All Fields]))* AND *("myocardial infarction"[MeSH Terms]* OR *("myocardial"[All Fields]* AND *"infarction"[All Fields])* OR *"myocardial infarction"[All Fields]))* AND *("adult"[MeSH Terms] OR "adult"[All Fields]* OR *"adults"[All Fields]))*

After that, we did a manual search through selected articles.

Inclusion and exclusion criteria

We included all cohort works found in the databases, with humans over 18 years of age, published in the last 10 years, in Portuguese, English and Spanish, with OSA diagnosis through polysomnography, with MI as one of the analysed outcomes. We excluded those in which 100% of the patients were under treatment for sleep disturbances (SD), all groups of treated apneic individuals, pregnant patients, those with other SDs, neurological or psychiatric diseases, and studies in which 100% of the population had previous coronary artery disease (CAD). We also excluded works whose population was approached in more than one study and that also had similar outcomes. In those cases, we considered the first work.

Identification and selection of the studies

Two independent researchers read the titles and abstracts of each pre-selected work, separately identifying articles that met the inclusion and exclusion criteria. After this stage, each researcher read the complete articles that respected the criteria exposed in the abstract and selected only those compatible to

the systematic review criteria. When there was doubt, a third researchers would be called in, but there was no disagreement between the first two researchers in this study.

Data extraction

Two researchers were responsible for the data collection. Characteristics extracted from the studies were: title, authors, year of publication, science journal where it was published, publication medium, key-words, geographical origin, study design, sample size, supervision, financing, methods, research time, OSA diagnosis criterion, other results of the research, and conclusions. Moreover, participants' characteristics of each work were registered: number, gender, age, use of medication, comorbidities, number of patients who suffered a MI, and who received an OSA diagnosis, as well as the apnea and hypopnea index (AHI).

Evaluation of methodological quality of selected articles

Two researchers read the articles, and each of them filled out a check list based on Strengthening the Reporting of Observational Studies in Epidemiology (STROBE).[9] The selected articles were evaluated as having fulfilled each item completely or partially, or not at all. Articles considered as having acceptable quality were those that satisfactorily contemplated at least 11 aspects. In case of a disagreement between the two researchers, a third researcher would have been called in to assess the article, but that was not necessary. This systematic review also followed the recommendations of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[10] and the step-by-step suggested by Cochrane Handbook,[11] produced by The Cochrane Collaboration.

Results

Study identification and selection

The present systematic review gathered 76 articles through the search strategy outlined in electronic databases. Four of these articles were repeated in more than one source, as were two articles of the 66 found in the manual selection. Thus, from the 148 articles found, we counted 142 (Figure 1).

Methodological evaluation of the studies

After complete reading of the selected works, we observed that all the articles satisfactorily fulfilled at least 16 aspects of the check-list.[9] In the study by Buchner et al.[8], 72.7% of the aspects were satisfactorily fulfilled; the one by Marin et al.[6] were at 77.3%, and Gottlieb et al. reached the highest percentage (95.4%).

Characteristics of the selected studies

The objective of the work by Gottlieb et al.,[6] The Sleep Heart Health Study (SHHS), was to evaluate the relation between OSA, CAD incidence, and heart failure in a sample of the general community of men and women. The study included patients at 40 years of age or older, recruited among participants of population base studies about cardiovascular and pulmonary diseases, including Atherosclerosis Risk in Communities Study, Cardiovascular Health Study, Framingham Heart Study, Strong Heart Study, Tucson Health and Environment Study, and The New York University-Cornell Worksite and Hypertension Study. We excluded individuals whose polysomnography was inconclusive, those under treatment for OSA, with low quality data, previous CAD or heart failure, without follow-up data, or incomplete data on body mass index (BMI), smoking, blood pressure and use of medications that refused to participate in the study.

SHHS[6] analyzed a total sample of 4,422 patients, including 2,434 who had AHI < 5. We also observed that 5.5% of 3,794 patients were diagnosed with OSA five years after the beginning of the study, and of those, 2.1% referred treatment for OSA, but were excluded without significantly altering the results. In this study, 43.3% of the population was male. Among the patients from the sample AHI ≥ 5, the male population represented 55.23% of the sample.

The objective proposed by Marin et al.[7] was to compare the incidence of fatal and non-fatal cardiovascular events in simple snorers, untreated OSA patients, patients with continuous positive airway pressure (CPAP) and healthy men recruited among the general population. In this study, the sample included only men with OSA or simple snoring from sleep clinics, and a population base sample of healthy men matched by age and BMI with severe apneic patients, between January 1992 and December 1994. The healthy men were recruited from the database of Zaragoza Sleep Apnea Study.

Buchner et al.[8] prospectively investigated cardiovascular outcomes in treated versus non-treated OSA patients. For this study, recruitment included all patients with suspected sleep-related obstructive respiratory disturbances, admitted in a sleep clinic through non-selected referral by primary or secondary care physicians between 1993 and 1998. Snorers without apnea and patients with central sleep apnea, Cheyne-Stokes breathing, hypoventilation syndromes or periodic limb movement during sleep were excluded from the analysis. The patient sample in this study was predominantly male – 85.5% of a total of 449, and 83.5% were untreated apneic individuals.

Mean follow-up among the three studies was 8.2 ± 0.99 years. The number of patients varied between 449 and 4,422. Only Gottlieb et al.[6] evaluated OSA impact on the appearance of CAD and, thus, none of the participants received OSA treatment.

Comorbidities and risk factors common to the three studies were systemic arterial hypertension (SAH), diabetes mellitus (DM), dyslipidemia, and smoking. However, only DM and smoking were related in the same way by all three studies (number of DM patients and smokers). Treatment with insulin or oral antidiabetic drugs was mentioned only by Buchner et al.[8] Regarding SAH and dyslipidemia, Gottlieb et al.[6] reported only the number of patients on antihypertensives or lipid-lowering drugs, and Marin et al.[7] registered only the total number of SAH and dyslipidemia patients. On the other hand, Buchner et al.[8] reported the initial number of SAH patients from those who started follow-up with antihypertensives, and the number of patients, at the end of follow-up, under treatment for this pathology; the same happened for cases of dyslipidemia.

