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Sociedade Brasileira de Cardiologia • ISSN-0066-782X • Volume 109, Nº 2, August 2017

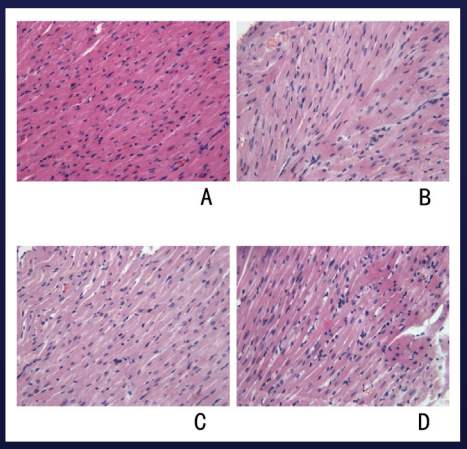

Figure 2 – *Myocardial changes after doxorubicin treatment at the light microscopy level (× 400). Vacuolization of the cytoplasm, loss of myofibrils was more severe in rats from group 4 (D). A: rat from group 1; B: rat from group 2; C: rat from group 3; D: rat from group 4. Page. 135*

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Contents

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Arquivos Brasileiros de Cardiologia

Volume 109, Nº 2, August 2017

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

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SUPPORT

Calcium Score Use in Isolation in Acute Chest Pain Setting – Is it Sufficient?

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The clinical usefulness of any test in Medicine depends on the population studied, because even an accurate test will yield no benefit if applied to the wrong population. While sensitivity and specificity are characteristics inherent in the diagnostic method, the individual probability of having a disease when the test is positive (positive predictive value, PPV) and the probability of not having the disease when the test is negative (negative predictive value, NPV) depend on the disease prevalence in the population and the individual probability of having the disease before undergoing the test, the pretest probability.

The PPV and NPV are the information that matters for clinicians. In the presence of a positive test, clinicians are interested in the patient's real probability of having the disease. However, in the presence of a negative test, Clinicians want to know the true probability of the disease even with the negative result. Thus, PPV and NPV should be considered before requesting a test, because some cases a positive test might not sufficient to confirm the presence of disease, while a negative test might not be able to exclude it safely.

The investigation of chest pain of possible cardiac origin is one of the most common examples of that duality between pretest probability of disease and sensitivity and specificity for the use of ancillary tests. The first discussion about that approach for the diagnosis of coronary artery disease (CAD) was published by Diamond and Forrester in the *New England Journal of Medicine* in 1979.[1] The pretest probability assessment before choosing the best diagnostic test for patients with chest pain continues to be recommended in current guidelines.[2-4]

While tests coronary computed tomography angiography, exercise test and functional tests associated imaging (stress myocardial perfusion scintigraphy) are clearly indicated for chest pain investigation in different scenarios,[2,5] the use of coronary calcium score (CCS) is not recommended to the routine assessment of chest pain in any clinical situation in Brazilian guidelines.[5] However, some studies have suggested that CCS should be considered for individuals with chest pain and low-to-moderate pretest probability of DAC, and those studies led to CCS incorporation into the *National Institute of Health and Clinical Excellence* (NICE) guidelines in 2010.[6] That indication was based on the use of Bayes theorem and considers the low pretest probability of the population studied and the substantial NPV of CCS. However, even the NICE recommendations, which strongly consider the cost-effectiveness of the test to choose the diagnostic method, have been recently modified. In their new version, the NICE no longer recommends CCS as part of the investigation for individuals with chest pain of possible cardiac origin, and currently recommends coronary computed tomography angiography as the first-choice test for the large majority of individuals.

Some reasons for the strong appeal of CCS use are as follows: CCS is easily performed; requires a very low radiation dose, neither stressor nor contrast agents, and no patient preparation; and has no absolute contraindication. In addition, the test has a short duration (less than 5 minutes), provides almost immediate analysis and results, and requires minimal image processing.

To extend the indication of methods originally designed or validated for a specific purpose should be carefully assessed. Even more when that extension is aimed at replacing an already established or clearly more accurate method, or avoiding its use, to achieve an important diagnostic definition.

One reason for controversy is related to the pathophysiology of acute coronary syndrome. Patients presenting to the emergency unit with acute coronary syndrome findings have a lower load of calcified plaque and culprit lesions with predominance of the non-calcified component;[7] therefore, to base a screening test on the presence or absence of coronary calcification to assess chest pain in the emergency room might not have a proper pathophysiological rationale. In addition, the CORE64 study, assessing symptomatic patients referred for coronary computed tomography angiography, has shown that one out of five individuals presenting to the emergency unit with acute chest pain and a CCS of zero (no coronary calcification) had significant stenosis of at least one coronary segment on invasive coronary angiography.[8] Such data do not support any decision-making based on CCS results for patients with acute chest pain.

In the present edition of *Arquivos Brasileiros de Cardiologia*, Correia et al.[9] have assessed the possibility of extending this controversial indication of CCS use for patients admitted to a coronary unit of a Brazilian tertiary hospital with higher pretest probability of obstructive CAD. Those authors have

Keywords

Coronary Artery Disease; Chest Pain; Predictive Value of Tests; Angiocardiography; Tomography, X -Ray Computed.

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DOI: 10.5935/abc.20170116

concluded that despite the limited PPV associated with a CCS over zero, CCS had a NPV of 90% for obstructive CAD. As expected, the CCS ability to exclude disease was higher in individuals with lower pretest probability (under 50%), whose NPV reached 95%. Finally, those authors have suggested that up to one out of four individuals would have the probability of obstructive CAD sufficiently low to allow discarding that differential diagnosis based on the presence of a CCS of zero.

The PPV and NPV estimation and Bayes theorem use in clinical practice require a tool to estimate CAD pretest probability for every patient. Despite possible problems of calibration,[10] pretest probability scores have been well established to assess stable chest pain. However, there is no validated pretest probability score for acute chest pain. Correia et al.[9] should be congratulated for using a local sample to derive a pretest probability score for acute CAD in patients admitted to a coronary unit. However, although the pretest probability used is adequate for the present study, it has important limitations. First, the performance of that score might be overestimated, and those results most likely do not maintain that performance when replicated to other populations. Thus, external validation of the probability score is required before extrapolating the results of the present study to clinical practice.

Although waiting for that external validation, the present study has other results that justify a deeper discussion. The authors defined that a probability of obstructive CAD lower than 10% allows discarding that diagnosis. Recommendations for stable CAD consider that probability low enough to not justify further investigation.[3,4] However, with that approach, one out of ten patients with obstructive CAD can be discharged without the right diagnosis, which might be considered inappropriate by the team in charge of patients' care in urgency and emergency settings, such as the coronary unit.

On the other hand, if a disease probability of 10% could be considered low enough to rule out disease, 8% of the patients in this study could have been discharged without undergoing any test, because none of them would have had obstructive CAD, but one in every four patients was incorrectly classified due to a CCS over zero.

In the present study, it is worth noting the inclusion of individuals with disease probability higher than previously studied. In addition, the authors were careful enough to stratify the results according to the pretest probability and the presence or absence of alterations in resting electrocardiogram and troponin levels. For individuals with disease probability higher than 50% or for those with troponin or electrocardiographic alterations, the CCS ability to rule out disease was only reasonable (NPV of 63% and 83%, respectively). On the other hand, in those with pretest probability lower than 50%, and particularly in patients with normal electrocardiogram and troponin levels, the CCS ability to rule out disease was stronger (NPV of 95% and 100%, respectively).

Those data suggest that, in patients with normal electrocardiogram and troponin levels and a pretest probability of disease between 10% and 50%, CCS can be considered an alternative in the investigation of possible anginal acute chest pain, particularly in situations in which other assessment methods, such as coronary computed tomography angiography and functional tests, are not available or are contraindicated. However, before the routine incorporation of that strategy into clinical practice, validation and calibration of the probability score adapted to this scenario are necessary, as is the replication of the results in larger cohorts to ensure the reproducibility of the CCS ability to rule out disease in that population.

Considering the pros and cons of CCS use as a gatekeeper in chest pain assessment at the emergency unit, it is worth emphasizing the current non-adoption of CCS use in isolation, aimed at ruling out significant obstructive CAD in patients with acute chest pain, by most guidelines on cardiology worldwide.

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Zero Calcium Score as a Filter for Further Testing in Patients Admitted to the Coronary Care Unit with Chest Pain

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Abstract

Background: The accuracy of zero coronary calcium score as a filter in patients with chest pain has been demonstrated at the emergency room and outpatient clinics, populations with low prevalence of coronary artery disease (CAD).

Objective: To test the gatekeeping role of zero calcium score in patients with chest pain admitted to the coronary care unit (CCU), where the pretest probability of CAD is higher than that of other populations.

Methods: Patients underwent computed tomography for calcium scoring, and obstructive CAD was defined by a minimum 70% stenosis on invasive angiography.

Results: In 146 patients studied, the prevalence of CAD was 41%. A zero calcium score was present in 35% of the patients. The sensitivity and specificity of zero calcium score yielded a negative likelihood ratio of 0.16. After logistic regression adjustment for pretest probability, zero calcium score was independently associated with lower odds of CAD (OR = 0.12, 95%CI = 0.04–0.36), increasing the area under the ROC curve of the clinical model from 0.76 to 0.82 (p = 0.006). Zero calcium score provided a net reclassification improvement of 0.20 (p = 0.0018) over the clinical model when using a pretest probability threshold of 10% for discharging without further testing. In patients with pretest probability < 50%, zero calcium score had a negative predictive value of 95% (95%CI = 83%–99%), with a number needed to test of 2.1 for obtaining one additional discharge.

Conclusion: Zero calcium score substantially reduces the pretest probability of obstructive CAD in patients admitted to the CCU with acute chest pain. (Arq Bras Cardiol. 2017; 109(2):97-102)

Keywords: Acute Coronary Syndrome / diagnosis; Chest Pain, Calcinosis / diagnosis; Predictive Value of Tests; Emergency Medical Services.

Introduction

The majority of patients presenting to the hospital with acute chest pain do not have obstructive coronary artery disease (CAD). These patients often undergo imaging tests before discharge.[1] The efficiency of this strategy is challenged by the low yield for positive results.[2] Furthermore, many imaging tests are only available in business hours, are time-consuming and costly, have several contraindications and require expert interpretation. For example, computed tomography (CT) coronary angiography should be avoided in patients with renal failure or known allergies to dye; functional tests require pharmacological or physical stress and are usually not available 24 hours a day.

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Manuscript received June 26, 2016, revised manuscript December 10, 2016, accepted January 19, 2017

DOI: 10.5935/abc.20170076

A filter is a simple test where a negative result obviates the need for more complex tests. Previous studies have suggested that zero calcium score has a sufficiently low negative likelihood ratio to play a filtering role in patients with chest pain.[3] However, these studies have focused on patients presenting to the emergency room and the outpatient clinic, a population with low prevalence of CAD.

Our aim was to study the diagnostic performance of zero calcium score as a filter for other imaging tests in patients with acute chest pain admitted to the coronary care unit (CCU) of a tertiary-care hospital, where the prevalence of disease is expected to be at least intermediate. We explored the negative predictive value of zero calcium score in the whole group and according to strata of pretest probability.

Methods

Sample selection

Between September 2011 and October 2013, consecutive patients admitted to the CCU of a tertiary-care hospital were asked to participate in the Chest Pain Registry, a prospective, and observational study. Among 370 patients included in the

Registry, a subgroup of 146 underwent coronary calcium scoring based on the following entry criteria: at least 18 years of age, no implanted coronary stents, no coronary artery bypass grafts and willingness to sign a written informed consent. Of those who did not undergo calcium scoring, 71 had coronary stents, 24 had previous coronary artery bypass graft surgery and 129 refused radiation exposure.

Coronary calcium score

All patients were imaged with a commercially available 64-multidetector CT scanner (Aquilion, Toshiba Medical Systems, Tochigi, Japan). The scans were obtained using slice collimation 4 x 3.0 mm, 300 mA, 120 kV and gantry rotation time 0.4 s. Calcium scoring using the Agatston method was performed in remote workstations (Vitrea2 Version 3.0.9.1, Vital Images, Minnetonka, Minnesota). A sole radiologist unaware of patient's characteristics or presence of obstructive CAD scored all scans. Calcium scoring was performed within a week of other noninvasive imaging and invasive coronary angiography.

Obstructive CAD

Patients underwent invasive coronary angiography or a provocative noninvasive test (perfusion magnetic resonance imaging or nuclear single-photon emission computed tomography) at the discretion of the cardiologist. Invasive angiography was performed whenever the ischemic defect size was ≥ 5% of the left ventricular myocardium on noninvasive imaging. The readers of invasive and noninvasive images were blinded to the calcium score. Obstructive CAD was defined as luminal stenosis ≥ 70% by invasive angiography. A normal or mildly abnormal noninvasive test, defined as ischemic defect size < 5% of left ventricular myocardium, was interpreted as negative for obstructive CAD and no further testing was required. Patients were classified as not having obstructive CAD if one of the following dominant diagnosis was confirmed by imaging: pericarditis, pulmonary embolism or aortic dissection.

Pretest probability of obstructive CAD

The entire cohort of 370 patients was used to generate a multivariate clinical model for predicting pretest probability of CAD based on admission data. Three sets of variables were studied as potential predictors for obstructive CAD: 13 variables of medical history, 14 characteristics of chest discomfort and 8 variables related to physical examination and laboratory tests. These included ischemia on electrocardiogram, positive troponin, physical and radiographic signs of left ventricular failure, 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. All serum specimens were collected at presentation to the emergency room. All variables, in case of normal distribution, were presented through mean and standard deviation and, in the case of non-normal distribution, were presented through median and interquartile range. By multivariate logistic regression analysis, variables remained independent predictors: age, male gender, chest pain relief with nitrate, signs of heart failure, ischemia on electrocardiogram and positive troponin. For discrimination of obstructive CAD, the area under the curve of this clinical model was 0.80 [95% confidence interval (CI) = 0.75–0.84] and calibration by Hosmer-Lemeshow's test led to a $\chi 2 = 1.95$ ($p = 0.98$). This was the reference model used to evaluate the incremental value of the calcium score and to define the pretest probability for the sensitivity analysis of the predictive value of calcium scoring.

Statistical analysis

The sample size was calculated to provide a maximum error of ± 12% for 95% CI of sensitivity and specificity. Assuming a sensitivity of 90% and a specificity of 50%, 25 patients with and 67 patients without obstructive CAD were required to provide this estimate precision. Anticipating a CAD prevalence of 50%, at least 134 patients had to be enrolled in the study.

A negative calcium score was defined as zero, while a positive score was defined as anything other than zero. Based on this predefined cut-off, sensitivity, specificity, positive and negative likelihood ratios were described as measures of accuracy with 95% CI. The incremental predictive value of zero calcium score over the pretest probability model was tested by comparing the area under the curve of this model versus the area under the curve of a second model containing clinical and binary calcium score information. This second model was derived from logistic regression analysis.

The accuracy of zero calcium score to reclassify the clinical model pretest probability to < 10% was evaluated by Pencina's method of net reclassification improvement (NRI).[4]

Finally, negative predictive values and number needed to test for discharging one additional patient were reported in the entire group and in subgroups of pretest probability < 50% or ≥ 50%. The same analysis was done in the groups of normal electrocardiogram and troponin versus either one of these tests abnormal.

The SPSS Statistical Software (Version 21.0, SPSS Inc., Chicago, Illinois, USA) was used for data analysis, and final statistical significance was defined as $p < 0.05$ in all cases.

Results

Sample characteristics

One hundred forty-six patients with acute chest pain were studied, aged 59 ± 16 years, 56% males. Ischemic electrocardiographic changes were present in 56% of patients, 42% had positive troponin and 71% had at least one of these two tests abnormal. Obstructive CAD was present in 60 patients (prevalence of 41%) and all cases were confirmed by invasive angiography. Among 86 patients without obstructive CAD, 28 had invasive angiography and 58 were deemed not to have obstructive CAD by noninvasive imaging only. The final diagnosis in patients without CAD was pericarditis (8), dyspepsia (4), muscular pain, pneumonia and pulmonary embolism (one each). The remaining 71 patients had chest pain of unclear etiology. Clinical and laboratory characteristics are depicted in Table 1.

Table 1 – Clinical and laboratory characteristics

Sample Size	146
Age (years)	59 ± 16
Male Gender	82 (56%)
Ischemic EKG	81 (55%)
Positive troponin	61 (42%)
CAD history	35 (24%)
Diabetes	43 (29%)
Systemic hypertension	108 (74%)
Smoking	19 (13%)
Total cholesterol (mg/dl)	183 ± 59
LDL-cholesterol (mg/dl)	112 ± 64
HDL-cholesterol (mg/dl)	43 ± 15
Triglycerides (mg/dl)	165 ± 152
Creatinine (mg/dl)	1.08 ± 0.85
Calcium score (Agatston)	66 (0 – 722)
Zero calcium score	51 (35%)
Obstructive CAD	60 (41%)

EKG: electrocardiogram; CAD: coronary artery disease; Ischemic EKG: T-wave inversion or ST-segment deviation. Positive troponin: troponin change to a level beyond the 99th percentile. Calcium score is described as median and interquartile range, and the remaining numeric variables as mean and standard deviation.

Diagnostic accuracy of zero calcium score

Calcium score had a non-normal distribution, with a median of 66 (interquartile range = 0–722). Zero calcium score was seen in 35% of patients. Among 60 patients with obstructive CAD, 55 had calcium score > zero, yielding a sensitivity of 92% (95% CI = 81%–97%). Among 86 patients without obstructive CAD, 46 had zero calcium score, specificity of 54% (95% CI = 43%–64%). The observed accuracy yielded a good negative likelihood ratio of 0.16 (95% CI = 0.07–0.37) and a poor positive likelihood ratio of 1.97 (95% CI = 1.55–2.50).

Incremental diagnostic value of zero calcium score

After adjustment for pretest probability based on the clinical model (OR = 1.04; 95% CI = 1.02–1.06; p < 0.001), zero calcium score was independently associated with absent CAD (OR = 0.12; 95% IC = 0.04–0.34; p < 0.001). The prediction based on the clinical model had an area under the curve of 0.76 (95% CI = 0.67–0.83), which improved to 0.82 (95% CI = 0.75–0.88; p = 0.006) when calcium scoring was added to the curve (Figure 1).

Net reclassification by calcium score

The target for theoretical discharge based on the clinical model with no further testing (CAD probability < 10%) was present in only 8.2% of patients. Upon inclusion of zero calcium score in the model, 23% of patients were classified as < 10% probability of CAD. Twenty-six of 86 patients without obstructive CAD were correctly reclassified to < 10% probability and 3 were incorrectly reclassified to ≥ 10%. Thus, the NRI for individuals with no CAD was 0.23 (p = 0.0001). Among 60 patients with CAD, none were correctly up-reclassified and 2 were incorrectly reclassified to < 10% probability.

The NRI of patients with CAD was –0.03 (p = 0.16). As a result, the overall NRI was 0.20 (p = 0.0018), indicating a reasonable proportion of patients with no CAD reclassified to < 10% probability (Table 2).

Sensitivity analysis of negative predictive values by pretest probability

The overall negative predictive value of zero calcium score for obstructive CAD was 90% (95% CI = 78%–96%). Ninety-two patients (63%) had a pretest probability < 50% with a disease prevalence of 27%. In this group, 43 patients had a zero calcium score, with a negative predictive value of 95% (95% CI = 83%–99%). Since 47% of the patients had zero calcium score, the number needed to test for obtaining one additional discharge (< 10% probability) was 2.1. In this group, calcium score had a high yield for a negative result.

On the other hand, 54 patients (37%) had a pretest probability of CAD ≥ 50% with a disease prevalence of 65%. In this group, only 8 patients (15%) had a zero calcium score, with a negative predictive value of 63% (95% CI = 23%–90%). In this group of high pretest probability, zero calcium score had a low yield for a negative result (Table 3).

Forty-two patients had normal electrocardiogram and troponin with CAD prevalence of 21%. Half of them had zero calcium score (number needed to test = 2), providing a negative predictive value of 100%. Of the remaining patients with either ischemic changes on electrocardiogram or positive troponin (CAD prevalence of 49%), 41% had zero calcium score but the negative predictive value was only 83% (95% CI = 70%–97%) (Table 3).

Discussion

The present study extends the validation of zero calcium score as a filter for further diagnostic testing to patients with acute chest pain admitted to the CCU. It is important to emphasize that our target population are individuals with an intermediate pretest probability of CAD, having undergone an initial clinical judgment in the emergency room. Usually, these patients perform provocative tests of coronary ischemia or CT coronary angiography. In this context, the calcium score could be used as a filter to perform more complex tests. The relatively high prevalence of coronary disease in this setting raises concern regarding the negative predictive value of the test. In fact, we found that 41% of patients had obstructive CAD. Since the prevalence of zero calcium score was 35%, roughly 3 patients had to undergo calcium scoring to avoid one additional diagnostic test. In addition, calcium score increased the accuracy of a clinical model of pretest probability by improving the area under the curve and net reclassifying 23% of patients from high to low probability of disease.

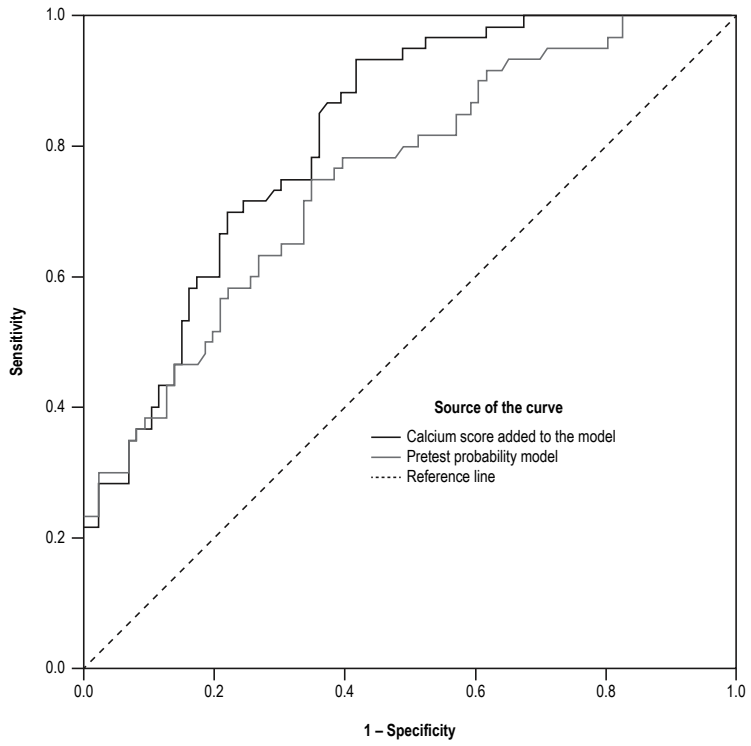

Figure 1 – *Incremental value of zero calcium score added to the reference model of pretest probability. The area under the curve increased from 0.76 to 0.82 (p = 0.006).*

Table 2 – Net reclassification (NRI) of low or high probability (cut-off 10%) according to zero calcium score.

CAD Status	Probability Threshold (10%)	Reclassification by Ca Score		NRI	Z Score	p Value
		Low	High			
Present = 60	Low = 0	--	--	- 0.03	1.41	0.16
	High = 60	2	58			
Absent = 86	Low = 12	9	3	+ 0.23	3.92	< 0.001
	High = 74	23	51			
			Global NRI	+ 0.20	3.12	0.002

CAD: coronary artery disease.

Table 3 – Negative predictive value and number needed to test for one additional discharge, according to pretest probability group and electrocardiogram/troponin results

	Sample	CAD Prevalence	Negative Predictive Value	Number Needed to Test
Pretest Probability < 50%	92	27%	95%	2.1
Pretest Probability ≥ 50%	54	65%	63%	6.6
Normal EKG and troponin	42	21%	100%	2.0
Positive EKG or troponin	104	49%	83%	2.4

Number needed to test: to avoid one further complex test. EKG: electrocardiogram; CAD: coronary artery disease.

We performed a sensitivity analysis to identify the subgroup better suited to calcium scoring according to the pretest probability of CAD. We suggest that if the pretest probability is less than 50%, a zero calcium score has a 95% negative predictive value. For every two patients tested, one would be discharged without the need for further testing. In the subgroup of normal electrocardiogram and negative troponin, the negative predictive value was 100%. Despite a general low case-fatality rate, there were no deaths in the group of zero calcium score.

Clinical interpretation of our findings suggests that there is a role for calcium scoring as a filter for other diagnostic tests in patients admitted to the CCU, provided the pretest probability for CAD is not high. However, our study brings initial data that need to be better tested in practice. A well-established filter for a potentially serious condition is the use of D-dimer in patients with low-to-intermediate probability of pulmonary embolism. D-dimer has a negative likelihood ratio of 0.13,[5] which is very similar to what we found in patients with suspected CAD and zero calcium score. Patients with low-to-intermediate probability of pulmonary embolism and a negative D-dimer comprise 24% of patients with suspected pulmonary embolism.[5] In our study, patients with CAD probability < 50% and zero calcium score comprised 29% of patients. The similarities between D-dimer (as a rule-out test for pulmonary embolism) and calcium scoring (as a rule-out test for CAD) highlight the potential of this approach in acute chest pain patients. One might be tempted to go directly to CT coronary angiography, instead of filtering it with a calcium score. While CT angiography is an option in some imaging centers, a few points should be addressed. First, CT angiography is not available 24/7 in most hospitals because it requires medical expertise for interpretation. The binary interpretation of coronary calcium on CT is simple and demands minimal training.

Second, there are technical difficulties and contraindications to CT angiography, which in the ROMICAT Study prevented this test in 1270 of 1869 (68%) patients with acute chest pain.[6] Third, despite a much better positive likelihood ratio of CT angiography, its negative likelihood ratio is very similar to zero calcium score. In the CORE-64 trial, the negative likelihood ratio of CT angiography was 0.19.[7] A reasonable approach would be to discharge patients with pretest probability < 50% and a zero calcium score. Patients with a positive calcium score would undergo CT angiography. This algorithm would not only reduce the time spent in the hospital to rule-out CAD, but also reduce costs and complications from more complex tests.

The diagnostic performance of zero calcium score described in the present study is in line with previous articles that reported good negative likelihood ratios and negative predictive values in emergency department patients.[8-12] However, their good negative predictive values were in part the result of a low pretest probability of disease. Our uniqueness relies on the study of patients admitted to the CCU of a tertiary-care hospital with a much higher prevalence of disease. We demonstrated a reasonable negative predictive value in this population, extending the findings already reported in emergency room patients to the CCU. Zero calcium score can be used to exclude obstructive CAD in patients with low-to-intermediate (< 50%) probability based on sensitivity analysis.

Limitation

The limitation of our work is a relatively small sample size, which provided only moderate precision according to our confidence intervals. Therefore, future studies should confirm our point-estimates of accuracy and predictive values. From the point of view of reliability of the scientific data, ideally, all patients should have undergone invasive coronary angiography. All patients labeled as positive for obstructive CAD had confirmation by invasive angiography, but most labeled as negative for obstructive disease had only non-invasive imaging.

Conclusion

In conclusion, our study suggests that the use of zero calcium score substantially reduces pretest probability of obstructive CAD in patients admitted to the CCU with acute chest pain.

Contribuição dos autores

Conception and design of the research: Correia LCL, Esteves FP, Carvalhal M, Souza TMB, Ferreira F, Noya-Rabelo M; Acquisition of data: Carvalhal M, Souza TMB, Sá N, Correia VCA, Alexandre FKB, Lopes F; Analysis and interpretation of the data: Correia LCL, Esteves FP, Carvalhal M, Souza TMB, Correia VCA, Ferreira F; Statistical analysis: Sá N, Correia VCA, Alexandre FKB, Lopes F; Writing of the manuscript: Correia LCL, Esteves FP, Carvalhal M, Souza TMB, Sá N, Correia VCA, Alexandre FKB, Lopes F, Ferreira F; Critical revision of the manuscript for intellectual content: Correia LCL, Esteves FP, Carvalhal M, Souza TMB, Noya-Rabelo M.

Potential Conflict of Interest

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

Sources of Funding

There were no external funding sources for this study.

Study Association

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

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Persistent Depressive Symptoms are Independent Predictors of Low-Grade Inflammation Onset Among Healthy Individuals

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Abstract

Background: Depressive symptoms are independently associated with an increased risk of cardiovascular disease (CVD) among individuals with non-diagnosed CVD. The mechanisms underlying this association, however, remain unclear. Inflammation has been indicated as a possible mechanistic link between depression and CVD.

Objectives: This study evaluated the association between persistent depressive symptoms and the onset of low-grade inflammation.

Methods: From a database of 1,508 young (mean age: 41 years) individuals with no CVD diagnosis who underwent at least two routine health evaluations, 134 had persistent depressive symptoms (Beck Depression Inventory – BDI ≥ 10, BDI+) and 1,374 had negative symptoms at both time points (BDI-). All participants had been submitted to repeated clinical and laboratory evaluations at a regular follow-up with an average of 26 months from baseline. Low-grade inflammation was defined as plasma high-sensitivity C-Reactive Protein (CRP) concentrations > 3 mg/L. The outcome was the incidence of low-grade inflammation evaluated by the time of the second clinical evaluation.

Results: The incidence of low-grade inflammation was more frequently observed in the BDI+ group compared to the BDI- group (20.9% vs. 11.4%; p = 0.001). After adjusting for sex, age, waist circumference, body mass index, levels of physical activity, smoking, and prevalence of metabolic syndrome, persistent depressive symptoms remained an independent predictor of low-grade inflammation onset (OR = 1.76; 95% CI: 1.03–3.02; p = 0.04).

Conclusions: Persistent depressive symptoms were independently associated with low-grade inflammation onset among healthy individuals. (Arq Bras Cardiol. 2017; 109(2):103-109)

Keywords: Depression; Cardiovascular Diseases; Inflammation; Patient Selection.

Introduction

Depression is a prevalent disease that leads to considerable global burden and disabilities.[1] The relationship between depressive symptoms and cardiovascular disease (CVD) has been well documented as it almost doubles the risk of developing coronary heart disease.[2,3] Although traditional cardiovascular risk factors tend to cluster in depressed patients as a consequence of an unhealthy lifestyle (e.g., poor diet, lack of exercise), these unhealthy behaviors do not adequately account for the impact of depression on CVD.

Inflammation may act as a possible mechanistic link between depressive symptoms and CVD. Elevation in plasma high-sensitivity C-reactive protein (CRP) is a marker of a low-grade inflammatory state that has been associated with the incidence of CVD[4] and all-cause mortality.[5] Studies have reported an association between depressive symptoms and plasma CRP elevation in cross-sectional analyses.[6-8]

The aim of this study was to assess the association of persistent depressive symptoms with a low-grade inflammatory process, taking into account potential explanatory factors such as physical activity, obesity and sex, in a group of young, healthy individuals. As persistent depression symptoms would be independent predictors of low-grade inflammation among healthy and young individuals, this condition should be included in routine health evaluations to avoid future cardiovascular events.

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Manuscript received September 19, 2016, revised manuscript March 09, 2017, accepted March 09, 2017

DOI: 10.5935/abc.20170080

Methods

Participants

From a dataset of 34,581 subjects, 4,222 individuals with at least two consecutive yearly exams were selected. All individuals had no background of CVD according to

self-report. Of those, 1,508 individuals who did not have signs of low-grade inflammation, defined as CRP values < 3 mg/L at baseline (time point 1), were included. Depressive symptom presence was defined as Beck Depression Inventory (BDI) ≥ 10 scale points assessed at times 1 and 2. Subjects were divided into those who had (BDI+, n = 134, 8.8%) or not (BDI-, n = 1,374, 91.2%) persistent depressive symptoms. Exclusion criteria were the presence of chronic or acute inflammatory (defined as CRP > 10 mg/L at either time points) or previous cardiovascular diseases (defined as myocardial infarction, angina, coronary revascularization, stroke, peripheral artery disease or heart failure) according to self-declaration of health conditions.

Independent Variables

As previously described, subjects were submitted to a routine mandatory health evaluation paid by their employers.[9] All conditions included in the evaluations potentially associated with future low-grade inflammation, such as clinical background, smoking, physical activity, laboratory analyses (cholesterol, triglycerides, glucose, uric acid, creatinine, liver transaminases) and the presence of hepatic steatosis were considered as independent variables. Demographics, medical history and medication use were routinely recorded. Smoking status was categorized as current smoker (at least 1 cigarette during the last 30 days) versus current nonsmoker. The International Physical Activity Questionnaire (IPAQ)[10] was used to assess physical activity level. Blood pressure was measured 3 times at the sitting position with an aneroid sphygmomanometer according to the standard method recommended by the American Heart Association.[11] Hypertension was defined according to current guidelines.[12,13] Height (meter) and weight (kilogram) were measured with a standard physician's scale and a stadiometer to calculate body mass index (BMI, kg/m^2). Waist circumference was recorded at the smallest diameter between the iliac crest and the costal margin with a plastic anthropometric tape held parallel to the ground.

Blood samples were collected after at least 12 hours fasting and processed at the Central Laboratory of the Preventive Medicine Unit of the Hospital Israelita Albert Einstein, Sao Paulo, Brazil. Total cholesterol, triglycerides (TG), HDL-cholesterol, glucose, uric acid, creatinine, and liver transaminases were determined using standardized automated laboratory tests (Vitros 5600, Johnson & Johnson Orthoclinical Diagnostics). When TG < 400 mg/dL, LDL-cholesterol was calculated by the Friedwald formula. When TG ≥ 400 mg/dL, LDL-cholesterol levels were measured directly. High-sensitivity CRP concentrations were determined by immunonephelometry (Dade-Behring). Hepatic steatosis was identified by the presence of an ultrasound pattern of bright liver, with evident contrast between hepatic and renal parenchyma as previously described.[14] Excess body weight was defined by the presence of a BMI > 25 kg/m^2, while abdominal obesity was characterized by elevated waist circumference (> 88 cm in women and > 102 cm in men). Metabolic syndrome was defined by the joint AHA/IDF consensus.[15]

Depression symptoms assessment

The BDI was used for depression symptom assessment[16] and repeated each time individuals underwent a new check-up survey. In brief, BDI is a 21-item self-administered scale with four alternative statements for each item, scoring from 0 to 3 points and a maximum score of 63 points. Similarly to other studies, scores ≥10 points were suggestive of depression (BDI+), with higher points indicative of increasing depression severity.[17,18] Those with scores < 10 were not considered with depressive symptoms (BDI-).

Outcome

The outcome was the incidence of new cases of inflammation according to onset of elevated CRP concentrations.

IRB approval

The Institutional Human Research Committee approved this study and all participants provided written informed consent to allow for the use of their medical information, as outlined by the 1975 Helsinki Declaration.

Statistical analysis

Continuous data with normal distribution are expressed as mean and standard deviation. Continuous data with non-normal distribution are represented as median and interquartile range. For normality hypothesis, the Kolmogorov-Smirnov test was adopted. Categorical data are expressed by percentage and comparison was made by the chi- square test. For normally distributed continuous data, Student *t* test was adopted. Non-normally data are analyzed by Mann-Whitney test. A BDI score ≥ 10 points was adopted to identify individuals with significant depressive symptoms.[18] CRP was considered as a dichotomous variable and results > 3 mg/L were considered positive for low-grade inflammation and cardiovascular risk.[19] The chi-square model was used to analyze the association between depressive symptoms and CRP. Logistic regression was used to determine the effect of depressive symptoms on low-grade inflammation after adjusting for potential confounding variables, such as age, sex, BMI, blood pressure, total cholesterol, smoking, diabetes, hepatic steatosis, physical activity and metabolic syndrome. Statistical significance was inferred at a two-tailed $p < 0.05$. All analyses were performed using SPSS v 20.0 (SPSS, Inc, Armonk, NY, USA).

Results

This was a predominantly young, Caucasian, male population with low calculated risk of CVD. The mean (standard deviation) follow-up time was 26 ± 10 months. Table 1 shows the clinical and laboratory characteristics of individuals presenting with and without depressive symptoms (BDI+ and BDI-) at time point 1. There was a greater prevalence of females (30.6% vs 18.4%; $p = 0.001$) and physical inactivity (25.5% vs 16.2%; $p = 0.015$) in the BDI+ group relative to the non-depressed group. Also, those with

Table 1 – Clinical and laboratory characteristics of subjects presenting (BDI+) or not (BDI-) persistent depressive symptoms at baseline

	No depressive symptoms (BDI-) n = 1,374	Depressive symptoms (BDI+) n = 134	p value
Age, mean (SD)	41.4 (8)	40.4 (6.5)	0.081*
Sex (%female)	18.4%	30.6%	0.001¥
Smoking (%)	6.3%	8.9%	0.245¥
Hypertension (%)	9.5%	7.5%	0.432¥
Diabetes (%)	2.3%	2,2%	0.999¥
BMI (kg/m^2)	26.1 (3.6)	26.6 (3.7)	0.114*
Waist circumference, mean (SD)	92.3 (11.1)	92.4 (12.2)	0.921*
Metabolic Syndrome (%)	14.7	20	0.111¥
Physical inactivity (%)	16.2	25.5	0.015¥
Lipid-lowering agents (%)	11.9	10.4	0.627¥
Oral antidiabetic drug or insulin use (%)	3.4	6.7	0.087¥
Glucose, mean (SD)	88 (12.8)	89 (14)	0.544*
LDL-C, mean (SD)	126 (34.4)	127 (39.8)	0.749*
HDL-C, mean (SD)	49 (12.7)	49 (12.5)	0.607*
Total cholesterol, mean (SD)	201 (37.3)	206 (42.5)	0.177*
Triglycerides, median (IQR)	108 (79; 154)	129 (92; 208)	0.003£
Creatinine, mean (SD)	0.87 (0.19)	0.82 (0.23)	0.009*
CRP, median (IQR)	1.00 (0.5;1.8)	1.15 (0.5; 2.3)	0.041£
Steatosis (%)	34.4	44.6	0.020¥

*Data were expressed as mean (SD) or median (IQR) for normal and non-normal data respectively. IQR: interquartile ranges; SD: standard deviation; age- in years; body mass index- BMI in kg/m^2; waist circumference- in cm; plasma lipids, glucose and creatinine- in mg/dL; C-reactive protein- in mg/L; * Student t test; ¥ Chi-square test, £ Mann-Whitney test.*

depressive symptoms had higher plasma TG (p = 0.008) and lower plasma levels of creatinine (p = 0.009) relative to those with no depressive symptoms. Of importance, no difference in age, BMI, waist circumference, smoking, metabolic syndrome prevalence and CRP levels was observed between the groups.

