

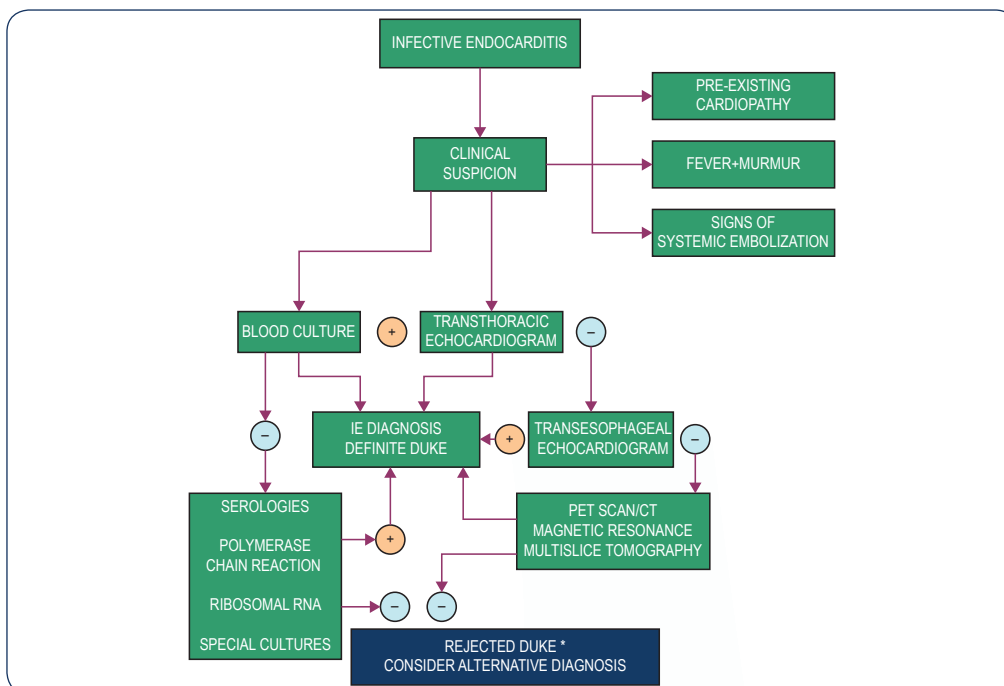


Figure 1 of Page 202.

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Study of myocardial perfusion in obese individuals without known IHD

Conversion to total cavopulmonary connection

Antiplatelet resistance in young MI patients

Prevalence of dyslipidemia in adolescents

Garlic and exercise increase cardiac angiogenesis

Cut-point for Dietary Sodium Restriction Questionnaire

Trimetazidine effects on diabetic hearts

Exercise effects on ovariectomized mice

The Year in Cardiology 2018: ABC Cardiol and RPC at a glance

Contents

Editorial

Physical Inactivity in Brazil and Sweden - Different Countries, Similar Problem

Ricardo Stein and Mats Börjesson

.....page 119

Original Article

Study of Myocardial Perfusion in Obese Individuals without Known Ischemic Heart Disease

Tufi Dippe Jr., Cláudio Leinig Pereira da Cunha, Rodrigo Julio Cerci, Arnaldo Lafitte Stier Jr., João Vicente Vítola

.....page 121

Short Editorial

Single Photon Computed Tomography-Myocardial Perfusion Scintigraphy. Diagnostic Tool Anticipating the Disease

Whady Hueb

.....page 129

Original Article

Outcomes of the Conversion of the Fontan-Kreutzer Operation to a Total Cavopulmonary Connection for the Failing Univentricular Circulation

Gabriel Carmona Fernandes, Guilherme Viotto Rodrigues da Silva, Luiz Fernando Caneo, Carla Tanamati, Aida Luiza Ribeiro Turquetto, Marcelo Biscegli Jatene

.....page 130

Short Editorial

Fontan-Kreutzer Conversion to Total Cavopulmonary Surgery due to Failing Univentricular Circulation. A Feasible Therapeutic Option?