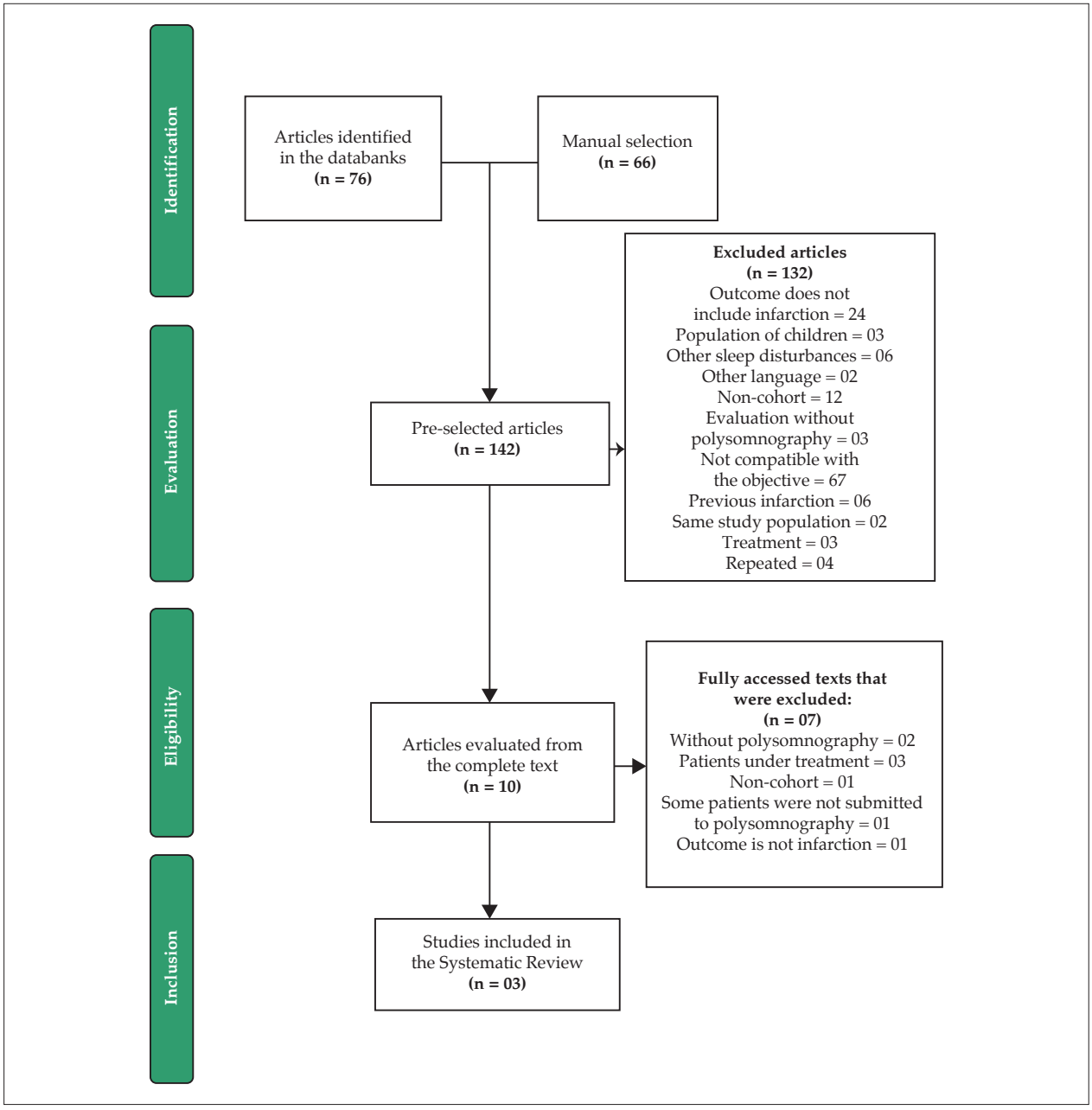

Figure 1 – *Flowchart of the study selection process.*

The sample of 5,067 patients analyzed in this systematic review must be considered in their isolated groups, considering there are important differences between the participants of each study regarding comorbidities. This situation must be illustrated with proportions, in the studies by Gottlieb et al.,[6] Marin et al.,[7] and Buchner et al.,[8] for the number of patients with AHI ≥ 5 and SAH or, in the case of SHHA,[6] the use of antihypertensives (39.2% vs 28.5% vs 69.4%)/ dyslipidemia or, in the case of SHHS,[6] the use of hypolipidemic drugs (7.5% vs 10.1% vs 57.6%). Gottlieb et al.[6] reported the percentage of patients who were on antihypertensives and hypolipidemic drugs, and, therefore, patients with untreated dyslipidemia or SAH were not registered. Marin et al.[7] did not report the number of patients under treatment (Table 1).

Clinical characteristics that compose the profile of groups common to all three studies are: participants' ages, BMI, number of DM patients, number of smokers and AHI, but no statistically significant conclusion was drawn on smoking (Tables 2-4).

Incidence of myocardial infarction

Gottlieb et al.[6] evaluated 4,433 individuals, of which 473 CAD cases were recorded – 76 deaths from CAD, 185 MI, 212

Table 1 – General characteristics of selected studies

Authors	Country Year	Journal	N initial	N AHI < 5	N AHI > 5	N Treated apneic	N Non-treated apneic	*Follow-up* Mean in years	% Male	Reported comorbidities
Gottlieb et al.[6]	USA. 2010	Circulation	4.422	2.434	1.988	79	1.988	8.7	43.5%	SAH. DM. Dyslipidemia. Smoking
Marin et al.[7]	Spain. 2005	The Lancet	1.651	264	1.010	372	638	10	100%	SAH. DM. Dyslipidemia. Cardiovascular Disease. Smoking. Alcoholism
Buchner et al.[8]	Germany 2007	American Journal of Respiratory and Critical care Medicine	449	0	449	364	85	6	85.5%	SAH. DM. Dyslipidemia. Coronary Disease. Peripheral Artery Disease. STROKE. Neoplastic Disease. COPD and Smoking

OSA: obstructive sleep apnea; AHI: apnea and hypopnea index; SAH: systemic arterial hypertension; DM: diabetes mellitus; COPD: chronic obstructive pulmonary disease.

Table 2 – Age of patients from studied samples according to apnea and hypopnea index

Authors	Median age (interquartile range); Mean ± Standard Deviation				p
	AHI < 5		AHI ≥ 5		
	< 5	5 a < 15	≥ 15 a ≤ 30	>30	
Gottlieb et al.[6] Men	61(54.7)	64(57.7)	64(57.7)	65(58.7)	NI
Gottlieb et al.[6] Women	60(50.7)	66(58.7)	66(58.7)	65(58.7)	NI
Marin et al.[7]	49.6 ± 8.1	50.3 ± 8.1		49.9 ± 7.2	NI
Buchner et al.[8]	NA	57.8 ± 10.2			NS

NS: non-significant; NI: not informed; NA: non-applicable; AHI: apnea and hypopnea index.

revascularization procedures – with an incidence of 20.1 events per 1000 person-years among men, while in women, this rate was 8.7 events per 1000 person-years. These data showed the increase, in men, of the incidence rate of revascularization according to the severity of OSA, whereas, in women, these values were less evident.

In the population of 403 men with mild to moderate OSA from the study by Marin et al.,[7] by associating the event rate to the severity of OSA, they observed 36 non-fatal cardiac events, with the incidence rate of events at 8.9 events per 1000 person-years, as well as 22 deaths from cardiovascular causes with a rate of 5.5 events per 1000 person-years. Among the 235 men with severe OSA, 50 non-fatal cardiovascular events were registered with an incidence rate of 21.3 events per 1000 person-years, and 25 deaths from cardiovascular causes at a rate of 10.6 events per 1000 person-years. This study did not differentiate the equivalent data of the different outcomes: fatal or non-fatal MI, fatal or non-fatal stroke, and acute coronary insufficiency requiring revascularization surgery or percutaneous transluminal coronary angiography, or both.

Buchner et al.,[8] evaluating 85 patients, concluded that 28.3% had the following outcome: five MIs, 25 revascularization procedures, five strokes, and three deaths from cardiovascular causes. Of these patients, 20 (23.5%) were diagnosed with mild-moderate OSA.[8]

Discussion

The present systematic literature review analysed three prospective works that followed a total sample of 5,067 patients, between men and women, of which 53.5% had different degrees of untreated OSA diagnosed by polysomnography. All the studies found an association between OSA and fatal and non-fatal cardiovascular outcomes. It was observed that 644 (12.7%) of the 5,067 patients suffered MI or stroke or required a revascularization procedure, and 25.6% of these events were fatal. This is a relevant number considering the main cause of death and disability, in Brazil and in the world, is constituted by cardiovascular diseases.[12]

According to the American Heart Association[13] (AHA), one in every seven deaths in the USA are caused by cardiac

Table 3 – Body mass index of patients from studied samples according to apnea and hypopnea index

Authors		BMI Kg/m² Median (interquartile range) ; Mean ± Standard Deviation				p
		AHI < 5	AHI ≥ 5			
		< 5	5 a < 15	≥ 15 a ≤ 30	> 30	
Gottlieb et al.[6]	Men	27.0(24.6. 29.3)	28.8(26.2. 31.4)	29.7(26.9. 33.5)	31.3(27.9. 34.9)	NI
	Women	26.3 (23.6. 29.8)	29.9(26.1. 34.1)	32.5(27.3. 36.9)	34.3(29.1.39.6)	NI
Marin et al.[7]		29.8 ± 4.4	27.5 ± 4.4*		30.3 ± 4.2	< 0.0001*
Buchner et al.[8]		NA	29.3 ± 5.4			0.003

NS: non-significant; NI: not informed; NA: non-applicable; AHI: apnea and hypopnea index; BMI: body mass index; (*) p < 0.0001 vs men with AHI < 5.

Table 4 – Number of patients with diabetes mellitus in the studied samples according to apnea and hypopnea index

Authors		Diabetes per group N(%)				p
		AHI < 5	AHI ≥ 5			
		< 5	5 a < 15	≥ 15 a ≤ 30	> 30	
Gottlieb et al.[6]	Men	73 (8.8)	77(12.0)	39(13.8)	29(16.9)	NI
	Women	123 (7.7)	82 (13.4)	33 (16.8)	14 (16.7)	NI
Marin et al.[7]		(6.1)	(8.5)		(9.9)	NI
Buchner et al.[8]		NA	13 (15.2)			NS

NS: non-significant; NI: not informed; NA: non-applicable; AHI: apnea and hypopnea index.

diseases – every 34 seconds, an American has a coronary event, and every minute and 24 seconds, there is a death from MI.[13] Thus, the data of the present study are in agreement with the current literature, since 190 MI cases were counted in the analyzed group (3.75%). However, this final number may be even higher, since we did not consider the percentage of the group studied by Marin et al.,[7] because there was no information on how many patients had MIs. These authors only reported that the type and frequency of the different outcomes did not differ between the studied groups.[7]

Some studies demonstrated an association between MI and OSA. [2,4,14-18] Shah et al.[4] concluded that OSA increases the risk of MI, revascularization procedures and cardiovascular death, regardless of risk factors, such as SAH, in patients over 50 years of age. However, this cohort did not exclude patients who received treatment for OSA during 2.9 years of follow-up.[4] With base on evidence that treatment with CPAP decreases the risk of fatal and non-fatal cardiovascular events, [1,14,19] Shah et al.[4] said that the study design did not allow them to work with adhesion to treatment and/or treatment effects, and such finding would polarize the results to zero. Nevertheless, in this study, 86 patients (6.1%) had some of the outcomes – 74 had OSA and, of those, 21 had MI, as well as 33 cases registered as cardiovascular death.[4]

In contrast, Kendzerska et al.,[2] in a study whose objective was to determine if OSA independently increases the risk of coronary events, concluded that AHI was associated to composite cardiovascular outcome in a univariate analysis, but not in a multivariate analysis. The argument used to explain this finding was that, possibly, studies with large community bases may not include important predictors related to OSA, or may selectively relate subgroup analyses conclusions. Kendzerska et al.[2] considered the history referred by the patients, such as smoking, MI, myocardial revascularization surgery, stroke, SAH and/or pulmonary disease. With the justification that recovery by CPAP was not associated to the risk of an event, the patients who needed treatment were not excluded, and in the analysis of the untreated patients, in relation to the complete sample, all predictors remained significantly associated to the outcome, except for daytime drowsiness.[2]