Table 2 shows the clinical and laboratory characteristics of subjects presenting with and without low-grade inflammation at time point 2. Low-grade inflammation was detected respectively in 20.9% and 11.4% of participants in the BDI+ and BDI- groups (OR = 2.05; 95% CI: 1.31–3.21; p < 0.001). In bivariate analysis, physical activity (p = 0.049), depressive symptoms (p < 0.001), metabolic syndrome (p = 0.017), waist circumference (p < 0.001), and BMI (p < 0.001) were also associated with low-grade inflammation.

Confounding factors and results obtained in the bivariate analysis were included in the multivariate analysis. In this analysis, the association of depressive symptoms and low-grade inflammation was adjusted for age, sex, waist circumference, BMI, levels of physical activity, smoking, presence of hepatic steatosis, and metabolic syndrome prevalence. New cases of inflammation were associated with depressive symptoms regardless those variables mentioned above (OR = 1.76; 95% CI: 1.03–3.02; p = 0.04). The statistical power to infer a difference on BDI+ group compared to BDI- was 56.5%, with a two-sided level of significance of 0.05.

Discussion

A positive association between persistence of depressive symptoms and low-grade inflammation after a 2-year average follow-up was observed. Findings were robust even after adjusting for risk factors associated with elevation in plasma CRP levels, such as abdominal obesity and metabolic syndrome.

Atherosclerosis, the main pathological substrate of CVD, is a chronic degenerative disorder with a low-grade inflammatory component. Persistent depressive symptoms over at least 2 years have been prospectively associated with coronary artery calcification detected by computed tomography,[20] a surrogate marker of atherosclerosis burden and a robust marker of cardiovascular event risk.[21] Robust evidence from prospective studies shows a clear and independent association of elevated CRP levels with cardiovascular events and mortality.[4,5] Indeed, increased CRP levels have been shown to add modest, but significant ability to improve risk reclassification over traditional risk markers in asymptomatic individuals.[22] The results of this study suggest that depressive symptoms are associated not only with atherosclerotic plaque burden, as previously shown,[20,23] but also with the low-grade inflammatory component of atherosclerosis. Therefore, detection of depressive symptoms might have prognostic information for CVD risk evaluation.

This is one of the largest longitudinal studies examining persistence depressive symptoms and subsequent

Table 2 – Bivariate clinical and laboratory characteristics associated with the presence or not of low-grade inflammation (CRP > 3 mg/L) at exam time point 2

Parameter	CRP ≤ 3	CRP > 3 mg/L	p value
Age, mean (SD)	41.4 (7.9)	40.8 (7.6)	0.395*
Sex, n (%)			
Female	248 (84.7)	45 (15.3)	0.077¥
Male	1074 (88.5)	140 (11.5)	
Smoking, n (%)			
No	1234 (87.8)	172 (12.2)	0.793¥
Yes	86 (86.9)	13 (13.1)	
Physical activity, n (%)			
Yes	876 (88.8)	111 (11.2)	0.049¥
No	171 (83.8)	33 (16.2)	
Depressive symptoms, n (%)			
BDI+	1217 (88.6)	157 (11.4)	0.001¥
BDI-	106 (79.1)	28 (20.9)	
Metabolic syndrome, n (%)			
No	1097 (88.6)	141 (11.4)	0.017¥
Yes	184 (82.9)	38 (17.1)	
Hypertension, n (%)			
No	1197 (87.6)	170 (12.4)	0.536¥
Yes	126 (89.4)	15 (10.6)	
Diabetes, n (%)			
No	1293 (87.7)	181 (12.3)	0.999¥
Yes	30 (88.2)	4 (11.8)	
Hepatic steatosis, n (%)			
No	839 (88.7)	107 (11.3)	0.115¥
Yes	443 (85.9)	73 (14.1)	
Lipid-lowering drugs, n (%)			
No	1166 (87.6)	165 (12.4)	0.676¥
Yes	157 (88.7)	20 (11.3)	
Antidiabetic drugs or insulin, n (%)			
No	1277 (87.9)	175 (12.1)	0.194¥
Yes	46 (82.1)	10 (17.9)	
BMI, mean (SD)	25.9 (3.5)	27.4 (3.9)	< 0.001*
Waist circumference, mean (SD)	91.9 (11.1)	95.2 (11.2)	< 0.001*
Total cholesterol, mean (SD)	201.3 (37.6)	204.4 (39.1)	0.291*
LDL-C, mean (SD)	126 (34.6)	128.5 (36.6)	0.372*
HDL-C, mean (SD)	49.3 (12.7)	48.4 (12)	0.368*
Triglycerides, median (IQR)	108 (80;154)	118 (95;174)	0.019£
Glucose, mean (SD)	87.9 (12.8)	88.3 (13.4)	0.677*
Creatinine, mean (SD)	0.87 (0.19)	0.86 (0.18)	0.692*

*Age: in years; body mass index: BMI in kg/m^2; waist circumference- in cm; plasma lipids, glucose and creatinine- in mg/dL; C-reactive protein- in mg/L; IQR: interquartile range; SD: standard deviation; * Student t test; ¥ Chi: square test, £ Mann-Whitney test.*

inflammation onset in a young non-CVD population. The strength of the current study is the comprehensive clinical, laboratory and behavioral factors that may be associated with the depression-inflammation relationship. These factors include physical inactivity, obesity and smoking. Another strength of this study was the enrollment of a poorly studied population, composed by subjects without previous CVD. Despite this sample of non-CVD individuals, persistent depressive symptoms were associated with subsequent inflammation. This finding highlights the importance of depression on cardiovascular primary prevention in a young population.

As previously described, depressive symptoms were associated with clinical characteristics associated with elevated plasma CRP levels, such as female sex and increased adiposity.[9,22] However, there is controversy if female sex may be associated with the process of low-grade inflammation as a result of depression.[24-26] While one study has found depressed white women to be more susceptible to inflammation,[24] other studies[25,26] have found an association between depression and inflammation only in men. In contrast, in this study the association of depressive symptoms and elevated CRP levels persisted even after adjustment for sex.

Some unhealthy behaviors are associated with depression and may interfere with the inflammation-depression relationship. In a cohort of 667 outpatients with established coronary heart disease from the Heart and Soul Study, depressive symptoms predicted inflammation after 5 years of follow-up.[27] However, this association was no longer significant after adjustment for physical inactivity, smoking and higher BMI, which suggests that other behavioral factors may be important modulators of the depression-inflammation process. The same inference was reached by the authors of a prospective study of 289 patients with atrial fibrillation,[28] in which obesity was the single strongest predictor of inflammation, eliminating the association between depression and inflammation in multivariate analyses. In contrast, in a group of 3,609 men and women with a mean age of 60.5 from the English Longitudinal Study of Ageing, Hamer et al. have found that baseline depression was associated with further inflammation 2 years later, even after taking into account other behavioral factors.[29] That study corroborates our own results, in which a persistent relationship between depressive symptoms and inflammation was observed after adjustment for age, sex, smoking status, physical inactivity, metabolic syndrome, fatty liver and excess body weight. Indeed, the adjustment for fatty liver is very important since we had previously shown a strong and independent association of hepatic steatosis, a highly active visceral fat depot, with elevated plasma CRP levels independently of markers of obesity in apparently healthy subjects.[9] Another point of interest in the current study was that most individuals had mild depressive symptoms. However, even this burden of depression symptoms may lead to future low-grade inflammation.

Limitations

The findings were limited to the inclusion of a predominantly Caucasian and young population. However, we did observe that the effects were robust across both sexes. Patients with depressive symptoms might have a lower adherence to subsequent exams, increasing the dropout of BDI+ subjects after the first time set. This lack of adherence during follow-up would have an impact on the statistical power of the study analysis. Finally, plasma CRP levels were measured only twice in this study, however in the absence of clear inflammatory diseases, high-sensitivity CRP assays have shown to have a good reproducibility and low variability.[30]

Conclusions

In summary, our results demonstrate that persistent depressive symptoms are an independent predictor of future low-grade inflammation onset in this population. These findings suggest that depressive symptoms should be considered among important factors contributing to subsequent health problems and should therefore be screened, even in apparently healthy individuals undergoing routine healthcare exams.

Author contributions

Conception and design of the research: Mello Franco FG, Laurinavicius AG; Acquisition of data: Mello Franco FG; Analysis and interpretation of the data: Mello Franco FG, Laurinavicius AG, Lotufo PA, Conceição RD, Morita F, Katz M, Wajngarten M, Carvalho JAM; Statistical analysis: Mello Franco FG; Writing of the manuscript: Mello Franco FG; Critical revision of the manuscript for intellectual content: Laurinavicius AG, Lotufo PA, Conceição RD, Morita F, Katz M, Wajngarten M, Carvalho JAM, Bosworth HB, Santos RD.

Potential Conflict of Interest

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

Sources of Funding

There were no external funding sources for this study.

Study Association

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

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Peripheral Vascular Resistance Impairment during Isometric Physical Exercise in Normotensive Offspring of Hypertensive Parents

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Abstract

Background: A family history of hypertension is associated with vascular and autonomic abnormalities, as well as an impaired neurohemodynamic response to exercise.

Objective: To test the hypothesis that normotensive individuals with a family history of hypertension present an impaired peripheral vascular resistance response to exercise.

Methods: The study included 37 normotensive volunteers of both sexes who were sedentary, eutrophic, and nonsmokers, comprising 23 with (FH+; 24 ± 3 years) and 14 without (FH-; 27 ± 5 years) a family history of hypertension. Blood pressure, heart rate (DIXTAL®), forearm blood flow (Hokanson®), and peripheral vascular resistance were simultaneously measured for 3 minutes during rest and, subsequently, for 3 minutes during an isometric exercise at 30% of maximal voluntary contraction (Jamar®).

Results: At rest, the FH+ and FH- groups present similar mean blood pressure (83 ± 7 versus 83 ± 5 mmHg, p = 0.96), heart rate (69 ± 8 bpm versus 66 ± 7 bpm, p = 0.18), forearm blood flow (3 ± 1 mL/min/100 mL versus 2.7 ± 1 mL/min/100 mL, p = 0.16), and peripheral vascular resistance (30 ± 9 units versus 34±9 units, p = 0.21), respectively. Both groups showed a significant and similar increase in mean blood pressure (Δ = 15 ± 7 mmHg versus 14 ± 7 mmHg, p = 0.86), heart rate (Δ = 12 ± 8 bpm versus 13 ± 7 bpm, p = 0.86), and forearm blood flow (Δ = 0.8 ± 1.2 mL/min/100 mL versus 1.4 ± 1.1 mL/min/100 mL, p = 0.25), respectively, during exercise. However, individuals in the FH+ group showed no reduction in peripheral vascular resistance during exercise, which was observed in the FH- group (Δ = –0.4 ± 8.6 units versus –7.2 ± 6.3 units, p = 0.03).

Conclusion: Normotensive individuals with a family history of hypertension present an impaired peripheral vascular resistance response to exercise. (Arq Bras Cardiol. 2017; 109(2):110-116)

Keywords: Vascular Resistance; Exercise; Patient Selection; Hypertension; Heredity.

Introduction

Hypertension is an independent risk factor for cardiovascular morbidity and mortality, affecting approximately 32.5% of the Brazilian population.[1,2] Therefore, primary prevention has been recommended for individuals at increased risk for the development of hypertension, notably those with a positive family history of the disease.[1]

Studies have shown that normotensive individuals with a hypertensive father and/or mother have an increased risk of development of hypertension.[3-5] Wang et al.[5] investigated the impact of parental hypertension on the risk of development of hypertension among 1160 normotensive men during a follow-up of 54 years. In the study, the relative risks of development of hypertension were 1.5, 1.8, and 2.4 among individuals with only the mother, only the father, and both parents with hypertension, respectively, compared with individuals whose parents were normotensive.[5]

The reason for the increased susceptibility to the development of hypertension among offspring of hypertensive parents has not been fully elucidated. However, vascular[6,7] and autonomic abnormalities,[8-10] present in this population even before changes in blood pressure level, have been considered relevant in the emergence of this pathology.

In fact, studies have demonstrated an increased sympathetic nervous activity both at rest and during physical exercise in offspring of hypertensive parents when compared with those of normotensive parents.[9,10] Similarly, it has been observed that individuals with a family history of hypertension have reduced nitric oxide bioavailability[11,12] and increased endothelin levels[10,12,13] (endothelial-derived vasodilatory and vasoconstrictor substances, respectively).

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Manuscript received September 30, 2016, revised manuscript January 09, 2017, accepted January 18, 2017

DOI: 10.5935/abc.20170096

During exercise, increased muscle blood flow, which occurs in response to increased metabolic needs, is dependent on vasodilatory mechanisms, especially endothelial and metabolic factors produced in the exercised muscle, which overcome the vasoconstrictor mechanisms.[14] However, exacerbated vasoconstrictor mechanisms, such as sympathetic hyperactivity, may impair the vasodilatory mechanisms during exercise.[14] Thus, due to changes in endothelial cells and exacerbated muscular sympathetic nervous activity response present in normotensive individuals with a family history of hypertension, it is possible that the vasodilatory response in this population may be impaired during physical exercise. In this regard, the objective of this study was to test the hypothesis that normotensive individuals with a family history of hypertension have an impaired peripheral vascular resistance response during physical exercise.

Methods

Cohort

Based on a sample size calculation using a difference of 2.2 units in peripheral vascular resistance between the means of both groups with and without a family history of hypertension,[15] standard deviations of 2 units, 5% alpha and 20% beta errors, 14 subjects would be required in each group. Thus, the cohort comprised 37 volunteers, subdivided according to their family history of hypertension among parents in a group with a positive family history (FH+, n = 23) and another with a negative family history (FH-, n = 14).

A positive family history of hypertension was defined as a diagnosis of hypertension in the father, mother or both, evaluated with a questionnaire. A negative family history was defined as the absence of hypertension (blood pressure lower than 140 X 90 mmHg) or a diagnosis of cardiovascular disease in both parents, also evaluated with a questionnaire.

We adopted as the inclusion criteria age between 18 and 40 years, systolic blood pressure below 140 mmHg, diastolic blood pressure below 90 mmHg, and lack of involvement in regular physical exercise for at least 6 months before the study. We did not include individuals with obesity, cardiometabolic diseases, smokers, or receiving treatment with drugs that could interfere with the cardiovascular system, as well as individuals with any bone, muscle or articular impairment that could interfere with the execution of the exercise protocol. We also did not include individuals whose parents had a diagnosis of any other disease besides hypertension.

After prior clarification and agreement, all volunteers signed a free and informed consent form. This study was approved by the Research Ethics Committee in Human Research at HU/UFJF under the number 0119/2010.

Measures and procedures

Anthropometry

To measure the participants' body mass and height, we used, respectively, a scale with a precision of 0.1 kg and a stadiometer with a 0.5 cm accuracy coupled to the scale (Leader®, Brazil). At the time of the evaluation, the volunteers wore light clothes and were barefoot. Body mass index (BMI) was calculated by dividing the participants' body weight by their squared height (kg/m^2). Their waist circumference was measured using an inextensible measuring tape (Cescorf®) with a 0.1 cm accuracy. All variables above were assessed according to the criteria established by the American College of Sports Medicine.[16]

Blood pressure and heart rate

Blood pressure was measured in the right lower limb by the automatic oscillometric method, using a multiparametric monitor (DIXTAL®, model 2023).[17] The heart rate was recorded continuously by five skin electrodes positioned according to the standard lead supplied with the five-way cable of the multiparametric monitor.[17]

Forearm muscle blood flow and local peripheral vascular resistance

Muscle blood flow in the forearm was evaluated with the venous occlusion plethysmography technique using the plethysmograph Hokanson® (Bellevue, WA, USA). The volunteer was positioned in the supine position with the nondominant forearm elevated above the heart level to ensure adequate venous drainage.

A silastic tube filled with mercury, connected to a low-pressure transducer and to the plethysmograph, was placed around the volunteers' forearms, 5 cm away from the humeroradial joint. A cuff was placed around the volunteers' wrists and another cuff was placed around their upper arms. The wrist cuff was inflated to a suprasystolic pressure level (200 mmHg) 1 minute prior to the measurements and was maintained inflated during the entire procedure. At 15-second intervals, the arm cuff was inflated to a supravenous pressure level (60 mmHg) for 7 to 8 seconds, and then quickly deflated and maintained for the same period of time. This procedure totaled 4 cycles per minute.

The increased tension in the silastic tube reflected the increased forearm volume, indirectly reflecting the increased muscle blood flow in the forearm, and was reported as mL/min/100 mL. The forearm muscle blood flow wave sign was acquired in real time by a computer using the program Non Invasive Vascular Program 3.

The local peripheral vascular resistance was calculated by dividing the mean blood pressure by the muscle blood flow in the forearm, and reported as units.

Protocol of isometric physical exercise

To evaluate the responses in blood pressure, heart rate, and forearm muscle blood flow, we used a handgrip isometric exercise protocol using a dynamometer (Jamar®, São Paulo, Brazil). Initially, with the volunteer in the supine position, the maximal handgrip isometric strength was calculated as the mean of three attempts of maximal voluntary contraction (MVC) performed on the dominant limb. Hemodynamic measurements were subsequently performed during 3 minutes at rest and, subsequently, during 3 minutes of isometric exercise at 30% of the MVC.

Experimental protocol

The evaluations were performed in the afternoon at the *Hospital Universitário da Universidade Federal de Juiz de Fora* (HU-CAS). The volunteers were instructed not to consume alcohol and/or caffeine or perform vigorous physical activity within 24 hours prior to the evaluations, as well as to not ingest fatty foods on the day of the data collection.

During history taking, the volunteers answered questions related to clinical information about themselves and their parents and underwent anthropometric assessment. After the MVC evaluation, the volunteers rested for 10 minutes in the supine position. After that, we simultaneously recorded their heart rate, blood pressure, and forearm blood flow for 3 minutes during rest and, subsequently, for 3 minutes during the handgrip isometric exercise.

Statistical analysis

The data are presented as mean ± standard deviation of the mean or as median and interquartile range. To verify the normal distribution of the data, we used the Shapiro-Wilk test. We also verified the homogeneity of variance assumption by the Levene test. Possible differences related to the characteristics of the groups were verified using unpaired Student's *t* test for data with normal distribution, and homogeneity of variance and Mann-Whitney U test for variables violating these assumptions. Sex distribution between the groups is presented in absolute values and percentages. The chi-square test was used to verify a possible difference in sex distribution between the groups.

To test for possible differences between the groups in regards to hemodynamic responses (deltas) during the protocol, we used two-factor analysis of variance for repeated measures (2 X 4 factorial ANOVA, intra- and intersubject; group X exercise time). The Mauchly test was performed and the Greenhouse-Geisser correction was applied in cases in which the sphericity was violated. The main effects and the interaction (group X time) were analyzed with adjustment of the confidence interval by Bonferroni correction. To measure the "effect size," we adopted eta-squared statistics (η^2), with subsequent classification of its strength according to the values of 0.01, 0.06, and greater than 0.15, as small, medium, and large, respectively.[18]

All statistical analyses were performed using the software IBM SPSS® 20 for Windows (Chicago, IL, USA). The statistical significance was set at $p < 0.05$.

Results

The demographic and anthropometric characteristics of the FH+ and FH- groups are described in Table 1. No differences were observed in terms of age, sex, weight, height, BMI, waist circumference, and MVC between both groups. In addition, the groups were similar in regards to systolic blood pressure, diastolic blood pressure, mean blood pressure, heart rate, percentage change in muscle blood flow, and forearm vascular resistance (Table 2).

During exercise, the responses in systolic, diastolic, and mean blood pressure, as well as the heart rate and forearm muscle blood flow were similar between the groups. In contrast, during the 3 minutes of the exercise, the forearm vascular resistance decreased significantly only in the FH-group (Table 3). The strength of the effect of the interaction between the factors group and time for this variable was average ($\eta^2 = 0.10$).

Discussion

The finding of this study indicate that normotensive individuals with hypertensive parents, when compared with their peers with normotensive parents, have a vascular dysfunction characterized by the absence of a decrease in peripheral vascular resistance during physical exercise. It is worth noting that the groups comprised individuals who were sedentary, nonsmokers, and with similar demographic, anthropometric, and hemodynamic characteristics.

Although there are a large number of studies on cardiovascular changes in individuals with a family history of hypertension, we found only one study whose objective was to assess the vasodilatory capacity of this population during physical exercise. This study, conducted by Borghi et al.,[19] also demonstrated impaired vasodilatory capacity during a handgrip isometric physical exercise at moderate intensity in normotensive participants with a positive family history of hypertension. However, the study did not control for variables influencing the vascular behavior during physical exercise, such as smoking,

Table 1 – Demographic and anthropometric characteristics of the FH+ and FH- groups

Variables*	FH+ (n = 23)	FH- (n = 14)	p value
Age (years)	24 ± 3	27 ± 5	0.09
Male sex (%)	5 (21.7%)	7 (50.0%)	0.07
Weight (kg)	64 ± 11	69 ± 13	0.17
Height (m)	1.67 (1.57 – 1.77)	1.64 (1.47 – 1.81)	0.68
BMI (kg/m²)	23 ± 3	24 ± 3	0.24
Waist circumference (cm)	74 ± 9	79 ± 11	0.13
MVC (kgf)	35.4 ± 9.5	41.3 ± 11.4	0.10

Values: mean ± standard deviation of the mean for age, weight, BMI, waist circumference, and MVC; median and interquartile range for height; absolute value and percentage for the male sex; BMI: body mass index; MVC: maximum voluntary contraction.

Table 2 – Comparisons of hemodynamic variables at rest between the groups FH+ and FH-

Variables	FH+ (n = 23)	FH- (n = 14)	p value
SBP (mmHg)	122 ± 11	121 ± 8	0.69
DBP (mmHg)	64 ± 5	65 ± 5	0.72
MBP (mmHg)	83 ± 7	83 ± 5	0.96
HR (bpm)	69 ± 8	66 ± 7	0.18
MBF (mL/min/100 mL)	3.0 ± 0.9	2.7 ± 0.9	0.16
FVR (units)	30 ± 9	34 ± 9	0.21

Values: mean ± standard deviation of the mean; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; HR: heart rate; MBF: variation in forearm muscle blood flow; FVR: forearm vascular resistance.

Table 3 – Hemodynamic responses (absolute deltas) during isometric exercise

Variable	Isometric Exercise			F	Interaction effect	η^2
	1st min	2nd min	3rd min			
SBP (mmHg)						
Hypertension+	1 ± 4	16 ± 8*	16 ± 10*	0.201	0.703	0.006
Hypertension -	0 ± 4	7 ± 7*	15 ± 10*			
DBP (mmHg)						
Hypertension +	3 ± 3*	9 ± 6*	15 ± 8*	0.234	0.753	0.007
Hypertension -	3 ± 4*	8 ± 6*	14 ± 7*			
MBP (mmHg)						
Hypertension +	3 ± 3*	9 ± 5*	15 ± 7*	0.098	0.863	0.003
Hypertension -	2 ± 3*	8 ± 6*	14 ± 7*			
HR (bpm)						
Hypertension +	4 ± 5*	9 ± 6*	12 ± 8*	0.169	0.858	0.005
Hypertension -	5 ± 6*	10 ± 7*	13 ± 7*			
MBF (mL/min/100 mL)						
Hypertension +	0.5 ± 0.8	0.6 ± 1.0*	0.8 ± 1.2*	1.409	0.251	0.039
Hypertension -	0.8 ± 0.9*	1.2 ± 1.0*	1.4 ± 1.1*			
FVR (units)						
Hypertension +	-2.1 ± 4.6	-2.1 ± 5.0	-0.4 ± 8.6	3.777	0.030	0.97
Hypertension -	-7.2 ± 6.4*	-7.9 ± 5.0*	-7.2 ± 6.3*			

*Values: mean ± standard deviation of the mean; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; HR: heart rate; MBF: variation in forearm muscle blood flow; FVR: forearm vascular resistance; *Significant difference relative to resting ($p < 0.05$; ANOVA).*

physical activity, BMI, presence of cardiometabolic diseases, and use of vasoactive medications,[20] which were controlled for in the present study. Therefore, we have expanded with this study the knowledge about the vascular function during physical exercise in normotensive individuals with hypertensive parents.

In addition, the inability to decrease peripheral vascular resistance among individuals with a family history of hypertension has also been demonstrated at reactive hyperemia peak,[6,7] a maneuver that despite being triggered by a distinctive mechanism, has a vasodilatory response that correlates with the response triggered by exercise.[21,22]

During physical exercise, the muscle blood flow depends on the balance between dilatory and constrictor forces. In this sense, exacerbation of the sympathetic nervous activity and functional changes in endothelial regulation have been identified as important vasoconstrictor mechanisms responsible for most peripheral vascular resistance observed during exercise in subjects with a history of hypertension.[12] Indeed, greater muscle sympathetic nervous activity has been reported in offspring of hypertensive parents when compared with those of normotensive parents during handgrip isometric exercise, when directly assessed by microneurography.[9,23]

In addition, we observed increased serum norepinephrine levels both at rest and during handgrip exercise in individuals with a positive family history of hypertension in relation to individuals with a negative history of this pathology.[10] These factors can explain the results of the present study related to peripheral vascular resistance during exercise.

With regard to the endothelial function, McAllister et al.[11] observed no differences in endothelium-dependent and endothelium-independent vasodilation evaluated with the dose-response curve induced by acetylcholine and sodium nitroprusside, respectively, among healthy young adults with and without a family history of hypertension. However, these authors verified in offspring of hypertensive parents a vasoconstrictor response mitigated by NG-monomethyl-L-arginine (L-NMMA), an endothelial nitric oxide synthase (eNOS) inhibitor, demonstrating impaired baseline release of nitric oxide in this population. Additionally, Ciolac et al.[12] evaluated women with a family history of hypertension and observed, both at rest and during a maximal incremental treadmill test, reduced levels of nitrite and nitrate, the end products of degradation of nitric oxide, which also suggests a reduction in the production/bioavailability of this important vasodilator.

In addition to reducing the bioavailability of nitric oxide, increased levels of endothelin, an endothelium-derived vasoconstrictor substance, have also been observed in offspring of hypertensive parents when compared with offspring of normotensive parents, both during handgrip exercise,[10,13] as well as during incremental exercise test on a treadmill.[12] Therefore, it may be hypothesized that the increased vascular resistance observed in offspring of hypertensive parents during exercise may be related, at least in part, to a reduced endothelial production of vasodilatory substances and increased production of vasoconstrictor substances.

Declines in vascular function are associated with the development of atherosclerosis and future cardiovascular events.[24] Moreover, with the increased sympathetic tone, vascular dysfunction is involved in the development of hypertension and may be related to the greater predisposition of offspring of hypertensive parents to developing this disease.[12]

In this study, the responses in blood pressure and heart rate during exercise were similar between the groups. In addition, the groups presented a physiological increase in these variables throughout the test. Our results reproduce the findings of other authors,[9,10,25] who also observed similar responses in blood pressure and heart rate during handgrip physical exercise. On the other hand, the study by Greaney et al.[23] observed an exacerbated response of the mean blood pressure during exercise in young women with a positive history of hypertension. The different results found may be related to the characteristics of the study population. The sample in the present study comprised sedentary individuals of both sexes, whereas the sample in the study by Greaney et al.[23] comprised sedentary and active women. It is worth noting that the studies investigating blood pressure levels during physical exercise involving large muscle groups, such as exercise on a cycle ergometer[26,27] and knee extension isokinetic exercise,[8] have observed increased blood pressure levels in offspring of hypertensive parents, suggesting that the increased amount of muscle mass involved could be related to the cardiovascular hyperreactive responses observed in this population during these types of exercises.

This study showed that healthy young individuals without cardiovascular risk factors besides a family history of hypertension have impaired vasodilation during exercise. The increased peripheral vascular resistance during physical exercise may explain, at least in part, the blood pressure hyperreactivity in normotensive individuals during physical exercise. It has been documented that the exacerbated blood pressure response during exercise stress testing associated with increased total peripheral vascular resistance[28,29] is a prognostic factor for cardiovascular events and mortality in middle-aged men[30] and hypertensive individuals,[29] in addition to being related to cardiac remodeling in pre-hypertensive individuals.[28] However, until the present moment, there have been no longitudinal studies designed with the intention of investigating the prognostic application of the vascular behavior in offspring of hypertensive parents during physical exercise and the possible development of hypertensive disease.

Thus, the results of this study emphasize the importance of a preventive intervention with measures aimed at reducing vascular resistance and, consequently, acting in the prevention of hypertension in this population. In this regard, physical exercise has been implicated as an important strategy for prevention of hypertension in offspring of hypertensive parents,[3] considering the beneficial results of training on pathophysiological factors involved in the emergence of this pathology, such as the sympathetic hyperactivity and vascular dysfunction,[31] which are often present in susceptible individuals, even before the increase in blood pressure levels.

Limitations

This study has some limitations that should be mentioned. The diagnosis of hypertension in the volunteers' parents was reported by the volunteers themselves (self-report). Although this information has been self-reported in several studies,[6,23,32] future research should include a detailed medical assessment of the parents. In addition, the women in this study were not evaluated during the same period of the menstrual cycle, a fact that could also configure a limitation of this study. However, Jarvis et al.[33] and Carter et al.[34] found no influence in young women of the ovarian cycle phase on sympathetic nervous activity, heart rate, and blood pressure during handgrip exercise and mental stress, respectively.

Conclusion

We conclude that young normotensive individuals with hypertensive parents have impaired vasodilation during isometric physical exercise.

Acknowledgments

We thank the *Hospital Universitário da Universidade Federal de Juiz de Fora*, for the availability of space to conduct this study and the volunteers, for their contribution.

Author contributions

Conception and design of the research: Portela N, Souza LV, Laterza MC; Acquisition of data: Portela N, Souza LV; Analysis and interpretation of the data: Portela N, Amaral JF, Mira PAC, Martinez DG, Laterza MC; Statistical analysis: Amaral JF; Writing of the manuscript: Portela N, Amaral JF; Critical revision of the manuscript for intellectual content: Portela N, Amaral JF, Mira PAC, Souza LV, Martinez DG, Laterza MC.

Potential Conflict of Interest

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

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the especialization course submitted by Natália Portela, from Universidade Federal de Juiz de Fora.

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The Diterpene Sclareol Vascular Effect in Normotensive and Hypertensive Rats

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Abstract

Background: The diterpene Sclareol has antimicrobial action, cytotoxic and cytostatic effects and anti-tumor activities. However, researches on the cardiovascular system are scarce.

Objective: This study was designed to investigate the mechanisms involved in the Sclareol cardiovascular effect in normotensive and hypertensive rats.

Methods: The arterial hypertension was promoted using 2-kidneys 1-clip model in rats. The effect of sclareol on blood pressure was performed by using three dose (10, 20 and 40 mg/kg). Cumulative dose–response curves for Sclareol were determined for endothelium-intact and endothelium-denuded aortic rings in presence or absence of L-NAME and ODQ. The NOx levels were measure in the plasma sample.

Results: The Sclareol administration in vivo caused a significant reduction in blood pressure in both groups. In vitro the sclareol promoted relaxation in aorta, with endothelium, pre-contracted to Phe. The inhibitors of the nitric oxide synthase and soluble guanylate cyclase were as efficient as the removal of endothelium, in inhibiting the Sclareol-induced relaxation. Otherwise, it was no change of NOx. Also, for unknown reasons, the Sclareol is not selective for hypertensive animals.

Conclusion: 1) The diterpene Sclareol showed in vivo hypotensive and in-vitro vasodilator effects; 2).The chemiluminescence plasmatic NO analysis showed no significant difference between groups and 3) The Sclareol exhibit better effect on normotensive than hypertensive animals to reduce blood pressure. It is concluded that the diterpenes metabolites would be a promising source prototype for the development of new agents in the cardiovascular therapy. (Arq Bras Cardiol. 2017; 109(2):117-123)

Keywords: Diterpenes / therapeutic use; Rats; Hypertension; Anti-Infective Agents; Cytotoxins.

Introduction

The plant kingdom has contributed in a significant way to provide substances useful in the treatment of diseases that affect humans. In this context, diterpenes are a large class of secondary metabolites produced by plants and have many important biological activities.[1] Several studies sighted these substances as a promising source of new leads for the discovery and development of new agents for use in cardiovascular therapy, and have shown that many diterpenoid classes exert the significant effect on the cardiovascular system.[2-5] These studies suggest that metabolites class as a promising source prototype for the development of new agents in the cardiovascular therapy. The diterpenes are synthesized in plants located in plastids, but can also be synthesized by certain insects and marine organisms.

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Manuscript received July 07, 2016, revised manuscript November 21, 2016, accpeted January 24, /2017

DOI: 10.5935/abc.20170086

The diterpene Sclareol (Figure 1) is extracted from inflorescences Salviasclarea L., relatively easy to grow grass and high throughput.[6] Studies using this compound showed its antimicrobial action, cytotoxic and cytostatic effects on leukemic cell lines and anti-tumor activities.[7-10] However, studies about this compound on the cardiovascular system are scarce, or maybe have never been studied. So it is crucial that such investigations are carried out, considering that this compound is highly available and secure for testing. Therefore, this study was designed to investigate the mechanisms involved in cardiovascular effect (in vitro and in vivo) of diterpene Sclareol in normotensive and hypertensive rats.

Methods

Ethics statement and animals

The experimental procedures and policies for animal handling were reviewed and approved by the Institutional Committee for Animal Care and Use of the School of Medicine of Ribeirão Preto, the University of São Paulo, and were by the European Commission's Directive 2010/63/EU. Twenty male Wistar rats (180–220 g) were housed under standard laboratory conditions (12 h light/dark cycle at

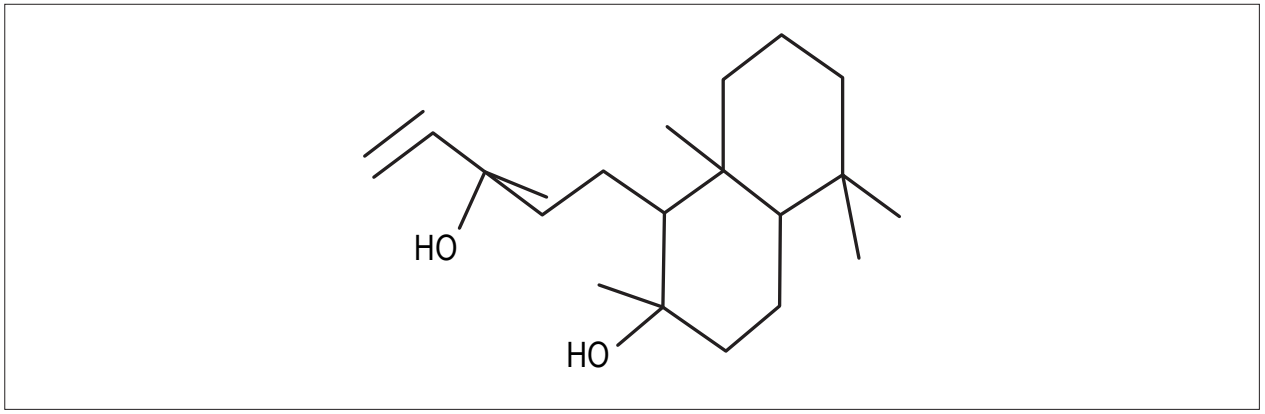

Figure 1 – *Sclareol molecular structure.*

21°C) with free access to food and water. The animals were randomly by lot and divided into two groups of 7 animals: normotensive and hypertensive for blood pressure protocols and 6 control animals for vascular reactivity protocol. The rats of the hypertensive group underwent the surgical procedure 2K1C for hypertension induction while the animals of the normotensive group were sham-operated.

Drugs

Acetylcholine (ACh), 1H-[1,2,4] oxadiazole [4,3-a] quinoxalin-1-one (ODQ), phenylephrine (Phe) and Sclareol were purchased from Sigma Chemical Company (St. Louis, MO, USA). Nω-nitro-L-arginine methyl ester (L-NAME) was obtained from Calbiochem (San Diego, CA, USA). Isoflurane from Abbott. All the salts used for Krebs solution preparation were furnished by Vetec Química Fina Ltda. Almost all the drugs were prepared with distilled water, except for indomethacin (which was dissolved in ethanol) and Sclareol (solubilized in dimethyl sulfoxide and diluted in ethanol + water).

Induction of the hypertension

The animals were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) intraperitoneally. After complete anesthesia, the rats were submitted to a laparotomy: half of the animals had partial constriction of the main left renal artery with a silver clip with a 0.10 mm gap (2K1C) (Goldblatt et al.,[11]) and half of them had the main left renal artery isolated but did not receive the clip (sham). In order to monitor the hypertension development, the SBP was non-invasively measured by means of a tail cuff, once a week. (Kent Scientific Corporation, Connecticut, USA).[12] The 2K1C rats were considered hypertensive if they had tail SBP ≥ 160 mmHg at 3th week after the surgical procedures. The 2K1C rats with SBP < 160 mmHg at 3th week were euthanatized. The sham operated rats constituted the normotensive group. Three weeks after hypertension induction, the animals were anesthetized, and the femoral artery and vein were cannulated for continuous measurement of systolic blood pressure (SBP) and drugs administration, respectively.

Sclareol effect on the systolic blood pressure

After anesthesia (urethane, 2 g/kg, intraperitoneal), vascular cannulation and stabilization period (20 minutes) with continuous real time SBP recording, three doses of Sclareol (10, 20 and 40 mg/kg) or vehicle (DMSO and water+ethanol) were administered to the normotensive and hypertensive rats. The vehicle administration was performed before sclareol curve in the both groups and because we didn't have difference between normotensive and hypertensive, we mixed then. Each dose was given in a 200 μL intravenous bolus and the interval between two consecutives doses was 6 minutes (time required for the SBP return to baseline values). For each animal, the variation in systolic blood pressure (ΔSBP) was calculated subtracting the mean of the lowest SBP values immediately after Sclareol administration from the mean of the baseline SBP values before Sclareol bolus. The monitoring of mean arterial blood pressure was measured using MP System 100 A (BioPac System, Inc., Santa Barbara, CA, USA).