Isabel Cristina Britto Guimarães

.....page 136

Original Article

Long Term Cardiovascular Outcome Based on Aspirin and Clopidogrel Responsiveness Status in Young ST-Elevated Myocardial Infarction Patients

Mustafa Umut Somuncu, Ali Riza Demir, Seda Tukenmez Karakurt, Huseyin Karakurt, Turgut Karabag

.....page 138

Original Article

Dyslipidemia in Adolescents Seen in a University Hospital in the city of Rio de Janeiro/Brazil: Prevalence and Association

Nathalia Pereira Vizentin, Paula Mendonça Santos Cardoso, Camila Aparecida Gomes Maia, Isabela Perez Alves, Gabriel Lunardi Aranha, Denise Tavares Giannini

.....page 147

Short Editorial

The Importance of Identifying Risk Factors in Childhood and Adolescence

Ana Paula Marte Chacra

.....page 152

Original Article

The Effect of Garlic and Voluntary Exercise on Cardiac Angiogenesis in Diabetes: The Role of MiR-126 and MiR-210

Roya Naderi, Gisou Mohaddes, Mustafa Mohammadi, Alireza Alihemmati, Amirmahdi Khamaneh, Rafighe Ghyasi, Rana Ghaznavi

.....page 154

Short Editorial

Exercise and Garlic Modulate microRNAs Involved in Diabetic Cardiopathy

Aline Regina Ruiz Lima

.....page 163

Original Article

Cut-Point for Satisfactory Adherence of the Dietary Sodium Restriction Questionnaire for Patients with Heart Failure

Karina Sanches Machado d'Almeida, Sofia Louise Santin Barilli, Gabriela Corrêa Souza, Eneida Rejane Rabelo-Silva

.....page 165

Short Editorial

Evaluating Sodium Restriction in Heart Failure

Pedro Pimenta de Mello Spinetti

.....page 171

Original Article

The Effects of Trimetazidine on QT-interval Prolongation and Cardiac Hypertrophy in Diabetic Rats

Fatemeh Ramezani-Aliakbari, Mohammad Badavi, Mahin Dianat, Seyed Ali Mard, Akram Ahangarpour

.....page 173

Short Editorial

Cardiac Effects of Trimetazidine in Diabetic Rats

Alfredo J. Mansur

.....page 179

Original Article

Evaluation of Collagen Fibers, MMP2, MMP9, 8-OHdG and Apoptosis in the Aorta of Ovariectomized LDL Knockout Mice Submitted to Aerobic Exercise

Laura Beatriz M. Maifrino, Nathalia E. A. de Lima, Mara R. Marques, Clever G. Cardoso, Lidiane B. de Souza, Tabata de Carvalho Tomé, Hananiah Tardivo Quintana, Flavia de Oliveira, Beatriz da Costa Aguiar Alves Reis, Fernando Luiz Affonso Fonseca

.....page 180

Brief Communication

Analysis of Iron Metabolism in Chronic Chagasic Cardiomyopathy

Carla Paixão Miranda, Fernando Antônio Botoni, Maria do Carmo Pereira Nunes, Manoel Otávio da Costa Rocha

.....page 189

Review Article

The Year in Cardiology 2018: ABC Cardiol and RPC at a glance

Ricardo Fontes-Carvalho, Gláucia Maria Moraes de Oliveira, Lino Gonçalves, Carlos Eduardo Rochitte

.....page 193

Viewpoint

Early Diagnosis and Treatment in Infective Endocarditis: Challenges for a Better Prognosis

Daniely Iadocico Sobreiro, Roney Orismar Sampaio, Rinaldo Focaccia Siciliano, Calila Vieira Andrade Brazil, Carlos Eduardo de Barros Branco, Antônio Sergio de Santis Andrade Lopes, Flávio Tarasoutchi, Tânia Mara Varejão Strabelli

.....page 201

Anatomopathological Correlation

Case 1/2019 – A 51-year-old Man with Arterial Hypertension, Aortic Dissection and Aortic Valve Regurgitation, in addition to Heart Failure with Unchanged Clinical Course After Surgical Intervention

Desiderio Favarato and Vera Demarchi Aiello

.....page 204

Case Report

Large Bilateral Coronary Artery Fistula: 10-year follow-up in Clinical Treatment

Rodrigo Melo Kulchetscki, Luka David Lechinewski, Luciana Oliveira Cascaes Dourado, Whady Armino Hueb, Luiz Antonio Machado César

.....page 211

Erratum

.....page 214



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Volume 112, Nº 2, February 2019

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



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Physical Inactivity in Brazil and Sweden - Different Countries, Similar Problem

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Physical inactivity is one of the major risk factors for non-communicable disease, such as cardiovascular diseases, depression, breast and colon cancer, and type 2 diabetes. It is the fourth leading cause of death worldwide.¹ People who are insufficiently active have a 20% to 30% increased risk of death compared to active individuals. International recommendations on physical activity (PA) for the general population have been developed, including at least 150 minutes of moderate intensity aerobic exercise, preferably divided into 5 days per week for at least 30 minutes.^{2,3} Most scientists agree that physical inactivity has been increasing globally, but figures for fulfillment of PA recommendations vary between studies and countries.⁴ The main reason for this is that in older studies, using self-reported activity, PA levels are overestimated. However, when PA measurements are validated by more objective methods, such as accelerometry,⁵ the number of sedentary individuals increases. In this regard, it is important to point out that current recommendations are built on self-reported PA.