Regarding the inclusion of cardiovascular risk factors, the only comorbidities equally studied by the authors of the works, in this systematic review, were DM and smoking. Age, BMI, and AHI were also mentioned by all the authors. Only Gottlieb et al.[6] excluded, at the beginning of the study, patients with previous CAD or heart failure, while the other two studies[7,8] included and registered these cases. Several factors, including the strict relation between obesity and OSA, make it difficult to understand the effect of each pathology and the synergy between them.[14] Moreover, multiple comorbidities are present in OSA patients with metabolic syndrome, DM, and cardiovascular disease itself – a situation that creates the challenge of explaining if secondary abnormalities are caused by OSA or other pre-existing conditions.[14]

Kendzerska et al.[2] and Shah et al.[4] also included potential factors of confusion in their works. What can be observed, in fact, is that there are several cardiovascular risk factors that are seldom seen together in only one study, including

family history - a target topic for the study by Gami et al.[15] These authors performed a cross-section study with over 500 apneic individuals, diagnosed by polysomnography, and found a strong and independent association between OSA and family history of premature death from cardiovascular disease.[15] This association shows important implications for the understanding of cardiovascular risk in these patients and raises this hypothesis so that future cohort works can be performed.[15]

In the current literature, the association between OSA and MI is shown by the proportion of events that occur throughout the years. Gottlieb et al.[6] reported that the association shown by them is considerably weaker that that of previous studies. The authors demonstrated the curves for the rate of survival free of coronary disease and heart failure, highlighting a drop in these rates throughout the years, according to the severity of the OSA. This weak association can be attributed to three main aspects: studies that work with cerebrovascular diseases, together with cardiovascular diseases, have higher rates of outcomes; studies that overestimate untreated patients such as those who refuse treatment, and thus neglect other health issues; and the study by Gottlieb et al.[6] that selected a sample from a community that did not seek sleep medicine services and, therefore, did not present signs or symptoms of OSA, with no clinical profile of these participants having been registered. It is possible that OSA, in such individuals, may bring a cardiovascular risk that is inferior to that of individuals who go to a sleep clinic for suspected OSA.[6]

Indeed, there is evidence that, in OSA patients without daytime drowsiness, treatment with CPAP does not offer a significant reduction in the incidence of SAH or cardiovascular events, though Barbe et al.[20] have admitted low power to detect differences between the groups with or without complaints. Regarding cardiovascular events incidence rates, Marin et al.[7] registered but did not distinguish gender and separated values by OSA severity degree and fatal or non-fatal outcome. On the other hand, Buchner et al.[8] did not record this information.

Free survival rate was also not checked in the study by Marin et al.,[7] whereas Buchner et al.[8] estimated a survival free of disease in patients with mild to moderate OSA without pre-existing cardiovascular disease after 10 years at 90.7% in groups of treated patients, and at 68.5% in non-treated patients groups.

AHI translates the frequency of apneas and hypopneas per hour of sleep and works as a measurement of OSA severity often related to advanced age, male gender, obesity, daytime drowsiness, and the presence of comorbidities.[14] Regarding this variable, it was observed that, in the study by Marin et al.,[7] mean value of AHI in patients with mild to moderate OSA was 18.2 ev/h and in those with severe apnea it was 43.3 ev/h. In the sample of untreated patients from the study by Buchner's et al.[8], mean value of AHI for all OSA patients groups was 15.3% ev/h, compatible with the frequency of mild, moderate, and severe apneic individuals: 56.7%, 28.2%, and 7.1%, respectively. In the group from Gottlieb et al.,[6] AHI median was 6.2 ev/h (men) and 2.7 ev/h (women), apparently including the values of patients with AHI < 5. This inclusion of 829 healthy men and 1605 healthy women may have interfered in AHI values of SHHA.[6] The authors also observed that the association of AHI with heart failure and CAD occurred in patients with AHI ≥ 30.

The patient sample, in this systematic review, was mostly composed by adults over 40 years of age, and this was also the inclusion criteria used by Gottlieb et al.[6] In the study by Marin et al,[7] the mean age of severe apneic patients was 49.9 years, the lowest mean registered among the groups; Buchner et al.[8] reported a mean age of 57.8 years among all untreated apneic patients. It is important to keep those age values in mind, because He et al.[21] suggested that OSA can have more severe cardiovascular consequences in individuals under 50 years of age. Studies have also demonstrated that younger individuals, with OSA, can be more prone to SAH,[22] atrial fibrillation,[23] and have a higher risk of death for any other cause.[24] With this evidence, it is necessary to understand if an aggressive therapeutic and diagnostic strategy would benefit younger and middle-aged individuals with OSA.[14] For that, other characteristics must be considered in future studies, such as ethnicity, gender and other demographic data.[14]

The relative consideration to the age factor allowed Gottlieb et al.[6] to show that cardiovascular risk associated to OSA decreases with age.[25] The SHHS[6] cohort, whose mean age was 62 years, may have underestimated the true cardiovascular risk in these patients. The authors of SHHS[6] argued that cardiovascular risk can decrease with age due to biological differences in OSA's pathophysiology between patients of different ages. The authors reported that the "healthy survivor" effect is a probable cause for a bias towards a null result, since apneic individuals, more susceptible to OSA's cardiovascular effects, are also more prone to cardiovascular diseases and have a higher risk of death than those with OSA who are resistant to cardiovascular consequences.[6]

In this work, 51.23% of the 5,067 analysed patients were male – Marin et al.[7] included only men in their study. By only observing the population of patients with AHI ≥ 5, we can see that the percentage increases to 65.57%. After statistical analysis and matching for age, ethnicity, smoking, and BMI, there was a strong association of AHI with heart failure in men, but not in women, according to Gottlieb et al.[6] In the same way, event rates increased with the severity of OSA in men, but that was not demonstrated in women.[6]

Buchner et al.[8] had only 16.5% of women in the non-treated group, and stated they could not extrapolate their results to this population. Considering the abovementioned facts, the present work will also restrict itself to the analysis of OSA in the male population – in the general population, the ratio between men and women with OSA is estimated at 2:1 to 3:1.[8]

Regarding factors of confusion, such as SAH, DM, dyslipidemia, we can observe that treatment of these pathologies has a relevant impact on outcomes such as MI.[26-28] They are part of the metabolic syndrome, which represents an important risk factor for CAD,[26-28] and it is important to know the therapeutic status of the population.

Gottlieb et al.[6] worked only with patients under treatment, an aspect that may have interfered in the weak association found between OSA and MI. Buchner et al.[8] also registered the number of patients under treatment, and it was also the study with the highest number of comorbidities, an aspect that may have interfered in the higher number of non-fatal cardiovascular events, revascularization procedures, and infarctions observed in the three articles. Marin et al.,[7] in turn, did not report the therapeutic status of the population, and it was also the only study that registered a large number of fatal cardiovascular events.

Studies show the prevalence of DM type II in the population of apneic individuals.[29-31] Catecholamine elevation together with sleep deprivation[32] are associated to insulin resistance. There are also data that suggest an association between OSA and glucose intolerance regardless of BMI.[33,34] Chen et al.[35] concluded, in a meta-analysis, that treatment with CPAP, even though it does not alter glycated hemoglobin levels, it significantly improves insulin resistance, positively impacting on DM symptoms. In the present systematic review, 12.7% of the 2,711 patients with AHI ≥ 5 also had DM, whereas Gottlieb et al.,[6] Marin et al.,[7] and Buchner et al.[8] registered 13.7% vs 9% vs 15.2%, respectively.

Regarding BMI, the highest median in the study by Gottlieb et al.[6] was 31.1 kg/m^2, in apneic men with AHI ≥ 30 ev/h. Marin et al.[7] registered a mean of 30.3 kg/m^2 in the untreated severe OSA group, and 27.5 kg/m^2 for mild to moderate OSA. Buchner et al.[8] reported a mean of 29.3 kg/m^2 for the entire sample, 55% of which had mild apnea. Resta et al.[36] and Newman et al.[37] observed a higher frequency of OSA among obese individuals, and, similarly, Silva et al.[38] concluded that obesity is a determining factor in OSA. Newman et al.[37] estimated that obese individuals can have approximately two times more chances of developing OSA. An elevated BMI is also associated to an increase in mortality from several chronic pathologies, especially cardiovascular diseases.[39] Framingham demonstrated that an elevated weight increases the risk of CAD, regardless of other risk factors.[40] These observations help us understand the importance of the high BMI value registered by the articles, in this systematic review, as well as the direct relation between BMI and OSA severity. It is also important to know the personal history of cardiovascular disease of the individuals in the analyzed sample in this systematic review. Gottlieb et al.,[6] Marin et al.,[7] and Buchner et al.[8] registered 0% vs 6.3% vs 70.5%, respectively.

From the exposed factors, we can observe that Buchner et al.[8] gathered conditions that favored cardiovascular outcomes: SAH, DM, dyslipidemia, as well as the high percentage of patients with a history of cardiovascular disease not being simple independent confusion factors of OSA. These factors can contribute to the adverse effects of OSA in cardiovascular outcomes; therefore, the higher proportion of cardiovascular risk factors and diseases could explain the number of cases and treatment benefits in patients with mild to moderate OSA, as reported by the authors.

The understanding of OSA effects may suggest explanations for the association of this pathology with MI. The prevalence of SDs in CAD patients is up to two times higher than in individuals without CAD. Bhama et al.[41] reported a prevalence of up to 30% of apneic individuals among patients with CAD.