Vascular reactivity

Five male Wistar rats (280–300 g) were anesthetized with inhalational isoflurane, followed by laparotomy for exsanguination via abdominal aorta and thoracotomy for thoracic aorta harvesting. The thoracic aorta was carefully dissected, confirmed to be free of connective tissue, and immediately immersed in Krebs solution. The Krebs solution was composed of NaCl (118.0 mM), KCl (4.7 mM), CaCl2 (2.5 mM), KH2PO4 (1.2 mM), MgSO4 (1.66 mM), glucose (11.1 mM), and NaHCO3 (25.0 mM); the solution had a pH 7.4. The thoracic aorta immersed in Krebs solution was cut into rings that were 4–5 mm in length. The endothelium was removed from some of the rings by gently rubbing the intimal surface of the blood vessel with a pair of watchmaker's forceps. This procedure effectively removes the endothelium, but it does not affect the ability of the vascular smooth muscle to contract or relax. The aortic rings were placed in isolated organ baths (10 mL) filled with Krebs solution, maintained at 37°C, and bubbled with 95%

O_2/5% CO_2 (pH 7.4). Each arterial ring was suspended by two stainless steel clips that were inserted through the lumen. One clip was anchored to the bottom of the organ bath while the other was connected to a strain gauge to measure the isometric force with the aid of the Grass FT03 equipment (Grass Instrument Company, Quincy, MA, USA). Each ring was stretched to the optimal length-tension of 2.0 g, which had been determined in a pilot study, and was allowed to equilibrate for 60 min. During this period, tissues were washed every 15 min. The endothelium was considered to be present (E+) when the Ach-induced relaxation was at least 80% after pre-contraction with Phe (10−6 M). Endothelium was deemed to be absent (E−) when the relaxation response did not occur. Next, each ring was washed and re-equilibrated for 30 min. The aortic rings were pre-contracted with Phe (10−6 M) after a stable plateau was reached, and dose-response curves of Sclareol were obtained. The concentration-response assays in the organ baths were carried out in the presence or absence: L-NAME (10−4 M), a nonspecific nitric oxide synthase inhibitor and ODQ (10−4 M), a guanylyl cyclase inhibitor. The preparations were incubated with the inhibitors for 30 min.

Indirect plasma measurements of NO

Blood samples were collected through the femoral vein after sclareol administration and placed into tubes containing heparin. After blood centrifugation (3000 × g, 10 minutes, 4°C), plasma aliquots were immediately immersed in liquid nitrogen and stored at –70°C until nitrite and nitrate (NOx) measurements. Samples were analyzed in duplicates for NOx using an ozone-based chemiluminescence assay. Briefly, the plasma samples were treated with cold ethanol (1 volume of plasma: 2 volumes of ethanol for 30 minutes at −20°C) and centrifuged (4000 × g, 10 minutes). The NOx levels were measured by injecting 25 μL of the supernatant in a glass purge vessel containing 0.8% of vanadium (III) in HCl (1 N) at 90°C, which reduces NOx to NO gas. A nitrogen stream was bubbled through the purge vessel containing vanadium (III), then through NaOH (1 N), and then into an NO analyzer (Sievers® Nitric Oxide Analyzer 280, GE Analytical Instruments, Boulder, CO, USA).

Statistical analysis

The data are expressed as means ± the standard error of the mean (SEM). We performed statistical analyzes with two-way repeated-measures analysis of variance (ANOVA) and the Bonferroni post-test, or test t Student was carried out to detect possible differences between the values in the study. $P < 0.05$ was considered significant. (Prism 5.0, GraphPad Software, San Diego, CA, USA). A sample size of ($N = 5$) animals per group provided 95% power with a 0.05 significance level to detect a relative 10% reduction in the maximal contraction in pre-contracted vessels and a sample size of ($N = 7$) animals per group provided 95% power with a 0.05 significance level in protocols of in vivo blood pressure measurement. The number of animals was chosen based on literature.

Results

Before surgical procedures, there were no differences in the SBP between normotensive and hypertensive groups (sham: 120.7 ± 3.5 mmHg versus 2K1C: 133.8 ± 3.6 mmHg, $p > 0.05$). However, from the 1st to 3th week after the hypertension induction, the SBP significantly increased in the hypertensive rats (sham at 3th week after surgical procedures: 130.6 ± 3.8 mmHg versus 2K1C group at 3th week after surgical procedures: 192.9 ± 10.2 mmHg, $p < 0.001$) (Figure 2).

All the three doses of Sclareol (10, 20 and 40 mg/kg) significantly decreased the SBP in the normotensive rats (vehicle: –10.7 ± 6.7 mmHg versus normotensive sclareol: –43.1 ± 7.1 mmHg at 10 mg/kg, $p < 0.01$; vehicle: –4.8 ± 2.8 mmHg versus normotensive sclareol: –45.5 ± 6.0 mmHg at 20 mg/kg, $p < 0.01$; vehicle: –2.8 ± 2.3 mmHg versus normotensive Sclareol: –33.3 ± 7.0 mmHg at 40 mg/kg, $p < 0.01$). Nevertheless, only 20 mg/Kg dose of sclareol change the SBP in the hypertensive animals (vehicle: -4.8 ± 2.8 mmHg versus hypertensive sclareol: –39.1 ± 15.8 mmHg at, $p > 0.05$) (Figure 3).

In the case of Phe pre-contracted arteries, Sclareol promoted a dose-dependent relaxation only in intact rings (E+ 52.9 ± 5.0 % versus E- 6,9 ± 4.0%). Incubation with either L-NAME or ODQ totally blocked the relaxation induced by Sclareol in both endothelium-intact rings (Figures 4 and 5).

The plasma NOx concentration did not change between all groups group (vehicle: 55.4 ± 7.4 μM; normotensive sclareol: 52.5 ± 3.9 μM and hypertensive vehicle: 68.7 ± 8.3 μM). (Figure 6).

Discussion

The in vivo results obtained after administration of three escalating doses of Sclareol demonstrate that it promoted a reduction in BP, both in the normotensive and hypertensive animals. The mechanisms involved in this relaxing effect remain unknown. Nevertheless, this effect may be connected with the fact that these compounds are possibly responsible for activation of NO pathways. Looking more deeply into the data collected in 2K1C model, the renin-angiotensin-aldosterone system (RAAS) is the primary factor in the development of hypertension.[13] In hypertension, there is an activation of the RAAS and in turn, a greater inhibition of kallikrein-kinin system (CMS) by ACE,[14,15] this can result in a smaller reduction in SBP induced by the Sclareol in the hypertensive group. The largest reduction in SBP in the normotensive group, in response to administration of Sclareol, may be indicative of an interaction between the RAAS and the SCC.[14,15] However, it takes more experiments to determine the actual cause.

The second mechanism possible involved in the hypotensive effect of sclareol, is the vasodilator property. We tested the Sclareol vasorelaxant effect, in vitro, using isolated rat aortic rings pre-contracted with phenylephrine. The relaxant effect observed from sclareol dose-response curve, in rat aorta denuded-rings, was completely blocked by incubation with L-NAME (non-selective NOS inhibitor) and ODQ (inhibitor of guanylate cyclase), which indicates that Sclareol promotes vasorelaxation via NO/cGMP pathway.

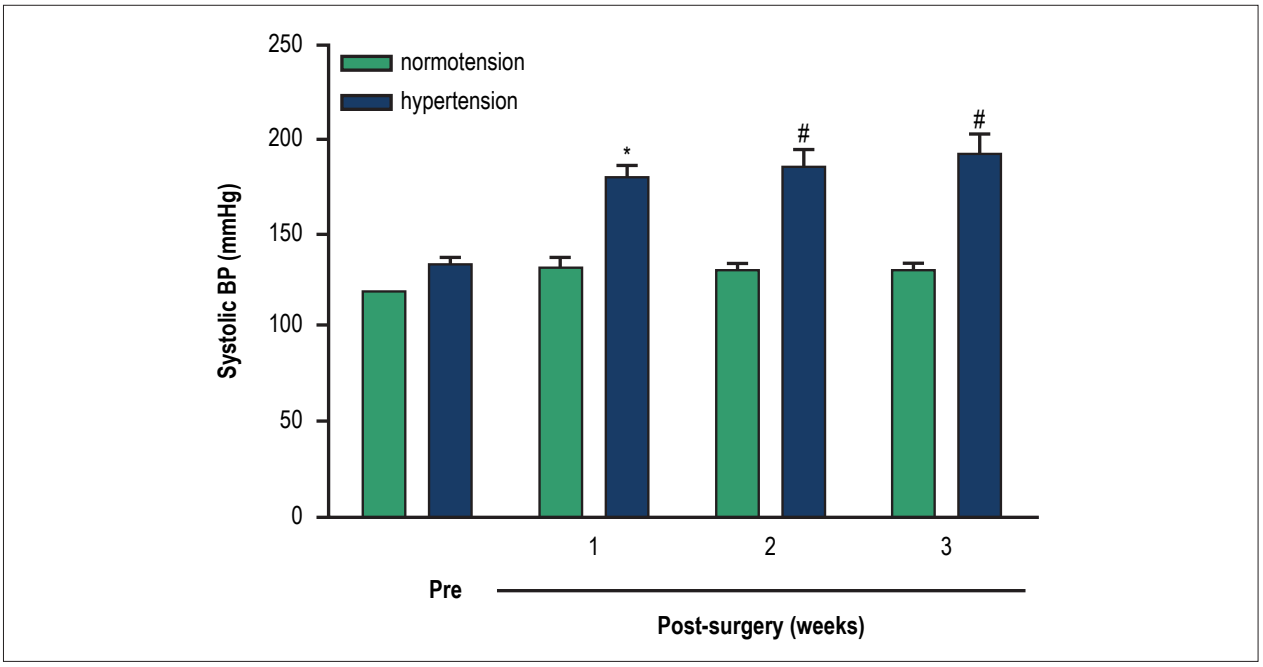

Figure 2 – *Temporal evolution of noninvasive systolic blood pressure in normotensive and hypertensive animals. The values represent the mean ± standard error of the mean (n = 7). SBP 2K1C before surgery (pre-operative) and at the 3 weeks following surgery. * (p < 0.01) and # (p < 0.001) indicated significant difference in the hypertensive group compared to the normotensive group.*

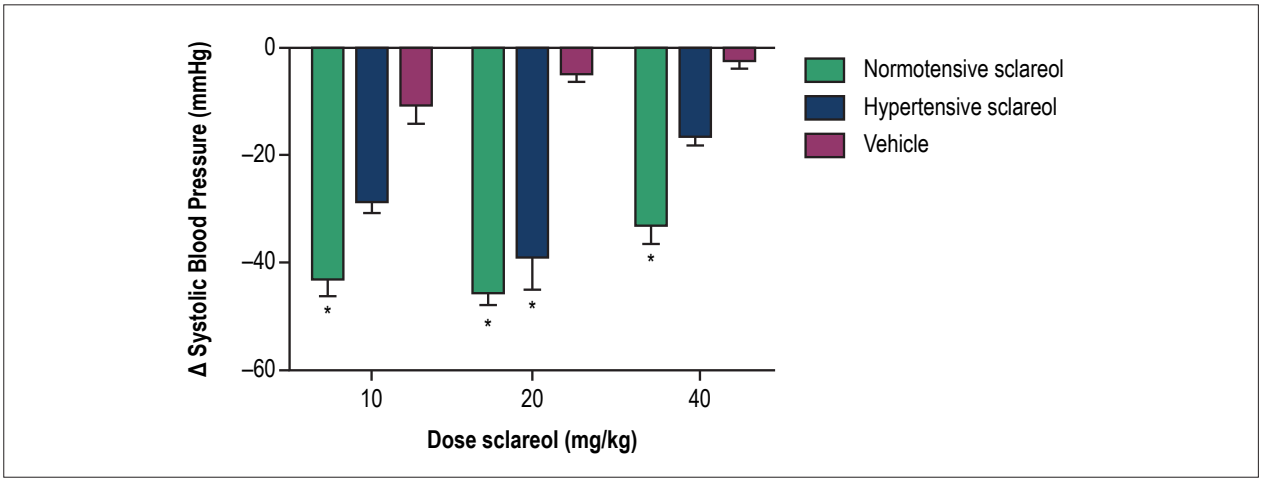

Figure 3 – *Representative image of the SBP variation, after three doses of Sclareol or vehicle. (n = 7). * (p < 0.01) between vehicle and normotensive or hypertensive groups.*

In the present study, indirect plasma measurements of NO were carried out by determination of serum levels of nitrite and nitrate using the SieversNOAnalizer 280i. There were no significant differences between the group treated with Sclareol and the vehicle group. However, the analysis of NO in plasma can be influenced in different stages of the process, because it is a very fine analysis. From this result, the ideal would be measured in real time of NO in isolated endothelial cells stimulated with the compounds. This protocol has been tested in different ways, but we were unsuccessful. After several attempts, we believe that the compounds, in any way interfere with the reading of sly (DAF) used.

Conclusion

The diterpene Sclareol showed in vivo hypotensive and in-vitro vasodilator effects; 2).The chemiluminescence analysis of the plasmatic NO showed no significant difference between groups and 3) For unknown reasons, the Sclareol

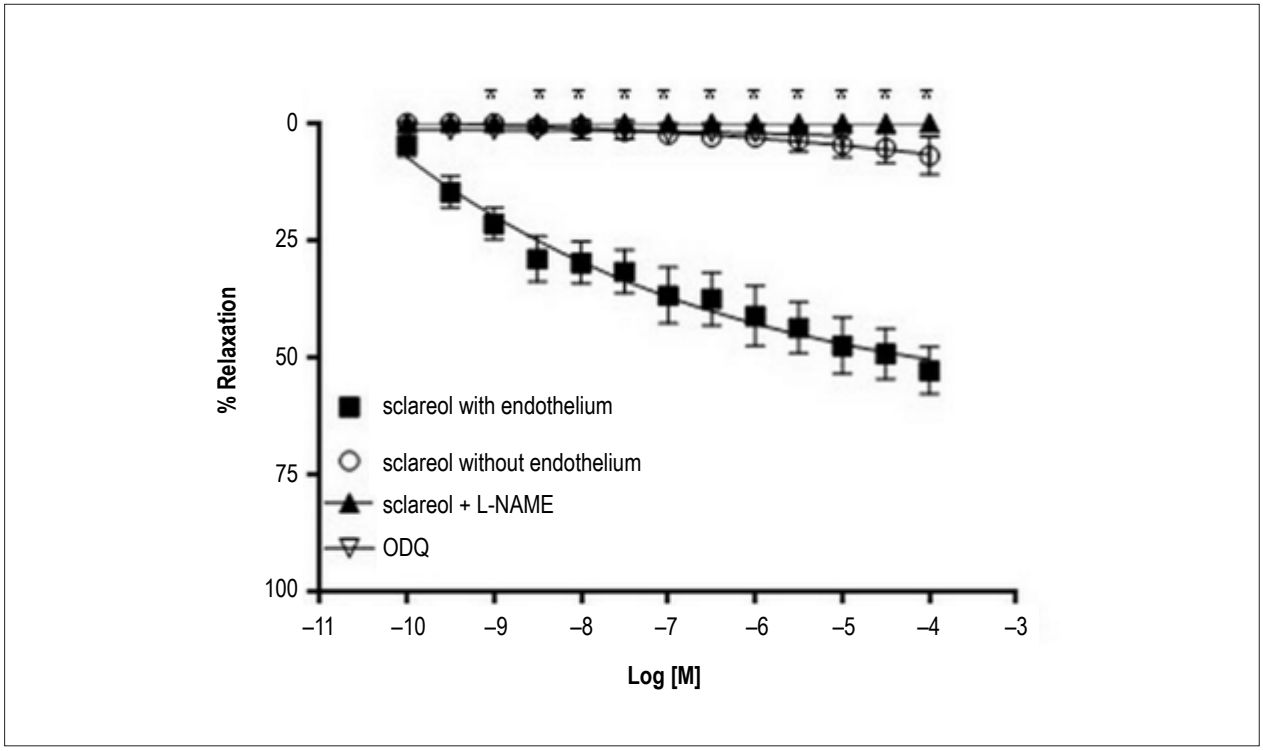

Figure 4 – *Dose response curve Sclareol in the presence of inhibitors. After the pre- contraction with 10^{-7}M Phe, the rings were subjected to a dose response curve from 10^{-10} to 10^{-4} in the presence of L-NAME and inhibitor ODQ. * (p < 0.001) indicate a significant difference between the groups with inhibitors and control. (n = 6).*

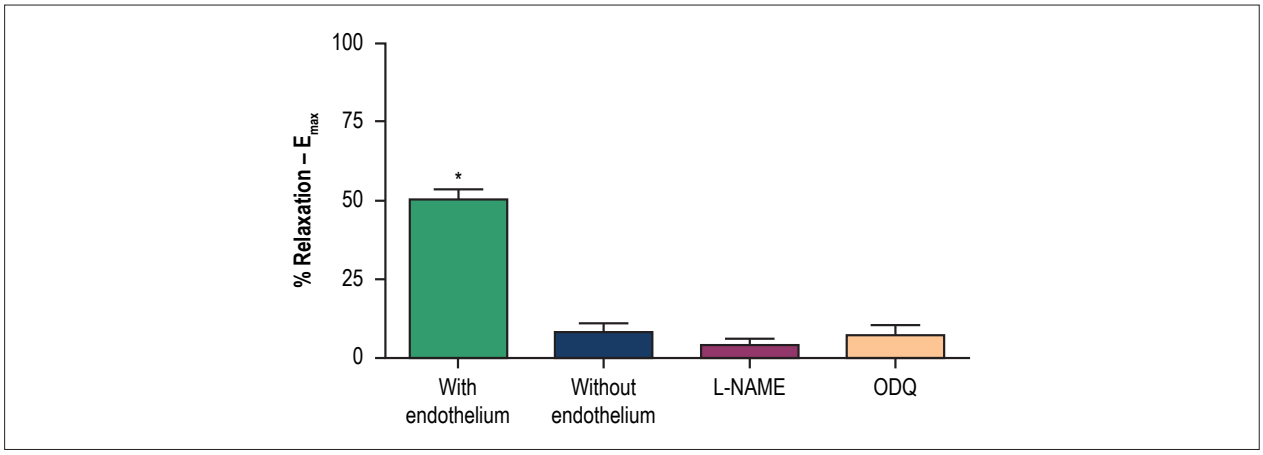

Figure 5 – *Maximum relaxing effect in the presence of inhibitors. The E_{max} was obtained from dose-response curves, using non-linear regression. * (p < 0.001).*

is not selective in hypertensive animals. So it is important that further research involving the diterpene Sclareol in the cardiovascular function can be explore more detail about mechanisms of action. From the data obtained in this study, it is concluded that the diterpenes metabolites class would be a promising source prototype for the development of new agents in the cardiovascular therapy.

Author contributions

Conception and design of the research and Writing of the manuscript: Campos DR, Celotto AC, Évora PRB; Acquisition of data and Critical revision of the manuscript for intellectual content: Campos DR, Celotto AC, Albuquerque AAS, Ferreira LG, Monteiro ASN, Coelho EB, Évora PRB; Analysis and interpretation of the data: Campos DR, Celotto AC,

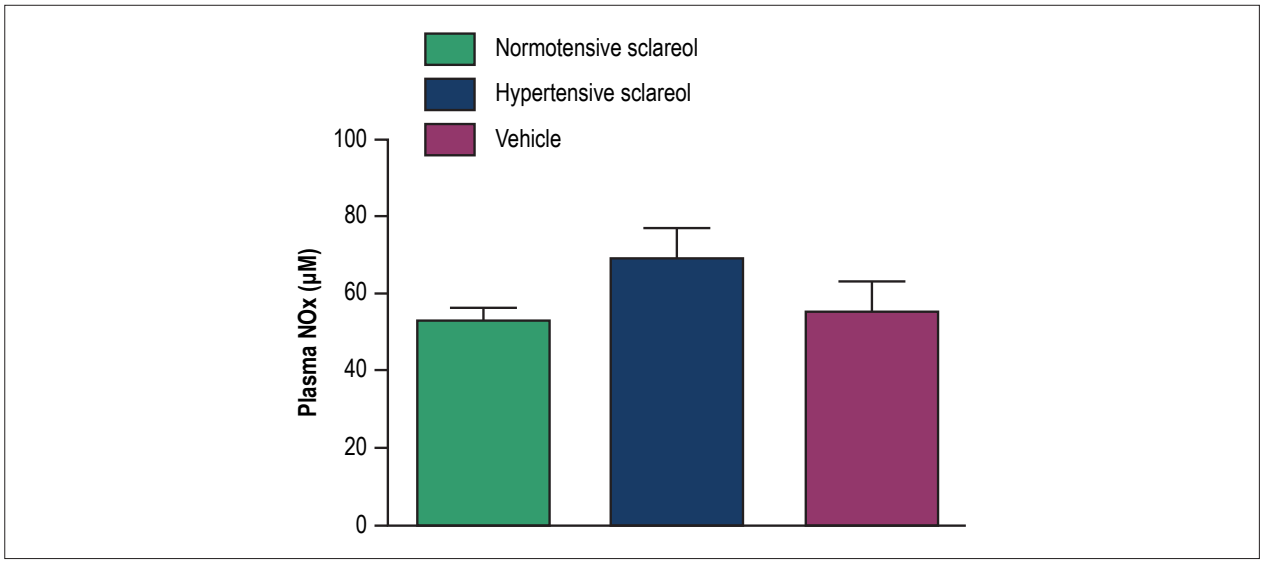

Figure 6 – *Plasmatic nitrite and nitrate levels (NOx) in normotensive and hypertensive animals. The animals were pretreated with vehicle or Sclareol. (N = 7).*

Albuquerque AAS, Monteiro ASN, Évora PRB; Statistical analysis: Campos DR, Celotto AC, Albuquerque AAS, Évora PRB; Obtaining funding: Évora PRB

Potential Conflict of Interest

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

Sources of Funding

This study was funded by CNPq.

Study Association

This article is part of the thesis of master submitted by Debora Ribeiro Campos, from Universidade de São Paulo.

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Ruthenium Complex Improves the Endothelial Function in Aortic Rings From Hypertensive Rats

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Abstract

Background: The endothelium is a monolayer of cells that extends on the vascular inner surface, responsible for the modulation of vascular tone. By means of the release of nitric oxide (NO), the endothelium has an important protective function against cardiovascular diseases.

Objective: Verify if cis- [Ru(bpy)$_2$(NO$_2$)(NO)](PF$_6$)$_2$ (BPY) improves endothelial function and the sensibility of conductance (aorta) and resistance (coronary) to vascular relaxation induced by BPY.

Methods: Normotensive (2K) and hypertensive (2K-1C) Wistar rats were used. For vascular reactivity study, thoracic aortas were isolated, rings with intact endothelium were incubated with: BPY(0.01 to10 μM) and concentration effect curves to acetylcholine were performed. In addition, cumulative concentration curves were performed to BPY (1.0 nM to 0.1 μM) in aortic and coronary rings, with intact and denuded endothelium.

Results: In aorta from 2K-1C animals, the treatment with BPY 0.1μM increased the potency of acetylcholine-induced relaxation and it was able to revert the endothelial dysfunction. The presence of the endothelium did not modify the effect of BPY in inducing the relaxation in aortas from 2K and 2K-1C rats. In coronary, the endothelium potentiated the vasodilator effect of BPY in vessels from 2K and 2K-1C rats.

Conclusion: Our results suggest that 0.1 μM of BPY is able to normalize the relaxation endothelium dependent in hypertensive rats, and the compound BPY induces relaxation in aortic from normotensive and hypertensive rats with the same potency. The endothelium potentiate the relaxation effect induced by BPY in coronary from normotensive and hypertensive rats, with lower effect on coronary from hypertensive rats. (Arq Bras Cardiol. 2017; 109(2):124-131)

Keywords: Rats; Hypertension, Renal; Ruthenium; Endothelium / physiopathology; Nitric Oxide.

Introduction

Endothelial dysfunction is characterized mainly by decreasing the ability of endothelial cells to release nitric oxide (NO),[1] and it has been associated with hypertension as well as other cardiovascular diseases, furthermore, it includes release and superoxide anion (O_2^-) increased bioavailability generating to peroxinitrite ($ONOO^-$) join reaction with NO. This reaction is present in dysfunctional endothelial cells 2K-1C animals, due to the current Angiotensina II increase.[2]

NO is involved in diverse pathophysiological process that encourages the emergence of researches about drugs that can be able to modulate NO concentration for therapeutic purpose,[3] including NO donors.

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Manuscript received August 19, 2016, revised manuscript December 16, 2016, accepted March 15, 2017

DOI: 10.5935/abc.20170090

On preliminary results, we have observed that the ruthenium complex *cis*-[Ru(H-dcbpy)$_2$(Cl)(NO)] (dcbpy) improved the relaxation endothelium dependent induced by acetylcholine in aortic rings from hypertensive rats[4]. This compound also is able to induce relaxation by NO release in higher concentration, and the improvement in endothelial function was attributed to inactivation of O_2^-.[4]

The NO donors are pharmacologically active substances that release NO. The NO donors most widely used in medical practice are organic and inorganic nitrates, nitroglycerine and sodium nitroprusside, respectively. However prolonged treatment with these drugs have induced adverse effects, such as intolerance, endothelial dysfunction, release of toxic compounds, reflex tachycardia and other adverse effects that are limiting factors to the use of these NO donors.[5-8]

Thus, the macrocyclic nitrosyl ruthenium complexes are being studied as NO donors, [9-14] which are attractive because they have active forms that are stable and have low toxicity under physiological conditions.[10,12,13] Another important feature displayed by these compounds is the sustained release of NO, as we noted in prolonged hypotensive effect generated in hypertensive animals [15,16] and that was also observed in studies of release kinetics NO *in vitro*.[17,18]

Exogenous NO donors agents based on ruthenium-derived metal nitrosyl complexes have been developed as strategy to reduce side effects and cytotoxicity. They have not displayed any toxic effects and they are able to induce vascular relaxation and decrease blood pressure in normotensive and hypertensive rats[15,19] being the cis- [Ru(bpy)$_2$ (NO$_2$)(NO)] (PF$_6$)$_2$ (BPY) able to induce aortic relaxation and decrease blood pressure in normotensive rats.[20]

Thus, drugs in which the center of the metal is ruthenium, as BPY, have good clinical application, especially considering that the low toxicity of the metal ion is similar to the physical and chemical properties present in the iron metal ion.[21] The body can protect from the effects caused by excess of iron ions with the formation of transferrin and albumin, therefore it is believed that the mechanism of protection against the toxicity of ruthenium would be the same.[21,22] Thus, based on literature existing surrounding this issue, it appears that the BPY is more attractive to present active form under physiological conditions predicting a good future clinical application.[11-13]

Objective

This study was made to evaluate if BPY improves endothelial function, and the sensibility of conductance (aorta) and resistance (coronary) to vascular relaxation induced by BPY.

Methods

Materials used (Drugs and chemicals), Acetylcholine (Ach) and phenylephrine (Phe) were purchased from Sigma–Aldrich (St.Louis, MO, USA); Compound cis- [Ru(bpy)$_2$ (NO$_2$)(NO)] (PF$_6$)$_2$ (BPY) was synthesized by a partner in University of Pharmaceutical Sciences of Ribeirão Preto.

Experimental animals

Male Wistar rats were used weighing between 180-200 grams. The animals were maintained on a standard diet with a 12 h cycle light/dark and free access to food (standard diet) and water. The animals were anaesthetized with Tribromoethanol (2.5 mg/kg, ip) after a midline laparotomy a silver clip with an internal diameter of 0.20 mm was placed around the left renal artery as previously described for 2K-1C by Goldblatt et al.[23], where only one renal artery is restricted to reduce chronic renal perfusion. Normotensive two-kidney rats (2K, n = 6) were only submitted to laparotomy. Systolic blood pressure (SBP) was measured by a method of indirect tail plethysmography (MLT125R pulse pressure transducer/Cuss coupled to PowerLab 4/S-digital converter; AD Instruments Pty Ltd, Castle Hill, Australia) in animals not anaesthetized. The animal were considered hypertensive when systolic blood pressure was greater than 160 mmHg six weeks after surgery.

Ethical aspects

Experimental protocols followed standards and policies of Animal Care and Use Committee of the Federal University of São Carlos (CEUA: 012/2013).

Vascular reactivity study

Six weeks after surgery, rats were killed by decapitation and the thoracic aorta or coronary were dissected, cut into rings and placed in bath chambers containing Krebs solution at 37°C, pH 7.4, continuously bubbled with 95% O_2 and 5% CO_2, in an isometric myograph (Mulvany-Halpern-model 610 DMT-USA, Marietta, GA) and recorded by a PowerLab8/SP data acquisition system (ADInstruments Pty Ltd., Colorado Springs, CO).

Endothelial integrity was assessed by the degree of relaxation induced by 1 μmol/L acetylcholine after contraction of the aortic ring by phenylephrine (0.1 μmol/l). The ring was discarded if relaxation with acetylcholine was lower than 80% in 2K and 60% in 2K-1C rat aortas. After the endothelial integrity test, aortic rings were pre-contracted with phenylephrine (0.1 μM) and then were constructed concentration–effect curves to acetylcholine (0,01 μM to 10 μM) and BPY (1,0 nM to 0.1 μM), similarly in coronary artery rings, with and without intact endothelium, pre-contracted contractile agent (serotonin 10 μM) cumulative concentration curves were performed for the purpose BPY compound.

Aortic rings from 2K and 2K-1C were treated for 30 min with BPY (at concentrations: 0.1 μM) or PBS (control). The concentration of BPY chosen (0.1 μM) is close to EC_{50}. After incubation, aortic rings were washed three times to remove drugs, pre-contracted and concentration–effect curves to acetylcholine were constructed. The potency values (pD2) and maximum relaxant effect (ME) were analyzed. The curves concentration effect for BPY were realized without previous incubation.[29]

Statistical analysis

Normality of distribution was checked with the Kolmogorov-Sminorv test, differences in means were compared by ANOVA. When significance was indicated, a Newman-Keuls post hoc analysis was used with statistical significance set at $p < 0.05$ (Software Prisma 3.0, Graphpad Software Inc, La Jolla, CA, USA). Data are expressed as mean ± S.D.

To calculate the sample size was followed the statistical formula for the calculation of the sample in an infinite population. In preclinical studies, we found that the standard deviation in the power of relaxation induced by acetylcholine in normotensive rat arteries was 0.31. We consider a tolerable sampling error of 0.25, thus define the size of the sample used in accordance with the formula: n = (1.96X0.31/0.25) 2 = 5.9 animals.

Results

Vascular reactivity studies

As can be seen at Figure 1, acetylcholine induces relaxation in pre-contracted aortic rings. However, the potency and the maximum relaxant effect was lower in aortic rings from hypertensive rats 2K-1C (Tables 1 and 2) when compared to aortic rings of normotensive 2K rats (Tables 1 and 2), indicating endothelial dysfunction in aortic rings of hypertensive rats 2K-1C.

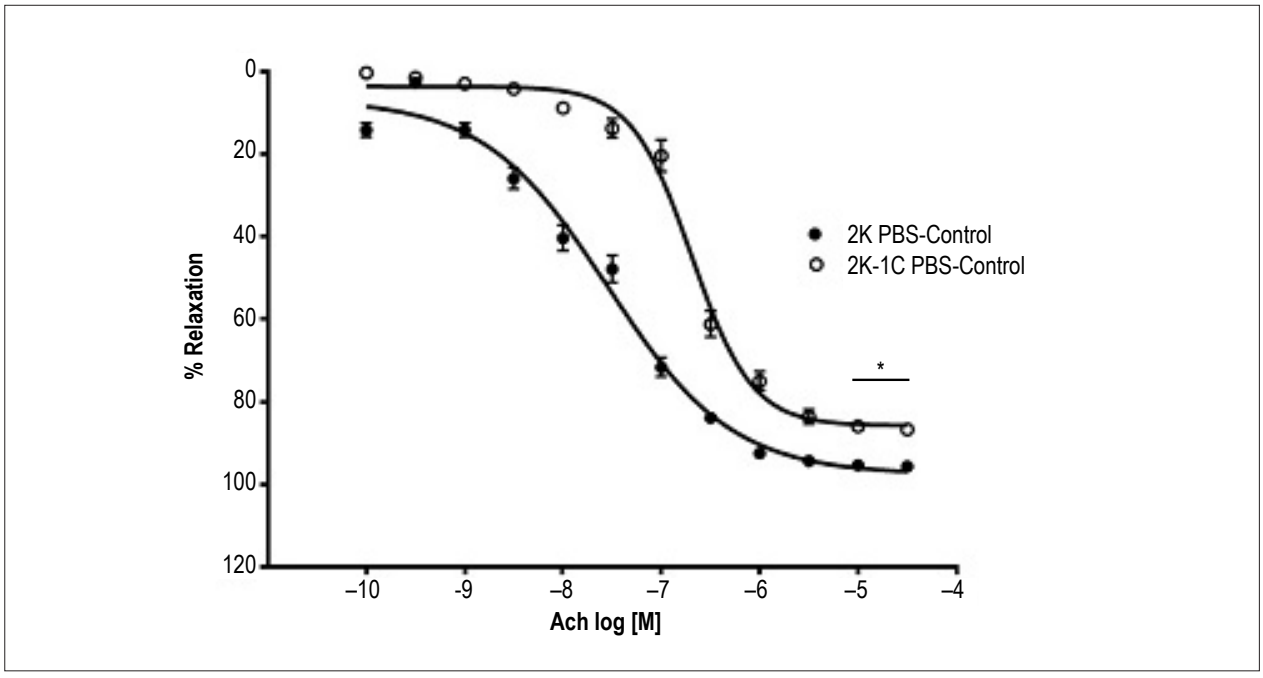

Figure 1 – *Concentration–response curves (n = 8) for acetylcholine in intact endothelium- aortic rings contracted with phenylephrine. Values are mean ± S.D of experiments performed on preparations obtained from different animals. *** indicates significant difference (p < 0.001) in pD2 value for 2K vs. 2K-1C.*

Table 1 – Potency (pD2) and Maximum relaxant effect (ME) to acetylcholine in endothelium intact aortic rings from 2K and 2K-1C rats incubated with PBS and BPY (0.1μM), and ME to acetylcholine in coronary rings from rats with intact (E+) and denuded (E-) endothelium from 2K and 2K-1C incubated with BPY (0.1μM). Values are mean of *n* experiments performed on preparations obtained from different animals, and number of animals used

		2K-1C	2K
PBS	pD2 Mean; Number of animals (n)	6.34; n = 6	7.07; n = 7
	Emax Mean; Number of animals (n)	71.01, n = 6	93.90; n = 7
BPY 0.1 μM	pD2 Mean; Number of animals (n)	7.74; n= 7	7.32; n = 7
	E_{max} Mean; Number of animals (n)	90.85; n = 6	98.64; n = 7
Intact endothelium (E+)	E_{max} Mean; Number of animals (n)	66.90; n = 5	86.97; n = 5
Denuded endothelium (E-)	E_{max} Mean; Number of animals (n)	34.72; n = 7	34.88; n = 5

Table 2 – Potency (pD2) and Maximum relaxant effect (ME) to acetylcholine in endothelium intact aortic rings from 2K and 2K-1C rats incubated with PBS and BPY (0.1 μM), and ME to acetylcholine in coronary rings from rats with intact (E+) and denuded (E-) endothelium from 2K and 2K-1C incubated with BPY (0.1 μM). Values are ± S.D of *n* experiments performed on preparations obtained from different animals.

		2K-1C	2K
PBS	Standard Deviation of pD2	± 0.07	± 0.22
	Standard Deviation of E_{max}	± 2.58	± 2.79
BPY 0.1 μM	Standard Deviation of pD2	± 0.08	± 0.11
	Standard Deviation of E_{max}	± 1.34	± 2.33
Intact endothelium (E+)	Standard Deviation of E_{max}	± 2.11	± 5.65
Denuded endothelium (E-)	Standard Deviation of E_{max}	± 6.89	± 5.45

Treatment of aortic rings with BPY at 0.1 μM was able to increase the potency of acetylcholine (Ach) in aortic rings of 2K-1C animals (Tables 1 and 2, $p < 0.001$) when compared with control 2K-1C -PBS (Tables 1 and 2) (Figures 2 and 3).

In addition, the treatment with 0.1 μM of BPY increased the maximum relaxant effect in aortic rings of 2K-1C rats (table 1 and 2, $p < 0.001$) when compared to the control – 2K-1C PBS (Tables 1 and 2) (Figure 4).

However, the treatment with 0.1μM BPY 2K-1C in aortic rings was able to normalize the potency and the maximum relaxation effect to acetylcholine. In other words, the potency and ME to 2K-1C aortic rings treated with 0.1 μM BPY were similar to that obtained in aortic rings of 2K animals (Tables 1 and 2), suggesting a reversion of endothelial function in 2K-1C aortic ring by treatment with 0.1 μM of BPY (Figures 2, 3 and 4).

As can be seen at Figure 5, the NO donor BPY promoted concentration-dependent relaxation in isolated aortic rings from normotensive (2K) and hypertensive (2K-1C) rats with (E+) and without (E-) endothelium. Moreover, the presence of the endothelium did not change the vasodilating effect induced by BPY compound.

The NO donor *cis*-$[Ru(bpy)_2(NO_2^-)(NO)](PF_6)_2$ (BPY) induced concentration-dependent relaxation in isolated rat coronary with intact (E+) and denuded (E-) endothelium from 2K and 2K-1C animals. As can be seen at figure 6, in coronary arteries of hypertensive (2K-1C) rats, the presence of endothelium potentiated relaxation induced by BPY (Tables 1 and 2) compared to the absence of the endothelium (Tables 1 and 2, $p < 0.001$).

In coronary from normotensive (2K) rats, the endothelium also increased the relaxation induced BPY (Tables 1 and 2, $p < 0.001$) (Figure 7).

In the absence of the endothelium, BPY compound is able to induce relaxation in coronary from normotensive (2K) rats (Tables 1 and 2) and hypertensive rats (Tables 1 and 2), with no significant difference between the two groups (Figure 7). In intact endothelium coronary arteries, the relaxation induced by BPY was more effective in normotensive animals (Tables 1 and 2) when compared to hypertensive (Tables 1 and 2, $p < 0.05$) (Figures 6 and 7).