For example, in one study, 1 in 4 adults was not active enough, and more than 80% of the world's adolescent population was deemed insufficiently active. Interestingly, adolescent girls were less active than adolescent boys, with 84% vs. 78% not meeting the World Health Organization (WHO) recommendations.³

According to the US Department of Health and Human Services, only approximately 1/3 of adults and 1/5 of teenagers, fulfill the new Physical Activity Guidelines for Americans, released in the 2018 American Heart Association meeting.⁶

Does Sweden and Brazil have the same problem?

Low or decreasing PA levels often correspond with a high or rising gross national product. In high-income countries, 26% of men and 35% of women were insufficiently physically active, as compared to 12% of men and 24% of women in low-income countries.⁴ This drop is partly due to inactivity during leisure time and sedentary behavior at home or during work. Also, an increase in car, bus and train use has contributed to insufficient PA. Besides, fear of violence and crime in outdoor areas, pollution, high-density traffic, lack of parks, sidewalks and sports/recreation facilities discourage people from becoming more active.

Keywords

Lifestyle; Physical Fitness; Exercise; Physical Conditioning.

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DOI: 10.5935/abc.20190010

There is a known socioeconomic division regarding PA levels in Europe; the Eurobarometer (https://ec.europa.eu/sport/news/2018/new-eurobarometer-sport-and-physical-activity_en), a survey series based on self-reported activity levels and sports participation, shows that 91% of Swedes of all ages, but only 22% of Bulgarians report to exercise. Populations from highly industrialized countries from northern and western Europe tend to practice more exercise/sports activities, compared with southeastern European countries. This, somehow, illustrates the well-known socioeconomic difference, in which higher education is associated with more sitting time, but also more “gym-cards” and higher fitness, typical of northern Europe, in contrast with other European countries.

In Brazil, since 2002, the rate of physical inactivity has grown more than 15% and data from 2016 indicate that more than 47% of Brazilians are sedentary.⁷ Interestingly, in Sweden, the relationship between socioeconomic status and PA level can be seen within major cities. Populations living in low socioeconomic areas show more sitting, less PA level and less fitness.⁸ Thus, certain vulnerable populations, often outside the workforce, will have the worst PA patterns and be at high-risk population for an unhealthy future. Industrialized countries are already sitting much and are likely to be physically inactive in the future.³ However, sedentary time is expected to increase considerably in developing countries, such as India, which has remained active until now, but already showed a tendency of increasing sitting time.⁴

What can we do?

Although Brazil and Sweden present very different statistics, these countries share a similar problem.⁸ Methods to increase PA in the general population, but also in health care need to be developed and implemented, which has been seen in recent years. The healthcare system must face the growing problem of lifestyle-related disease, both in Brazil and in Sweden. The traditional and simple doctor-patient advice, to be more physically active, has been shown to have limited effect. A program with a more complex design is the Swedish PA on prescription (PAP) program, which was recently shown, in a systematic review,⁹ to increase PA level in inactive patients. This method uses individualized exercise prescription, using the reference book FYSS (www.fyss.se), which lists the optimal and most evidence-based exercise prescriptions for around 40 major diseases. In the program, exercise prescription is followed in healthcare services, as any other medical treatment offered to the patient. The Swedish FYSS book was recently translated to English and also into Vietnamese, as part of a national Vietnamese campaign to introduce Swedish PAP. The European community has now supported a project to spread the Swedish PAP to nine other European Union-countries in the next three

years. Similar initiatives are needed in both Sweden and Brazil, to overcome the future challenge of physical inactivity and increasing lifestyle-related diseases.

Conclusion

Non-communicable diseases are very prevalent and their frequency increases with population aging. In this scenario, urgent action is needed. In Brazil the barriers have not been broken and the price to be paid due to physical inactivity will be even higher in the coming years. In this context, Brazilians should learn from the Swedes, who already gave

the first steps, although they are also still far from what is considered ideal.