There are pathophysiological mechanisms that suggest the contribution of OSA in the origin and progression of CAD: severe intermittent hypoxemia, acidosis, increase of blood pressure and sympathetic vasoconstriction, together with simultaneous changes in transmural, intrathoracic and cardiac pressures.[14] These factors strengthen the argument that OSA has a strong potential to trigger cardiac ischemia.[14] In the long run, the mechanisms of cardiac and vascular diseases, including endothelial dysfunction and systemic inflammation, can damage the structures of coronary arteries.[14] Sorajja et al.,[42] when studying patients with no history of CAD, observed the presence of an important calcification in the coronary arteries of OSA patients, through the calcification score = 9 (Agatston units) and zero score in patients without OSA ($p < 0.001$).

There are reports that the reduced number of cardiovascular events separated amongst themselves, and the variation of definitions used by research limit the conclusions of studies that address this theme.[18] The present systematic review also had as limitations the heterogeneity of the selected studies in relation to aspects such as objective, clinical and polysomnographic profile of the patients, as well as the difference in the presentation of patient groups and their classification regarding AHI.

This context demonstrated the challenges of investigating the causal relation between OSA and CAD, considering both conditions are chronic and have long latency periods before the appearance of complaints.[14] Moreover, both pathologies also have multifactorial origins with an overlap of common risk factors such as gender, age, obesity, and smoking.[14,43]

Defining the causal relationship between CAD and OSA means clarifying the necessary care apneic individuals must have regarding the prevention of MI. Making sleep apnea a marker for cardiac disease implies early tracking of these patients, as well as an incentive to treat this disease that is associated to numerous cardiovascular consequences. Endothelial, neuro-hormonal and metabolic alterations cannot be overlooked, even if it seems complex to dissociate the onset of CAD and OSA, because it is only thus that we can understand if OSA can interfere in the development or aggravation of CAD. OSA can be treated and, therefore, if this is confirmed, it can be a controllable determinant of CAD.

Conclusion

This systematic review has shown that there is an association between OSA and MI. We were able to observe that this association was higher among men, and that AHI was considered one of the markers for this relationship.

Author contributions

Conception and design of the research, Acquisition of data, Analysis and interpretation of the data, Writing of the manuscript and Critical revision of the manuscript for intellectual contente: Porto F, Sakamoto YS, Salles C

Potential Conflict of Interest

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

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Challenges for the Implementation of the First Large-Scale Rheumatic Heart Disease Screening Program in Brazil: The PROVAR Study Experience

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Introduction

Rheumatic Heart Disease (RHD) is the cardiac consequence of acute rheumatic fever (ARF), an inflammatory disease triggered by streptococcal pharyngitis. Although the prevalence of RHD has decreased in high-income countries, lack of social and economic development and poor primary prevention – mainly in in low- and middle-income countries – perpetuate an environment where RHD remains endemic. It is estimated that RHD continues to affect nearly 33 million people worldwide.[1] According to the World Health Organization (WHO), RHD is responsible for 1-1.5% of all cardiovascular deaths and 3-4% of cardiovascular Disability-Adjusted Life Years (DALYs).[2] In Brazil, according to the Unified Health System (SUS), there were 26,054 hospital admissions for ARF (45% with cardiac compromise) between 2008 and 2015, and the total cost to SUS was US$3.5 million, a number that is most likely underestimated.[3]

The main burden of RHD to public health systems consists of repeated hospital admissions and cardiac surgeries in the following decades after initial cardiac damage. If RHD is detected in its early stages, secondary prophylaxis (regular penicillin injections) can be initiated to prevent new episodes of ARF, avoiding further valve damage and progression of RHD. In high prevalence regions, RHD meets the traditional screening criteria defined by Wilson and Jungner,[4] although the long-term clinical significance of latent RHD is not entirely clear. Previous studies have demonstrated, however, that in 38 to 68% of asymptomatic RHD patients, echocardiographic findings show that abnormalities persist, and progress in 4 to 16%,[5] reinforcing the importance of early diagnosis in susceptible populations.

The PROVAR study (*Programa de Rastreamento da Valvopatia Reumática* – Rheumatic Heart Disease Screening Program) is the first large-scale echocardiographic screening program in Brazil, using echocardiography to estimate the prevalence of latent RHD in asymptomatic children between 5 and 18 years old attending public schools of underserved areas of the cities Belo Horizonte, Montes Claros and Bocaiúva, in the Brazilian State of Minas Gerais. Minas Gerais is the second most populous Brazilian state (>20 million inhabitants) and has a large territory, great geographical diversity and is marked by economic discrepancies between its different regions. This project is a clinical and research collaboration between the University Hospital of *Universidade Federal de Minas Gerais* (UFMG), Brazil, and the Children's National Health System (CNHS) in Washington DC, United States of America (USA).

Implementation of the study

The regulatory process started in the end of 2013, and the study was approved by the institutional review boards of UFMG and CNHS, as well as the State Boards of Health and Education. Legal sectors then analyzed the proposal and approved it, and there was no extra cost for the government or patients. The Department of Education selected schools with the highest socioeconomic vulnerability, with a special interest in areas with limited access to healthcare. Prior to screening, an educational curriculum was implemented, including lectures and printed material, for students and their parents, teachers and school staff regarding the importance of streptococcal pharyngitis, ARF and RHD. Parents were asked to sign an informed consent form – a requirement of Brazilian research regulations. Non-physician personnel started echocardiographic screening in July 2014 after a 12-week hands-on training supervised by an expert cardiologist and online RHD education modules (WiRED International, http://www.wiredhealthresources.net/EchoProject/index.html). They used portable (GE Vivid Q®) and handheld (GE VSCAN®) machines. Images were uploaded into dedicated cloud computing systems or Dropbox® and remotely interpreted by experienced cardiologists at UFMG (board certified by the Brazilian Society of Echocardiography) and CNHS using telemedicine resources (Figure 1).[6] We used the World Heart Federation criteria for the diagnosis of RHD.[7] Two experts blindly interpreted 10% of all acquired studies including 100% of the studies initially flagged as abnormal. In case of discrepancies during this process, a third expert blindly reviewed the images and a consensus diagnosis was reached.

When abnormalities were detected during the screening echocardiogram, a medical student called the child's parents to schedule a follow-up standard exam, which

Keywords

Rheumatic Heart Disease; Mass Screening; Echocardiography; Child; Adolescent.

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Figure 1 – *Operational flowchart of the PROVAR study: a) selection of schools in low-income areas; b) educational process; c) acquisition of echocardiographic images by non-physicians, using portable and handheld echo machines; d) image upload to cloud computing solutions with image viewing and measurement capability (LifeImage® (Newton, MA, USA) and ViTel Net®, (McLean, VA, USA)) for DICOM images from standard portable machines and secure Dropbox® plus dedicated Gateway® software for VSCAN; e) download and interpretation via telemedicine by cardiologists in Brazil and the United States (Sable, C. and Nascimento, B.R.).*

was performed by an experienced pediatric cardiologist at the university's hospital. Borderline RHD cases had a clinical consult and a follow up echocardiogram scheduled within one year. For patients found to have definite RHD based on the screening and follow-up echocardiogram, in addition to the referral to a specialized outpatient clinic, penicillin prophylaxis was initiated and more frequent follow-up echocardiograms were recommended based on the observations of subsequent visits.

Initial results

The PROVAR team has educated over 20,000 children on RHD, and has performed over 9,000 echocardiograms in 32 schools.[8] In the published analysis of 5,996 asymptomatic children from 21 schools, the overall prevalence of RHD was 4.2%, including 3.7% borderline (N = 221) and 0.5% definitive (N = 30). The inclusion of borderline patients as positive screening may raise some doubts, considering the available data. However, we believe this should be done, since this group seems to have increased the risk of progression to clinical RHD (Figure 2).[5,9] Children with cardiovascular symptoms (self-reported or reported by teachers/parents) that eventually presented to the program were directly referred to tertiary care. It is important to note that non-physician personnel performed the screening echocardiograms. Only physicians are allowed to perform and read echocardiograms in Brazil, and we were able to conduct the study because it was a research protocol.