Discussion

Our results have shown that the endothelium-dependent relaxation induced by acetylcholine is impaired in aortic rings from hypertensive rats (2K-1C). Hypertension model (2K-1C) is mediated by activation of the Renin Angiotensin Aldosterone System, occurring high concentration of circulating Angiotensina II. In accordance with Santeliz et al.,[24] vascular cells stimulated by angiotensin II show high concentration of superoxide anion (O_2-) due to activation of NADPH complex, which is responsible for the reduction in the vascular relaxation, since this species produced react with the released NO to form peroxynitrite, thus generating

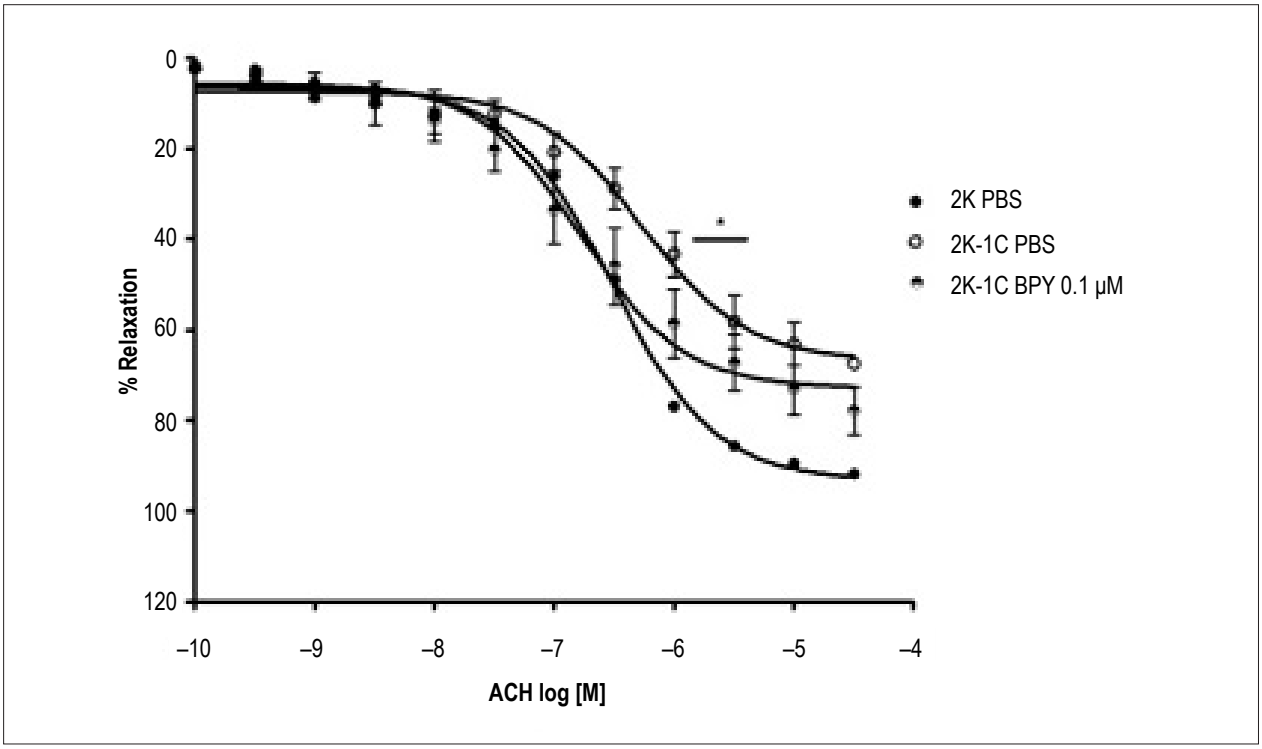

Figure 2 – *Concentration–response curves for acetylcholine (BPY) in aortic rings with intact endothelium and incubated with different concentrations of cis-$[Ru(bpy)_2(NO_2^-)(NO)](PF_6)_2$ and contracted with phenylephrine. Values are mean ± S.D of experiments performed on preparations obtained from different animals.* indicates significant difference 2K-1C PBS vs 2K-1C BPY 0.1 µM ($p < 0.001$) e 2K-1C PBS vs 2K PBS ($p < 0.001$) in pD2.*

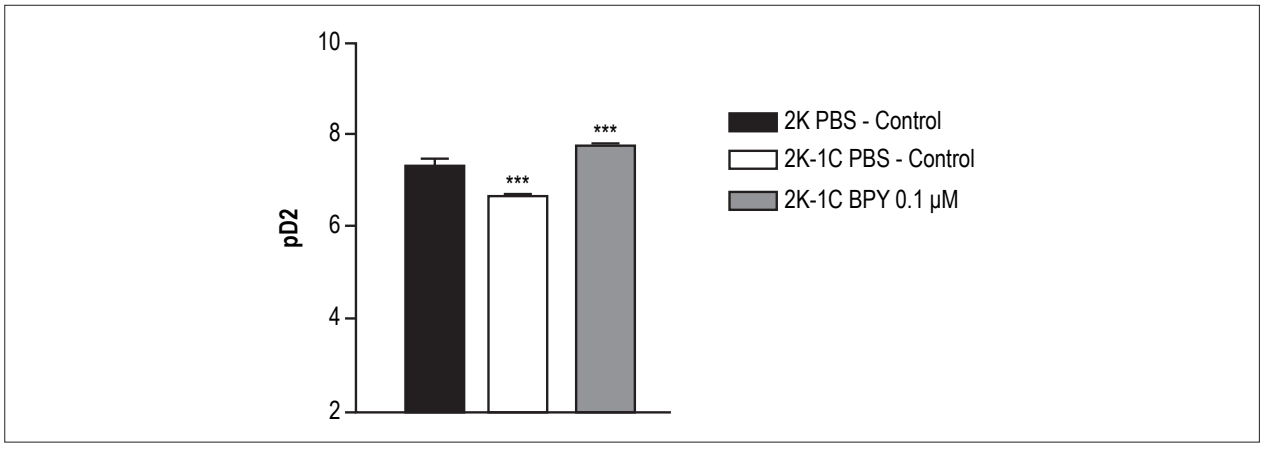

Figure 3 – *Presents differences in the potency (pD2) of acetylcholine in inducing relaxation in aortas with and without cis-[Ru(bpy)$_2$(NO$_2^-$)(NO)](PF$_6$)$_2$ treatment. The concentration 0.1 nM normalized relaxation in 2K-1C aortic rings compared to 2K aortic rings. *** - Indicates statistical difference between 2K-1C PBS vs. 2K-1C BPY 0.1 µM (p < 0.001) and 2K-1C PBS vs. 2K PBS (p < 0.001).*

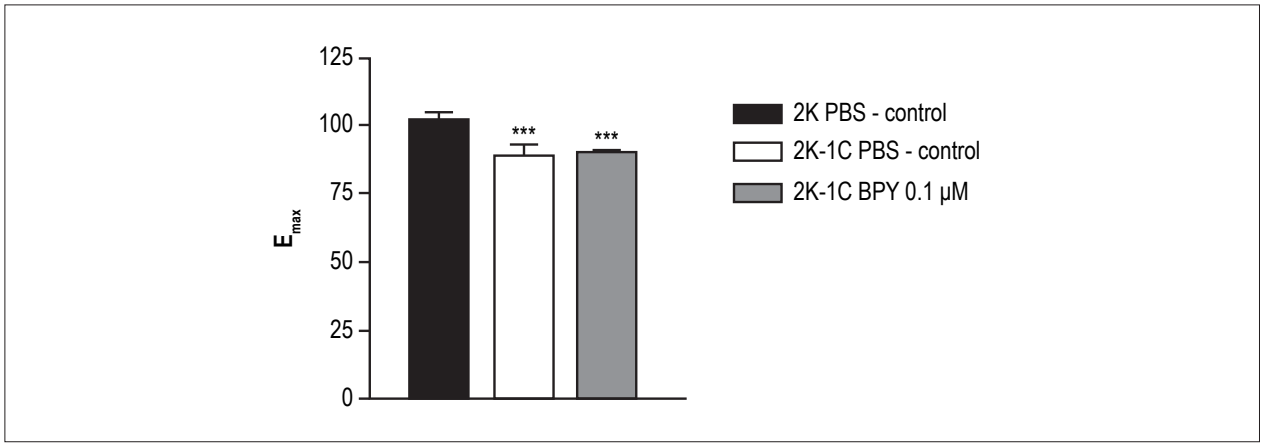

Figure 4 – *Presents differences in the efficiency (E$_{max}$) of acetylcholine in inducing relaxation in aortas with and without cis-[Ru(bpy)$_2$(NO$_2^-$)(NO)](PF$_6$)$_2$ treatment. The concentration 0.1 nM normalized relaxation in 2K-1C aortic rings compared to 2K aortic rings. *** - Indicates statistical difference between 2K-1C PBS vs. 2K-1C BPY 0.1 µM (p < 0.001) and 2K-1C PBS vs. 2K PBS (p < 0.001).*

smaller amount of NO available. Furthermore, in hypertensive animals occurs a malfunction in endothelial cell layer due to shear stress and activation of the renin-angiotensin-aldosterone system. This dysfunction is characterized mainly by the decreasing ability of endothelial cells to release NO[1]. The NO produced in the endothelial cell diffuses to a lesser extent into the vascular lumen and for vascular cells smooth muscle[25-28] causing a failure to control the modulation of vascular tone by NO.

The main finding of the present manuscript was that the treatment with BPY (at concentration 0.1 μM) in hypertensive aortic rings improved the endothelium-dependent relaxation, and was able to normalize the relaxation in 2K-1C aortic rings. These results suggest that a punctual concentration of BPY is able to induce improvement on endothelial function, which could be because of some enzymatic activation or an inhibition generating an increasing effect of endothelium dependent relaxation. It seems that the tonus modulation by endothelial can be improved by BPY.

These results are in accordance with previous study, that have shown an improvement on endothelial function by aortic rings treatment with 0.1μM of another ruthenium compound (*cis*-[Ru(H-dcbpy$^-$)$_2$(Cl)(NO)]).[4] Thus, some results have suggested that ruthenium compounds can release NO and improve the endothelial function, which is a desirable effect on vascular system when endothelial dysfunction is present.

The endothelium and hypertension did not change the vasodilator effect induced by BPY compound in aortic rings. Rodrigues et al.,[9] demonstrated that NO donors, TERPY (ruthenium complex) and SNP as well as BPY promoted concentration-dependent relaxation on isolated aorta from hypertensive (2K-1C) rats and normotensive (2K) rats, without altering the percentage of the maximum relaxation. However the potency of both NO donors (TERPY and SNP) was lower in the aorta from hypertensive rats (2K-1C), different from that observed to BPY, which generated the same potency of relaxation in 2K and 2K-1C aortas. The lower potency to TERPY and SNP was attributed to

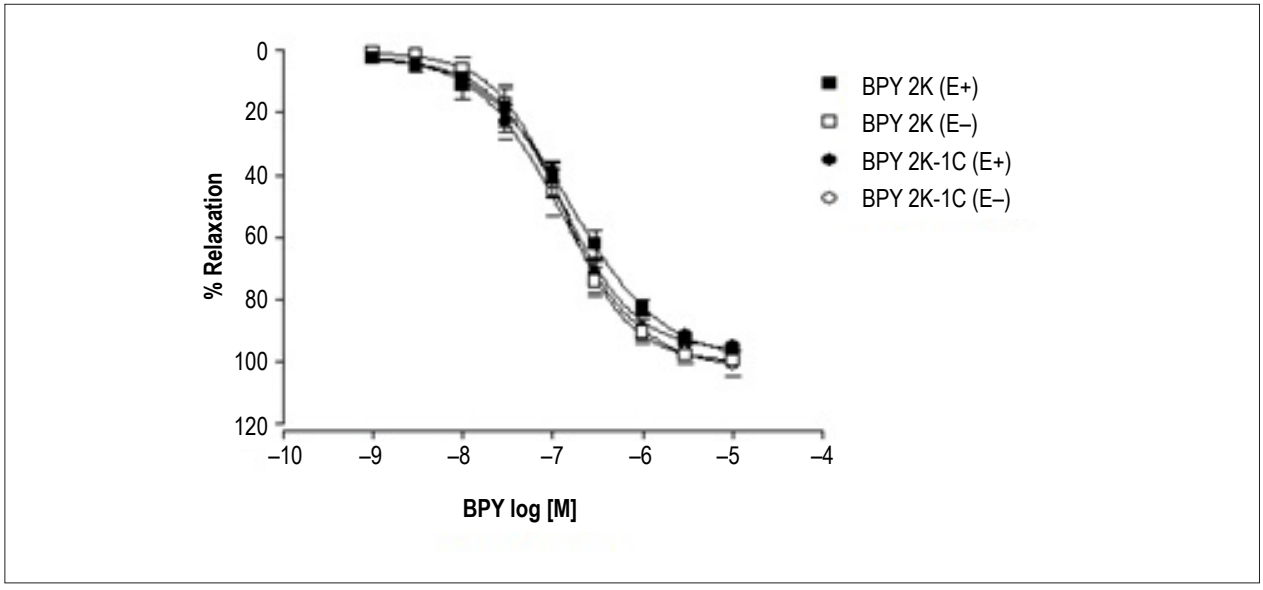

Figure 5 – *Concentration-response curves for acetylcholine in aortic rings with (E+) and without (E-) intact endothelium, from rats 2K and 2K-1C and incubated with different concentrations of cis-[Ru(bpy)$_2$(NO$_2^-$)(NO)](PF$_6$)$_2$ and contracted with phenylephrine. Values are mean ± S.D of experiments performed on preparations obtained from different animals. There was no statistical difference.*

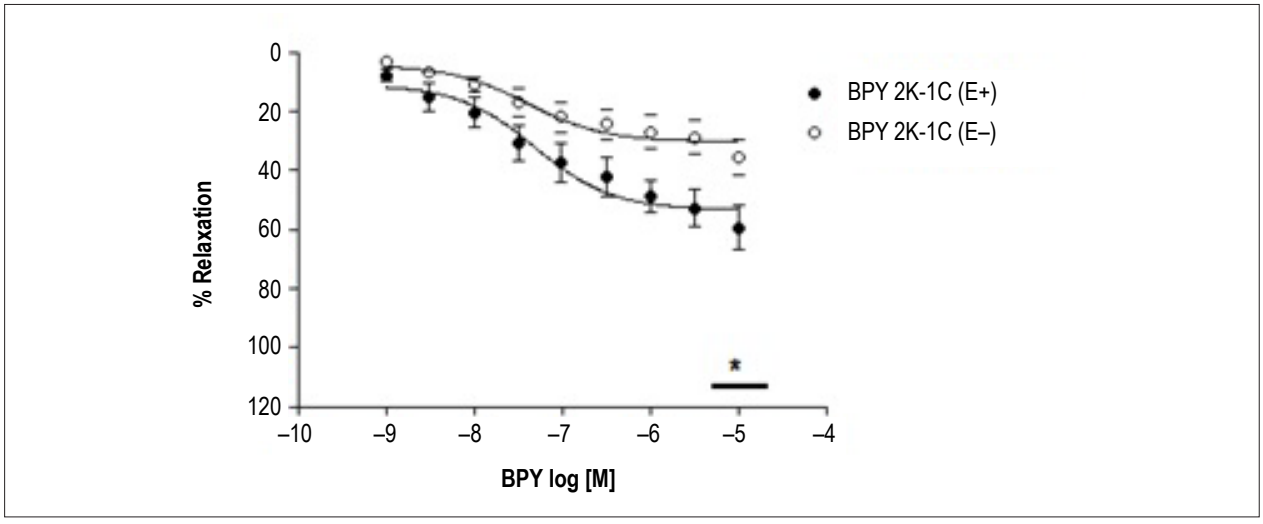

Figure 6 – *Relaxation coronary artery of rats (2K-1C) with (E +) and without (E-) form endothelium induced by compound cis-[Ru(bpy)$_2$(NO$_2^-$)(NO)](PF$_6$)$_2$ in rings pre-contracted with serotonin (SE). Curves cumulative concentration-effect were performed for BPY compound. Each point represents the mean ± S.D of data obtained from 5-7 independent determinations. * Indicates difference in the value of Emax.*

elevated concentration of O_2^- in aortic rings.[2] Thus, our results indicate that the vascular effect of BPY is not modified by endothelium or by O_2^- present in aorta 2K-1C.[29]

The endothelium potentiated the relaxation in coronary from normotensive (2K) and hypertensive (2K-1C) rats. This effect was observed just in coronary and not in aorta. In previous study, it was found that the endothelium also potentiated the relaxation induced by SNP in aortic rings,[18] and we have not found coronary study evaluating the effect of endothelium on relaxation induced by SNP. However, the relaxation induced by BPY is impaired in 2K-1C coronary rings with endothelium, with no difference in the absence. The impaired relaxation is in accordance to our previous study in aortic rings with another ruthenium compound.[2] but we have not verified any description in coronary. In our opinion, the potentiation of the effect generated on the relaxation was greater in coronary suggesting that in resistance vessels, the endothelium participates in inducing relaxation, and it does not happen in conductance vessels such as the aorta.[30]

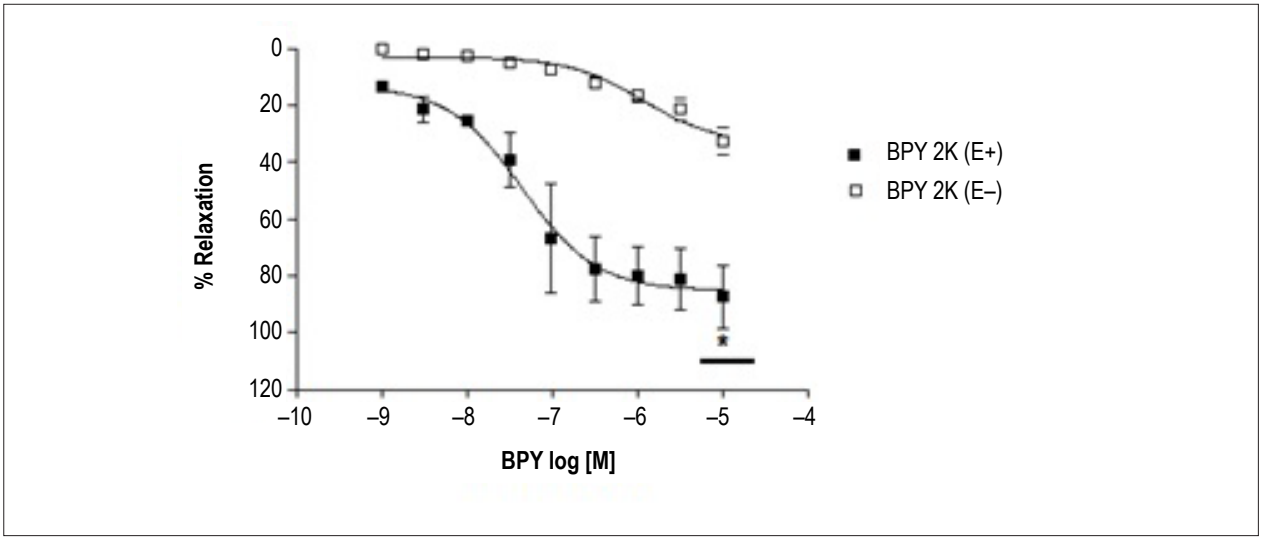

Figure 7 – *Coronary artery relaxation of normotensive rats (2K) with (E +) and without (E-) form endothelium induced by compound cis-[Ru(bpy)$_2$(NO$_2^-$)(NO)](PF$_6$)$_2$, in coronary rings contracted with serotonin. Curves cumulative concentration-effect were performed for BPY compound. Each point represents the mean ± S.D of data obtained in five independent determinations. *Indicates difference in the value of E_{max}.*

Conclusion

Taken together, our results suggest that 0.1 uM of BPY is able to normalize the endothelium dependent relaxation in hypertensive rats, and the compound BPY induces relaxation in aortic rings from normotensive and hypertensive rats with the same potency. In addition, the endothelium potentiate the relaxation effect induced by BPY in coronary rings from normotensive and hypertensive rats, with lower effect on coronary from hypertensive rats.

Limitations

The short period of time, corresponding to the duration of a master degree.

Author contributions

Conception and design of the research, Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content: Vatanabe IP, Rodrigues GJ, Silva RS; Acquisition of data: Vatanabe IP, Rodrigues CNS, Buzinnari TC, Moraes TF; Statistical analysis and Writing of the manuscript: Vatanabe IP; Obtaining funding: Vatanabe IP, Rodrigues GJ.

Potential Conflict of Interest

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

Sources of Funding

This work was supported by grants from São Paulo Research Fundation (FAPESP grant 2012/24477-8 and 2014/02231-2) and National Counsel of Technological and Scientific Development (CNPq grant 478849/2013-3).

Study Association

This article is part of the thesis of master submitted by Izabela Pereira Vatanabe, from Universidade Federal de São Carlos.

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Assessment of Subclinical Doxorubicin-induced Cardiotoxicity in a Rat Model by Speckle-Tracking Imaging

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Abstract

Backgrounds: Despite their clear therapeutic benefits, anthracycline-induced cardiotoxicity is a major concern limiting the ability to reduce morbidity and mortality associated with cancers. The early identification of anthracycline-induced cardiotoxicity is of vital importance to assess the cardiac risk against the potential cancer treatment.

Objective: To investigate whether speckle-tracking analysis can provide a sensitive and accurate measurement when detecting doxorubicin-induced left ventricular injury.

Methods: Wistar rats were divided into 4 groups with 8 rats each, given doxorubicin intraperitoneally at weekly intervals for up to 4 weeks. Group 1: 2.5 mg/kg/week; group 2: 3 mg/kg/week; group 3: 3.5mg/kg/week; group 4: 4mg/kg/week. An additional 5 rats were used as controls. Echocardiographic images were obtained at baseline and 1 week after the last dose of treatment. Radial (Srad) and circumferential (Scirc) strains, radial (SRrad) and circumferential (SRcirc) strain rates were analyzed. After the experiment, cardiac troponin I (cTnI) was analyzed and the heart samples were histologically evaluated.

Results: After doxorubicin exposure, LVEF was significantly reduced in group 4 ($p = 0.006$), but remained stable in the other groups. However, after treatment, Srads were reduced in groups 2, 3 and 4 (p all < 0.05). The decrease in Srads was correlated with cTnI (rho = –0.736, $p = 0.000$) and cardiomyopathy scores (rho = –0.797, $p = 0.000$).

Conclusion: Radial strain could provide a sensitive and noninvasive index in early detection of doxorubicin-induced myocardial injury. The changes in radial strain had a significant correlation with myocardial lesions and serum cardiac troponin I levels, indicating that this parameter could accurately evaluate cardiotoxicity severity. (Arq Bras Cardiol. 2017; 109(2):132-139)

Keywords: Cardiotoxicins; Oxidative Stress; Doxorubicin; Echocardiography, Doppler; Troponin I.

Introduction

Cardiotoxicity, which may result from cardiac oxidative stress, is the main limiting factor of the anticancer therapy using anthracycline.[1] Noninvasive techniques for the identification of patients who are at high risk of developing anthracycline-induced cardiomyopathy are critically important for the prevention and management of this complication.

Currently, two-dimensional speckle-tracking imaging (STI), based on tracking local image details from frame to frame throughout the cardiac cycle, has been reported as a simple and accurate method for the assessment of left ventricular mechanics.[2-5] It has been applied for early detection of myocardial injury in ischemic heart disease or various cardiomyopathies in both humans and experimental animals, allowing more accurate measurements of regional myocardial systolic performance.[6-10]

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Manuscript received October 12, 2016, revised manuscript January 20, 2017, accepted February 20, 2017

DOI: 10.5935/abc.20170097

The purpose of this study was to determine, by means of an experimental rat model using doxorubicin, whether STI could provide a more sensitive and accurate measurement in detecting left ventricular injury.

Methods

Animal treatment

This protocol was approved by the Animal Care and Use Committee of the Shanghai Jiaotong University and was in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Academy Press. Thirty-seven adult male Wistar rats, weighing 250.4 ± 4.3 g, were housed at constant temperature, with freely available food and water. The sample size calculation was performed based on the following assumptions: after anthracycline exposure, the difference in strain values was 20% between groups, the standard deviation within the group was 10%, power was 0.80, and the significance level was 0.05. As a result, we calculated a required sample size of 8 rats in each treatment group. Rats were randomized into 4 groups with 8 rats each, based on the process published by Martin RA et al.,[11] given doxorubicin intraperitoneally at weekly intervals for up to 4 weeks. Group 1: 2.5 mg/kg/week, total dose 10 mg/kg; group 2: 3 mg/kg/week,

total dose 12 mg/kg; group 3: 3.5mg/kg/week, total dose 14 mg/kg; group 4: 4 mg/kg/week, total dose 16 mg/kg. An additional 5 rats were used as controls, which received 1 mL of 0.9% saline solution intraperitoneally.

Echocardiography protocol

Images were obtained at baseline and 1 week after the last dose of anthracycline treatment. Rats were anesthetized by an intraperitoneal injection of 10% chloral hydrate at a dose of 0.3 ml/Kg. The rats were put in left lateral decubitus position and scanned using a commercially available echo-scanner, the Vivid ultrasound cardiovascular system (GE Healthcare Inc., Horten, Norway.), using a 10S (11.5MHz) phased array pediatric transducer and a cardiac application with high temporal and spatial resolution. The transmission frequency was 10MHz, the depth 2.5 cm, and the frame rate was 225 frames per second. The standard two-dimensional (2D) short-axis images acquired at the papillary muscle level were digitally stored for further off-line analysis. Left ventricular dimensions were measured using M-mode echocardiography through the short-axis view of the mid-papillary level and left ventricular ejection fraction (LVEF) was calculated using the Teicholz method.

EchoPAC 11.0 (GE Healthcare In,. Norway) was used for radial strain (Srad), circumferential strain (Scirc), radial strain rate (SRrad), and circumferential strain rate (SRcirc) analysis. This 2D-strain program tracked the movement of strong reflectors that were observed in the B-mode images, frame by frame, after segmenting the ventricular silhouette into six segments. The endocardial border was marked, while the outer border was adjusted to fit the epicardial contour. The software automatically tracked and computed strain and strain rate in radial and circumferential directions throughout the cardiac cycle. Peak systolic Srad, Scirc, SRrad and SRcirc were obtained from 6 segments of the papillary muscle levels. Data of at least three distinct cardiac cycles were averaged.

Histological study

One week after the end of the doxorubicin administration, the animals were euthanized with an overdose of chloral hydrate. Blood samples were collected for determination of serum levels of cardiac troponin I (cTnI). Left ventricles at the level of papillary muscles were fixed in phosphate-buffered 10% formalin, embedded in paraffin, and sectioned at a thickness of 5μm. These sections were stained with hematoxylin and eosin. The frequency and severity of myocardial lesions induced by doxorubicin were assessed semiquantitatively by light microscopic examination. The changes were graded based on the number of myocytes showing myofibrillar loss and cytoplasmic vacuolization (score from 0 to 3 according to Billingham.[12]) Animals that died spontaneously during the study also underwent necropsy, but they were not included in the data analysis.

Serum levels of cTnI

Blood samples were centrifuged and the serum samples were frozen at –80°C until analyzed. Serum concentrations were determined by immunoassay (Denley Dragon Wellscan MK 3, Thermo, Finland). The cardiomyopathy scores were calculated by an expert and cTnI levels were recorded by a technician, who were blinded to the experimental process and echocardiographic data.

Statistical analysis

Continuous variables close to a normal distribution were expressed as the mean ± standard deviation. Non-normal, skewed data were expressed as medians and boundaries of interquartile ranges. One sample K-S test was used to determine the normality of data. Differences in echocardiographic data before and after treatment, and between each group were determined using repeated measure ANOVA analysis. Values of cTnI levels and cardiomyopathy scores between each group were analyzed by Kruskal Wallis test. Spearman analysis was used in determining the correlation between strain values, cTnI and cardiomyopathy scores. Data were analyzed using the SPSS software, version 16.0 (SPSS, Inc, Chicago, IL, USA). A value of $p < 0.05$ was considered significant.

Results

General toxicity and gross anatomic changes

One of the rats from group 3 died after the third dose of doxorubicin. No terminal blood sample of this animal was available, and thus it was excluded from the study. At the necropsy, excessive amounts of pericardial and peritoneal fluids were observed in 4 of 8 animals from group 2, in 6 of 7 animals from group 3 and all animals from group 4. Excess fluid was also observed in the animal that died spontaneously. Accumulation of fluid was not found in the animals from group 1 and animals that received saline solution.

Systolic functions

There was no significant difference in LVEF at baseline between the groups. After doxorubicin exposure, LVEF reduced from 85.50 ± 1.06% to 82.50 ± 1.85% ($p = 0.006$) in animals given 16mg/kg doxorubicin. However, LVEF in the other animals receiving lower doses of doxorubicin showed no statistical difference before and after treatment (Table 1).

Strain analysis

Data on the strain and strain rate values in animals in the various treatment groups were summarized in Table 1. Baseline characteristics of doxorubicin-treated animals were similar to those of controls. Radial strains reduced after treatment in animals of group 2, group 3, and group 4 (from 52.1 ± 5.6% to 43.2 ± 5.7%, 52.5 ± 5.1% to 38.6 ± 4.8% and 52.3 ± 7.3% to 34.6 ± 7.4% respectively, all p values < 0.05). Circumferential strain reduced from –17.4 ± 2.1% to –14.1 ± 1.8% in group 4 after treatment ($p = 0.004$). The reduction of radial strains induced by doxorubicin was dose-related ($p = 0.000$). Radial strain rate and circumferential strain rate remained stable after exposure, regardless of the doxorubicin doses (Table 1, Figure 1).

Table 1 – LVEF and speckle-tracking indices in doxorubicin-treated and control rats

	Control group			Group 1			Group 2			Group 3			Group 4		
	Baseline	After	P value	Baseline	After	P value	Baseline	After	P value	Baseline	After	P value	Baseline	After	P value
LVEF (%)	85.4 ± 0.9	84.8 ± 2.9	0.591	86.2 ± 1.9	86.4 ± 2.3	0.890	84.6 ± 3.3	84.3 ± 2.9	0.714	83.9 ± 2.4	83.3 ± 2.3	0.220	85.5 ± 1.2	82.5 ± 1.8*	0.006
Srad (%)	52.2 ± 3.6	52.6 ± 3.1	0.730	51.2 ± 6.8	49.4 ± 5.2	0.061	52.1 ± 5.6	43.2 ± 5.7*	0.000	52.5 ± 5.1	38.6 ± 4.8*	0.000	52.3 ± 7.3	34.6 ± 7.4*	0.000
Scirc (%)	–17.2 ± 3.1	–18.2 ± 4.6	0.551	–16.1 ± 2.0	–17.0 ± 2.2	0.113	–17.2 ± 2.4	–16.7 ± 2.4	0.578	–17.0 ± 2.7	–17.9 ± 2.0	0.634	–17.4 ± 2.1	–14.1 ± 1.8*	0.004
SRrad (sec^{-1})	5.7 ± 1.1	5.5 ± 1.2	0.821	5.9 ± 0.8	6.1 ± 1.2	0.617	6.0 ± 0.9	6.0 ± 1.1	0.983	5.6 ± 1.1	5.6 ± 1.1	0.987	5.6 ± 1.0	5.5 ± 1.1	0.786
SRcirc (sec^{-1})	4.5 ± 0.7	4.9 ± 0.5	0.137	4.5 ± 1.2	4.7 ± 0.9	0.556	4.3 ± 1.0	4.3 ± 0.8	0.571	4.6 ± 0.9	4.3 ± 0.5	0.409	4.7 ± 0.7	4.5 ± 0.7	0.179

*LVEF: left ventricular ejection fraction; Scirc: circumferential strain; Srad: radial strain; SRcirc: circumferential strain rate; SRrad: radial strain rate. *: p < 0.05 compared with that of baseline.*

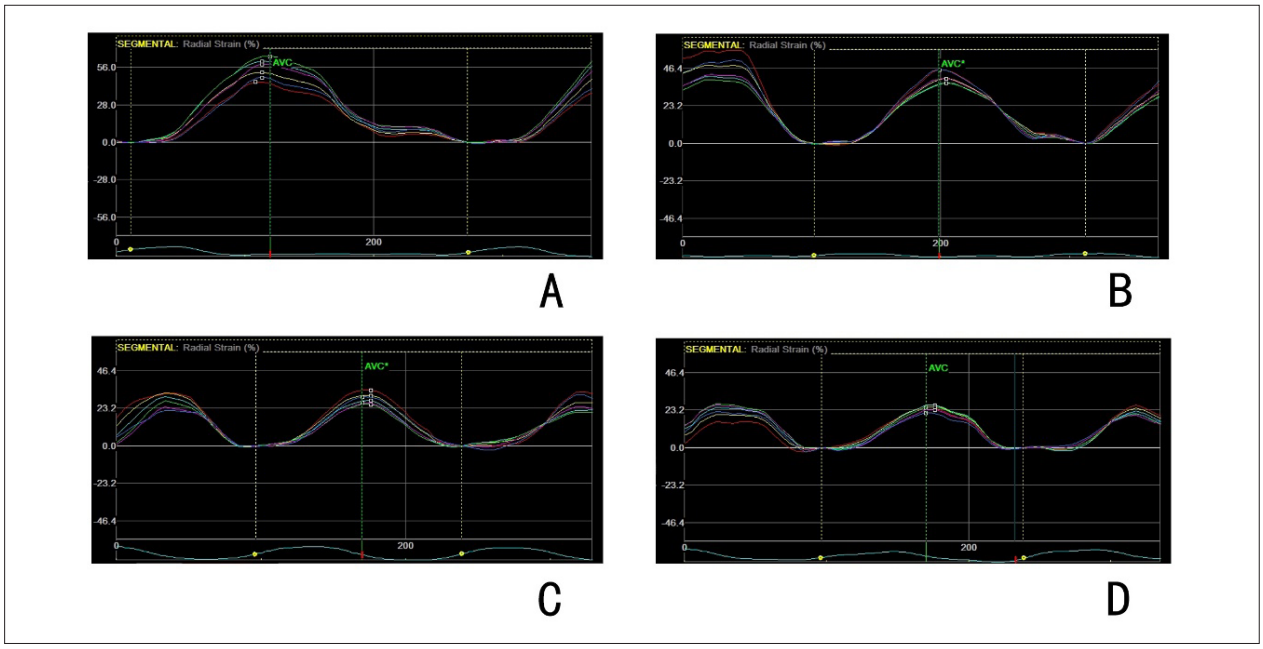

Figure 1 – *Radial strain curves obtained at the short-axis view of rats after doxorubicin treatment. A: rat from group 1, radial strain = 55.23%; B: rat from group 2, radial strain = 41.63%; C: rat from group 3, radial strain = 29.71%; D: rat from group 4, radial strain = 24.95%.*

Table 2 – Cardiomyopathy scores in Wistar rats treated with doxorubicin for 4 weeks

Dose of doxorubicin (mg/kg/w)	Nº. of animals	Cardiomyopathy score					
		0	1	1.5	2	2.5	3
4*	8	0	2	3	3	0	0
3.5†	7	2	2	2	1	0	0
3	8	4	2	2	0	0	0
2.5	8	6	2	0	0	0	0
Saline control	5	5	0	0	0	0	0

Cardiomyopathy scores are based on the percentage of myocytes showing cytoplasmic vacuolization and/or myofibrillar loss and are graded from 0 to 3 as follows: 0 = no alterations, 1 ≤ 5%, 1.5 = 5% to 15%, 2.0 = 16% to 25%, 2.5 = 26% to 35%, and 3 ≥ 35%.
**: Cardiomyopathy scores were significantly (p < 0.05) higher than in those receiving 3 mg/kg/w or less doxorubicin. †: Cardiomyopathy scores were significantly (p < 0.05) higher than in those receiving 2.5 mg/kg/w or less doxorubicin.*

Myocardial pathology

Animals treated with doxorubicin developed cardiac lesions that could be identified on light microscopy evaluation. The characteristics of these lesions, cytoplasmic vacuolization and myofibrillar loss, have been previously observed in animal models, as well as in human patients who received anthracycline chemotherapy.[13,14] Data on the incidence and severity of the myocardial lesions were summarized in Table 2. The severity of these lesions was significantly higher in group 3 and 4 (who received 14 mg/kg and 16 mg/kg doxorubicin, respectively) than those who received either 12 mg/kg or 10 mg/kg doxorubicin. The hearts of all animals from the control group were normal (Figure 2).

Levels of cTnI

The levels of cTnI in the control group and groups 1,2,3, and 4 were 7.62 (3.06) ng/mL, 6.92 (4.04) ng/mL, 17.03 (8.46) ng/mL, 22.57 (12.21) ng/mL and 34.93 (11.24) ng/mL, respectively. As shown in Figure 3, the serum cTnI levels in group 1 were not significantly different from those of the control group. However, compared with those of the animals that received saline, cTnI levels significantly increased with the rise of total cumulative doses of doxorubicin (Figure 3).

Correlations between strain values, cTnI levels and histological lesions

The decrease in radial strains exhibited a clear correlation with the cTnI levels (Spearman's correlation rho = –0.736, p = 0.000) (Figure 4) and with cardiomyopathy scores (Spearman's correlation rho = –0.797, p = 0.000) (Figure 5).