High-quality research is needed to promote good long-term cardiorespiratory fitness in long term. In parallel, sustainable and feasible programs to decrease physical inactivity are needed aiming to reduce different types of non-communicable diseases and improve global health.

Finally, the government, policy makers and research community need to help build societies in which the choice of being physically active is not only healthy, but also enjoyable, affordable and safe.

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Study of Myocardial Perfusion in Obese Individuals without Known Ischemic Heart Disease

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Abstract

Background: Obesity is associated with an increased risk of type 2 diabetes mellitus (DM), ischemic heart disease (IHD) and cardiovascular mortality. Several studies have demonstrated the diagnostic and prognostic value of single photon computed tomography-myocardial perfusion scintigraphy (SPECT-MPI) in the evaluation of patients with suspected IHD, including in obese population. Data on clinical risk factors and their association with abnormal myocardial perfusion in obese patients are scarce in the Brazilian population.

Objective: To determine the factors associated with abnormal myocardial perfusion in obese individuals without known IHD.

Methods: We studied obese patients without known IHD who were referred for evaluation through SPECT-MPI between January 2011 and December 2016. Clinical variables and results of SPECT-MPI were obtained systematically. The distribution of continuous variables was assessed using the Shapiro-Wilk and Shapiro-Francia tests. We used the unpaired Student t test to compare the means of continuous variables with normal distribution and the Chi Square test for binomial variables analysis. A p value < 0.05 was considered statistically significant. The association of the clinical variables for the presence of factors associated with abnormal myocardial perfusion was determined by univariate and multivariate logistic regression analysis, and respective odds ratios (OR) and 95% confidence intervals (CI).

Results: The study sample consisted of 5,526 obese patients. Mean body mass index (BMI) of our patients was 33.9 ± 3.7 kg/m², 31% had DM, and myocardial perfusion abnormalities was observed in 23% of the total sample. The factors associated with abnormal myocardial perfusion on multivariate analysis were: age (OR: 1.02, 95% CI 1.01-1.03, p < 0.001), DM (OR: 1.57, 95% CI 1.31-1.88, p < 0.001), typical angina before the test (OR: 2.45, 95% CI: 1.82-3.31, p < 0.001), need for pharmacologic stress test (OR: 1.61, 95% CI: 1.26-2.07, p < 0.001), less physical effort evaluated in metabolic equivalents (METs) during the exercise treadmill test (OR: 0.89, 95% CI: 0.85-0.94, p < 0.001) and a lower post-stress left ventricular ejection fraction after stress (LVEF; OR: 0.989, 95% CI: 0.984-0.994, p < 0.001).

Conclusion: The factors associated with abnormal myocardial perfusion in obese patients without known IHD were age, DM, presence of typical angina, ventricular dysfunction, and inability to undergo physical stress as clinical variables, in addition to functional capacity during physical stress. (Arq Bras Cardiol. 2019; 112(2):121-128)

Keywords: Obesity; Diabetes Mellitus; Myocardial Perfusion Scintigraphy; Coronary Artery Disease.

Introduction

According to the World Health Organization (WHO), obesity is defined as a body mass index (BMI) ≥ 30 kg/m².¹ In 2016, more than 1.9 billion were overweight, 650 million of them obese.²

In Brazil, Vigitel 2016, a nationwide telephone survey of protective and risk factors for chronic diseases, sponsored by the Ministry of Health, revealed that 53.8% of Brazilian adults were above ideal body weight. The proportion of obese individuals older than 18 years was 18.9%.³

Obesity is an independent risk factor for cardiovascular disease. Besides, it increases the risk of traditional risk factors, such as systemic arterial hypertension (SAH), type 2 diabetes mellitus (DM) and dyslipidemias, leading to an increased incidence of ischemic heart disease (IHD), cardiovascular mortality and risk of sudden death.⁴⁻⁶ Evidence from cohort studies have indicated that obesity is also an independent risk factor for coronary artery disease (CAD).⁷⁻⁹

Many studies have shown the diagnostic and prognostic value of single-photon emission computed tomography – myocardial perfusion imaging with (SPECT-MPI) in patients with suspected or confirmed IHD,¹⁰⁻¹² including obese patients.¹³⁻¹⁵ Nevertheless, information on the predictive role of SPECT-MPI among Brazilian obese subjects are scarce.

The aim of this study was to determine factors associated with abnormal SPECT-MPI in a large population of obese subjects without known IHD.