The accuracy of these non-physicians (nurses, technicians and medical students) for basic interpretation of simplified screening exams was tested and had good results: overall sensitivity of 83% and specificity of 85%.[10] Moreover, the educational process has proven to be effective, with a median 20% increase in knowledge about RHD, evaluated through structured pre- and post-tests applied to more than 1,100 school children.[11]

Main challenges

There were several challenges during the implementation and conduction of the PROVAR study. During the initial ethics approval – which took almost 90 days in Brazil – some resistance from the Board of Education and Health was

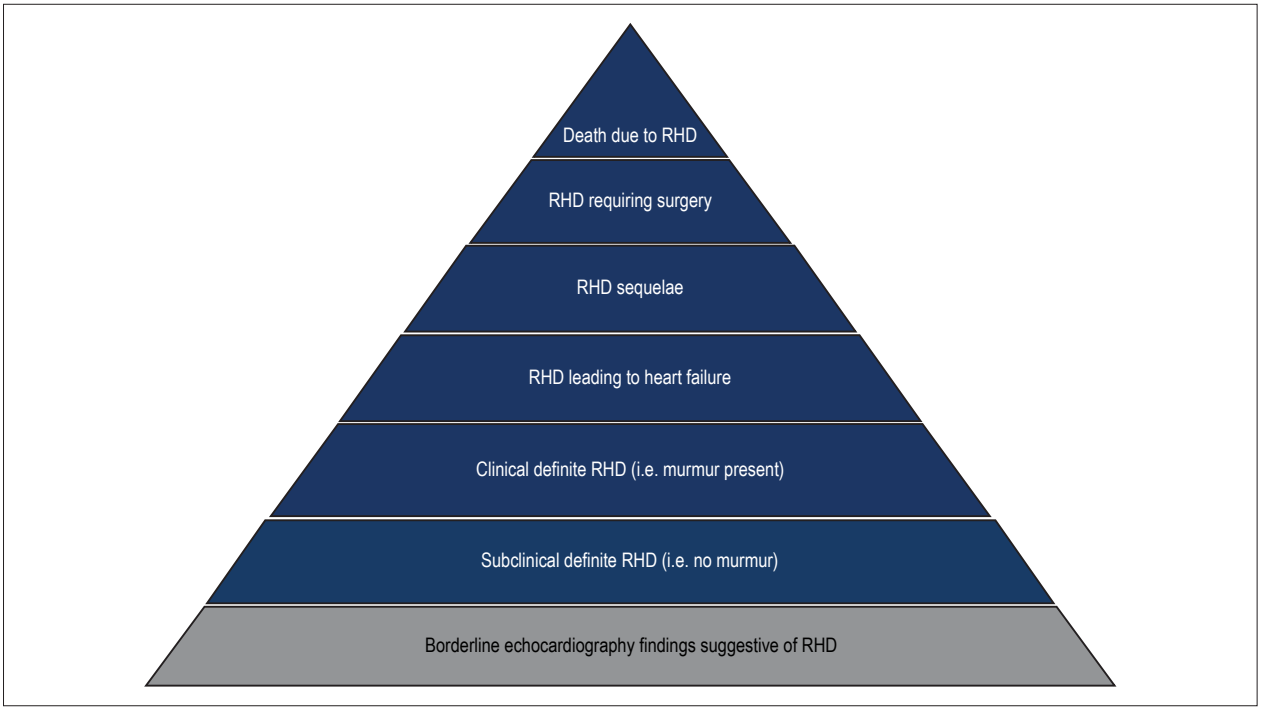

Figure 2 – *Progressive presentation of Rheumatic Heart Disease over time (adapted by Carmo, G.A.L. from Zühlke LJ and Steer AC.[5]).*

observed, mainly related to research procedures and the impact on the school routine. Some modifications to the consent forms were required, which took an extra 4 months. There was also questioning by the Board of Health, related to concerns about task-shifting (which could be questioned by medical and nursing councils) and the impact of screening on the population: referral strategies, availability of penicillin for all positive cases – considering the shortage observed in Brazil – and information for families with children with positive exams to avoid stigmatization.

During the initial steps of the field study, the PROVAR team faced several challenges. At first, the main challenges were related to the lack of involvement of school representatives with the program and lack of understanding possible benefits. Overall, there was low parental engagement with the project with the poorest attendance to educational sessions seen in the lowest socioeconomic status areas. The proportion of signed informed consents was low (about 35%: 5,996 out of 17,000 children)[8] especially among older students, even after the educational process. Also, some school principals refused to participate despite approval by the ethics committee and government authorities. Finally, organizing the kids for screening, especially the youngest age groups, was a challenge: removing the children from the classroom, getting them to undress/dress and organized in a line, collecting demographic data, etc. Follow-up meetings with the Boards of Education and local delegates were scheduled on a regular basis to assess weaknesses and special needs of different communities, and to encourage school staff to support the project locally. Screening in primary care centers with population education through the Family Health Program – a plan for the following years – may also be an effective strategy to increase the scope of the program.

There were also several challenges for follow-up visits. Phone contact with parents was often not possible because the children did not have the contact information, numbers changed frequently, and schools are not always authorized to disclose contact information. In addition, 35% of families failed to show up for scheduled appointments. This may presumably be, in part, due to patients being asymptomatic and families not being convinced of the importance of monitoring. Based on conversations with parents who have not returned for the follow-up, our team hypothesizes that financial constraints, living in distant neighborhoods and impossibility to miss a workday also contributed to the lack of compliance with recommendations for follow up in the study protocol. However, data about the reasons for the low compliance were not systematically collected. Some strategies, such as subsequent educational calls and flexible follow-up dates have been recently tested, with relative success. There is also a plan to provide follow-up echocardiograms in the schools, immediately after the screening process, to improve follow-up and referral rates. Additionally, educational and public awareness materials for different scenarios are under development, considering the questions posed by the families.

Future directions

Fighting RHD is a challenge for Brazil. Understanding the burden of the disease and how it affects the health system is the primary objective of PROVAR. The PROVAR program will continue to screen for RHD in underserved

areas, but we are now expanding our efforts to private schools, where students' socioeconomic conditions are much better. A much lower prevalence is anticipated in this "control" population.

Authorities should be prepared to effectively eradicate RHD. To do so, we believe ARF and RHD should be included in the list of notifiable diseases, since these conditions have a considerably high incidence in Brazil. Group A streptococcal infections (triggers for ARF) are communicable from patient to patient and transmission can be eliminated with eradication.[12] Also, by identifying early RHD, secondary prophylaxis can be initiated to prevent disease progression and late consequences such as heart failure, endocarditis, arrhythmias, stroke and heart valve surgeries.

The next steps of PROVAR are related to the diagonal integration of RHD screening in primary care. This strategy is now being implemented in Montes Claros (MG, Brazil) (2 primary care centers have already been enrolled) and will be launched in Belo Horizonte and Nova Lima (MG – Brazil) in the following months, also including screening for other degenerative and congenital valve conditions. For this purpose, primary care physicians will also be trained in basic echocardiographic skills for routine evaluation of different age groups, utilizing the same telemedicine infrastructure for uploads and remote interpretation. We believe this integration is a crucial step for long-term sustainability of echocardiographic screening and for its integration into healthcare policies, and will allow assessment of the ideal screening strategies, including cost-effectiveness analysis. If RHD screening in these scenarios proves to be feasible and cost-effective, the final step would be to include it as a priority for the discussion of the Public Health System budget in the long run.

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Author contributions

Conception and design of the research: Santos JPA, Carmo GAL, Beaton AZ, Sable C, Nascimento BR; Acquisition of data: Santos JPA, Carmo GAL, Lourenço TV, Diamantino AC, Nunes MCP, Nascimento BR; Analysis and interpretation of the data: Beaton AZ, Nunes MCP, Sable C, Nascimento BR; Statistical analysis: Carmo GAL, Beaton AZ, Nunes MCP, Nascimento BR; obtaining funding: Beaton AZ, Sable C, Nascimento BR; Writing of the manuscript: Santos JPA, Lourenço TV; Critical revision of the manuscript for intellectual content: Carmo GAL, Beaton AZ, Sable C, Nascimento BR.

Potential Conflict of Interest

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Study Association

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Case 3/2017 - High Origin of the Right Coronary Artery at the Sinotubular Junction, in a 14-Year-Old Teenager, in Diagnostic Imaging Diversity

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Clinical data

He reports that, six months ago, after discreet exercise (having run about 500 meters) he felt tiredness and dizziness, malaise and skin paleness. Repeated migraines accompany the clinical status. Recent bi-Doppler echocardiography revealed the high origin of the right coronary artery at the sinotubular junction. There was no morbid past of importance.

Physical examination: eupneic, acyanotic, normal pulses. Weight: 66 Kgs, Height: 169 cm, BP: 110/65 mm Hg, HR: 57 bpm, O2 saturation = 96%. The aorta was not palpated at the suprasternal notch.

In the precordium, without systolic impulses, the *ictus cordis* was not palpated. Cardiac sounds were normal and heart murmur was not heard. The liver was not palpated.

Additional Examinations

Electrocardiogram showed sinus rhythm and signs of electrical normality. The duration of the QRS complex was 0.092` with morphology rS in V1 and qRs in V6, with negative T wave in V1. AP: + 40°, AQRS: + 75°, AT: + 25°.

Chest X-ray shows normal cardiac area and pulmonary vascular markings (Figure 1).

Image exams: **Bi-Doppler echocardiogram** showed cardiac cavities of normal size and function. The dimensions were, in Ao = 24; RA = 28; RV = 20; LV = 49; ventricular septum and posterior wall of LV = 7; Ventricular ejection fraction = 65%. The right coronary artery in the parasternal long axis view showed high origin at the sinotubular junction in clear oblique orientation between the aorta and the pulmonary trunk (Figure 1).

Tilt-test showed no significant changes in blood pressure (103/66 to 104/65 mmHg) and heart rate (from 77 to 94 bpm).

Dynamic 24-hour electrocardiogram (Holter): showed no changes in heart rhythm and / or waves, complex and electrical segments.

Keywords

Myocardial Ischemia; Coronary Vessels; Echocardiography, Doppler; Sinus of Valsalva; High origin of right coronary artery.

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Angiotomography of the coronary arteries revealed normal and usual origin of them in the different sinuses of Valsalva and both at the same height (Figure 2).

Myocardial scintigraphy with physical exertion did not reveal any myocardial ischemic changes.

Clinical diagnosis

High origin of the right coronary artery at the right sinotubular junction by echocardiogram, in a teenager with nonspecific symptoms, not confirmed by angiographic study.