Discussion

Anthracycline remains a commonly used chemotherapy agent. However, the clinical efficacy is undermined by potential life-threatening cardiotoxicity.[1] An accurate and noninvasive method for early monitoring of cardiac injury is of vital importance to guide preventive and therapeutic strategies in reducing cardiotoxicity. In this study, we proposed a novel use of speckle-tracking imaging for the assessment of subclinical myocardial injury after anthracycline treatment. In clinical practice, LVEF monitoring is the most important clinical diagnostic tool in the recognition of cardiac dysfunction.[15,16] However, they are somewhat insensitive in detecting early signs of cardiac stress, myocardial injury, and changes in myocardial compliance. In the present study, even though myocardial lesions and elevation of serum cTnI levels were observed after the treatment, LVEF remained stable and

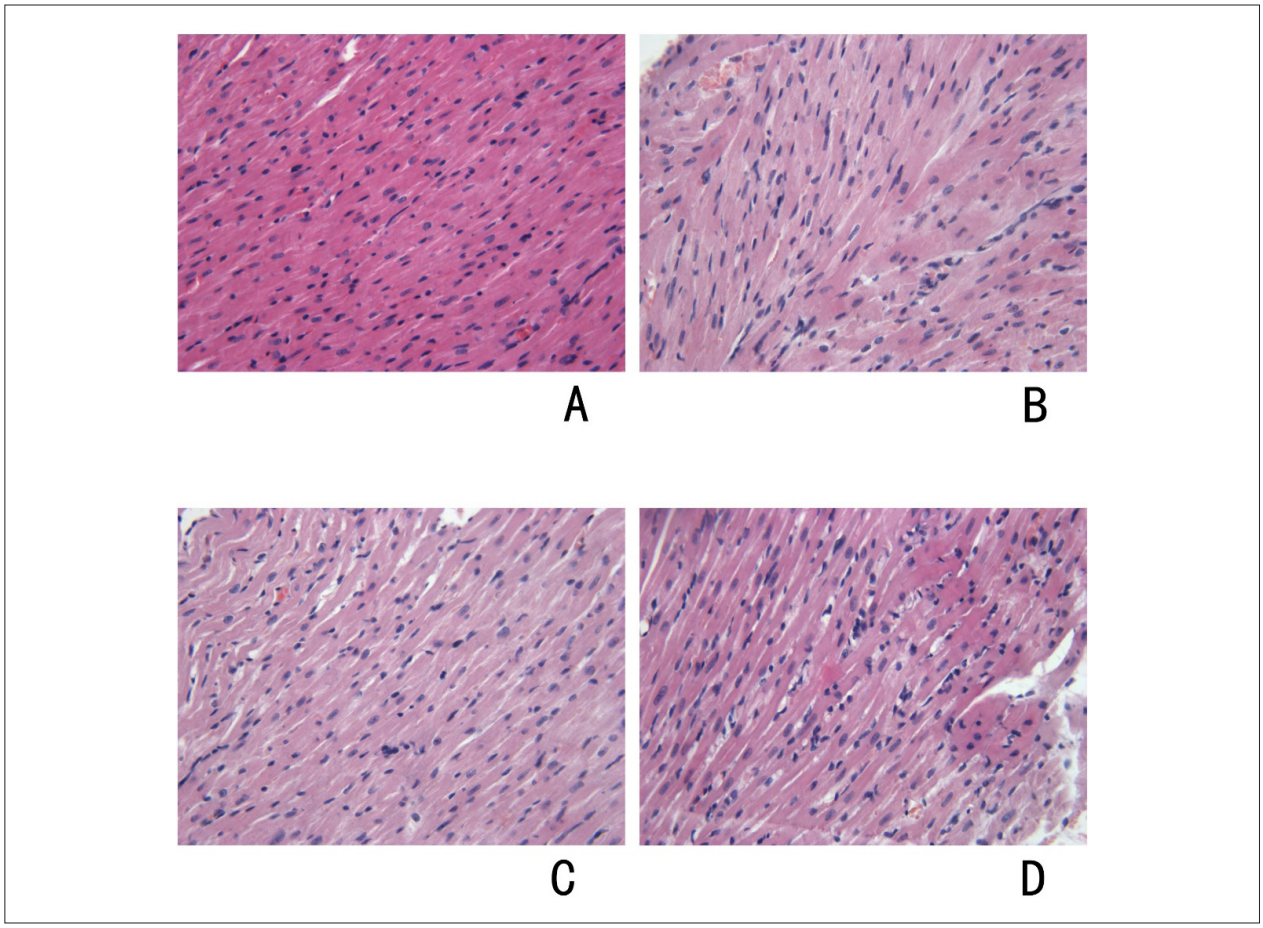

Figure 2 – *Myocardial changes after doxorubicin treatment at the light microscopy level (× 400). Vacuolization of the cytoplasm, loss of myofibrils was more severe in rats from group 4 (D). A: rat from group 1; B: rat from group 2; C: rat from group 3; D: rat from group 4.*

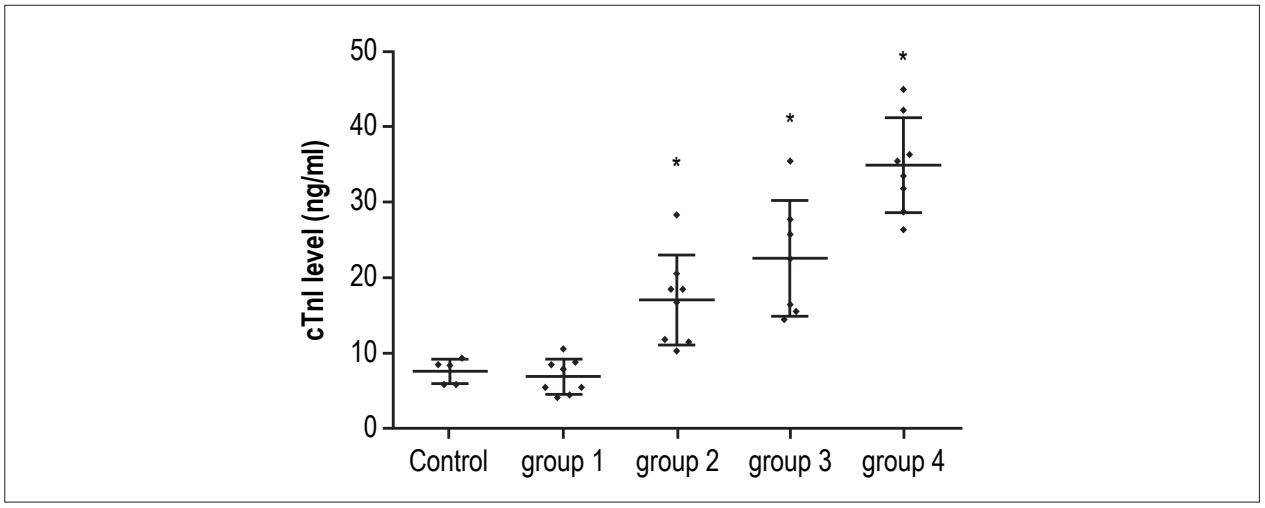

Figure 3 – *Scatter diagram of serum levels of cTnI in individual rat. *: p < 0.05 compared with that of baseline.*

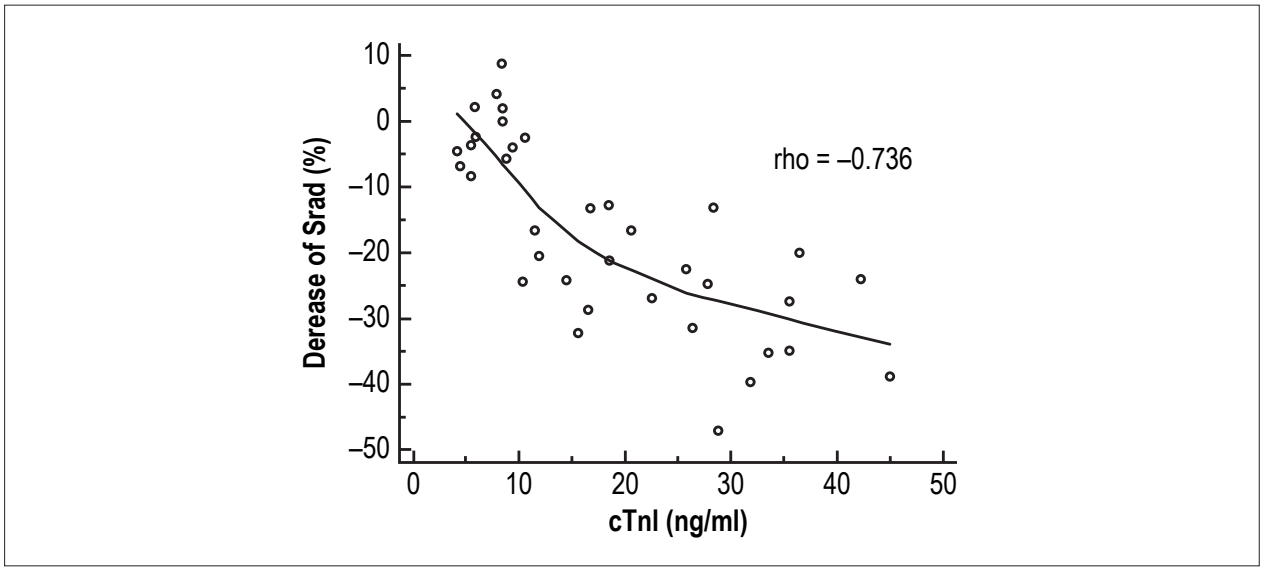

Figure 4 – *Correlations between radial strains and cTnI levels.*

within the normal range, showing that LVEF was insensitive in early detection of myocardial injury.

Strain is a dimensionless parameter representing the deformation of a myocardial segment in relation to its original dimensions within a systolic time-frame. Early studies reported that the reduction in left ventricular function, caused by anthracycline, could be assessed by strain and strain rate indices when measured by Doppler imaging.[17] With the advantages of angle independence, speckle-tracking imaging, a relatively new and more comprehensive technique, could assess both regional and global left ventricular myocardial deformation in three dimensions, providing reliable and sensitive parameters for early cardiac injury detection. Shi et al.[18] found that radial strain analysis based on STI could detect acute allograft rejection in a rat heart transplant model, which was more sensitive than conventional echocardiographic parameters. In a rat model of athlete's heart, speckle-tracking based strain values correlated well with pressure-volume loop-deprived contractility indices.[8] Previous studies observed that strains decreased significantly after epirubicin treatment, although conventional echocardiographic parameters remained stable and within normal range.[19]

We demonstrated that in our animal model, radial strain was more sensitive than left ventricular ejection fraction in the assessment of cardiac injury at an early stage, which was confirmed by histological examination and serum cTnI. Chemotherapy-induced cardiotoxicity has a regional pattern,[20,21] which could explain the increased sensitivity of strain values compared with the LVEF in the detection of early cardiotoxicity. In addition, we found that the changes

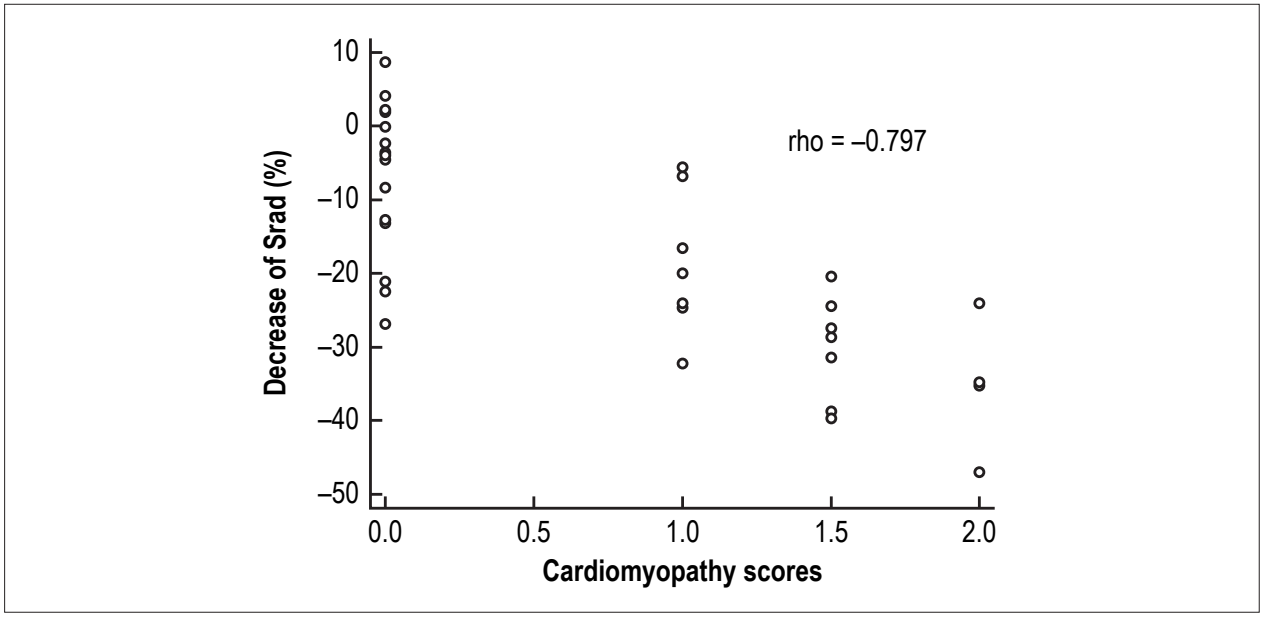

Figure 5 – *Correlations between radial strains and cardiomyopathy scores.*

in radial strain exhibited a clear correlation with histological lesions and elevation of cTnI levels, indicating that radial strain could accurately evaluate cardiotoxicity severity.

STI has a better spatial resolution in comparison with the tissue-Doppler imaging-based technique.[22] In clinical use, frame rates > 90 frames/s often lead to poor speckle-tracking.[23] However, because of their faster heart rates, higher frame rates are necessary in rodents. Transducer frequency, sector width and depth, as well as the number of crystals within the transducer will have an impact on scan line resolution, which will in turn affect the quality of speckle-tracking. With high crystal density over a very small sector width and depth, our 11.5 MHz transducer can obtain good images at high frame rates, with no loss of scan line resolution.

Limitations

Cardiac imaging of the left ventricle in rodents is limited to a few echocardiographic views. Although it is possible to obtain an apical 4-chamber view, the lateral wall is rarely visualized.[24,25] The image quality of the longitudinal view was poor and, therefore, we could not provide data about the longitudinal function.

The type of anesthesia can influence heart rate and intrinsic myocardial contractility. However, in this study, all animal including the treated and control groups underwent the same anesthesia procedure in order to limit the effect of anesthesia on cardiac function analysis. The frame rate related to the heart cycle duration used in this study was lower than studies performed in humans or large animals. Of note, we had similar frame rates as in recent experiments in a rat model of myocardial infarction and acute rejection.[18]

Conclusion

Radial strain based on speckle-tracking imaging can provide a sensitive and noninvasive strategy in early detection of doxorubicin-induced myocardial injury.

Author contributions

Conception and design of the research: Kang Y, Shen X; Acquisition of data: Kang Y, Wang W, Zhao H, Qiao Z; Analysis and interpretation of the data: Kang Y, Wang W, Zhao H; Statistical analysis: Kang Y, Wang W; Obtaining funding, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Kang Y; Supervision: Shen X, He B.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by National Natural Science Foundation of China (N° 81401411) and Shanghai Natural Science Foundation (N° 14ZR1425200, 16ZR1420600).

Study Association

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

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Plasma Total Antioxidant Capacity and Cardiometabolic Risk in Non-Obese and Clinically Healthy Young Adults

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Abstract

Background: The oxidative biomarkers play an important role in the genesis of cardiometabolic risk-related processes.

Objective: To investigate the total antioxidant capacity of plasma and its association with cardiometabolic risk in non-obese and clinically healthy young adults.

Methods: University students of the state of Sergipe, Brazil, aged between 18 and 25 years, were recruited for this study from May of 2013 and October of 2014. Anthropometric, clinical and biochemical parameters were measured and analyzed using protocols which were previously standardized and described in the literature. The measurement of plasma total antioxidant capacity was based on the ability that all the antioxidants present in the sample (plasma) have to inhibit the oxidation of the oxidizable substrate ABTS (2,2`- Azino-di-[3-ethylbenzthiazoline sulphonate]) to ABTS•+ by metmyoglobin.

Results: Approximately 25% of the sample presented more than one component of cardiometabolic risk. Low HDL-cholesterol was the most prevalent component. Compared to absence of components, the subjects with at least one component presented greater body weight and waist circumference, higher levels of diastolic blood pressure and fasting glucose, greater total cholesterol/HDL-c ratio, and lower levels of HDL-c ($p < 0.05$). Fasting glycemia was the only parameter which was associated with total antioxidant capacity ($R^2 = 0.10$; $\beta = 0.17$; $p = 0.001$).

Conclusions: The plasma total antioxidant capacity was not able to predict the cardiometabolic risk components due possibly to the establishment of compensatory mechanisms that become activated in physiological conditions. (Arq Bras Cardiol. 2017; 109(2):140-147)

Keywords: Cardiovascular Diseases; Risk Factors; Metabolic Syndrome; Oxidative Stress; Antioxidants; Young Adult.

Introduction

The presence of cardiometabolic risk (CMR) components, such as systemic arterial hypertension, hyperglycemia, abdominal obesity, and lipid profile alterations (e.g. high triglycerides and low HDL-c)[1,2] has been strongly associated with oxidative stress (OS) which is established by the increased expression of oxidizing substances to the detriment of antioxidants.[3]

The association between OS and the CMR components has been evidenced due to intense production of reactive oxygen species (ROS) from metabolic alterations, such as increased insulin resistance and visceral adiposity.[4]

Total antioxidant capacity (TAC) of plasma is an important biomarker of OS since it defines the synergistic effect between the various antioxidant compounds in the sample.[5] The presence of CMR components and chronic non-communicable diseases (NCD) has been associated to lower TAC levels in the population.[6] Studies have demonstrated that this decrease in the TAC occurs because of the greater amount ROS as compared to the antioxidant compounds.[7,8]

The presence of CMR components in individuals with NCD is predictable and largely described in the literature since these components are predisposing factors of NCDs.[9] OS and inflammation have also been associated with CMR and NCD.[4] However, there is still a dearth of investigations with representative data about frequency of CMR components and their predisposing factors among healthy populations in Brazil.

Therefore, the association between TAC and anthropometric, clinical, and biochemical components of the CMR in non-obese, clinically healthy young adults is still not well elucidated in the literature. The hypothesis of this study is that decreased TAC increases CMR, even in non-obese, healthy young adults. Evaluating changes in CMR components, as well as in their predisposing factors, may be a preventive measure to the development of NCDs, since clinical consequences of NCDs, such as clinical complications, increasing prevalence of premature death, and its social and economic costs may be

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Manuscript received July 29, 2016, revised manuscript November 10, 2016, accepted March 15, 2017

DOI: 10.5935/abc.20170095

prevented by interventions.[10] The objective of the study was to evaluate the association between TAC of plasma and CMR components in non-obese and clinically healthy young adults.

Methods

Study design

This is a cross-sectional study on a convenience sample. The volunteers' recruitment occurred through invitations by email, posters, and classroom visits. The data collection was performed between May of 2013 and October of 2014 at two universities, one public and one private, located in the city of Aracaju, Northeast, Brazil.

Participants

Non-obese, clinically healthy young adults, who were students of schools of health sciences, aged between 18 and 25 years of both sexes, participated in the study. Exclusion criteria included the evidence of any disease related to OS, chronic inflammation, gestation, lactation, water-electrolyte imbalance, and self-reported changes in body composition or in absorption and/or metabolism of nutrients. Exclusion criteria still included recent use of medicaments and/or dietary supplements, follow-up of nutritional treatment that may affect energy balance, food consumption, lipid profile, plasma insulin levels, and metabolism of glucose; regular use of birth control pills in the 2 months before the participation in the study; unstable body weight in the past 6 months (10% variation above or below allowed); being an elite athlete or planning to change lifestyle during the period of the study; and follow-up of special diets (e.g. vegetarian diet, Atkins diet, etc.) in the 3 months prior to the participation of the study.

The sample was calculated according to Miot (2011),[11] considering a prevalence of 9.9% for high waist circumference among university students,[12] significance level of 5.0%, sampling error of 5.0% and population size of 8,951, considering the number of university students enrolled in health majors. A minimum sample size of 135 individuals was estimated.

Anthropometric and body composition parameters

Height was measured to the nearest 1 mm using a stadiometer (Altura Exata, Minas Gerais, Brazil). Weight was measured to the nearest 100 grams using an electronic digital balance (Líder, P 180M, São Paulo, Brazil) with maximum capacity of 180 kilograms. Body Mass Index (BMI) was calculated by dividing body weight (kg) by height squared (m) and classified according to the cutoff points proposed by the World Health Organization (WHO).[13]

Waist circumference was measured to the nearest 1 mm between the last rib and the iliac crest using a flexible and inelastic tape measure.[13]

Triceps, biceps, subscapular, and suprailiac skinfolds thickness were measured to the nearest 1 mm using a skinfold caliper (*Lange caliper, Cambridge Scientific Industries Inc., Cambridge, Maryland, USA*). Truncal fat percentage was calculated from the ratio of the sum of subscapular and suprailiac skinfolds and the sum of the four skinfolds.[14]

Body fat percentage was obtained by bioelectrical impedance analysis using a quadrupole device (Biodynamics model 310, Washington, USA), from which body fat and fat-free mass were calculated in kilograms.

Biochemical measures

Blood collection was performed by venipuncture after fasting of 12 hours and no intake of alcohol, coffee or tea for 24 hours. Samples of heparin and plasma were separated by centrifugation at 2,465 g to 5°C for 15 minutes and stored at –80°C.

Serum concentration (mg/dL) of glucose, total cholesterol, high-density lipoprotein (HDL-c), and triglycerides (TG) were analyzed by a colorimetric or turbidimetric assay by an automatic analyzer using specific assay kits.

TAC in plasma was determined by colorimetric assay using a specific assay kit (Cayman Chemical, Ann Arbor, MI, catalog no. 709001). The assay was based on the ability that all the antioxidants present in the sample (plasma) have to inhibit the oxidation of the oxidizable substrate ABTS (2,2`- Azino-di-[3-ethylbenzthiazoline sulphonate]) to ABTS•+ by metmyoglobin. The amount of oxidized substrate (ABTS•+) was monitored by absorbance reading at 750 nm. The decrease in absorbance at 750 nm was directly proportional to the concentration of antioxidants in plasma expressed as mM Trolox equivalents, a synthetic water-soluble analogue of vitamin E.

Blood pressure

Systolic and diastolic blood pressure levels were measured to the nearest 2 mmHg using a mercury sphygmomanometer according to Perloff et al.[15]

CMR components

The CMR components were diagnosed according to the criteria of the International Diabetes Federation (2005):[1] abdominal obesity (waist circumference >80 cm for women and > 94 cm for men); fasting hyperglycemia (> 100 mg/dL); hypertriglyceridemia (> 150 mg/dL); low HDL-C (< 50 mg/dL for women and < 40 mg/dL for men); and hypertension (systolic pressure > 130 mmHg; diastolic pressure > 85 mmHg).

Food Consumption and lifestyle variables

Usual dietary intake was obtained by the application of a semiquantitative food frequency questionnaire (FFQ), developed for this study population. The Virtual Nutri software was used to quantify energy and nutrient intake. Inadequate intake was evaluated according to the recommendation proposed by the Dietary Reference Intakes (DRI) (National Research Council, NRC, 2011),[16,17] using the Estimated Average Requirement (EAR) and the Adequate Intake (AI) values as cutoff points. Inadequate energy intake was determined by the intake < 90% or > 110% from the Estimated Energy Requirement (EER), calculated by predictive equations proposed by the Institute

of Medicine (2005).[15] Some methodological precautions were adopted around the food consumption assessment, such as the use of visual aids to assist the participants estimate the portion sizes during the FFQ application, training of interviewers, pilot test to clarify questions and inadequacies in the FFQ, and standardization of recipes.

We collected information on vitamin supplements, smoking, number of cigarettes per day, regular physical activity, and its intensity. To characterize and quantify physical activity, we used the short version of the International Physical Activity Questionnaire (IPAQ), which is recommended by the World Health Organization and has been validated in Brazil by the Center of Studies of the Physical Fitness Research Laboratory of São Caetano do Sul – CELAFISCS.[18]

Statistical analysis

Continuous variables were presented as mean ± standard deviation while categorical variables as absolute (n) or relative frequency (%).

Kolmogorov-Smirnov test was used to verify the normality of the distribution. Unpaired Student's t-test was adopted to compare the categorized groups by the presence of the CMR components. To track the correlation between TAC and other variables of interest related to the components of the CMR, we used Pearson test. Multivariate linear regression was performed with the fasting glucose values (mg/dL) as being the dependent variable and TAC value (mM), sex and age as being the independent variables. A 95% confidence interval was used to describe the values of the linear regression coefficient (β).

Statistical significance was accepted at $p < 0.05$. All analyses were conducted using Statistical Package for Social Science, SPSS version 20.0, for Windows.

Ethical aspects

The study was approved by the Human Research Ethics Committee of the Federal University of Sergipe (C.A.A.E.: 0113.0.107.000-11).

In accordance with the principles of the declaration of Helsinki, all volunteers were informed about the study protocol and then signed the consent form. The volunteers were informed about the methods and procedures used in the data collection, the possible benefits and inconveniences, the privacy of results and the voluntariness of participation.

Results

A total of 139 non-obese and clinically healthy young individuals, aged 21.4 ± 1.9 years, participated of the study. Women predominated in the distribution of gender (77%). The anthropometric, clinical and biochemical parameters are described in Table 1.

Although the individuals of this study were non-obese and clinically healthy, they already presented CMR components. About 15% (n = 20) of the sample had low HDL-c concentrations followed by high diastolic blood pressure levels (n = 9; 7%), triglyceride (n = 8; 6%), glucose (n = 6; 4%), and abdominal obesity (n = 3; 2%). Almost one-quarter of the sample (n = 34; 24.5%) had at least one component of the CMR followed by 2 (n = 11; 8%) and 3 or more CMR components (n = 1; 0.7%), respectively.

Among the nutrient intake inadequacies were considerable those relating to the consumption of saturated fat (n = 114; 92%), fiber (n = 89; 72%), and vitamin D (n = 107; 86%).

The individuals were categorized by the presence of the CMR components (Table 2). Those with at least one component showed greater body weight, TG/HDL-c ratio, and total cholesterol/HDL-c ratio when compared with the individuals who did not present any of the CMR components ($p < 0.05$).

Glycemia was the only CMR component which correlated with plasma TAC (Figure 1).

After adjusting the multivariate linear analysis by sex and age, fasting glycemia was positively associated with the TAC values ($R^2 = 0.10$; $\beta = 0.17$; $p = 0.001$). TAC had a positive predictive effect on the serum glucose levels. An increase by 1 unit in the levels of TAC was associated with an increase by 0.17 mg/dL in fasting glycemia. It is noteworthy that 10.0% of the increase in glycemia was due to the effect of TAC (Table 3).

Discussion

The study about the CMR components has attracted attention since they are strongly related to the development of diseases associated with insulin resistance and cardiovascular events, which are the major causes of morbidity and mortality in the population.[19] These diseases have direct and indirect impact on social and state economy, including low productivity, loss of working days, damage to the productive sector, and intangible costs of people affected by these conditions.[10,20]

Determining the presence of CMR components in young populations is relevant for the early diagnosis, and establishment of specific interventions and preventive measures. In our study population, although the prevalence of alterations in the outcome measures was low – according to the proposed reference values – almost one-quarter of the sample had at least one component of the CMR. Low HDL-c was the most prevalent component (15%). On the other hand, studies on college students, both in Brazil[20] and in other countries,[21,22] have found higher prevalence. This controversy may be related to the characteristics of our population, composed of physically active (65%), non-smoking, university students from health sciences area, mainly nutrition (39%), and with low prevalence of alcohol consumption.

The prevalence of CMR components in college populations in national[23] and international[21,22] studies varied from 30 to 77% among individuals with at least one risk component, from 12 to 13% for those with two, and from 3 to 16% for those with three components. Low HDL-c and high blood pressure are the most prevalent CMR components.

The early development of these components in young adults has been attributed to poor eating habits, commonly observed in young populations, due to factors related to this life stage, including independence, inability to make healthy food choices, lack of time, convenience, costs, and influence

Table 1 – Demographic, anthropometric, clinical and biochemical characteristics (mean and standard deviation) of non-obese and clinically healthy young adults

Total (n=139)	X	SD
Age (years)	21.4	1.9
Weight (kg)[a]	55.9	7.4
BMI (kg/m²)[a]	20.6	2.1
WC (cm)[a]	71.1	5.6
TSF (mm)[a]	18.6	6.8
BSF (mm)[a]	9.6	5.4
SISF (mm)[a]	16.1	6.5
SSF (mm)[a]	14.8	4.4
Total fat (%)[a]	23.0	9.7
Truncal fat (%)[a]	53.2	7.0
Fat mass (kg)[a]	12.7	5.5
Fat-free mass (kg)[a]	43.1	8.6
SBP (mmHg)[b]	108.8	8.0
DBP (mmHg)[b]	74.8	7.8
Fasting glucose (mg/dL)[c]	85.6	8.4
Total cholesterol (mg/dL)[d]	170.7	38.5
HDL-c (mg/dL)[d]	56.0	10.9
LDL-c (mg/dL)[d]	98.9	33.0
Triglyceride (mg/dL)[d]	78.4	35.2
Total cholesterol/HDL-c[d]	3.0	0.7

BMI: body mass index; WC: waist circumference; TSF: triceps skinfold; BSF: biceps skinfold; SISF: suprailiac skinfold; SSF: subscapular skinfold; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-c: high-density lipoprotein; LDL-c: low-density lipoprotein. [a]n = 123; [b] n = 122; [c] n = 136; [d] n = 137. Reference values: waist circumference < 80 cm for women and < 94 cm for men; fasting glucose ≤ 100 mg/dL; triglyceride ≤ 150 mg/dL; HDL-c > 50 mg/dL for women and > 40 mg/dL for men; SBP ≤ 130 mmHg; DBP ≤ 85 mmHg.

Table 2 – Demographic, anthropometric, clinical and biochemical characteristics (mean and standard deviation) according to the presence of cardiometabolic risk components among non-obese, clinically healthy young adults (n = 139)

	No component		≥ 1 component		
	X	SD	X	SD	p
Age (years)	21.4	2.0	21.9	1.8	0.73
Weight (kg)[a]	54.5	6.7	60.6	7.7	< 0.01
BMI (kg/m²)[a]	20.5	2.0	29.0	2.3	0.28
Total cholesterol (mg/dL)[d]	171.9	39.5	166.8	35.5	0.50
LDL-c (mg/dL)[d]	99.1	34.9	98.3	26.7	0.90
Triglyceride/HDL-c (mg/dL)[d]	1.2	0.5	2.0	0.9	0.00
Total cholesterol/HDL-c[d]	2.9	0.6	3.5	0.8	< 0.01
TAC (mM)[d]	3.1	0.6	2.9	0.8	0.23

BMI: body mass index; TAC: total antioxidant capacity; LDL-c: low-density lipoprotein. Data present as mean ± standard deviation (X ± SD); statistical signicance level of 5%; Student's t-test. [a]n = 123; [b] n = 122; [c] n = 136; [d] n = 137

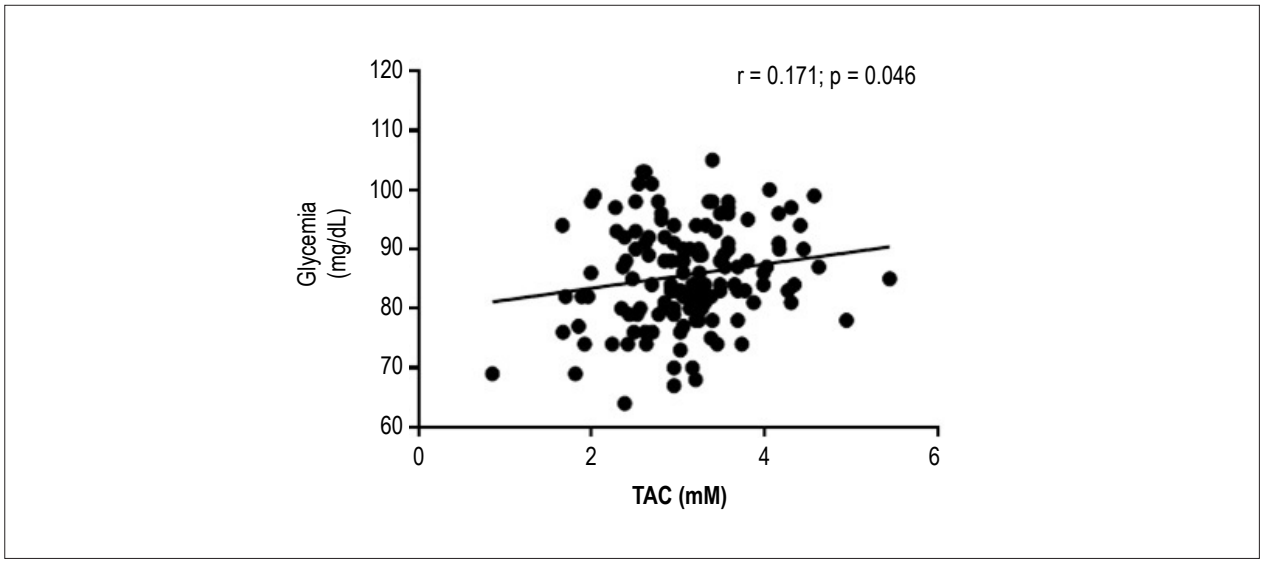

Figure 1 – *Pearson correlation between plasma total antioxidant capacity (TAC) and fasting glycemia values (n = 139).*

Table 3 – Multivariate linear regression analysis with the glycemia (mg/dL) as dependent variable adjusted by sex and age (n = 139)

	β (95% confidence interval)	p
TAC (mM)	0.174 (0.135-3.957)	0.030
Sex	0.267 (2.099-8.553)	0.001
Age	–0.132 (-1.243-0.128)	0.110

R^2 = 0.100, p = 0.001. TAC: total antioxidant capacity.

of both physical and social environments.[24] The result of this combination is the greater weight gain in the first year of college when compared to adulthood.[25]

In this study, it was observed that the presence of at least one risk component in the study population was associated with greater anthropometric (body weight) and biochemical (TG/HDL-c and total cholesterol/HDL-c ratios) parameters. These results are corroborated by studies conducted with college students from Brazil[23] and from other countries.[21,22]

TG/HDL-c ratio has been largely discussed as an atherogenic risk indicator for predicting the development of coronary artery disease,[26] acute myocardial infarction,[27] and atherosclerosis.[28] Studies have showed that high values of TG/HDL-c are correlated with increased CMR in the population.[22,29] In addition, TG/HDL-c ratio has been positively correlated with biochemical (total cholesterol, LDL-c, and TG) and anthropometric parameters (BMI, waist circumference, and body fat percentage) and negatively with HDL-c.[30]

The presence of the CMR components, such as abdominal obesity,[31] hypertriglyceridemia, low HDL-c and hypertension,[32,33] has been associated to the development of NCDs, especially, type 2 diabetes, cardiovascular diseases, and cancer. In Brazil, a study was conducted to evaluate the association between cardiovascular risk factors and anthropometric indicators in patients with NCD.[34] The authors found that 74% of the sample presented glycemia > 100 mg/dL, 56% low HDL-c, 82% high waist circumference, and 78% overweight.[34]

Excessive ROS production is another factor that has been associated with the presence of CMR components and, consequently, to the development of NCDs.[4,6] Several studies have identified the association between plasma TAC –an important biomarker of OS for expressing the synergistic action between various antioxidant compounds[5] – and alterations in the anthropometric, clinical and biochemical CMR components.[6] It is worth mentioning that all these studies were conducted with individuals with NCDs. There is still a dearth of studies with healthy individuals.

Although the individuals of the present study were non-obese and clinically healthy, it was possible to identify an association between TAC and fasting glycemia after adjustment by sex and age (R^2 = 0.10, β = 0.17, p = 0.001). The positive correlation between TAC and fasting glucose found in the study does not corroborate with the literature.[35] Hyperglycemia increases the expression of OS by the increase of NADPH concentrations and ROS production due to the intense mitochondrial metabolism of glucose.[36] This results in an increased production of electron donors (FADH2 and NADH) in the Krebs cycle and, hence, in a high mitochondrial membrane potential

(DmH+) by pumping protons across the inner membrane, inhibiting electron transport at complex III, and increasing the half-life of free-radical intermediates of coenzyme Q (ubiquinone) which reduces O_2 to superoxide. Thus, studies have shown a negative correlation between fasting glycemia and plasma and dietary TAC,[37] as well as greater amount of products from oxidative reactions, which reduce the level of the substances that make up the antioxidant system.[38] However, all these studies were conducted with individuals with NCD already established.

Due to the characteristics of the participants of this study –young, clinically healthy, and non-obese, and the high TAC, one may suggest the establishment of an adaptive mechanism based on the evidence that the increase of 1 unit in the TAC levels (1mM) is associated to the increase of 0.17 mg/dL in the fasting glycemia levels, *i.e.*, increased glycemia in homeostasis would determine a compensatory increase of the TAC. This occurs through negative feedback which may activate the enzymatic pathways of the antioxidant system to reduce the intracellular levels of ROS, thereby minimizing oxidative damage.[39] The findings by Demirbag et al.[35] corroborate this assumption. The increase in TAC becomes impracticable in pathologic conditions already set in, different from what occurs in health individuals.

The lack of associations between TAC and the other anthropometric, clinical and biochemical variables in the study may be explained by the low prevalence of alterations on these parameters and by the characteristics of the studied population: young, predominantly women, non-obese, clinically healthy, physically active, students of health sciences, low alcohol consumption, non-smokers, and markers of adiposity – waist circumference (71.1 ± 5.6 cm) and body fat percentage (23.0 ± 9.7%) – below the risk for triggering metabolic alterations. Nevertheless, it is worth pointing out the high TAC value found in the present study (3.10 ± 0.71; median = 3.09 mM) compared to the values found by Barbosa et al.[40] in young adults (1.60 mM). This result may be associated with the low prevalence of behavioral risk factors, such as sedentary life style (36%), low consumption of alcohol, being non-smoker, in addition to be university students in health sciences.

Some limitations in this study must be acknowledged: the sample loss of some variables due to incomplete information and/or study dropout; the assessment of food consumption by instruments available in the literature are subject to error because of their large inter- and intra-individual variability, as well as the dependence on the respondents' memory about past habits, low accuracy in quantifying the intake due to the use of standardized measures and food lists. Finally, the methods used to evaluate plasma TAC strictly reflect chemical reactions *in vitro*, without similarity to biological systems. Their results should therefore be interpreted with caution since they do not measure bioavailability, *in vivo* stability, retention of antioxidants in the tissues, and *in situ* reactivity.

Conclusions

In this study, CMR components were present in some young, clinically healthy, non-obese, and high-TAC adults.

The observed positive correlation between plasma TAC and fasting glycemia suggests the establishment of an adaptive mechanism. The increase in glycemia in a biological system, in homeostasis, would determine a compensatory increase of the plasma TAC.

Thus, different from what occurs in populations with NCD already set in, TAC was not associated with CMR components in this sample of young, non-obese and clinically healthy individuals due possibly to the establishment of compensatory mechanisms that become activated in physiological conditions.

Author contributions

Conception and design of the research: Jesus AMR, Barbosa KBF; Acquisition of data: Costa JO, Vásquez CMP, Santana GJ, Silva NJ, Braz JM, Cunha LCS, Barbosa KBF; Analysis and interpretation of the data: Costa JO; Statistical analysis: Silva DG, Barbosa KBF; Obtaining funding: Barbosa KBF; Writing of the manuscript: Costa JO; Critical revision of the manuscript for intellectual content: Costa JO, Barbosa KBF.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by Fundação de Apoio à Pesquisa e à Inovação Tecnológica do Estado de Sergipe.

Study Association

This article is part of the thesis of master submitted by de Jamille Oliveira Costa, from Universidade Federal de Sergipe.

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Primary Mitral Valve Regurgitation Outcome in Patients With Severe Aortic Stenosis 1 Year After Transcatheter Aortic Valve Implantation: Echocardiographic Evaluation

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Abstract

Background: Mitral valve regurgitation (MR), present in up to 74% of the patients with severe aortic stenosis (AS), can be a negative prognostic factor when moderate or severe. The outcome of MR after percutaneous transcatheter aortic valve implantation (TAVI) and predictors associated with that outcome have not been well established in the literature.

Objective: To assess the outcome of primary MR in patients submitted to TAVI and to identify associated factors.