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Manuscript received April 25, 2018, revised manuscript July 16, 2018, accepted July 23, 2018

DOI: 10.5935/abc.20180250

Methods

Patients

Obese patients without known IHD who had undergone SPECT-MPI were studied between January 2011 and December 2016.

The following clinical data were prospective collected using a standardized questionnaire – age, sex, weight, height, BMI, symptoms before the SPECT-MPI test (typical, atypical or no pain, and tiredness), previous heart disease or procedures (coronary cineangiography, acute myocardial infarction, myocardial revascularization surgery and coronary angioplasty), SAH, DM, dyslipidemia, smoking, use of medications and family history of IHD).

Regarding SPECT-MPI, we assessed the type of stress used during the test, treadmill test (TT) alone or combined with pharmacological stress test. Physical exertion during the tests was quantified by metabolic equivalents (METs). We also analyzed myocardial perfusion patterns (normal, ischemia alone or associated with fibrosis), and post-stress left ventricular ejection fraction (LVEF).

All tests were performed using a CardioMD (Philips, Milpitas, CA, USA) or a Vertex (ADAC, Milpitas, CA - USA) gamma camera. All images were reviewed immediately after acquisition, and an additional prone imaging was always obtained when the presence of artifacts was suspected. Both images were considered to define the type of myocardial perfusion defect and the final report also.

Statistical analysis

All continuous variables are shown as mean and standard deviation, and all categorical variables as absolute values and percentages. Normal distribution of continuous variables was tested by Shapiro-Wilk and Shapiro-Francia tests.

Unpaired Student's t test was used to compare the means of continuous variables with normal distribution, and the chi-square test used for analysis of binominal variables. A p-value < 0.05 was considered statistically significant.

The association of clinical variables, type of the test stress, and left ventricular function with abnormal SPECT-MPI was analyzed by univariate logistic regression, followed by multivariate analysis. The respective odds ratio (OR) and 95% confidence intervals were also calculated.

All analyses were performed using a specific software, the Stata Statistical Software, Release 11 (College Station, TX: StataCorp LP).

Results

Demographic characteristics of the patients

From January 2011 to December 2016, a total of 5,526 obese patients were referred for SPECT-MPI. Table 1 shows demographic characteristics of the patients.

Table 1 – Demographic characteristics of the patients without known ischemic heart disease and body mass index (BMI) ≥ 30 kg/m² (n = 5,526)

Characteristics	Mean (standard deviation) or number (percentage)
Age	59.4 (12.2)
BMI (kg/m ²)	33.9 (3.7)
Male sex	2,605 (47.1%)
Diabetes mellitus	1,727 (31.5%)
Systemic arterial hypertension	4,106 (74.3%)
Family history of IHD	1,081 (19.5%)
Smoking	466 (8.4%)
Dyslipidemia	2,996 (54.2%)
Symptoms before SPECT-MPI	
Asymptomatic	2,996 (55.0%)
Atypical angina	1,210 (22.3%)
Typical angina	362 (6.6%)
Tiredness	878 (16.1%)
Stress protocol	
Physical	3,576 (64.7%)
Pharmacological	1,950 (35.3%)
Physical exertion, in METs	8.52 (2.37)
LVEF	59.2 (17.6)
LVEF > 50%	4,821 (92.9%)
LVEF 30 - 49%	330 (6.4%)
LVEF < 30%	38 (0.7%)
Abnormal SPECT-MPI	1,288 (23.3%)
Ischemia alone	1,228 (22.2%)
Ischemia > 10% of the LV	74 (1.3%)
Fibrosis alone	22 (0.4%)
Fibrosis and ischemia	38 (0.7%)

BMI: body mass index; IHD: ischemic heart disease; METs: metabolic equivalents; LVEF: post-stress left ventricular ejection fraction; SPECT-MPI: myocardial perfusion imaging with single-photon emission computed tomography; LV: left ventricle.

Demographic characteristics of the patients by sex

The total sample was composed of 2,921 women and 2,605 men. Table 2 shows demographic characteristics of the patients by sex.

Distribution of patients by BMI

Most patients (70.2%) were class I obese. Table 3 shows the distribution of the patients by BMI.