Clinical reasoning

The clinical elements were compatible with cardiovascular normality with nonspecific symptoms. The finding of an echocardiographic examination with a high origin of the right coronary artery led to a more accurate investigation of the existence of myocardial ischemia, not evidenced in myocardial scintigraphy and angiotomography.

Differential diagnosis

The anatomical and ischemic findings of the heart in teenagers and young adults occur in several other situations, such as the origin of coronary artery from the contralateral sinus of Valsalva (most common coronary anomaly of all), as well as in hypoplastic coronary arteries, in stenosis in the coronary ostium-*slitlike shape*, in aortic and also interarterial intramural tracts, between the aorta and the pulmonary artery, besides the acute angle formed between the coronary and the aorta in descending straight path, and finally in the early atherosclerotic disease. All these conditions are well recognized as causing ischemia and sudden death after physical exertion, and with prodromes of palpitations, syncope or precordial pain.

Conduct

As the findings of the echocardiogram did not find any correspondence with the normal images revealed by angiotomography and consolidated by the functional study of myocardial scintigraphy, there was the recommendation of periodic follow-up without limitation of the usual activities. It was difficult to explain the echocardiographic finding that was characterized by a diagnostic error of this coronary anomaly. Hence, the need for an ever more accurate assessment in order to confirm the diagnosis, having as support the analysis of all the elements that were exhaustively pursued in the case in question.

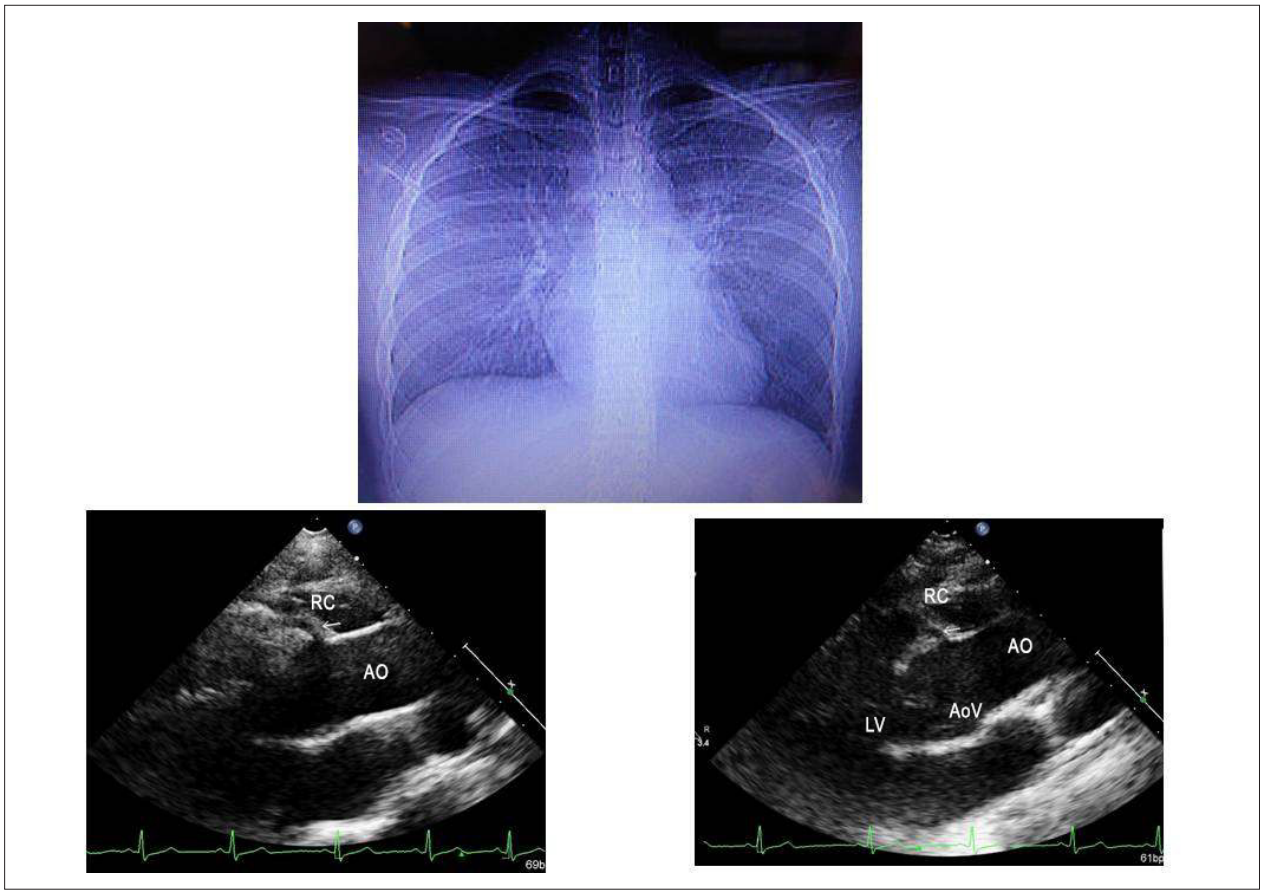

Figure1 – *Chest X-ray shows normal cardiac area and pulmonary vascular markings and parasternal long-axis view echocardiogram clearly points to the high origin of the right coronary artery at the sinotubular junction. Ao: aorta; RC: right coronary; AoV: aortic valve, LV: left ventricle.*

Comments

High origin of the coronary artery is very rare (0.1% of the right coronary artery and 0.7% of the left of all coronary abnormalities) and in the reported cases, associated ischemia is systematically correlated with the presence of other additional abnormalities. Therefore, it is difficult to implicate high coronary origin as a cause of ischemic event and hence as being definitely pathological. It may predispose to myocardial ischemic alterations in the presence of associated abnormalities, such as in a single coronary artery, in a vertical and oblique path between the aorta and the pulmonary artery, allowing obstruction by compression and with consequent reduction of coronary flow, as well as a narrow coronary ostium[1] and intramural aortic. Thus, in findings of high origin of the coronary artery we should immediately eliminate the presence of myocardial ischemia due to decreased ostium (50% of the diameter), interarterial pathway or presence of another abnormality.However, there are authors who believe in the hypothesis of myocardial ischemia only because of the high coronary origin due to the reduction of the diastolic filling of the coronary, being able in addition to theoretically cause chronic myocardial damage with consequences in adulthood. Thus, the question is if the high origin of the coronary artery constitutes a benign or malignant anomaly.[2,3]

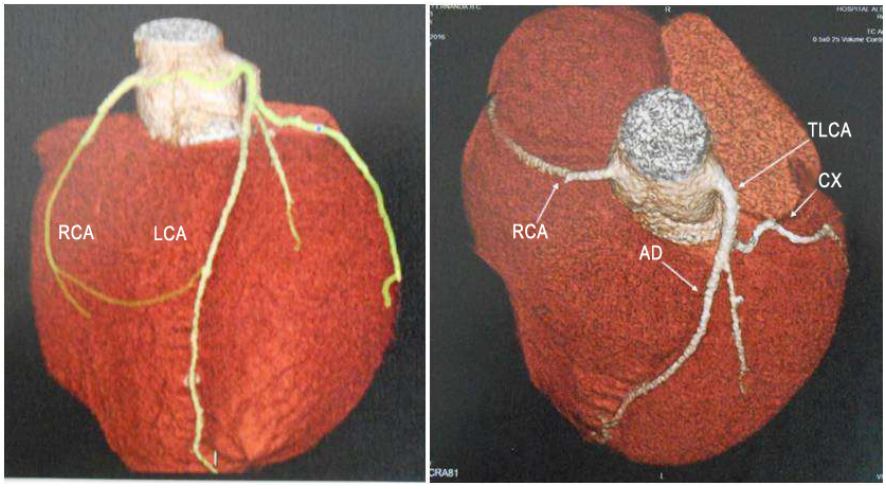

Figure 2 – *Angiotomographies of the coronary arteries, in two distinct views, show normal origins and at the same height of both. AD: anterior descending; Cx: circunflex artery; LCA: left coronary artery; RCA: right coronary artery; TLCA: trunk of left coronary artery.*

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Heyde's Syndrome and Transcatheter Aortic Valve Implantation

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Introduction

Aortic Stenosis (AoS) is the valvular pathology most frequently acquired in developed countries, present in 4% of individuals over 85 years of age. Patients with severe AoS may have comorbidities, presenting greater risk of thrombotic and bleeding events.

Heyde's Syndrome was described in 1958 by E. C. Heyde[1] as he observed the relation between aortic valve stenosis and gastrointestinal bleeding. In 1992, Warkentin et al.[2] observed the loss of the largest Willebrand factor multimers, characterizing the Acquired type 2A[3,4] von Willebrand syndrome. Von Willebrand Factor (vWF) is a multimeric protein, secreted by endothelial cells and platelets.[5] It promotes adherence of platelets to vascular lesion sites through glycoprotein Ib-vWF interactions. A change in the shape of vWF occurs in AoS, making such protein more susceptible to proteolytic cleavage. As a consequence, vWF is degraded specifically by protease ADAMTS13, hindering vWF-mediated platelet adhesion, thus generating a lack of these multimers and causing bleeding, especially in pre-existing lesions such as gastrointestinal angiodysplasia.[6-8]

Treatment for this syndrome may be obtained with surgical aortic valve replacement, and percutaneous implantation of aortic valve (TAVI), whose effect is still under investigation.[9]

Case Report

Patient MNS, male, 81 years of age, sought medical treatment for tiredness, black feces, edema of the lower limbs, and worsening of functional class (FC) to NYHA III one month ago.