Methods: Observational study of patients with symptomatic severe AS submitted to TAVI from January 2009 to April 2015 at two specialized centers. Echocardiographic outcome was assessed with data collected before and 1 year after TAVI.

Results: Of the 91 patients with MR submitted to TAVI and followed up for at least 12 months, 67 (73.6%) had minimum/mild MR before the procedure and 24 (26.4%) had moderate/severe MR. Of those with minimum/mild MR, 62 (92.5%) had no change in the MR grade ($p < 0.001$), while 5 (7.5%) showed worsening. Of those with moderate/severe MR, 8 (33.3%) maintained the same grade and 16 (66.7%) improved it ($p = 0.076$). Patients with moderate/severe MR who improved MR grade had lower EuroSCORE II ($p = 0.023$) and STS morbidity ($p = 0.027$) scores, as compared to those who maintained the MR grade.

Conclusion: MR grades change after TAVI. This study suggests a trend towards improvement in moderate/severe MR after TAVI, which was associated with lower preoperative risk scores. (Arq Bras Cardiol. 2017; 109(2):148-155)

Keywords: Mitral Valve Insufficiency; Aortic Valve Stenosis; Transcatheter Aortic Valve Replacement; Echocardiography.

Introduction

Aortic stenosis (AS) is one of the most prevalent heart valve diseases worldwide, being increasingly frequent because of the population ageing.[1] Data from the American Heart Association have shown a prevalence of AS of 0.4% in the North American population, and of moderate or severe AS of 2.8% in patients older than 75 years.[2]

A new therapeutic option for those patients appeared in 2002, when Cribier et al. performed the first percutaneous transcatheter aortic valve implantation (TAVI).[3] TAVI has been established as a safe, effective and less-invasive treatment for patients with severe AS and high surgical risk, who used to have no therapeutic alternative for a highly lethal disease.[4]

Mitral valve regurgitation (MR) is commonly associated with AS, whose prevalence can reach up to 74% in the MR population. Literature has shown that approximately 15% of the patients submitted to TAVI have significant MR. The presence of moderate or significant MR can have important implications in deciding between percutaneous or surgical treatment.[5] While in some studies MR has proved to be an important negative prognostic factor, it has shown no interference with mortality in patients submitted to TAVI in others.[2,6-8] In most reference centers, significant MR (> 3+) can be a contraindication to TAVI.[9]

Retrospective studies with a limited number of patients have suggested a reduction in MR after TAVI, with better prognosis in patients with smaller residual MR.[10,11] Some factors have been associated with that improvement, such as low ejection fraction, pulmonary artery pressure under 60 mmHg, and secondary etiology of MR (no structural lesion of the leaflets).[12-15] However, data on the impact of TAVI in patients with AS and MR in the Brazilian population still lack. In addition, there is no study including only patients with MR of primary etiology.

This study aimed at assessing patients submitted to TAVI who had primary MR associated with AS. We analyzed the clinical and echocardiographic findings of those patients 1 year after TAVI to identify possible factors associated with MR improvement or worsening.

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Mansucript received May 15, 2016, revised manuscript February 06, 2017, accepted March 13, 2017

DOI: 10.5935/abc.20170094

Methods

This is an observational study including all patients with severe symptomatic AS submitted to TAVI from January 2009 to April 2015 at two centers, where the same multidisciplinary team works, in the city of São Paulo – SP, Brazil. The study project was approved by the Ethics Committee of both institutions. All patients provided written informed consent prior to the TAVI procedure.

Clinical data, such as age, sex, functional class (NYHA) and associated comorbidities, were obtained via complete clinical exam, and the following complementary tests were performed: resting electrocardiography, chest X-ray, laboratory tests, transthoracic echocardiography with protocol to measure the aortic complex, computed tomography angiography of the heart and total aorta, and coronary angiography. On a second assessment, a team of cardiologists specialized in several areas decided which procedure should be performed, its access route and most suitable prosthesis. Intra-operative transesophageal echocardiogram was performed routinely.

In the population studied, the presence of primary MR prior to the transcatheter implantation of the aortic prosthesis and its outcome 1 year after that procedure were assessed. In a secondary analysis, that outcome was correlated with other variables considered to be of clinical importance. Primary MR was defined as that resulting from changes in the tissue constituting any of the mitral valve elements, such as leaflets, ring and subvalvular apparatus, corresponding to valvular calcification, valvular prolapse or rheumatic disease. Secondary MR was defined as that related to left ventricular systolic dysfunction, with no impairment of the valvular tissue itself.

We obtained data from 250 patients classified based on the MR grade. To define the MR severity, effective regurgitant orifice (ERO) and regurgitant volume were determined by using the proximal isovelocity surface area method (PISA), according to the latest American Society of Echocardiography recommendations.[16]

To calculate the ERO and regurgitant volume, the baseline of color flow mapping was lowered to values between 30 and 40 cm/s. The velocity-time integral of the regurgitant jet and the peak velocity of the regurgitant jet were obtained with continuous-wave Doppler, which was also used to measure the mitral transvalvular gradients. The linear measures of the cardiac chambers were obtained in the left parasternal acoustic window (long-axis view), using two-dimensional echocardiography. Left ventricular systolic function was assessed by use of the ventricular volume measures, obtained from the images of the orthogonal apical planes, in an acoustic window from four- and two-chamber view (Simpson's method). The mitral valve area was calculated by measuring pressure half time (PHT) or with the continuity equation, depending on the case. Pulmonary artery pressure was measured based on the gradient between the right ventricle and the right atrium, obtained with continuous Doppler, and that difference was added to the estimate of the right atrial pressure, determined from the diameter and collapse of the inferior vena cava.

The patients were divided according to the MR severity before and after TAVI into two large groups: trace/mild MR, composed of patients with ERO < 0.2 cm^2 and regurgitant volume < 30 mL/beat; and moderate/severe MR, composed of patients with ERO > 0.2 cm^2 and regurgitant volume > 30 mL/beat. Of those groups, we selected 91 patients with primary MR on the pre-procedure echocardiogram who completed 1-year follow-up for analysis of clinical and echocardiographic data.

In all cases, the following characteristics of the procedure were registered: access route; bioprosthesis type and size; and angiographic and echocardiographic results. The patients on vasoactive drugs and/or with hemodynamic instability signs were considered as being critically ill. All patients were cared for by the same medical team, the Heart Team of both hospital centers.

The patients were further divided into four subgroups according to the MR grade before and after TAVI: group 1, patients with moderate/severe MR, who maintained the MR grade after TAVI; group 2, patients with moderate/severe MR prior to the procedure, who changed to trace/mild MR; group 3, patients with trace/mild MR, who remained with the same MR grade after TAVI; group 4, patients with trace/mild MR prior to the procedure, whose MR worsened after the procedure.

Statistical analysis

Data were recorded in appropriate forms developed for this study, stored in electronic sheets and submitted to statistical analysis. The continuous variables were presented as median and difference between the 25th and 75th percentiles. The categorical variables were presented as absolute numbers and percentages. The continuous variables were compared using the Mann-Whitney test for independent samples, while the categorical variables, by using Fisher exact test or chi-square test. The McNemar test was used to assess the binary categorical variables and their proportion throughout time. All statistical analyses were performed with the SPSS 19 and R programs, 3.1.2 version. The level of statistical significance adopted was 5%.

Results

This study sample comprised 91 patients with MR and submitted to TAVI, who underwent a minimum follow-up of 12 months (Figure 1).

Table 1 shows the demographic data, comorbidities and prognostic scores, and Table 2 shows the echocardiographic parameters of the population studied. Of the 91 patients, 54 (59.3%) were of the female sex, and the median age was 84 (8.25) years. Regarding comorbidities, 33 (36.26%) had significant pulmonary arterial hypertension (systolic pulmonary artery pressure - sPAP > 55 mm Hg), 7 (7.69%) had chronic obstructive pulmonary disease (COPD) and 11 (12.1%) had atrial fibrillation. The medians were as follows: EuroSCORE I, 21.69 (15.39); EuroSCORE II, 5.7 (4.23); STS mortality, 5.65 (4.22); and STS morbidity, 27.25 (11.65); mean aortic gradient, 53 (22.5) mm Hg; left ventricular ejection fraction (LVEF), 62.5 (19)%; left atrial diameter, 45 (9) mm; sPAP, 47.5 (20.75) mmHg;

Table 1 – Demographic and clinical data of patients

	Patients (n = 91)	Moderate/severe MR			Trace/mild MR		
		Group 1 (n = 8)	Group 2 (n = 16)	p	Group 3 (n = 62)	Group 4 (n = 5)	p
Age	84 (8.25)	85.5(7.50)	85(10.5)	0.55	84(9.5)	80(21)	0.32
BMI (kg/m²)	26.45 (6.01)	23.83(3.87)	26.44(8.10)	0.27	26.67(6.14)	27.73(6.64)	0.82
Female	54(59.3)	3(37.5)	12(75)	0.09	38(61.3)	2(40)	0.64
Cardiovascular risk factors, n(%)							
Hypertension	75(82.4)	5(62.5)	13(81.3)	0.36	53(86.9)	3(60)	0.16
Diabetes	23(25.3)	0	2(12.5)	0.53	20(32.8)	0	0.31
Dyslipidemia	60(65.6)	6(75)	9(56.3)	0.65	41(67.2)	3(60)	1.0
Cardiovascular conditions, n(%)							
PVD	17(18.6)	1(12.5)	4(25)	0.63	12(19.3)	1(20)	1.0
Carotid lesion > 50%	13(14.2)	2(25)	0	0.10	10(16.1)	1(20)	1.0
PAH > 55 mm Hg	33(36.2)	3(37.5)	7(43.8)	1.0	19(33.8)	3(60)	0.32
Previous stroke	7(7.6)	0	1(6.3)	1.0	5(8.2)	1(20)	0.38
CAD > 50%	43(47.2)	4(50)	6(37.5)	0.67	29(46.7)	4(80)	0.19
Atrial fibrillation	11(12.1)	2(25)	4(25)	1.0	4(6.5)	1(20)	0.33
NYHA, n(%)				1.0			1.0
NYHA FC I/II	20(22.2)	1(12.5)	2(12.5)		16(25.8)	1(20)	
NYHA FC III/IV	71(78.0)	7 (87.5)	14(87.5)		46 (74.2)	4 (80)	
Non-cardiac conditions, n(%)							
COPD	7(7.6)	1(12.5)	1(6.2)	1.0	3(4.8)	2(40)	0.04
CrCl < 50 ml/min	59(64.8)	6(75)	9 (56.2)	0.65	40(64.5)	2(40)	1.0
Critical illness	3 (3.3%)	0	0	-	2(3.23%)	1(20%)	0.21
Risk scores							
EuroSCORE I	21.69(15.39)	26.91(26.02)	25.13(18.17)	0.35	19.75(11.96)	32.14(19.48)	0.89
EuroSCORE II	5.7(4.23)	8.95(9.84)	4.91(5.23)	0.02	5.63(4.31)	6.6(4.35)	0.63
STS mortality	5.65(4.22)	6.06(6.79)	4.21(5.49)	0.14	5.7(3.40)	5.36(2.23)	0.56
STS morbidity	27.25(11.65)	33.81(14.67)	22.22(10.41)	0.02	26.4(11.29)	31.45(12.76)	0.50
Type of aortic prosthesis, n(%)				0.85			0.07
Accurate	24(26.4)	4(50)	5(31.3)		12(19.4)	3(60)	
CoreValve	35(38.5)	2(25)	4(25)		26(41.9)	2(40)	
Sapien XT	32(35.1)	2(25)	7(43.7)		24(38.7)	0	

Data expressed as median (interquartile interval) or frequency (%); MR: mitral regurgitation; Group 1 - patients whose moderate/severe MR remained after transcatheter aortic valve implantation (TAVI); Group 2 - patients whose moderate/severe MR improved to trace/mild after TAVI; Group 3 - patients whose trace/mild MR remained after TAVI; Group 4 - patients whose trace/mild MR worsened to moderate/severe after TAVI; BMI: body mass index; PVD: peripheral vascular disease; PAH: pulmonary arterial hypertension; CAD: coronary artery disease; CrCl: creatinine clearance; COPD: chronic obstructive pulmonary disease; NYHA: New York Heart Association; FC: functional class; STS: Society of Thoracic Surgeons.

and aortic valve area, 0.7 (0.23) cm². The etiology of primary MR was valvular tissue calcification, including ring and leaflets, in all cases. Mitral stenosis, when present, was mild and related to calcification of the ring and leaflet base, and all patients had a valvular area > 1.5 cm².

The access routes used were as follows: femoral, 77 patients (84%); transaortic, 7 patients; apical, 6; and iliac, 1. The prosthesis types used were as follows: CoreValve, 38.5% of the patients; Sapien XT, 35.1%; and Accurate, 26.4%.

During the 1-year follow-up, 99.9% of the patients were in functional class (FC) I or II, and only one patient was in FC III.

Of the 91 patients, 67 (73.6%) had trace/mild MR before the procedure, and 24 (26.4%) had moderate/severe MR. Considering the entire group of patients, there was a significant change in the MR grade after TAVI (p = 0.013) (Figure 2).

Of the patients with moderate/severe MR, 8 (33.3%) maintained the same grade and 16 (66.7%) improved their MR (p = 0.076) as shown in Table 3.

Table 2 – Echocardiographic data of 91 patients with mitral regurgitation (MR) submitted to transcatheter aortic valve implantation and followed up for 1 year

	Patients (n = 91)	Moderate/severe MR		p	Trace/mild MR		p
		Group 1 (n = 8)	Group 2 (n = 16)		Group 3 (n = 62)	Group 4 (n = 5)	
LVEF (%)	62.5(19)	47(35.5)	59.5(14.75)	0.358	64(16.50)	67(43)	0.848
LVEDD (mm)	50(10)	53(8.5)	49.5(9.75)	0.326	50(10)	45(24.5)	0.905
LVESD (mm)	31.5(10.25)	31.5(22.25)	32(11.5)	0.620	32(10.25)	27.5(19.75)	0.45
LA (mm)	45(9)	50(6.25)	46.5(9.5)	0.539	43(8)	48(8.5)	0.135
Maximum AoG (mm Hg)	87(34.75)	76.5(42)	81(45.5)	0.603	89(33.5)	78(28)	0.133
Mean AoG (mm Hg)	53(22.5)	46.5(30.75)	49(29.75)	0.520	56(21)	50(18.5)	0.115
AoVA (cm^2)	0.7(0.23)	0.7(0.33)	0.65(0.30)	0.458	0.7(0.2)	0.7(0.25)	0.578
SPAP (mm Hg)	47.5(20.75)	49(29)	59.5(20.75)	0.391	45(16)	56(14)	0.130
Mitral stenosis (%)	9 (9.9%)	1 (12.5%)	2 (12.5%)	1.0	4 (9.3%)	2 (40%)	0.063
Aortic regurgitation (%)				1.0			1.0
Trace/mild	83 (91%)	7 (87.5%)	15 (93.75%)		56 (90.3%)	5 (100%)	
Moderate/severe	8 (9%)	1 (12.5%)	1 (6.25%)		6(9.7%)	0	
Tricuspid regurgitation (%)				0.829			0.269
Trace/mild	76 (83.5%)	5 (62.5%)	12 (75%)		55 (88.7%)	4 (80%)	
Moderate/severe	12 (13.1%)	3 (37.5%)	4 (25%)		4 (6.5%)	1 (20%)	
Not available	3 (3.4%)	-	-		3 (4.8%)	-	

Data expressed as median (interquartile interval) or frequency (%); LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; LA: left atrium; AoG: aortic gradient; AoVA: aortic valve area; SPAP: systolic pulmonary artery pressure.

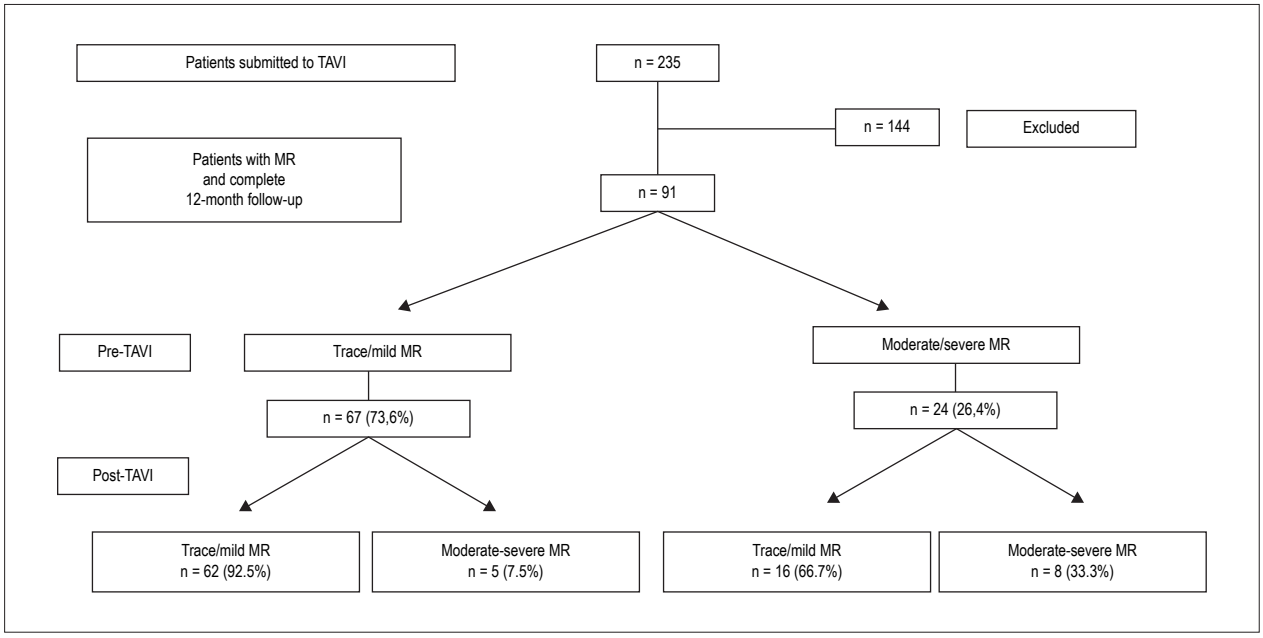

Figure 1 – *Study design. TAVI: Transcatheter aortic valve implantation; MR: mitral regurgitation.*

The patients with moderate/severe MR showed an association between surgical risk, based on the scores, and MR improvement after TAVI. The subgroup with persistent moderate/severe MR had the following medians: EuroSCORE I, 26.91 (26.02); EuroSCORE II, 8.95 (9.84); STS morbidity, 33.81 (14.67); and STS mortality, 6.06 (6.79). The subgroup that improved MR had the following medians: EuroSCORE I, 25.13 (18.17); EuroSCORE II, 4.9 (5.23); STS morbidity, 4.21 (5.49); and STS mortality, 22.22 (10.41). The p values for that difference were 0.35, 0.023, 0.027 and 0.14, respectively.

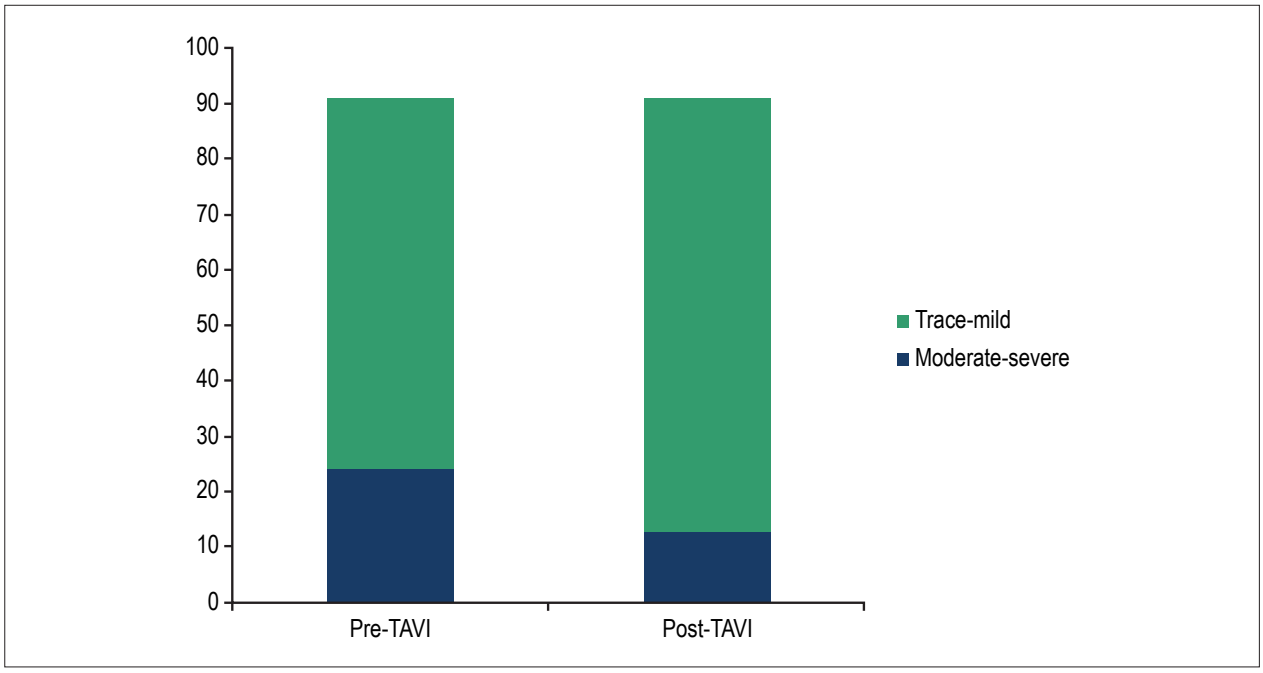

Figure 2 – *Change in mitral regurgitation grade. TAVI - transcatheter aortic valve implantation.*

Table 3 – Change in mitral regurgitation (MR) grade in the minimum/mild MR and moderate/severe MR groups

Pre-TAVI	Post-TAVI	N	%	p
Trace/mild (n = 67)				
	Trace/mild	62	92,5	< 0.001
	Moderate/severe	5	7,5	
Moderate/severe (n = 24)				
	Trace/mild	16	66,7	0.076
	Moderate/severe	8	33,3	

TAVI: transcatheter aortic valve implantation.

Regarding the patients with moderate/severe MR, variations in the following echocardiographic parameters were assessed: LVEF; left ventricular end-systolic diameter (LVESD); left ventricular end-diastolic diameter (LVEDD); and left atrial diameter. In group 1, a 0.5-mm reduction in the left atrial diameter was observed on the echocardiogram 1 year after the procedure, and, in group 2, a 4-mm reduction in the left atrial diameter was observed, with statistical significance ($p = 0.023$ - Table 4).

Of the patients with –/mild MR before the procedure (n = 67), 92.5% maintained the same MR classification. Worsening to moderate/severe MR was observed in 7.5% of the patients, with a $p < 0.01$ for remaining in the group with –/mild MR.

Regarding the clinical parameters, in group 3, 4.8% of the patients had COPD. In group 4, 40% of the patients had COPD ($p = 0.042$), that being the only clinical variable associated with MR change in those patients.

Analyzing the variation in echocardiographic parameters after 1 year, patients with trace/mild MR showed no significant variation between the subgroups (Table 4).

Discussion

Although some studies have assessed the behavior of MR in patients submitted to TAVI, their results are controversial in establishing if MR improves after aortic prosthesis implantation.[2,6-8] In addition, no study on that subject has assessed a Brazilian population. A retrospective study analyzing 101 patients with AS undergoing TAVI or surgical valve replacement has reported an improvement in MR grade regardless of the MR etiology.[17]

The present study assessed the behavior of MR in 91 patients submitted to TAVI, who underwent a minimum 12-month follow-up at two large Brazilian centers with the same multidisciplinary team involved in the percutaneous treatment of patients with AS.

Table 4 – Echocardiographic parameters pre-procedure and after 1 year

	Moderate/severe MR			Trace/mild MR		
	Group 1	Group 2	p	Group 3	Group 4	p
LVEF (%)	7(27.75)	3.5(12)	0.51	–0.5(10)	–5(16.5)	0.09
LVEDD (mm)	–1.5(4.25)	0.5(8.75)	0.83	–2(6)	2(11)	0.40
LVESD (mm)	–0.5(3.25)	–1(8.75)	0.31	–0.5(6.63)	6(4.5)	0.06
LA (mm)	–0.5(10)	–4(7.5)	0.02	–1(7)	–1(2.5)	0.54

Data expressed as median (interquartile interval); MR: mitral regurgitation; Group 1 - patients whose moderate/severe MR remained after transcatheter aortic valve implantation (TAVI); Group 2 - patients whose moderate/severe MR improved to trace/mild after TAVI; Group 3 - patients whose trace/mild MR remained after TAVI; Group 4 - patients whose trace/mild MR worsened to moderate/severe after TAVI; LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; LA: left atrium.

The population studied had a median age of 84 years, relatively preserved LVEF, similarly to those of major international studies.[18] The presence of associated comorbidities, such as systemic arterial hypertension, diabetes *mellitus*, dyslipidemia, peripheral vascular disease, significant carotid disease, coronary artery disease, chronic kidney disease, pulmonary arterial hypertension, COPD and stroke, was similar to that of the major studies.[17] The median STS score was 5.65. The mean aortic transvalvular gradient was 54.38 ± 16.55 mm Hg (median, 53 mm Hg), higher than that reported in the major studies (mean aortic gradient of 40-50 mm Hg).[17]

The surgical risk assessment of the subgroups was performed by using EuroSCORE I and II, STS mortality and morbidity. In the PARTNER study, the mean STS was 11.8 ± 3.3 in the A cohort and 11.2 ± 5.8 in the B cohort,[19,20] values relatively higher than the ones observed in this study. Likewise, we observed that the patients selected had lower values in EuroSCORE II. However, it is worth noting that those risk scores are not specific for heart valve disease, and they do not contemplate several comorbidities that influence directly or indirectly the surgical outcome. Thus, those patients can be at high surgical risk and/or have technical difficulties for the traditional approach via sternotomy, even having a low score.[6]

In this study population, 67 (73.6%) patients had –/mild MR before the procedure, while 24 (26.4%) had moderate/severe MR. After valve replacement, 78 (85.7%) patients had minimum/mild MR and 13 (14.3%) had moderate/severe MR. The change in MR severity after TAVI was statistically significant (p = 0.013), showing the impact of aortic valve replacement on MR severity, with a general trend towards MR improvement.

When assessing the subgroup of 24 patients with moderate/severe MR before the procedure, 16 (66.7%) improved MR severity to minimum/mild after TAVI. Although lacking statistical significance, MR improvement can be identified in that specific group. Prospective studies with larger samples are necessary to confirm this trend.

In addition, MR improvement was accompanied by a significant reduction in the left atrial diameter, which can be explained by the reduction of intracavitary filling pressures and regurgitant volume into the atrium. In the general population, the left atrial size is associated with mortality, heart failure and stroke.[21-24] New studies are required to determine if the left atrial reduction in patients undergoing TAVI, either associated or not with MR change, has prognostic value.

An association was observed between patients with moderate/severe MR who maintained the same MR grade after the procedure and higher surgical risk scores. This might result from the fact that individuals with higher scores have a higher number of chronic mitral valve changes. Studies with larger samples are required to confirm risk scores as independent predictors of the persistence of severe MR after TAVI.

The subgroup with trace/mild MR that worsened after the procedure had a higher prevalence of COPD. A previous publication by the same team has reported COPD as an independent cause of mortality in patients submitted to TAVI, and can represent a critically-ill subgroup,[25] although the reasons for that finding are not clear. Thus, new studies might clarify that association.

In a recent publication, Kiramijyan et al. have compared the progression of secondary versus primary MR in 70 patients submitted to TAVI, 30 of which had primary MR. The population was assessed 1 month and 1 year after the intervention, and similar survival was evidenced in both groups in the short and long term. Similarly to our findings, in that study MR improved in both groups. However, patients with primary MR had a less marked improvement in MR as compared to those with secondary MR (p = 0.0008).[26] This emphasizes that patients with primary or secondary MR should be assessed in different ways.

The main limitation of this study relates to the fact that it is a retrospective cohort, with a relatively small number of patients. Therefore, a multivariate analysis to determine independent associations that could justify MR improvement could not be performed. However, we believe this is an important Brazilian study on the subject showing that the percutaneous treatment can be an acceptable therapeutic option in patients with AS, even when there is primary MR associated.

Conclusion

In this group of patients, a significant change in MR grade was observed after TAVI, those with trace/mild MR maintaining it and those with moderate/severe MR showing a trend towards improvement. In patients with moderate/severe MR, MR grade improvement correlated with lower preoperative risk scores. However, the presence of COPD associated with MR worsening in patients with mild MR before the procedure.

Author contributions

Conception and design of the research, Acquisition of data, Analysis and interpretation of the data, Statistical analysis, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Florentino TM, Le Bihan DCS, Abizaid AAC, Cedro AV, Corrêa AP, Santos ARM, Souza AC, Bignoto TC, Rego e Sousa JEM, Rego e Sousa AGM.

Potential Conflict of Interest

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

Sources of Funding

There were no external funding sources for this study.

Study Association

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

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Review Article

A Meta-Analysis of Circulating Microvesicles in Patients with Myocardial Infarction

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Abstract

Background: Cell-derived microvesicles (MVs) are vesicles released from activated or apoptotic cells. However, the levels of MVs in myocardial infarction have been found inconsistent in researches.

Objective: To assess the association between MVs and myocardial infarction by conducting a meta-analysis.

Methods: A systematic literature search on PubMed, Embase, Cochran, Google Scholar electronic database was conducted. Comparison of the MVs levels between myocardial infarction patients and healthy persons were included in our study. Standard Mean Difference (SMD) and 95% confidence interval (CI) in groups were calculated and meta-analyzed.

Results: 11 studies with a total of 436 participants were included. Compared with the health persons, AMVs [SMD = 3.65, 95% CI (1.03, 6.27)], PMVs [SMD = 2.88, 95% CI (1.82, 3.93),] and EMVs [SMD = 2.73, 95% CI (1.13, 4.34)], levels were higher in patients with myocardial infarction. However, LMVs levels [SMD = 0.73, 95% CI (–0.57, 2.03)] were not changed significantly in patients with myocardial infarction.

Conclusions: AMVs, PMVs and EMVs might be potential biomarkers for myocardial infarction. (Arq Bras Cardiol. 2017; 109(2):156-164)

Keywords: Myocardiaol Infarction; Biomarkers; Cell-Derived Microparticles; Annexion A5; Blood Platelets; Leukocytes; Endothelium.

Introduction

Ischemic Heart Disease (IHD) is one of the cardiovascular diseases, which impairs human health.[1] Atherothrombosis, endothelial dysfunction and cell apoptosis are on the pathologic basis in these diseases. The relevant studies in this area have suggested that cell-derived microvesicles (MVs) are related with platelet activation, endothelial damage and inflammation associated with the existence of cardiovascular risk factors.[2-4] Since they are involved in the pathophysiologic process of diseases, attention has being focused on the relationship between MVs and myocardial infarction (MI).[5-8] Increasing evidences imply that MVs might be considered as novel biomarkers or mediators helpful in understanding the mechanisms of cardiovascular diseases.

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Manuscript received january 20, 2017, accepted March 15, 2017accepted March 15, 2017

DOI: 10.5935/abc.20170102

MVs are used to describe a population of sub-cellular vesicles released from plasma membrane during cell activation or apoptosis and identified by size range from 100 nm to 1.0 μm in diameter. MVs constitute a heterogeneous population, different in cellular origin, numbers, size, antigenic composition, and functional properties. Alterations in the amounts of different cell-derived MVs may provide information on the pathophysiologic changes. Although many studies have shown that myocardial infarction is associated with MVs, the information obtained shows heterogeneous result, with a high variation regarding MVs size, MVs type, MVs levels, inclusion criteria and methods. Thus, we performed a meta-analysis of the changes of Annexin V positive MVs (AMVs), platelet MVs (PMVs), endothelial MVs (EMVs) and leukocytes MVs (LMVs) in patients with myocardial infarction and healthy persons.

Methods

Data sources and Searches

We searched the databases of MEDLINE (pubmed), Embase, Cochrane and Google Scholar electronic database for articles from 2000 to 2013. All searches were applied with the following medical subject headings: "myocardial infarction", "primary percutaneous coronary intervention", "stenting", "balloon angioplasty", "acute coronary syndrome"

or "coronary ischemia", "microparticles", "cell-derived microparticles", "circulating microparticles", "microvesicles". These searches were restricted to publications limited to research on humans. A manual search for references cited in the published studies and relevant review articles was also performed to identify additionally suitable investigations for our purpose. For unpublished and published studies that were not exhaustively disclosed, the attempt through e-mail was made to contact principal investigators in order to retrieve missing data. Finally, well-known experts in this area were contacted to ensure that all relevant data were captured.

Study selection

Two of us performed the identification of relevant abstracts and the selection of studies based on the criteria described below independently, and a third investigator resolved any discrepancy. We selected studies comparing the levels of diverse MVs: total, platelet-, endothelial-, leukocyte-derived between healthy persons and patients with myocardial infarction.

Studies were included if they met the following criteria: (i) Study entitled circulating MVs correlated with MI; (ii) Design of study was case-control study or cohort study; (iii) The MI as a research subgroup independently extract relevant information MI. We excluded the following: (i) Review; (ii) Not full text, only a summary; (iii) Animal testing.

Data extraction and synthesis

We extracted information including study and population characteristics, sample size, study design, and outcomes relevant to this study. Means and standard deviations of MVs levels were extracted. When an article complied with the inclusion criteria but lacked information on parameters for analysis, or when outcomes were reported but not related to myocardial infarction, we contacted the authors to obtain raw data. The quality of studies was assessed using the Downs and Black checklist.

Statistical analysis

The data was analyzed using RevMan 5.0 statistical software provided by the Cochrane Collaboration analyzed. SMD and 95% CI were used as summary estimates. The presence of heterogeneity between studies was tested with the χ^2 test for heterogeneity and the I^2 statistic. Heterogeneity was significant when $p < 0.05$ or I^2 was more than 50%. A random-effects model was used in all analyses to test the stability of the results to the choice of the statistical model. If significant heterogeneity, results of the random-effects model are used. We defined a priori sensitivity analysis of high-quality studies for each clinical outcome. The potential for publication bias was evaluated using the funnel plot approach.

Results

Search results

172 articles identified from MEDLINE (pubmed), Embase, Cochrane and Google Scholar electronic database were analyzed; and then, 140 were excluded based on title and abstract. After detailed evaluation of potential eligibility, 11 studies met all the inclusion criteria and were retrieved for meta-analysis.[9-19] The trial flowchart is summarized in Figure 1.

Baseline characteristics of the studies

The characteristics of all included studies are presented in Table 1. These studies were published from 2004 to 2013. Sample size ranged from 5 to 61. A total of 436 participants (186 healthy controls and 250 MI patients) were included. Among these studies, the results of MVs were expressed differently. Six reports were expressed as numbers of MVs in plasma per microliter, milliliter and liter. Three reports were expressed as PS eq (phosphatidylserine equivalents), one report was expressed as numbers of MVs in platelet count, and one report was expressed as plasma concentrations of MVs. There were four reports only report the median, range and the size of the trial. In order to estimate the mean and the variance in these articles, we used the number of sample and elementary inequalities.[20]

Quality index

The majority of studies scored highly on reporting of the interventions used and outcome measures. Only one report scored lowly on the small sample size. The average score of all studies was 15.8 (Table 2).

Annexin V+ microvesicles in health control and patients with myocardial infarction

Four of the eleven studies showed changes in AMVs levels between patients with myocardial infarction and healthy controls. In three of these reports, AMVs levels in patients with myocardial infarction were higher than healthy controls. Only in one study, patients with myocardial infarction did not differ from healthy controls concerning AMVs levels. When results of all studies were combined, there was a significant difference between groups with higher AMVs levels in patients with a myocardial infarction [SMD = 3.65, 95% CI (1.03, 6.27), Z = 2.73 ($p < 0.00001$; Figure 2A)]. Furthermore, there was significant statistical heterogeneity across studies ($\chi^2 = 95.64$, df = 3, $p < 0.00001$, $I^2 = 97\%$). As shown in Figure 2B, no publication bias was found.

Platelet microvesicles in health control and patients with myocardial infarction

All of eleven studies found PMVs levels varied between myocardial infarction patients and healthy controls. Nine studies reported that level of PMVs were higher in MI patients, whereas the other studies showed no difference in groups. Combining the results of all studies, it was significantly increased in myocardial infarction patients [SMD = 2.88, 95% CI (1.82, 3.93), Z = 5.35 ($p < 0.00001$; Figure 3A)]. There was also significant statistical heterogeneity across studies ($\chi^2 = 235.02$, df = 10, $p < 0.00001$, $I^2 = 96\%$). As shown in Figure 3B, a little publication bias was found.