Percentage of abnormal perfusion according to the BMI

Among obese individuals (n = 5,526), there was no statistically significant difference in the number of patients

Original Article

Table 2 – Demographic characteristics of the patients by sex

	Men n = 2,605	Women n = 2,921	p value
Age; mean (SD)	56.7(11.8)	61.7(12)	< 0.0001
BMI (kg/m ²); mean (SD)	33.6(4.1)	34.2(3.3)	< 0.0001
Diabetes mellitus; n (%)	773 (29.7)	954 (32.7)	0.02
SAH; n (%)	1,843 (70.7)	2,263 (77.5)	< 0.001
Family history of IHD; n (%)	429 (16.5)	652 (22.3)	< 0.001
Smoking; n (%)	270 (10.4)	196 (6.7)	< 0.001
Dyslipidemia; n (%)	1,369 (52.5)	1,627 (55.7)	0.02
Symptoms before SPECT-MPI; n (%)			< 0.001
Asymptomatic	1,701 (65.8)	1,295 (45.2)	
Atypical angina	433 (16.7)	777 (27.2)	
Typical angina	108 (4.2)	254 (8.9)	
Tiredness	343 (13.3)	535 (18.7)	
Stress protocol; n (%)			< 0.001
Physical	1,895 (72.7)	1,681 (57.5)	
Pharmacological	710 (27.3)	1,240 (42.5)	
Physical stress in METs; mean (SD)	8.7 (2.2)	6.8 (2.1)	< 0.0001
%LVEF; mean(DP)	54.1 (18.4)	63.9 (15.5)	0.04
LVEF; n(SD)			< 0.0001
LVEF > 50%	2,126 (89.4)	2,695 (95.9)	
LVEF 30 - 49%	227 (9.5)	103 (3.7)	
LVEF < 30%	25 (1.0)	13 (0.5)	
Abnormal SPECT-MPI abnormal; n (%)	475 (18.2)	813 (27.8)	< 0.001
Ischemia	436 (16.7)	792 (27.1)	
Ischemia > 10% of the LV	45(1.7)	29 (0.9)	0.017
Fibrosis alone	13 (0.5)	9 (0.3)	
Fibrosis and ischemia	26 (1)	12 (0.4)	

SD: standard deviation; BMI: body mass index; SAH: systemic arterial hypertension; IHD: ischemic heart disease METs: metabolic equivalents; LVEF: post-stress left ventricular ejection fraction; SPECT-MPI: myocardial perfusion imaging with single-photon emission computed tomography; LV: left ventricle

Table 3 – Distribution of patients by body mass index

BMI Classification	30.0 - 34.9 kg/m ² Class I obesity*	35.0 – 39.9 kg/m ² Class II obesity*	≥ 40.0 kg/m ² Class III obesity*
Number (%) of patients	n = 3,880 (70.2%)	n = 1,207 (21.8%)	n = 439 (7.9%)

BMI: body mass index. *World Health Organization¹

with abnormal SPECT-MPI according to BMI. Figure 1 shows the percentage of abnormal SPECT-MPI according to BMI.

Factors associated with abnormal myocardial perfusion

Univariate analysis revealed that the following factors were associated with abnormal myocardial perfusion – age (OR: 1.04; 95%CI: 1.04-1.05. $p < 0.001$), female sex (OR: 1.18; 95%CI: 1.18-1.21; $p < 0.001$), DM (OR: 1.96; 95%CI: 1.72-2.23. $p < 0.001$); SAH (OR: 1.79; 95%CI: 1.53-2.10; $p < 0.001$),

dyslipidemia (OR: 1.19; 95%CI: 1.04-1.34. $p < 0.008$), typical angina (OR: 1.96; 95%CI: 1.55-2.48; $p < 0.001$) or tiredness (OR: 1.29. IC 95%: 1.08-1.54. $p = 0.004$) before SPECT-MPI, lower stress test duration (mean) (OR: 0.81, 95%CI: 0.78-0.84; $p < 0.001$) and lower (mean) LVEF (OR: 0.996, 95%CI: 0.993-0.999, $p < 0.041$).

After multivariate analysis (Table 4), age, typical angina before SPECT-MPI, need of using the pharmacological stress protocol, less physical exertion (METs), and post-stress LVEF were found to be associated with abnormal myocardial perfusion.