Patient presents with antecedent systemic arterial hypertension, heart failure, severe AoS, dyslipidemia, diabetes mellitus, chronic renal failure requiring dialysis, two myocardial revascularization surgeries, angioplasty with stent, and anemia.

Keywords

Stenosis Valve Aortic / therapy; Gastrointestinal Hemorrhage / complications; Angiodysplasia; Prosthesis Implantation of Aortic Valve; von Willebrand Diseases / therapy.

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Physical examination: pale 3+/4+, eupneic, acyanotic. Pulse: 66bpm, BP: 100x60 mmHg, heart rhythm was regular with two clicks with systolic murmur 4+/6+ in the aortic area radiating to the wishbone. Pulmonary auscultation with bibasilar crackles and edema of the lower limbs 2+/4+ with reduced peripheral perfusion.

Labs: Intense anemia with hemoglobin 6.4 g/dL. Initially, the anemia was related to bleeding in the digestive tract due to the black feces – melena. In view of "color anemia" with NYHA III HF, a red blood cell transfusion was requested.

Transthoracic echocardiography confirmed a double aortic lesion with significant stenosis, with valve area of 0.9 cm^2 and maximum gradient underestimated of 35 mmHg and mean gradient of 22 mmHg. Ejection fraction of 32%. Left ventricle with 63mm diastolic diameter and 53 mm systolic diameter.

Colonoscopy showed the presence of diverticula of sigmoid, descending and transverse colon polyp, and ascending colon angiodysplasia. Thus, the presence of bleeding from angiodysplasia associated to AoS suggested a diagnosis of Heyde's Syndrome (Figure 1).

Due to the patient's comorbidities, traditional surgical intervention was discarded due to high risk. A Transcatheter Aortic Valve Implantation (TAVI) was performed, with successful implantation of the transcatheter valve INOVARE® via transapical implantation (Figure 2).

On the fourth day following TAVI, the patient had an episode of enterorrhagia after extreme effort to evacuate his bowels, so blood transfusion was necessary. A colonoscopy and a high digestive endoscopy were then performed with no evidence of active bleeding. Afterwards, the patient was monitored in the clinic, with return visits 3 and 6 months after the surgery with no recurring episodes of bleeding.

Discussion

Heyde's Syndrome was described in 1958 by Edward C. Heyde as a combination of AoS and bleeding from gastrointestinal angiodysplasia.

The pathophysiology of the condition is explained by the passage of vWF through the stenotic valve, with multimer proteolysis through the enzyme ADAMTS13, a proteinase that acts especially in situations of high shear stress.[3-5,7,8] VWF is secreted by blood endothelial cells, contributing to the formation of platelet thrombi and acting as a mediator of platelet adhesion in the vascular lesion site.[5]

The relation between AoS and gastrointestinal angiodysplasia has yet to be established. The hypothesis is that AoS is related to a degree of chronic hypoxia, stimulating vasodilation and smooth muscle relaxation, progressing to ecstasia of the vessel wall.[10] Patients with Heyde's Syndrome treated with bowel resections generally continue to have bleedings in other

sites, while the valve approach cures coagulation disorder and anemia.[10]

Elderly patients may present several risks for the surgical replacement of the valve or refuse the procedure. Seniors often have comorbidities that require the use of anti-coagulants or antiplatelet drugs, but those must be avoided, especially in more severe cases. Another option for patients at high surgical risk is the TAVI.

Recently it was demonstrated that the presence of aortic regurgitation after TAVI can generate multimers proteolysis occurring in some cases the persistence of Heyde's syndrome being associated with a higher mortality at 1 year.[11]

Conclusion

The elimination of gastrointestinal hemorrhaging risk after calcific aortic valve replacement and valvular prosthesis is well demonstrated in literature.[3-5]

However, there is no evidence that this new approach by TAVI, without removal of the calcium block, can resolve the occurrence of new digestive bleedings. It is necessary, in the long run, to observe and check if the transcatheter valve implantation can correct digestive hemorrhages like the conventional valve replacement.

Author contributions

Conception and design of the research, Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content: Balbo CP, Seabra LP, Caputi G, Palma JH, Buffolo E; Acquisition of data and Writing of the manuscript: Balbo CP, Seabra LP, Galoro VG, Caputi G, Palma JH, Buffolo E; Statistical analysis: Buffolo E.

Potential Conflict of Interest

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Sources of Funding

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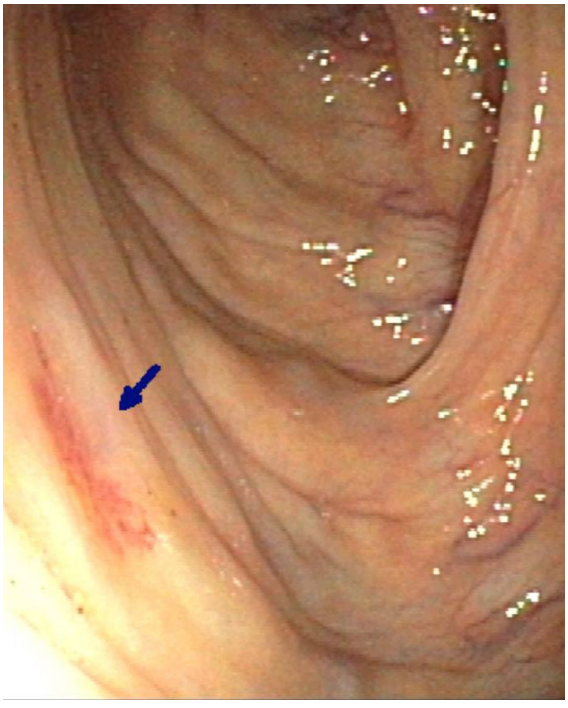

Figure 1 – *Angiodisplasia de cólon.*

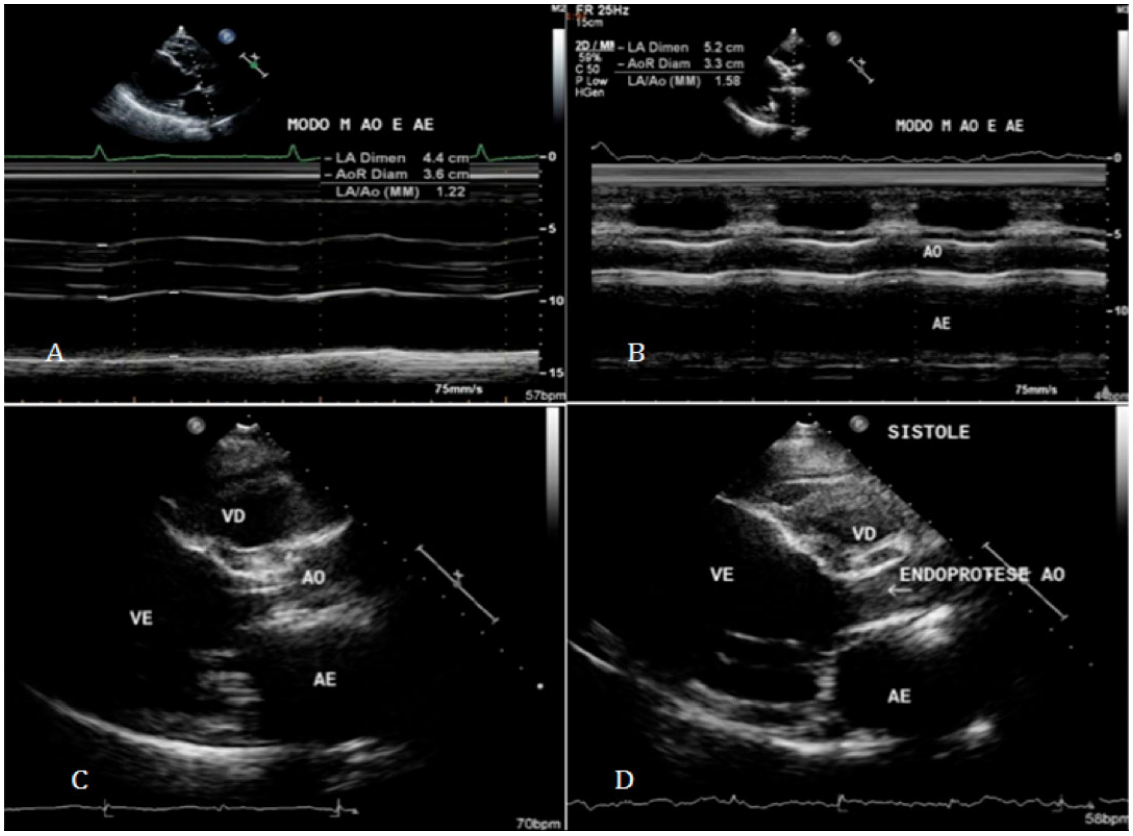

Figure 2 – *A) ECHO pre TAVI (M mode); B) ECHO post TAVI (M mode); C) ECHO pre TAVI (2D mode); D) ECHO post TAVI (2D mode). AO: aorta; LA: left atrium; RD: right ventricle; LV: left ventricle.*

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Giant Left Ventricle Outflow Tract Pseudoaneurysm after Ross Procedure

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A 33-year-old woman was admitted to our hospital because of dyspnea on exertion, orthopnea, cough and pedal edema for the past six months. Six years earlier she had been submitted to Ross procedure for correction of a bicuspid aortic valve.