Endothelial microvesicles in health control and patients with myocardial infarction

Six of the eleven studies reported alternation in EMVs levels between the two groups. Four reports concluded

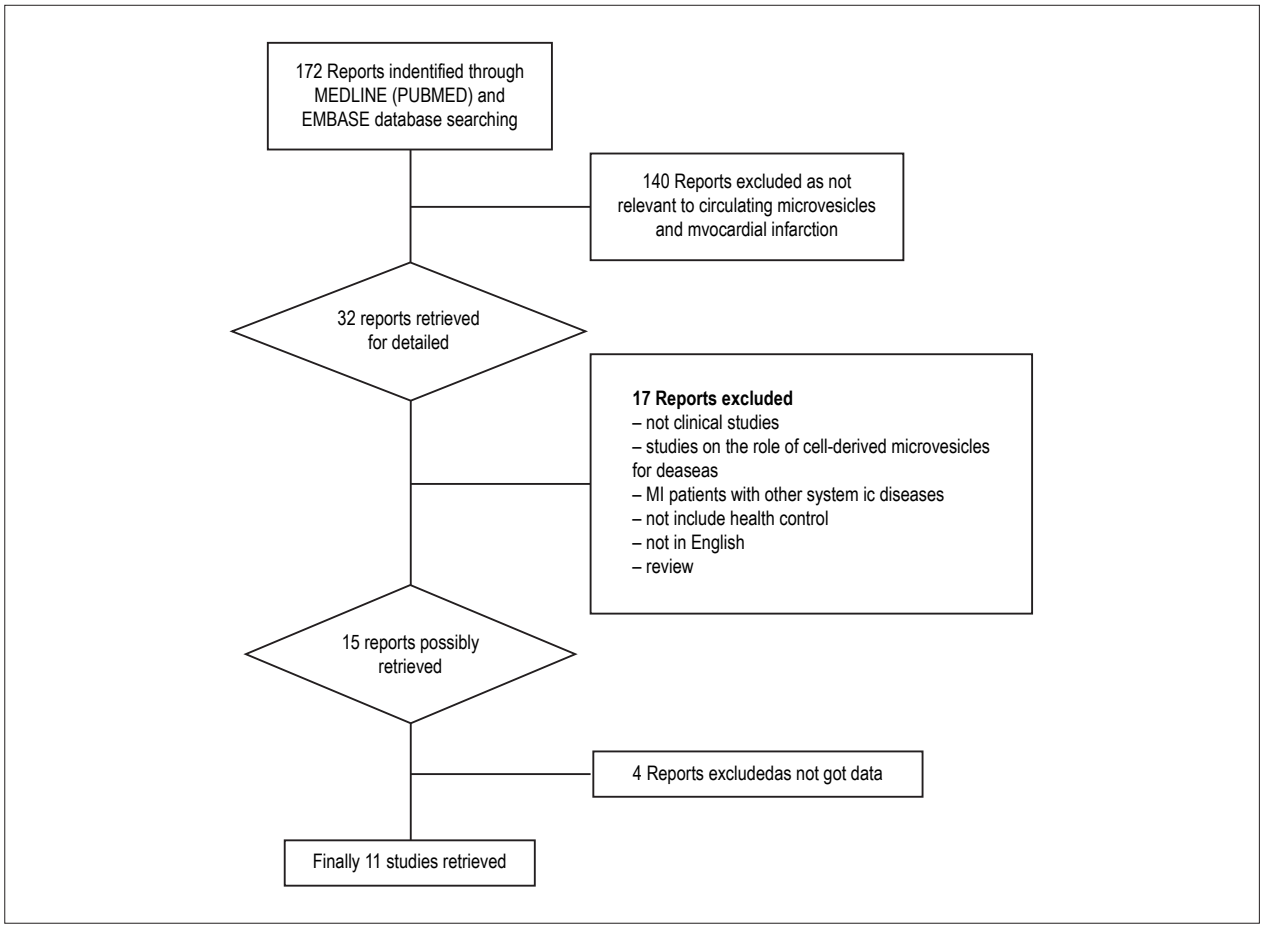

Figure 1 – *Flow diagram of search strategy and study selection.*

Table 1 – Characteristic of included studies

Author/Year	Study object		Measurement method of MVs	Units of MVs	Sample size
	MI	Control			MI/Control
Cui Y/2013	STEMI/NSTEMI	Health	Flow cytometry	10^5/mL	40/20
Del Turco S/2008	MI	Health	Flow cytometry	10^6/L	46/10
Leong H S/2011	AMI	Health	Flow cytometry	/μL	6/5
Matsumoto N/2004	ACS	Health	Flow cytometry	10^4/platelet count	41/20
Michelsen A E/2008	MI	Health	BCA Protein Assay	μg/L	61/61
Min P K/2013	STEMI	Health	ELISA	nM (phosphatidylserine equivalent)	45/16
Morel O/2004	STEMI	Health	ELISA	nM (PhtdSer equivalent)	50/50
Morel O/2005	STEMI	Health	ELISA	nM (PhtdSer equivalent)	9/50
Skeppholm M/2012	STEMI/NSTEMI	Health	Flow cytometry	10^6/L	51/61
Stepien E/2012	AMI	Health	Flow cytometry	/μL	12/9
Tan K T/2005	ACS	Health	Flow cytometry	10^5/mL	54/35

that a proportion of MI patients have elevated EMVs levels. But the other studies showed no significant difference. Combining all results of those studies, MI patients had a higher level of EMVs. [SMD = 2.73, 95% CI (1.13, 4.34), $Z = 3.33$ ($p = 0.0009$; Figure 4A)]. The statistical heterogeneity was significant across studies ($\chi^2 = 155.28$, $df = 6$, $p < 0.00001$, $I^2 = 96\%$). As shown in Figure 4B, no publication bias was found.

Leukocyte microvesicles in health control and patients with myocardial infarction

Five of the eleven studies exhibited differences in LMVs levels between myocardial infarction patients and health controls. Four reports showed that LMVs levels in myocardial infarction patients were higher than health controls. The finding of one report was, however, in the opposite direction, with patients having significantly lower LMVs levels than controls. When results of all studies were combined, there was no significant difference between the two groups [SMD = 0.73, 95% CI (–0.57, 2.03), Z = 1.11 (p = 0.27; Figure 5A)]. There was also significant statistical heterogeneity across studies (χ^2 = 90.69, df = 4, p < 0.00001, I^2 = 96%). As shown in Figure 5B, no publication bias was found.

Discussion

We conducted an exhaustive search to identify studies related to our question and gave the most comprehensive overview of MVs in MI to date. Systematic methods were applied to reduce bias in the identification of studies, data extraction and synthesis, and appraisal of study quality. This meta-analysis showed that higher level of AMVs, PMVs and EMVs in peripheral blood might be associated with patients with MI, indicating that these MVs may be helpful in the diagnosis of MI. However, the result of LMVs was negative.

Microvesicles (MVS) or microparticles (MPS) have relationship not only with inflamatory and thrombotic processes but with tissue regenerative process and angiogenesis, which can be a protective function. In addition, MVS can be a signaler of homeostasis balancing cell stimulus and apoptosis. Diabetic patients have increased release of MVS and this can be a biomarker of diabetic progression by retinopathy. Pharmacologic approach is helpful because of endothelial dysfunction. Renin-angiotensin system blocker and calcium channel blocker may be good options in type 2 diabetes mellitus.[21] Pinheiro et al.[22] have assessed the effect of the antiplatelet drug clopidogrel in association or not with rosuvastatin (40 mg) on the levels of EMP and PMP in patients with stable coronary disease on statins for at least three months. Those authors have identified an increase

Table 2 – Quality Index of included studies

Author/Year	Quality Index
Cui Y/2013	16
Del Turco S/2008	16
Leong H S/2011	14
Matsumoto N/2004	16
Michelsen A E/2008	16
Min P K/2013	16
Morel O/2004	16
Morel O/2005	16
Skeppholm M/2012	16
Stepien E/2012	16
Tan K T/2005	16

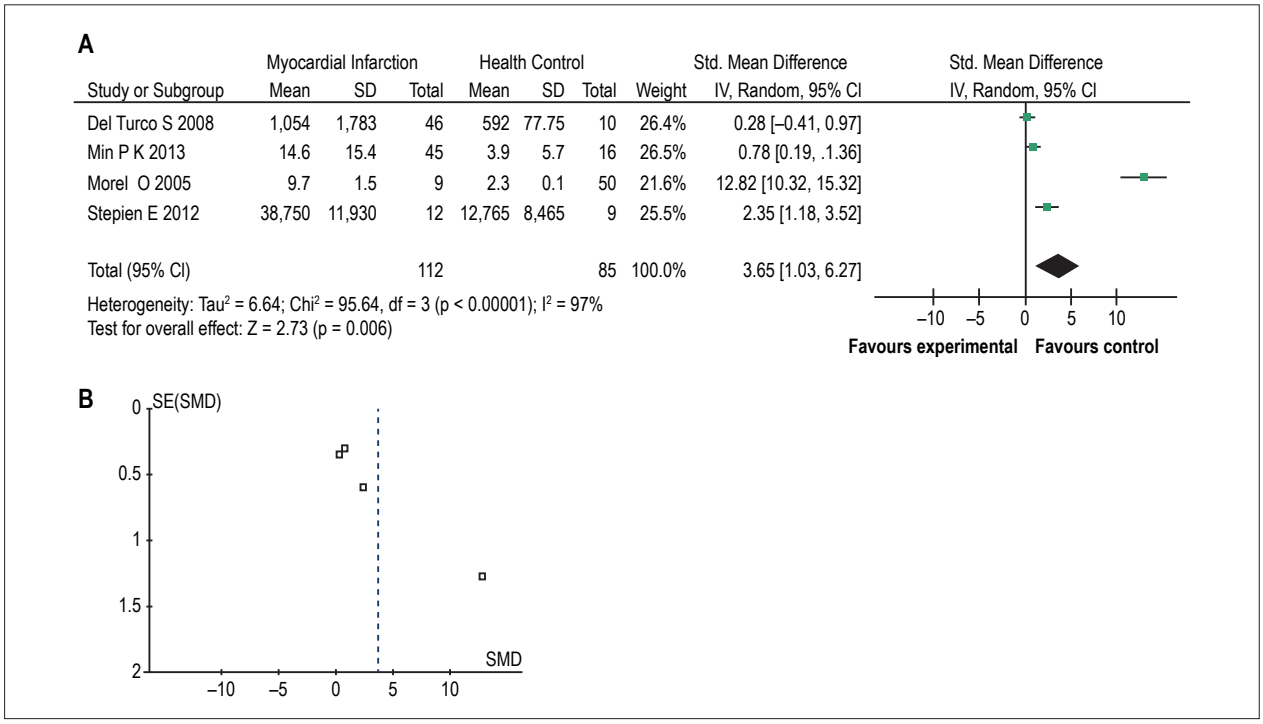

Figure 2 – *The forest plot (A) and funnel plot (B) of meta-analysis of Annexin V+ microvesicles in myocardial infarction.*

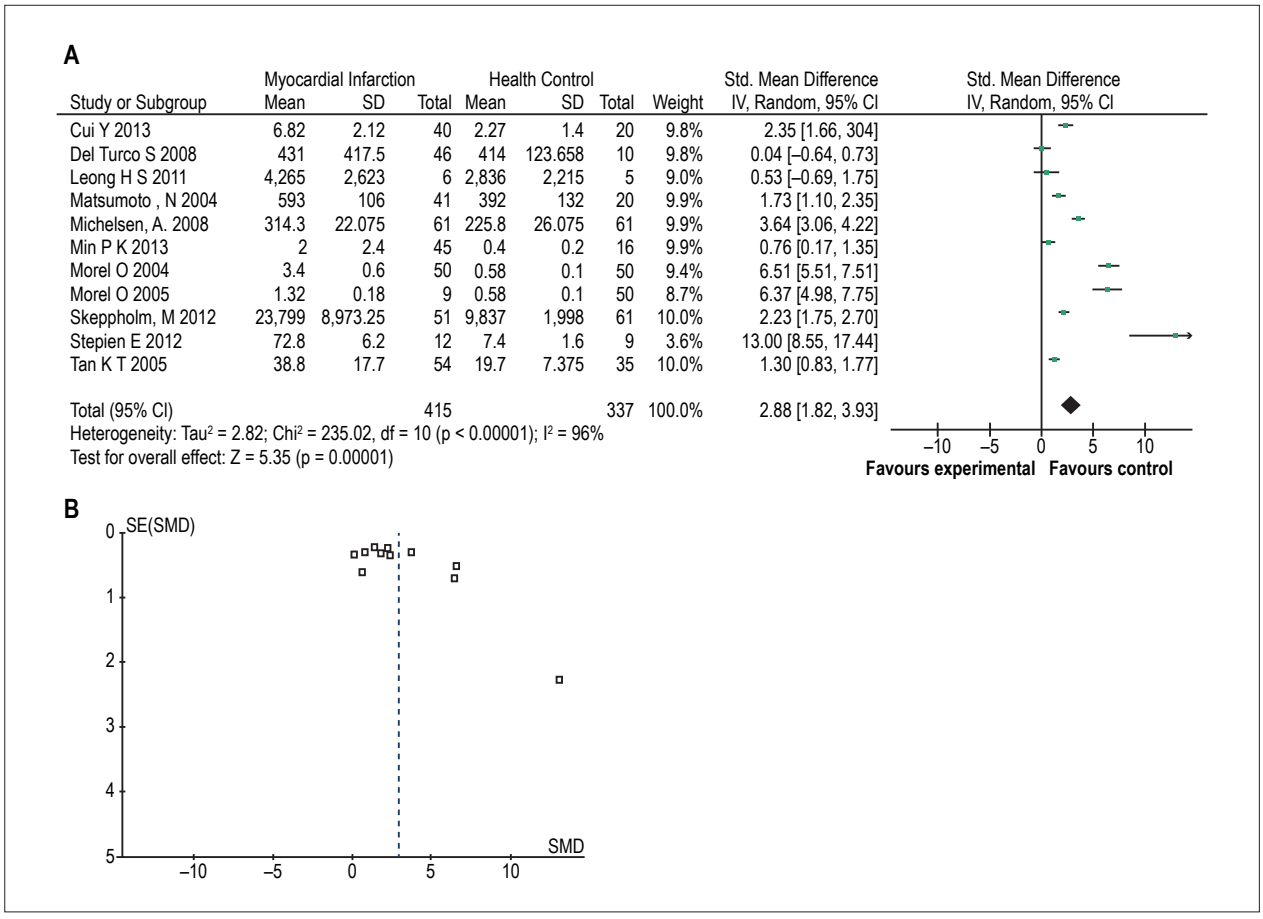

Figure 3 – *The forest plot (A) and funnel plot (B) meta-analysis of platelet microvesicles in myocardial infarction.*

in the levels of PMP after suspension of rosuvastatina and maintenance of only clopidogrel for four weeks and a tendency towards greater release of EMP in those patients. They have suggested that an increase in the apoptosis of platelets occurred, and that rosuvastatin might have a protective effect on the endothelium when associated with clopidogrel.[22] In a similar study, França et al.[23] have assessed the influence of atorvastatin (80 mg) in association or not with clopidogrel in patients with stable coronary disease. Those authors have suggested higher vascular stability promoted by atorvastatin after identifying an inverse relationship between the plasma concentration of atorvastatin and the levels of PMP.[23]

MVs have a bilayered phospholipid membrane.[24] The presence of externalized PS on MVs surfaces indicates an altered phospholipid distribution profile compared to the plasma membrane of a resting mammalian cell. Additionally, the molecules present in the outer surface, once defined, provide accessible markers for detecting and characterizing MVs using molecular probes.[25] AMVs express phosphatidylserine (PS) in their surface and are currently defined as apoptotic MVs. It is shown that AMVs levels were significantly higher in patients with MI than healthy controls. It is likely due to the myocardial ischemia and hypoxia which make cell apoptosis, thereby releasing large amounts of AMVs. In each case, the variation in AMVs levels shows that cell apoptosis occurs. A in vitro study uncovered that MVs extracted from the circulating blood of a patient with myocardial infarction and applied to the isolated aortic rings of a rat, led to severely damage of endothelial function.[26] Thus, the high level of AMVs and their effect may be the cause the further progression of myocardial infarction.

PMVs are defined as membranous vesicles derived from the platelet which are defined and identified by surface molecules CD62P and CD63.[27] With the ability to bind coagulation factors VIII, Va, and IX, PMVs not only reflect platelet activation but also contribute to the activation of the coagulation pathway and thrombogenesis.[28-29] It was found that there was high level of PMVs in the peripheral blood of the MI patients. The main cause may be the change of blood flow shear force induced by pathological changes in the blood vessels of the MI patients. These changes promote the aggregation and activation of platelets, thereby leading to the generation of a large number of PMVs. This suggests that increased PMVs may be further expanded by the coagulation reaction of the blood vessels in the MI patients.

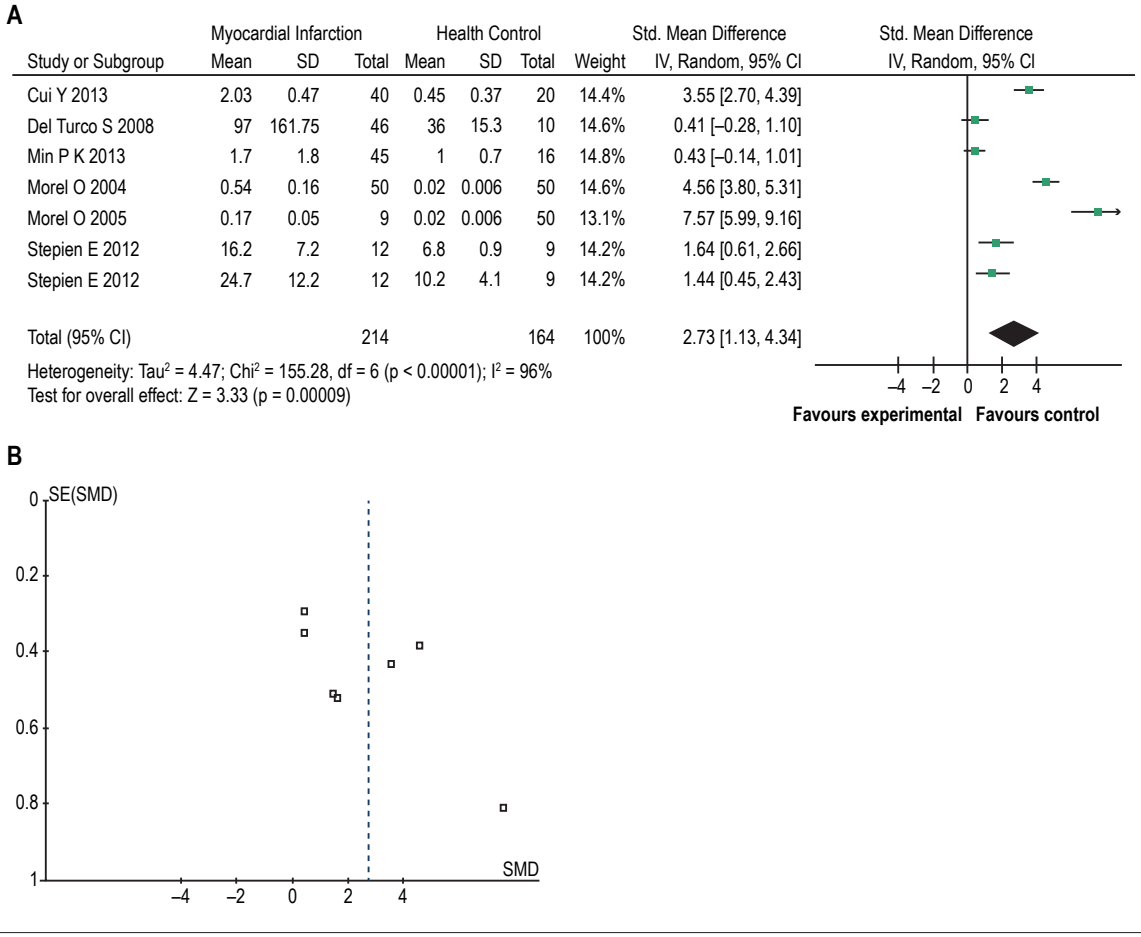

Figure 4 – *The forest plot (A) and funnel plot (B) meta-analysis of endothelial microvesicles in myocardial infarction.*

The dysfunction of endothelial cells plays an important role in the MI. EMVs are sub-cellular membrane vesicles released from endothelial cell during activation or apoptosis.[30] EMVs carry specific markers which originate from the maternal cells, including CD31, CD51, CD54, CD62E, CD105, CD144 or CD146. It is found that EMVs were significantly higher in the MI group. His could be because of the dysfunction of endothelial cells, which release lots of EMVs into the blood. When the EMVs were incubated with human umbilical vein endothelial cells (HUVECs), endothelial cells proliferation decreased and their apoptosis increased, then the capacity of angiogenesis went down dramatically.[31] The high level of EMVs is considered as a cause to worsen the condition by inducing endothelial dysfunction on the MI states.

Unlike the single source of EMVs or PMVs, LMVs may originate from neutrophils, monocytes/macrophages, and lymphocytes.[32] They also express markers from their parental cells. Various antibodies were used to capture LMVs, including CD4, CD14, CD11a and so on. Combined with the clinical results, this meta-analysis found that there was no significant variation in LMVs' level between the health controls and patients with MI. But the studies that used CD4 and CD14 antibodies, shown LMVs' level was higher in patients with MI. When CD11a antibody was used, a muddle of contradictory results was found. Therefore, additional studies are needed to further investigate the level of LMVs in MI. Meanwhile, LMVs measurement still requires elaborate techniques because of its lack of standardization.

Limitations of this meta-analysis must be considered. First, the quality of individual studies was not always optimal, as shown by the general lack of information on some studies. So we used simple and elementary inequalities in order to estimate the mean and the variance for such studies. But it is not exactly enough. Second, there is heterogeneity of SMD across studies, corresponding in part to heterogeneity in study definitions. Third, although the quality of included studies was judged overall to be adequate, the findings in the present study need to be interpreted with caution, given that not all studies reported on potential confounding variables and their adjustment in analyses. Finally, meta-analytic data also need to be cautiously interpreted, given the substantial heterogeneity among studies.

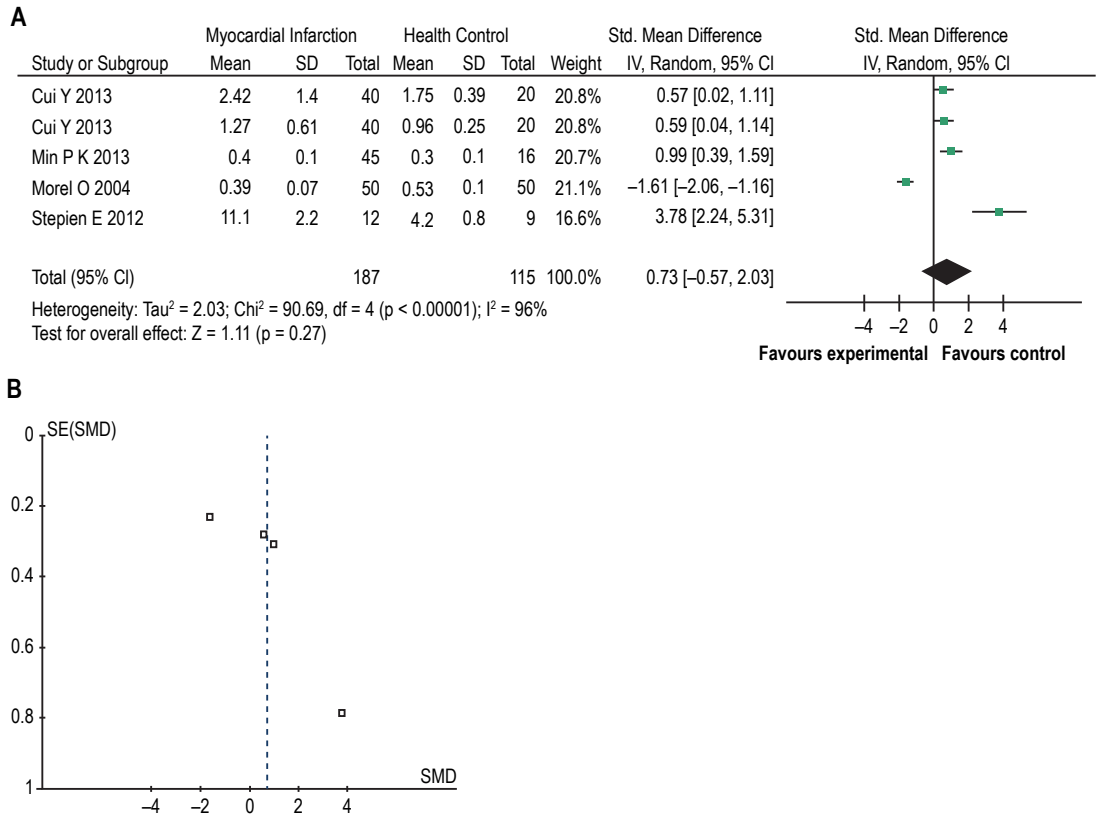

Figure 5 – *The forest plot (A) and funnel plot (B) meta-analysis of leukocyte microvesicles in myocardial infarction.*

Conclusion

This meta-analysis showed that higher level of AMVs, PMVs and EMVs in peripheral blood may be associated with patients with MI, indicating that these MVs may be helpful in the diagnosis of MI. However, the result of LMVs was negative.

Author contributions

Conception and design of the research and Writing of the manuscript: Wang Z, Cai W; Acquisition of data: Hu S; Analysis and interpretation of the data: Xia Y; Statistical analysis: Wang Y; Obtaining funding and Critical revision of the manuscript for intellectual content: Zhang Q, Chen L.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by National Natural Science Foundation of China through grant n°. 81602496; Science and Technology Foundation of Administration of Traditional Chinese Medicine of Tianjin, China, nos 2015022 and 13047; Science and Technology Foundation of Tianjin Municipal Commission of Health and Family planning, n°. 2013KZ054.

Study Association

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

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Review Article

Hypotension and Renal Dysfunction: The Ghosts of Heart Failure

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Hypotension, bradycardia and renal dysfunction as obstacles to the treatment of heart failure

"We can easily forgive a child who is afraid of the dark; the real tragedy of life is when men are afraid of light."

Plato

Heart failure (HF) is a chronic, high morbidity and high cost disease. The treatment of HF due to left ventricular (LV) systolic dysfunction is well determined and is listed in Medical Guidelines. However, innumerable situations may limit treatment, causing the physician to fail to implement the guidelines. Some serious patients may not tolerate medications or recommended doses; others may have side effects. In some cases, however, there is an excess of caution, failing to prescribe the recommended treatment, fearing complications. The purpose of this article is to demystify, based on the literature, some situations that may prevent the optimized drug treatment from being offered to the HF patient.

The two major side effects that may act as barriers to the treatment of HF are hypotension and worsening renal function. Besides these, we will comment on bradycardia and hyperkalemia.

Arterial Hypotension

The main limiting factor in the treatment of HF is the lack of understanding of the concept of hypotension in this scenario. Patients with HF due to LV systolic dysfunction, in New York Heart Association (NYHA) functional class III or IV, when adequately medicated, usually have systolic blood pressure (BP) levels as low as 90 mmHg, with no symptoms. In some cases, of non-ischemic etiology, up to 80 mmHg of systolic pressure may be tolerated. A patient of this type, when presented with "normal" BP, 120x80 mmHg, may be submedicated, although these parameters may change, in cases of hypertensive heart disease. Therefore, for the diagnosis of hypotension in these cases, we can not only rely on the absolute value of BP. Symptoms of hypotension, such as lightheadedness, dizziness, weakness, cold hands, asthenia, pre-syncope, or syncope, need to be present.

Keywords

Hypotension; Blood Pressure; Heart Failure; Renal Insufficiency.

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Manuscript received January 03, 2017, revised manuscript March 29, 2017, accepted April 17, 2017

DOI: 10.5935/abc.20170110

We must remember that patients with HF have several activated neurohormonal systems, resulting in vasoconstriction (renin-angiotensin-aldosterone system, sympathetic nervous system, endothelin, etc.).[1] It is therefore necessary that vasodilators be used, to antagonize these effects and reduce afterload, relieving cardiac work. In fact, it is well established in the literature that the use of drugs that combat such systems, such as beta blockers,[2] inhibitors of conversion enzyme (ACE)[3,4] or angiotensin receptor blockers (ARBs)[5] and mineralocorticoid receptor antagonists (spironolactone),[6] result in increased survival and should be prescribed for all HF patients at the doses recommended in the Medical Guidelines.[7] Other vasodilators, such as the nitrate-hydralazine combination, have also shown increased survival in a specific setting and may be added to the previous regimen or even replace ACE inhibitor in cases of intolerance or limitations due to renal function.[7,8]

A fall in BP accompanied by symptoms after drug prescription identifies patients of greater severity, since hypovolemia is removed. Nevertheless, an asymptomatic drop in BP with medications used to treat HF may not have a prognostic impact. Indeed, there are data in the literature that suggest that the "lower" BP is actually a marker that treatment is being effective. For example, in the SOLVD study, where enalapril was compared to placebo in patients with HF, systolic BP at study admission averaged 125.3 and 124.5 mm Hg in the enalapril and placebo groups, respectively. At the end of the study, BP fall was greater in the enalapril group than in the placebo group (4.7 vs 4.0 mmHg). However, the survival was higher in the enalapril group, despite a greater fall in PA.[4] The same was observed in the CONSENSUS study, also with enalapril.[3] More recently, we highlight the PARADIGM-HF study, where LCZ 696 (valsartan + sacubitril) was compared to enalapril. There was a higher incidence of hypotension in the LCZ 696 group, but the LCZ696 reduced 20% the outcome cardiovascular death and hospitalizations for HF, compared to enalapril.[9]

Therefore, we should not suspend or reduce doses of medications because BP is "low." Only if there are symptoms of hypotension the dose should be reduced. Even in these cases, hypotension is often due to diuretics and not to ACE inhibitors. Check the patient's fluid status. If there are no objective signs of congestion, discontinue the diuretic first, as there may be hypovolemia. Then reduce the dose or stop the nitrate-hydralazine combination. ACE and ARB should be the last ones on the list because their benefits are greater.

Worsening Renal Function

As observed in the previous section in relation to BP, ACE inhibitors promote increased survival, despite increasing creatinine. In the SOLVD study, the use of enalapril reduced

mortality, despite increasing the mean creatinine values[4] by 0.1 mg/dL. By the mechanism of action of ACEIs, they are expected to increase creatinine, since they promote dilatation of the efferent glomerular artery.[10] But the final effect is of cardio and renoprotection.[3,4,7,10,11] There is no definite creatinine value in the literature that contraindicates the use of ACE inhibitors and may even be used in patients on a hemodialysis program,[12] although they may cause hypotension in this situation. In the SOLVD study, patients were excluded if they had baseline creatinine greater than 2 mg/dL, but the CONSENSUS study included patients with up to 3.4 mg/dL. Increases in creatinine of up to 30% compared to baseline, after the introduction of ACE inhibitors, appear to be safe.[11] In patients with chronic HF, ACE inhibitors should be prescribed and maintained despite a moderate increase in creatinine provided there is no hyperkalemia or acute renal failure.

In the hospitalized patient with acute HF, it is common to observe transient creatinine elevations during the treatment of congestion with diuretics by reducing intravascular volume. However, as long as congestion has been adequately treated, these increases are not associated with a worse prognosis.[13,14] In our experience, creatinine, in these cases, usually falls at the end of hospitalization or about 30 days after discharge.[14,15] In other words, elevated creatinine at admission appears to be a reflection of congestion of the renal veins and transient increases are a consequence of the volume reduction process. Congestion, regardless of worsening of renal function, is associated with worse prognosis.[14,16] The intense and persistent increase in creatinine seems to indicate a worse prognosis, but transient increases do not.[16] Therefore, the diuretic and the ACEI should be maintained in this situation, in which there are still evident signs of congestion, despite the increase of slag. Diuretics should only be discontinued in cases of pre-renal renal failure, where creatinine is increased in patients with signs of hypovolemia and ACE inhibitors in cases of severe hyperkalemia or acute renal failure (anuria or oligoanuria associated with increased creatinine).

Bradycardia

The elevated heart rate (HR) is a marker of severity and is harmful to the patient with HF, and may even be the cause of HF (tachycardiomyopathy).[1,17] Since the publication of the study Systolic Heart Failure Treatment With the Inhibitor Ivabradine Trial (SHIFT) it is known that HR is not only a marker of severity, but a therapeutic target in HF, since patients treated with ivabradine, an exclusive HR reducer, showed a reduction in the combined outcome of cardiovascular mortality and hospitalization for HF.[18] Beta blockers prolong the survival of patients with HF and are medicines that reduce HR. Patients with HF should target HR between 50 and 60 bpm. It is not uncommon to find patients with HR above these values, where the maximum dose of the beta-blocker is not achieved, for fear of bradycardia. In another scenario, we see patients with sinus rhythm, already with maximum doses of beta-blockers, with HR above 70 bpm, where ivabradine would be indicated,[7] but the doctor does not prescribe because of fear of bradycardia. In the US Carvedilol study, there was a higher HR decrease in the carvedilol group compared to placebo (mean, 12.6 vs 1.4 beats, respectively) with a higher incidence of bradycardia (9% vs 1%). However, only 0.9% of patients needed to discontinue carvedilol because of bradycardia. In the SHIFT study, the incidence of asymptomatic and symptomatic bradycardia was, respectively, 6% and 5%. However, the drug was therefore suspended in only 1% of cases. Therefore, we must pursue this target of HR between 50 and 60 bpm. If this target is not reached with beta-blockers, ivabradine may be added if the patient is in sinus rhythm, with systolic dysfunction and HR above 70 bpm. We also recall that digoxin may be an option, used in more severe patients, who remain symptomatic despite treatment with the previous regimen and for frequency control in patients with atrial fibrillation.[7]

Hyperkalemia

The use of spironolactone in patients with NYHA class III and IV HF resulted in a 30% reduction in the risk of death from any cause.[6] Subsequently its use was extended to patients in class II, assuming the same benefit found with eplerenone, another aldosterone antagonist, in the EMPHASIS Study.[7] It is a low-cost medicine with a great impact on HF. Its main side effect is gynecomastia, which occurs in 9% of cases. Another complication that scares the doctor for the potential to cause arrhythmias and sudden death is hyperkalemia. In the RALES study, the incidence of severe hyperkalemia occurred in 10 (1%) patients in the placebo group and 14 (2%) in the spironolactone group, a difference with no statistical significance. However, it is common to hear that in the "real world" the incidence of hyperkalemia would be higher. Many point to a study in Canada that showed increased mortality from hyperkalemia following the publication of the RALES study[19] to justify their fears. However, a more detailed analysis reveals that often behind the hyperkalemia is inadequate use of spironolactone. For example, a study done in the United States before and after the publication of the RALES study in September 1999 showed that there was a 7-fold increase in the prescription of spironolactone after study publication.[20] However, in 31% of cases the patient did not meet RALES criteria (had creatinine> 2.5 mg/dL or serum potassium > 5.0 mEq/L). In addition, doses above the recommended dose (25 mg/day) are sometimes used in clinical practice, which increases the risk.

A study in the UK monitored prescriptions for spironolactone between the years 1994 to 2007. There was a marked increase in the prescription of spironolactone following the publication of the RALES Study, but unlike the Canadian study, there was no increase in hospitalizations for hyperkalemia and in outpatients there was a drop in the rates of hyperkalemia, due to the greater monitoring of serum potassium.[21] These data show the safety of the drug as long as the contraindications are respected and serum potassium and renal function are adequately monitored. The Brazilian guideline for chronic HF does not recommend starting spironolactone if baseline creatinine is above 2.5 mg/dL or serum potassium greater than 5 mEq/L.[7] Once the drug is started, potassium and creatinine should be monitored frequently. In the RALES study, this was done monthly in the first 3 months and every 3 months in the first year and then every 6 months. We should only suspend spironolactone if there is severe hyperkalemia. The European Society of Cardiology HF

Guideline recommends suspension of spironolactone if potassium levels exceed 6.0 mEq/L or if the creatinine exceeds 3.5 mg/dL. For potassium values between 5.6 and 6.0, or creatinine between 2.5 and 3.5 mg/dL, it is recommended to reduce the dose by half and to increase the frequency of monitoring tests.[22]

We hope this article will help to give more confidence to the doctor and increase prescription at the correct doses of medications with benefits in HF. Every medication has a built-in risk of complications, which must be weighed against its benefits. And in the case of HF, these benefits are very well proven.

Author contributions

Conception and design of the research: Villacorta Junior H; Writing of the manuscript and Critical revision of the manuscript for intellectual content: Villacorta Junior H, Villacorta AS.

Potential Conflict of Interest

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

Sources of Funding

There were no external funding sources for this study.

Study Association

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

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Viewpoint

Case 4/2017 - Young Male Marathoner with Heart Failure Due to Dilated Cardiomyopathy

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The patient was a 22-year-old male marathoner, from the town of Ranchinho, Bahia state, Brazil, coming from the city of São Paulo, São Paulo state, Brazil, hospitalized due to cardiogenic shock following chest pain and syncope after physical exertion.

According to his father, the patient had convulsions during childhood and an episode of rheumatic fever during adolescence, having undergone antibiotic prophylaxis with benzathine penicillin for 2 years, which was discontinued spontaneously, without disease recurrence. In addition, he reported recent alcohol abuse and depressive symptoms, but neither his family nor friends knew any illicit drug use.

In the preceding year, the patient had sporadic episodes of dyspnea and chest discomfort on exertion. He maintained his training and running practices, although less intensely, because of lower limb pain and weakness in past months, until 5 days ago (Aug 26, 2009), when, right after a training session, he experienced sudden chest pain, cough with hemoptysis, general weakness, shivering, mental confusion and syncope. The patient sought a hospital close to his dwelling, being admitted for observation.

His ECG (Aug 27) showed supraventricular rhythm, 125 beats per minute, indirect signs of right atrial overload, left ventricular overload, and secondary changes of ventricular repolarization (Figure 1).

His enzyme measurements were as follows: CPK 120 IU/L, CK-MB 29 UI/L, and troponin 0.77 ng/L.

His echocardiogram (Aug 29) revealed the following: diameters of the aorta 28 mm, left atrium 42 mm, right ventricle 20 mm, and left ventricle (diastole/systole) 75/66 mm; ejection fraction 25% (Teicholz); and interventricular septum and posterior wall thickness of 9 mm and 10 mm, respectively. There was left ventricular diffuse hypokinesis, with restrictive filling, and moderate mitral and tricuspid regurgitation.

The patient became lethargic with tachypnea. His physical exam (Aug 30) showed heart rate of 115 beats per minute, respiratory rate of 30 breaths per minute, blood pressure of 92/54 mmHg, and O_2 saturation of 90% with O_2 catheter at a 3 L/min flow rate. His jugular venous pressure was elevated (++/4), and his pulmonary auscultation, normal. His cardiac auscultation revealed mitral and tricuspid heart murmurs (+++/6) at systole and no accessory heart sound. His abdomen was flaccid and painless, without visceromegaly. His lower limbs showed no edema. His pulses were symmetrical and thin, and the peripheral perfusion was poor.

Intravenous dopamine was initiated to treat shock, and enoxaparin and clopidogrel were added.

The patient experienced a new episode of chest pain and dyspnea, with lowering of the level of consciousness, requiring orotracheal intubation for ventilatory support. The patient developed shock refractory to volume and noradrenaline administration, being transferred to the Instituto do Coração (InCor).

His physical exam on admission at InCor (Aug 31) revealed poor general state, cold extremities, mechanical ventilation with endotracheal intubation, and inaudible blood pressure. His pulmonary auscultation evidenced diffuse rhonchi. His cardiac auscultation revealed a third heart sound and mitral heart murmur (+++/6) at systole. The abdomen showed no abnormality, and the extremities, no edema.