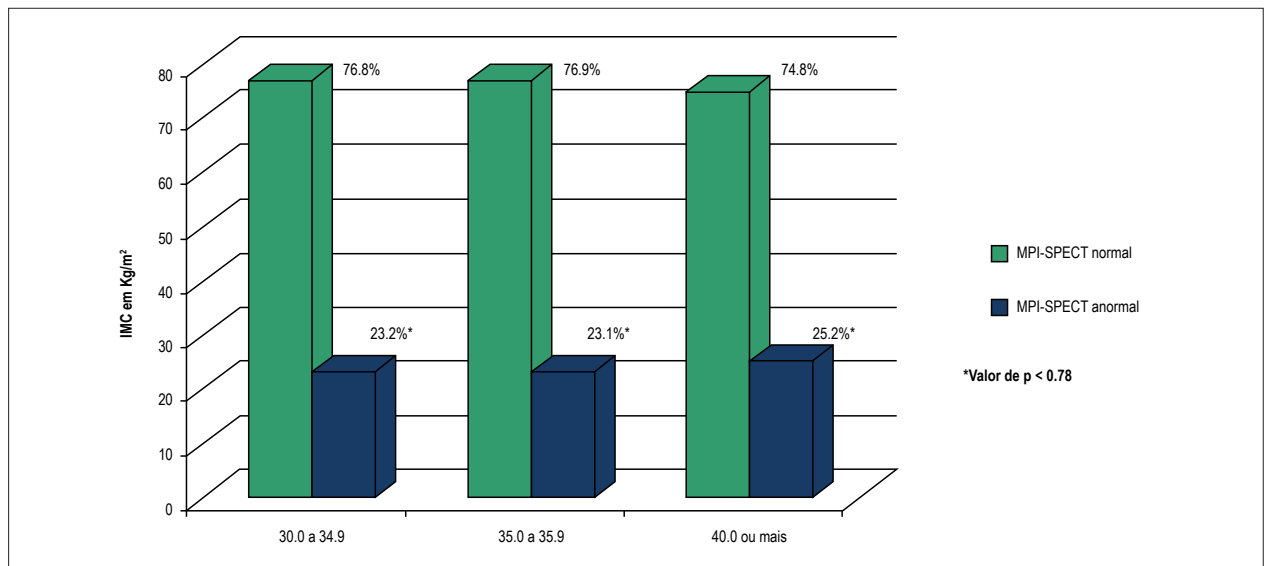


Figure 1 – Percentage of abnormal myocardial perfusion imaging with single-photon emission computed tomography (SPECT-MPI) according to BMI in the study population ($n = 5,526$) *a p-value lower than 0.05 was considered statistically significant.

Table 4 – Factors associated with abnormal perfusion after multivariate analysis in obese patients without known ischemic heart disease ($n = 5,526$)

	OR (95%CI)	Valor de p
Age, years	1.02 (1.01 - 1.03)	< 0.001
BMI		
30.0 - 34.9 kg/m ²	Reference	
35.0 - 39.9 kg/m ²	0.91 (0.73 - 1.12)	0.38
≥ 40.0 kg/m ²	0.99 (0.68 - 1.45)	0.97
Male sex	0.82 (0.67 - 1.01)	0.052
Diabetes mellitus	1.57 (1.31 - 1.88)	< 0.001
Systemic arterial hypertension	1.21 (0.98 - 1.50)	0.08
Dyslipidemia	1.14 (0.96 - 1.36)	0.13
Symptoms before the test		
Asymptomatic	Reference	
Atypical angina	1.21 (0.97 - 1.49)	0.08
Typical angina	2.45 (1.82 - 3.31)	< 0.001
Tiredness	0.93 (0.72 - 1.20)	0.59
Stress protocol; n (%)		
Physical	Reference	
Pharmacological	1.53 (1.18-1.98)	< 0.001
Physical exertion, in METs	0.89 (0.85-0.93)	<0.001
LVEF		
LVEF > 50%	Reference	
LVEF 30 - 49%	7.42 (5.3-10.4)	<0.001
LVEF < 30%	10.2 (2.6-40.3)	<0.001

BMI: body mass index; METs: metabolic equivalents; LVEF: left ventricular ejection fraction. A $p < 0.05$ was considered statistically significant.