Physical exam was unremarkable except by a grade 3 systolic murmur on left sternal border.

A chest X-ray revealed an opacity on left border of cardiac silhouette (Figure 1A). Transthoracic echocardiography presented a giant saccular structure, adjacent and connected to the left ventricular outflow tract through a neck located at 2 o´clock position, compatible with a pseudoaneurysm. This structure caused compression of the right ventricle outflow tract (RVOT) and pulmonary artery, causing mild obstruction (Figure 1 B and C).

Cardiac magnetic resonance imaging identified its origin on left ventricule outflow tract (LVOT), in close relation to left and non-coronary sinus. There were small thrombi inside the pseudoaneurysm, tapering the wall adjacent to the left ventricle (Figures 1D and E).

Patient was surgically treated. The pseudoaneurysm sack was opened through a transpleural access and the communication between the LVOT and the pseudoaneurysmal cavity was closed with a Teflon patch. Patient's post-operative recovery and follow-up were uneventful.

After 3 months of follow-up she is asymptomatic. The pseudoaneurysm is completely excluded from arterial circulation, without significant obstruction of RVOT (Figure 1F).

LVOT pseudoaneurysm is an uncommon but potentially life-threatening complication of Ross procedure. Follow-up with imaging techniques allows early identification and prompt intervention.

Author contributions

Conception and design of the research and Writing of the manuscript: Leão S; Acquisition of data: Leão S, Carvalho S; Analysis and interpretation of the data: Leão S, Carvalho S, Ribeiro H; Critical revision of the manuscript for intellectual content: Carvalho S, Ribeiro H, Fontes P, Moreira JI.

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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Keywords

Ventricular Outflow Obstruction / surgery; Cardiac Surgical Procedures / complications; Echocardiography.

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Image

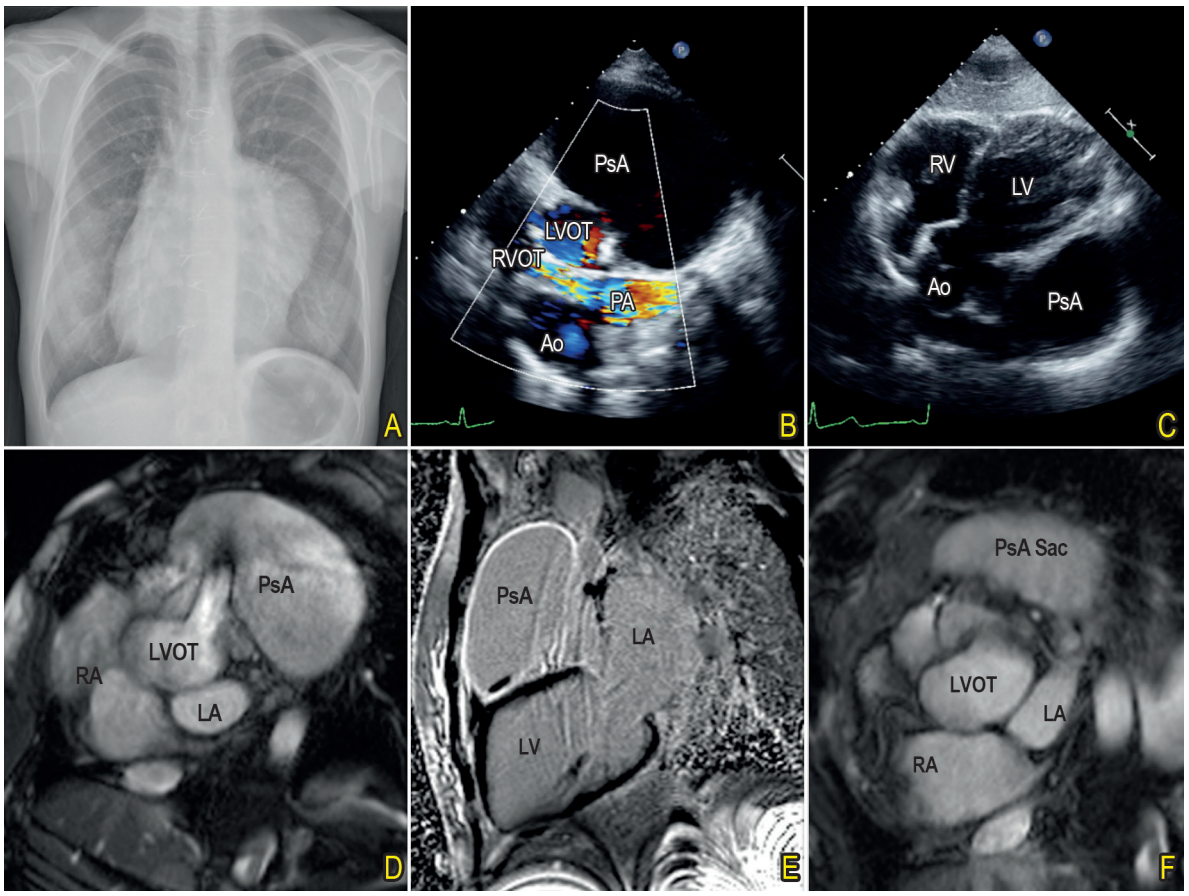

Figure 1 - *A) Chest radiograph; B and C) Preoperative transthoracic echocardiography, modified short axis and subcostal views; D and E) Preoperative magnetic resonance imaging; F) Follow-up magnetic resonance imaging. Ao: aorta; LA: left atrium; LVOT: left ventricle outflow tract; LV: left ventricule; RA: right atrium; RV: right ventricule; PA: pulmonary artery; PsA: Pseudoaneurysm; RVOT: right ventricle outflow tract.*

The Tip of The Iceberg: Non-Calcified Coronary Plague and Epicardial Adipose Tissue

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To the Editor,

I have read with great interest the article entitled "Relationship between Calcium Score and Myocardial Scintigraphy in the Diagnosis of Coronary Disease" by Siqueira et al.,[1] recently published in *Arquivos Brasileiros de Cardiologia* 2016; 107:367-74. The investigators reported the possibility of removing extensive coronary artery disease (CAD) by means of a zero calcium score, or by indicating the presence of an extensive disease when it is severely increased, which justifies the use of this method in the initial or joint evaluation in asymptomatic patients with suspected CAD and in cardiovascular risk stratification. The evaluation of symptomatic low-risk patients, despite suggestive evidence, should be re-evaluated in upcoming guidelines.[1]

Epicardial adipose tissue (EAT) is anatomically contiguous with the myocardium and several studies have shown it to be a potential contributing factor for coronary atherosclerosis.[2] EAT is a type of visceral adipose tissue with paracrine and endocrine effects.[3] EAT serves as an energy source for the myocardium and it is known to secrete proatherogenic cytokines.[3] Increased EAT is not only associated with a higher prevalence of CAD but it is also a prognostic parameter for future cardiovascular events, and, eventually, cardiovascular mortality.[4] Hwang et al.[5] have reported that a high epicardial fat volume index determined by computed tomography was an independent risk factor for the future development of non-calcified coronary plaque even after adjustment for traditional cardiovascular risk factors.

In the light of these findings, assessment of EAT by computed tomography might be beneficial as a part of further evaluation for future cardiovascular events.

Keywords

Adipose Tissue / pathology; Coronary Artery Disease; Calcium Signaling; Biomarkers / analysis; Radionuclide Imaging; Tomography, Emission Computed.

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Letter to the Editor

Reply

We would like to thank you for your interest and comments related to our recent article.[1] We believe the great scientific basis referent to coronary calcium quantification clearly demonstrates the importance of this method in the stratification of asymptomatic patients with low to intermediate cardiovascular risk.[2-7]

Recent publications related to calcium score (CS) continue to point to flaws in clinical score classifications and reinforce their ability to distinguish the different cardiovascular events risk groups. The role of clinical score reclassification, when CS is implemented, is a warning to its clinical applicability.[2-7] Population studies with long periods of follow-up[2-7] have demonstrated that the use of CS is one of the best tools to determine cardiovascular risk, even when compared to other markers.[7]

The potential use of CS is not limited to cardiovascular risk evaluation, for it has proven to be a useful tool in the primary prevention and adequate treatment of sub-clinical forms of coronary artery disease.[8-15] The presence of a zero CS is indicative of a very low risk, often exempting the patient from early preventive treatment with statins.[8-11] On the other hand, in the presence of a CS that is not zero, and especially > 100 Agatston, therapeutic introduction may be recommended, even in patients who do not fit the indication of current guidelines for the use of statins and other medications, such as anti-hypertensives.[10-15]

Important changes in the guidelines can already be seen, putting CS in the recommendation class I for some of its indications.[16] However, there still are some discrepant recommendations,[17-20] such as in orientations about the beginning of treatment for cardiovascular risk reduction, that still do not include coronary calcification data, even with robust data that support this positioning.[21-22]

Therefore, we believe that in the next few years, this method will take on a growing importance in clinical guidelines, aiding in a more adequate follow-up of low to intermediate risk patients.

Yours truly,

Fabio Paiva Rossini Siqueira,
Claudio Tinoco Mesquita,
Alair Augusto Sarmet M. Damas dos Santos,
Marcelo Souto Nacif

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