His laboratory tests (Aug 31) were as follows: hemoglobin, 17.4 g/dl; hematocrit, 59%; leukocytes, 20900/mm^3 (4% band neutrophils, 76% segmented neutrophils, 11% lymphocytes, 9% monocytes); platelets, 288000/mm^3; ferritin, 258 ng/mL; CK-MB, 12 ng/mL; troponin I, 4.11 ng/m L; CPK, 3168 U/L; AST, 145 U/L; ALT, 61 U/L; LDH, 387 U/L; C-reactive protein, 88.5 mg/L; urea, 45 mg/dL; creatinine, 3.1 mg/dL (GF = 27 mL/min/1.73 m^2); sodium, 140 mEq/L; potassium, 6.4 mEq/L; arterial lactate, 15 mg/dL; TP (INR) 6.6; TTPA incoagulable; D dimer, 2460 ng/mL. His arterial blood gas analysis revealed: pH 7.15; $paCO_2$ 60.2 mm Hg; paO_2 54.2 mm Hg; O_2 saturation 79.6%; bicarbonate 20.2 mEq/L and base excess (-)10 mEq/L. His protein electrophoresis revealed: total protein, 4.9 g/dL; albumin, 2.3 g/dL; and globulins: alpha 1, 0.3 g/dL; alpha 2, 0.6 g/dL; beta, 0.7 g/dL; gamma, 1 g/dL. Serologies for Chagas disease, hepatitis C, and HIV were negative, and IgG antibodies were positive for hepatitis A and B, cytomegalovirus and toxoplasmosis.

His ECG (Aug 31) showed atrial fibrillation, 140 beats per minute, left ventricular overload, pointed T wakes in V_1 and V_2, and inverted in V_5 and V_6 (Figure 2).

His new echocardiogram (Aug 31) evidenced: diameters of the aorta 27 mm, left atrium 45 mm, right ventricle 42 mm, left ventricle (diastole/systole) 74x68 mm; ejection fraction (Teicholz) 17%; and similar interventricular septum and posterior wall thickness, 9 mm. The ventricles were dilated and

Keywords

Heart Failure; Cardiomyopathy, Dilated; Physical Exertion; Athletes; Sports.

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DOI: 10.5935/abc.20170124

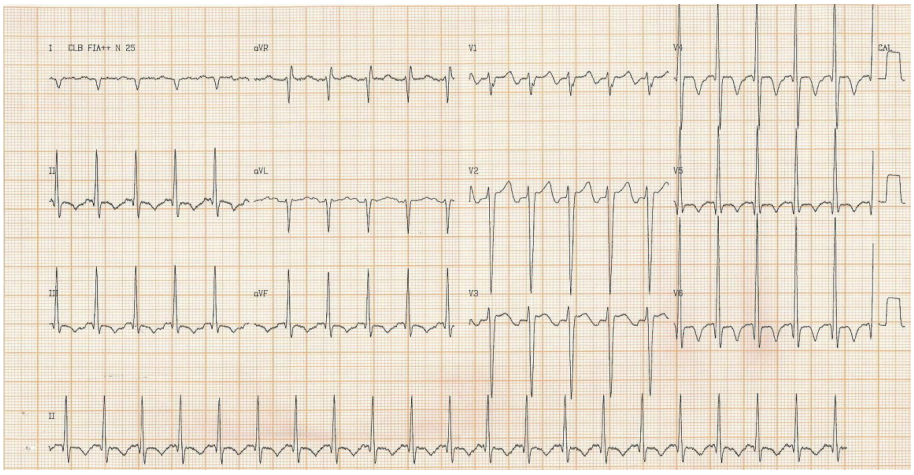

Figure 1 – *ECG (Aug 27): supraventricular tachycardia, axis shifted to the right, 125 beats per minute, indirect signs of right atrial overload, left ventricular overload, secondary changes of ventricular repolarization.*

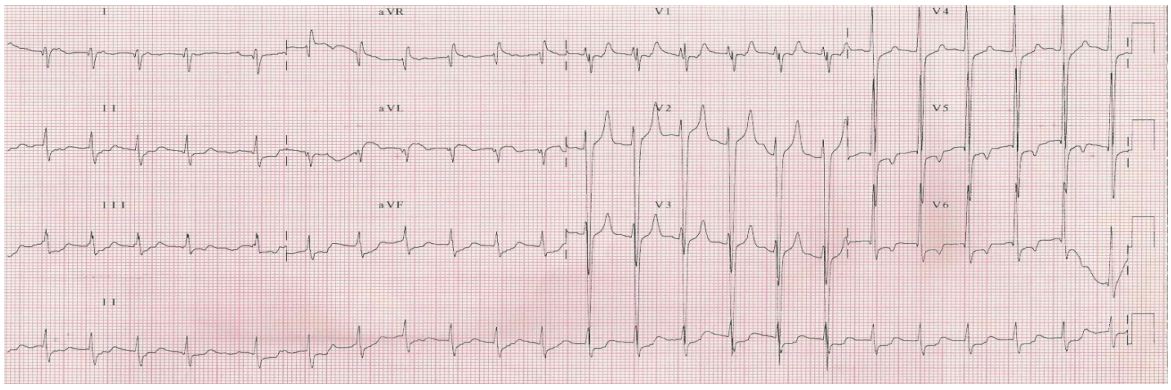

Figure 2 – *ECG (Aug 31): atrial fibrillation, axis shifted to the right, 140 beats per minute, left ventricular overload, pointed T waves in V_1 and V_2 and inverted in V_5 and V_6.*

diffusely hypokinetic, and there was no valvular abnormality, but signs of pulmonary hypertension. His transesophageal echocardiogram (Sept 2) showed a pedunculated thrombus adhered to the left ventricular anterior wall, measuring 2.18 x 1.16 cm.

Intraaortic balloon for circulatory support was initiated on August 31.

The patient improved his hemodynamic findings, but fever appeared.

The intraaortic balloon was removed on September 4, when the hemodynamic measures were as follows: blood pressure 150/72 mm Hg; pulmonary artery pressure 34/15 mm Hg; central venous pressure, 11 mm Hg; cardiac index 3.72 L/min.m^2; left ventricular systolic volume index 48 mL/m^2/beat; systemic vascular resistance index 1741 dyn.s.m^2.cm^{-5}.

Antibiotic therapy with vancomycin, piperacillin and tazobactam was initiated.

His laboratory tests (Sept 4) revealed: hemoglobin, 10.4 g/dL; hematocrit, 33%; leukocytes, 10400/mm^3 (75% neutrophils); platelets, 155000/mm^3; TP (INR) 1.1; TTPA (rel) 1.25; PCR, 130 mg/L; CPK, 1124 U/L; urea, 23 mg/dL; creatinine, 0.6 mg/dL; sodium, 139 mEq/L; potassium, 3.2 mEq/L; AST, 98 U/L; and ALT, 62 U/L.

In the following days, his renal function (creatinine, 2.6 mg/dL) and pulmonary congestion worsened.

His state of consciousness oscillated from agitation to somnolence. His neurological assessment comprised skull tomography and cerebrospinal fluid analysis. The latter was as follows: colorless and clear; negative for bacteria and fungi; glucose, 70 mg/dL; chloride, 129 mEq/L; proteins, 38 mg/dL; lactate, 9.2 mg/dL; cells, zero and 1 red blood cell/mL (Sept 9).

His skull tomographies (Sept 8 and 10) revealed hypoattenuating areas in the white and gray matters of the right parietal and occipital regions, suggesting acute ischemic lesions.

His chest tomography (Sept 8) revealed significant cardiomegaly, moderate bilateral pleural effusion, atelectasis of the adjacent parenchyma and diffuse ground glass opacity, more evident in the lower lobes.

His abdominal tomography (Sept 8) showed hepatomegaly, homogeneous liver with blunt borders, dilatation of the inferior vena cava and suprahepatic veins; hyperattenuating gall bladder content, suggesting biliary mud. The spleen, pancreas, kidneys, adrenal glands and abdominal aorta showed no abnormality.

The new echocardiographic assessments (Sept 9 and 17) showed no change as compared to the initial one, except for a reduction in right ventricular dilatation and hypokinesis.

On the 11th day of admission, the fever recurred and purulent sputum appeared. The latter improved with the addition of colistimethate to therapy. However, after three days, the patient had acute pulmonary edema with arterial hypertension, and persistent fever. Because of the presence of yeast cells with pseudohyphae in tracheal secretion, fluconazole was introduced.

The blood cultures collected (Sept 11) were positive for coagulase-negative *staphylococci* sensitive to teicoplanin, vancomycin and the sulfamethoxazole-trimethoprim association.

On the 20th day of admission, the patient had polymorphic ventricular tachycardia and cardiopulmonary arrest, which reversed with electrical defibrillation with 200 J, but recurred short after, degenerating to ventricular fibrillation and pulseless electrical activity, which also reversed. The patient received intravenous amiodarone (300 mg), but developed shock and acute pulmonary edema.

His laboratory tests (Sept 19) were as follows: hemoglobin, 12.1 g/dL; hematocrit, 39%; leukocytes, 26900/mm^3 (14% band neutrophils, 72% segmented neutrophils); platelets, 300000/mm^3; TP (INR) 7.5; TTPA (rel) 1.92; sodium, 155 mEq/L; potassium, 5.8 mEq/L; magnesium, 1.9 mmol/L; urea, 89 mg/dL; creatinine, 3.9 mg/dL; lactate, 145 mg/dL. His arterial blood gas analysis was as follows: pH 7.42; $paCO_2$ 35 mm Hg; paO_2 108 mm Hg; O_2 saturation 98%; bicarbonate 22 mEq/L and base excess (-)1.5 mEq/L.

The patient remained in shock with fever, which did not improve with the intraaortic balloon and change from fluconazole to amphotericin and introduction of the sulfamethoxazole-trimethoprim association. He died in pulseless bradycardia on the 22nd day of admission (Sept 21, 2009).

Clinical aspects

The patient was a 22-year-old male marathoner with fatal heart failure for 1 year. The clinical data reported were dyspnea and precordial discomfort on exertion. The patient maintained his trainings and running practice less intensely due to lower limb pain and weakness in the last months, until he was hospitalized due to chest pain, dyspnea, hemoptysis, mental confusion and syncope. During hospitalization, his clinical findings rapidly and progressively deteriorated. His electrocardiogram on admission revealed signs of right atrial overload, left ventricular overload and secondary ventricular repolarization changes. The left ventricle was diffusely hypokinetic and the diastolic function pattern was restrictive. There was moderate mitral and tricuspid valve regurgitation. These findings suggest cardiomyopathy with important hemodynamic repercussion.

The causes of cardiomyopathy in young individuals are: idiopathic cardiomyopathy, infectious myocarditis and autoimmune myocarditis.[1] Cardiotoxicity can also be observed in exposure to certain agents, such as alcohol, cocaine, heavy metals and antineoplastic drugs, such as anthracyclines and cyclophosphamide.

Idiopathic dilated cardiomyopathy is the most common cause of heart failure in young individuals, 30% to 50% of the cases being familial and associated with inherited genetic mutations.[2,3] Some patients have heart failure of rapid progression and refractory to treatment, one of the most frequently found etiologies in heart transplant lists.[4]

Lymphocytic myocarditis can be triggered by different infectious agents, viruses being the most frequent ones. Lymphocytic myocarditis can progress with acute, subacute or chronic heart failure. More than 20 viruses, such as Coxsackievirus B, adenovirus, parvovirus B19, cytomegalovirus and human herpesvirus 6, have been related to the risk for myocarditis.[5] Some groups, such as children and immunocompromised patients, are at higher risk to develop rapidly progressive or fulminant heart failure related to viral myocarditis. Other infectious agents, such as bacteria, rickettsia and fungi, are occasionally associated with myocarditis, but less commonly than viruses. In Brazil, where Chagas disease is endemic in some regions, some patients can have rapidly progressive myocardial impairment. Although our patient was born in an area potentially endemic for Chagas disease, his serology was negative.

Autoimmune myocarditis can lead to acute or subacute heart failure with rapid decompensation. Giant cell myocarditis is a rare disease, mediated by abnormalities in T lymphocyte function, affects mainly young and middle-aged individuals, has a rapid course and high mortality rate. The initial presentation in 75% of the cases is rapidly progressive heart failure, while the rest present with cardiac arrhythmias and findings similar to acute myocardial infarction. Around 20% of the patients with giant cell myocarditis have associated autoimmune conditions, such as inflammatory intestinal disease, thyroiditis, celiac disease, rheumatoid arthritis.[6] Similarly to giant cell myocarditis, eosinophilic myocarditis can be characterized by rapidly progressive and potentially fatal heart failure, being occasionally related to exposure to drugs or exogenous agents. Cardiac sarcoidosis is also a differential diagnosis, although there was no report of previous impairment of organs, such the lungs and liver. Usually, patients with cardiac sarcoidosis have changes in the cardiac conduction system, with total atrioventricular block in up to 30% of the cases, related to the presence of granulomas and scars in the basal region of the interventricular septum.[2]

Acute myocardial infarction is a frequent cause of acute heart failure, is more common in middle-aged and old patients with atherosclerotic risk factors and/or established

disease. In our case, the patient was young, had no classical risk factor for cardiovascular disease, and his clinical and laboratory findings did not suggest acute coronary syndrome.

Between the fifth and sixth days of hospitalization, the patient had a significant clinical deterioration, with respiratory and hemodynamic failure, change in his level of consciousness, requiring invasive ventilatory support and vasoactive drugs. His physical exam showed signs of acute heart failure with the third heart sound and pulmonary congestion, poor peripheral perfusion with elevated capillary filling time and increased central venous pressure, characterized by the presence of jugular venous distention. His laboratory tests on the occasion revealed several data of poor prognosis due to organic dysfunctions, such as elevation of nitrogen compounds, arterial lactate, liver enzymes, acidosis, hypoxemia, as well as increased levels of inflammatory markers, such as C-reactive protein, leukocytosis and D dimer. His electrocardiogram evidenced atrial fibrillation with high response, left ventricular overload and ventricular repolarization changes. The comparison of the new echocardiography with the previous one showed right ventricular dilatation and hypokinesis, and indirect signs of pulmonary hypertension. The transesophageal echocardiogram revealed a pedunculated thrombus on the left ventricular anterior wall, which increased his cardioembolic risk, being probably related to the presence of right frontal and parietal cerebral ischemic lesions later observed on skull tomography.

In some patients with acute or chronic heart failure, some conditions, such as underlying disease, infections, pulmonary thromboembolism (PTE), arrhythmias, myocardial ischemia and anemia, can trigger or worsen decompensation. Infection and PTE might have contributed to our patient's clinical deterioration. A recent study has reported a 17.8% PTE prevalence in individuals hospitalized due to syncope.[7] In our patient, the new right ventricular dilatation and dysfunction associated with worsening of the respiratory findings, hypoxemia and shock might suggest PTE as the possible cause of decompensation. Viral and bacterial infections are among the most common causes of decompensation in patients hospitalized with heart failure.[4] This higher incidence of infectious diseases in patients with heart failure is multifactorial, resulting from the interaction of different abnormalities, such as immunological changes in critically ill patients, nutritional deficiencies and higher need for invasive diagnostic and therapeutic procedures. Despite the treatment with large spectrum antibiotics because of fever and suggestive signs of sepsis, in addition to the hemodynamic support during hospitalization, the patient experienced hemodynamic instability and refractory shock. **(Hilda Sara Montero Ramirez, MD, and Rafael Amorim Belo Nunes, MD)**

Diagnostic hypotheses: congestive heart failure; myocarditis (autoimmune? viral?); cardioembolic ischemic stroke; nosocomial bronchopneumonia; cardiogenic and septic shock. **(Hilda Sara Montero Ramirez, MD, and Rafael Amorim Belo Nunes, MD)**

Postmortem examination

The heart weighed 590 g (normal, up to 350 g), was moderately enlarged and had a globose shape (Figure 3). The epicardial surface had small pericoronary fibrous thickenings. When opened, dilatation of all chambers with mild hypertrophy of the ventricular and atrial walls was observed (Figures 4 and 5). Neither the atrioventricular nor the arterial valves had abnormalities. There were neither cavitary thrombi nor endocardial thickening. The coronary arteries had usual origins and showed no significant obstructive lesion.

The microscopic exam showed moderate to marked hypertrophy of cardiomyocytes, with focal myocardial interstitial fibrosis (Figure 6) and areas of organizing microinfarcts (Figure 7). There were signs of terminal shock, such as centrilobular liver necrosis, renal acute tubular necrosis and recent pulmonary alveolar hemorrhage (Figure 8), in addition to signs of systemic embolism, such as recent infarcts in the brain (right parietal and occipital) and spleen. **(Prof. Vera Demarchi Aiello, MD)**

Diagnosis: Cause of death: cardiogenic shock. Main disease: idiopathic dilated cardiomyopathy. **(Prof. Vera Demarchi Aiello, MD)**

Comments

The patient was a marathoner with symptoms compatible with heart failure in the preceding year. He had syncope after physical training, being hospitalized. During his hospitalization, dilated cardiomyopathy was identified. The postmortem examination revealed moderate hypertrophy and dilatation of cardiac chambers. Clinically speaking, there was doubt regarding the etiology of the cardiomyopathy, and whether it would be related to the so-called "athlete's heart". Data from the literature have shown that, although some athletes, mainly practitioners of aerobic sports, can have dilated cardiac chambers, most have ventricular dimensions within the normal range. In around 10% of those athletes, the intensity of the dilatation is similar to that occurring in dilated cardiomyopathy, the differential

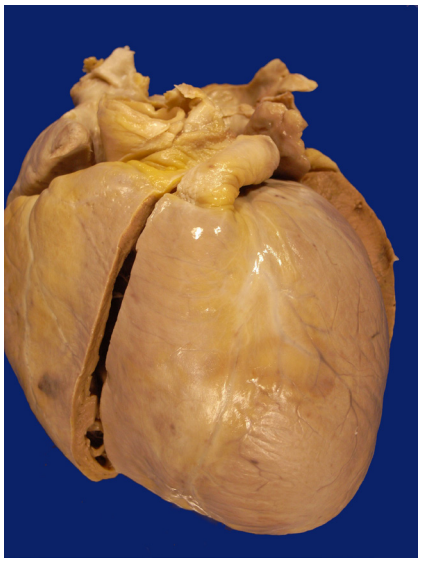

Figure 3 – *External view of the heart showing volume enlargement and globose shape, and bright epicardium.*

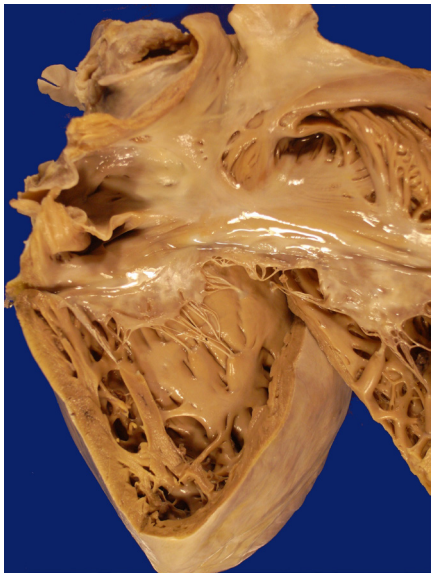

Figure 4 – *Opened right cardiac chambers showing atrial and ventricular dilatation, and mild myocardial hypertrophy.*

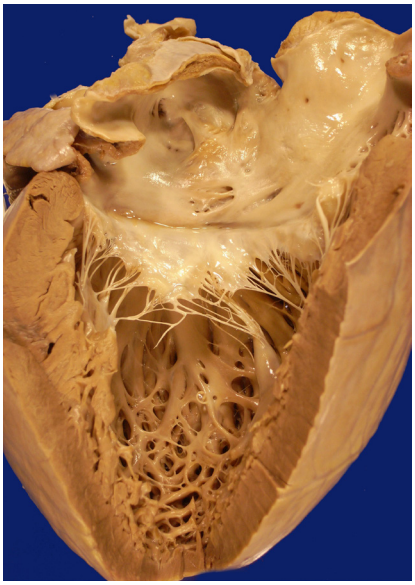

Figure 5 – *Opened left atrium and ventricle showing dilatation of both cardiac chambers and hypertrophic myocardium.*

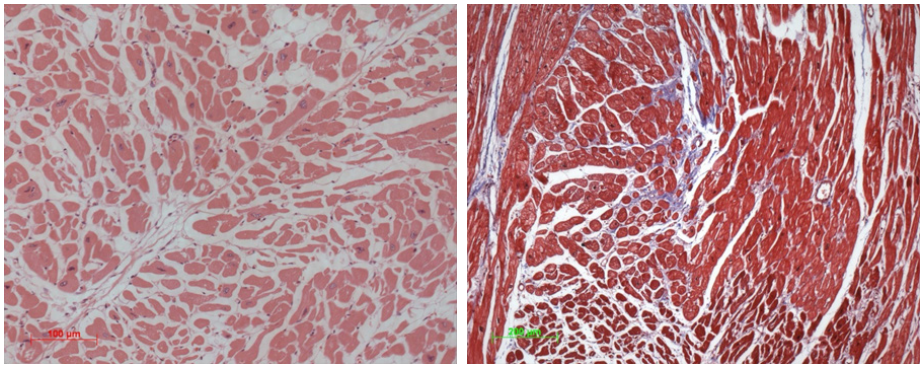

Figure 6 – *Photomicrographs of the myocardium showing hypertrophy of cardiomyocytes and interstitial fibrosis (blue areas in the right panel). Left panel: Hematoxylin-Eosin, 20X; right panel: Masson's trichrome, 10X.*

diagnosis being established by the lack of systolic dysfunction, with maintenance of ventricular ejection fraction or even its increase. The same European authors have demonstrated that ventricular dilatation is positively related to the athlete's body surface and height.[8,9] Therefore, although our patient was an athlete (marathoner) and had myocardial dilatation and hypertrophy, there was cardiac dysfunction, characterizing dilated cardiomyopathy. In addition, the microscopic exam evidenced marked pathological myocardial changes, such as cardiomyocyte hypertrophy and myocardial interstitial fibrosis, as well as organized or organizing microinfarcts.

Although infarcts were identified in the territories of the cerebral and splenic arteries, cardiac thrombi, a possible embolic source, were not evidenced. The postmortem exam showed no infection sign. Despite the report of rheumatic disease during childhood, his heart valves showed no lesion compatible with sequelae of that disease. **(Prof. Vera Demarchi Aiello, MD)**

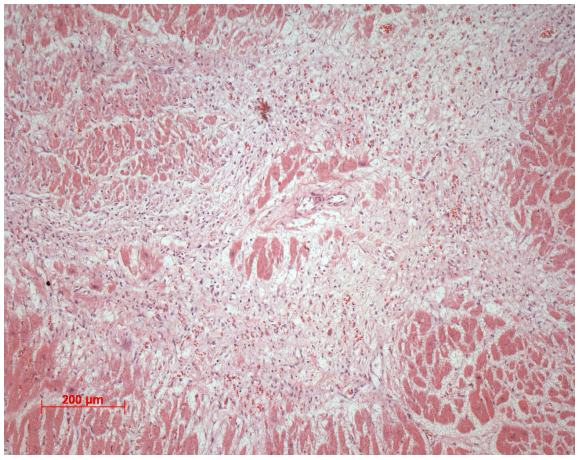

Figure 7 – *Photomicrograph of an area of organizing myocardial microinfarct. Hematoxylin-Eosin, 10X.*

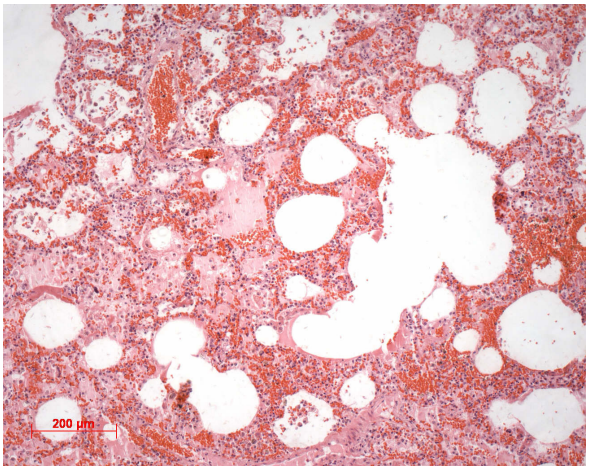

Figure 8 – *Photomicrograph of the lungs showing diffuse alveolar hemorrhage. Hematoxylin-Eosin, 10X.*

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Asymptomatic Coronary Spasm Due to Polytraumatism

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Introduction

Variant angina is a form of angina pectoris caused by vasospasm of an epicardic coronary artery that results in myocardial ischemia that might be manifested by transient ST segment elevation on the electrocardiogram.[1] Although it usually presents with chest pain, asymptomatic episodes are not rare.[2,3] We report a case of an incidental diagnosis of asymptomatic coronary vasospam in a patient with polytrauma.

Description

A 42-year-old man with a past medical history of smoking and no previous cardiovascular disease was admitted to the emergency department because of an accidental fall from 3 meters high with cranial, facial and thoracic traumatism. As part of the initial evaluation a 12-lead electrocardiogram was performed by emergency medical services showing a transient 3 mm ST-segment elevation in II, III, aVF, V5 and V6 leads which was not longer present in the first ECG performed at hospital admission (Figure 1). Patient denied any chest pain, dizziness or dyspnea prior to the accident nor after it. Physical examination, including neurological basic explorations, was completely unremarkable. Cranial, thoracic and abdominal computed tomography scans were performed without evidence of any organ damage. Blood analyses reported normal values of cardiac biomarkers. A transtorathic ecocardiogram showed a normal left ventricle size with preserved systolic function, without segmental wall motion abnormalities, and absence of pericardial effusion.

The patient was admitted to the cardiology ward remaining completely asymptomatic with no changes in serial ECGs, troponin determinations or ecocardiographic explorations. However, due to the high suspicion of coronary vasospasm, with the presence of transient ischemic changes on ECG, catheterization was planned and performed 24 hours later. Coronary angiography revealed mild lumen irregularities, mainly on the left circumflex coronary artery, but no significant coronary stenosis. An ergonovine test was performed. The test was clinically and electrically positive, with weak chest pain and 3 mm ST-segment elevation in II, III and aVF leads. A severe vasospasm in proximal segment of circumflex artery was also documented (Figure 2). Intracoronary nitroglycerin was given leading to a complete resolution of the angiographic changes and ST-segment normalization. Optical coherence tomography revealed an uncomplicated lipid-rich plaque at the target segment. No data suggestive of minor plaque rupture or intracoronary thrombus was revealed.

With the final diagnosis of vasospastic angina, the patient was discharged four days later under treatment with calcium channel blockers and low-dose of aspirin. At three months follow the patient remained completely asymptomatic without new cardiovascular events.

Discussion

This case meets the vasospasm guideline's criteria required for the diagnosis of "definite vasospastic angina".[4] We observed transient ischemic changes on the ECG that were reproduced in the catheterization laboratory during the provocation test with clear drug-induced angiographic coronary spasm that resolved after vasodilators. Pathophysiology of this syndrome includes endothelial disfunction and increased oxidative stress.[5] In addition, the important role of disbalance of autonomic nervous system has also been well defined in its development.[6] Smoking is also a well-known risk factor for vasospasm. However, in the case we present here, cathecolamine discharge after trauma may have played an important role which can have led to sudden excessive coronary vasoconstriction. Interestingly, coronary vasospasm has also been reported with other unusual presentations like severe arrhythmias or even Tako-Tsubo syndrome.[7] However, in our patient a completely normal left ventricular function was demonstrated. To the best of our knowledge, this is the first case of asymptomatic variant angina that is diagnosed incidentally as a consequence of other acute clinical entity that precipitates coronary vasospasm.

In addition, in our patient OCT was instrumental to rule out plaque rupture or intracoronary thrombus formation that have been described in patients with vasospastic angina. Prior studies have recently suggested that erosion of a fibrotic underlying plaque with superimposed white coronary thrombus can be identified in most patients with coronary vasospasm.[8-10] In our case, however, subtle images of rupture, erosion or thrombus were ruled out.

Our findings highlight the wide spectrum of this unique pathology. A better understanding of the pathophysiology of this challenging clinical entity is warranted to better identify potential precipitant factors of coronary vasospasm and thus obtain an early diagnosis.

Author contributions

Conception and design of the research: Aguilar R; Acquisition of data: Aguilera MC, Restrepo J, Rivero F, Bastante T; Analysis and interpretation of the data and Writing of the manuscript:

Keywords

Coronary Vasospasm; Angina Pectoris; Endothelium/physiopatolhology; Wounds and Injuries.

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Manuscript received March 13, 2016; revised manuscript June 03, 2016, accepted June 06, 2016

DOI: 10.5935/abc.20170093

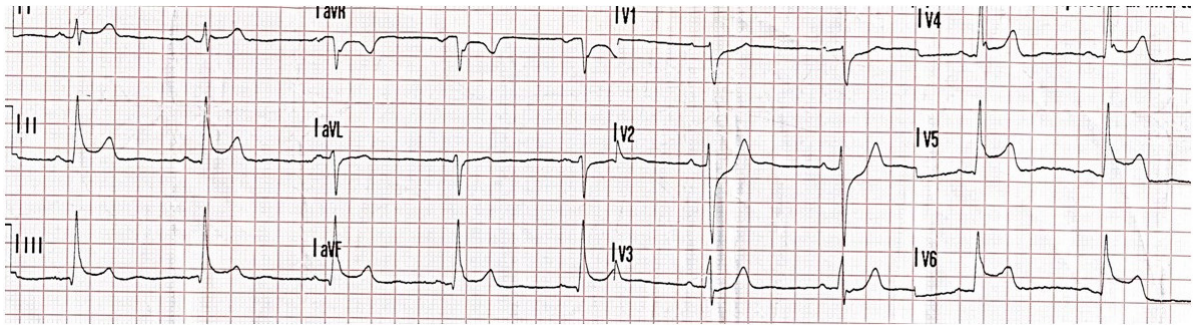

Figure 1 – *ST-segment elevation on first ECG performed by emergency medical services.*

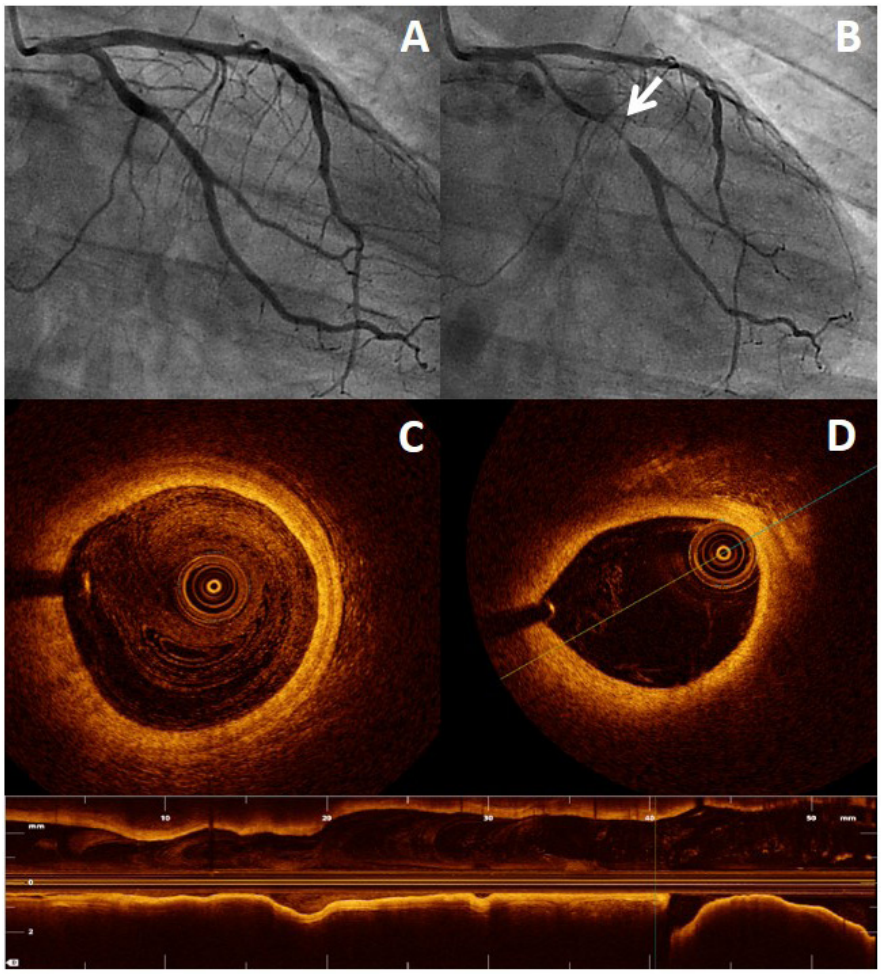

Figure 2 – *A) Basal coronary angiography with non-significant stenosis. B) Ergonovine-induced severe coronary vasospasm of the circunflex coronary artery (arrow) with a very mild diffuse calliber reduction of the entire coronary tree. C) Normal coronary wall structure. The characteristic 3-layered appearance is readily visualized. D) Uncomplicated atherosclerotic lipid plaque at the same coronary segment where vasospasm was induced.*

Aguilera MC; Critical revision of the manuscript for intellectual content: Aguilar R, Alfonso F.

Potential Conflict of Interest

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

Sources of Funding

There were no external funding sources for this study.

Study Association

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

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Crescent Moon Image as a Peculiar Complication During Percutaneous Coronary Intervention of an In-Stent Chronic Total Occlusion

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Case

55-year-old male with history of ischemic cardiomyopathy with previous percutaneous coronary intervention (PCI) in the anterior descending coronary artery (LAD) middle segment was admitted to our hospital for chest pains. A new coronary angiogram showed in-stent chronic total occlusion (ISCTO) of LAD receiving collaterals from right coronary artery (RCA). Left circumflex (LCX) was totally occluded and RCA presented a significant mid-segment lesion. Complete percutaneous revascularization was planned. First attempt to recanalize the LAD failed because the lesion obstructed the passage of the balloon, so a second dedicated attempt was planned. Bilateral injection using radial and femoral arteries was used, and a Confianza Pro 9 (Asahi Intecc, Japan) guidewire was progressively advanced through the ISCTO (Figure 1a) and the wire's position in true lumen was verified by contralateral injection. Considering the lesion, which impeded the balloon's passage, a microcatheter Tornus (Asahi Intecc, Japan) was utilized to penetrate and advance through and past the occlusion (Figure 1b). After balloon predilatation, intravascular ultrasound sonography (IVUS) verified the position of the wire in a very short segment into true lumen, but outside the previously implanted stent (Figure 1c). Several drug eluting stents (DES) were implanted and the artery was successfully recanalized, although a very distal embolization was detected (Figure 1d). A new IVUS examination showed partial crush of the previous stent in the shape of a crescent moon (Figure 1e).

This rare complication is likely due to an underexpanded stent point in the first procedure. The guidewire in this point got out through a stent strut but remained within the true lumen (Figure 1f). After balloon predilatation and stent implantation, the former stent was crushed in its underexpanded point. This is a potential complication which could happen during ISCTO-PCI and a careful examination by IVUS before stent implantation can localize the wire bias. There is a difficult but feasible manoeuvre consisting of reintroducing a new wire into the stent lumen guided by IVUS, which can potentially avoid the above complication.

Keywords

Percutaneous Coronary Intervention; Coronary Occlusion; Drug Eluting Stents; Ultrasonography, Interventional; Cardiac Catheters.

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Manuscript received November 01, 2016; revised manuscript November 08, 2016; accepted November 23, 2016.

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Potential Conflict of Interest

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

Sources of Funding

There were no external funding sources for this study.

Study Association

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

DOI: 10.5935/abc.20170073

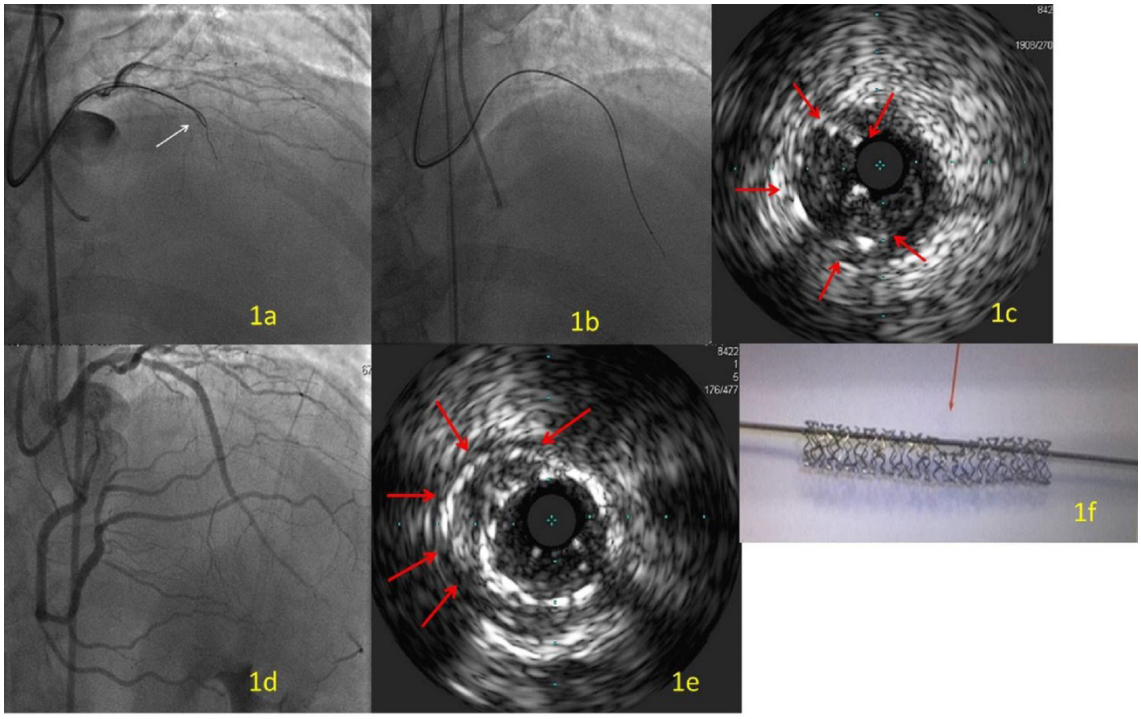

Figure 1 – *1a) Confianza Pro 9 guidewire penetrated in-stent occluded segment using parallel wire technique. 1b) Tornus microcatheter successfully passed through the in-stent occluded segment and advanced up to the distal portion of the artery. 1c) Arrowheads limit the stent's underexpanded point with further distortion after balloon dilatation. IVUS probe in this point is positioned into coronary true lumen but out of the previously implanted stent. 1d) successful recanalization of left anterior descending coronary artery after implantation of several drug eluting stent, although distal embolization is observed. 1e) crescent moon image after implantation of new stent crushing the previous one. 1f) on-bench model of an underexpanded stent shows how the guidewire could get out of the stent and re-enter again into the stent lumen.*

Erratum

Arq Bras Cardiol. 2017; [online].ahead print, PP.0-0

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DOI: 10.5935/abc.20170117