Discussion

Our study reveals a strong association between obesity and other cardiovascular risk factors. Obesity is known to lead to insulin resistance, SAH, dyslipidemia, thromboembolism and sleep apnea and increase inflammatory markers, all known to be risk factors for CAD.⁴ Obesity is an important factor in the pathogenesis and progression of CAD, with an almost linear relationship between BMI above 25 kg/m² and the risk of CAD.⁷

Xingping et al.¹² evaluated the relationship between BMI and the prognostic value of SPECT-MPI in 2,096 obese subjects without known CAD (mean age 62 ± 12 years). The authors reported a high prevalence of DM (22%), dyslipidemias (47%) and SAH (61%).¹² More recently, researchers of The Southern Community Cohort Study investigated the relationship between BMI and late mortality in young adults. At the end of the study, the total sample of obese individuals was 6,276 (mean age 50 ± 7.8 years). In this group, the authors also observed a high prevalence of risk factors – DM in 35.9%, dyslipidemias in 38.8% and SAH in 66.4%.^{13,14}

The World Health Organization (WHO) believes that overweight and obesity are responsible for 44% of the risk for DM.¹ The International Diabetes Federation (IDF) estimates a prevalence of 10-12% of DM among adults in Brazil, which corresponds to 14.5 million people. In addition, the IDF estimates a 60% increase of new cases of DM in Latin America in the next 15 years.¹⁵

In the DIAD (Detection of Ischemia in Asymptomatic Diabetics) study, the authors assessed whether the screening for CAD could decrease the occurrence of cardiovascular events in symptomatic diabetic patients. A total of 1,123 patients were randomized to screening with SPECT-MPI or to no screening. After a mean follow-up of 4.8 years, the authors did not find any significant differences in cardiovascular event rate between the two groups.¹⁶ The presence of DM significantly increases cardiovascular risk, and the need for diagnostic methods, including the rational use of nuclear medicine.¹⁷⁻¹⁹

A significant percentage of our study group (55%) were asymptomatic before the test, especially men. The high proportion of asymptomatic patients in our sample may be explained by several factors, including stratification of future events in patients at high cardiovascular risk, previous altered or inconclusive cardiologic tests, patients referred for preoperative assessment, and the presence of electrocardiographic abnormalities that limit the performance of TT (left branch bundle block, artificial pacemaker rhythm or ventricular preexcitation).^{20,21}

Regarding preoperative assessment, the II Guidelines for Perioperative Assessment of the Brazilian Society of Cardiology suggests that indications for SPECT-MPI are similar to those of TT, i.e., patients at estimated intermediate risk of vascular surgery complications, without severe cardiovascular conditions in the perioperative period. Also, SPECT-MPI would be the best choice for patients with physical impairment, changes in the ST segment that affect electrocardiography analysis, and in case of possible false positive results in TT.²²

The decision to screen for IHD among obese patients should be similar to that in the general population, based mainly on clinical symptoms, chest pain and tiredness, and/or the presence of other associated risk factors. Besides, patients' ability to exercise and the presence of an interpretable electrocardiogram guide us in making decisions about the methods to be used.

Obese subjects are more likely to be screened for IHD, due to the higher presence of associated risk factors, tiredness, low functional capacity and musculoskeletal impairments.^{23,24}

In 35% of our patients, a pharmacological stress was used, and this percentage was higher among women than men (42.5% versus 27.3%). This frequency was similar to that reported by Xingping et al.¹² (38%).

Duvall et al.,²⁵ evaluating the prognostic and diagnostic value of SPECT-MPI in 433 morbidly obese patients, observed that 77.4% of the patients used the pharmacological stress protocol, indicating a decreased functional capacity with increase of BMI. The use of pharmacological stress protocols is associated with low functional capacity, non-cardiac physical limitations, low motivation to exercise, left ventricular dysfunction, pulmonary diseases, abnormal electrocardiographic findings at rest (above mentioned), and inappropriate discontinuation of medications prior to the test (e.g., beta-blockers).^{20,21}

With respect to demographic differences by gender, most of our patients were women, who showed a more severe cardiovascular risk profile – higher mean BMI, and higher prevalence of associated risk factors (DM, SAH and dyslipidemias). In women, the rates of typical angina were lower, the use of pharmacological stress protocols was more common, and less physical effort during the test compared with men. The percentage of abnormal perfusion in SPECT-MPI was also higher in women than in men (27.8% versus 18.2%).

Studies have shown that women with diagnosis of CAD tend to be older, and present diffuse disease and a worse prognosis than men, including higher acute myocardial infarction and myocardial revascularization surgery. The use of effective diagnostic and prognostic methods, including nuclear medicine, is essential to reduce IHD morbimortality in this group.^{18,26} In a previous study of our group, Cerci et al.,²⁷ in a study with 2,250 women, reported a strong, independent association between abnormal SPECT-MPI and mortality among women in Brazil.²⁷

In our country, there is little information available about factors associated with abnormalities in myocardial perfusion in obese patients. Our data showed that age, DM, typical angina prior to the test, use of pharmacological stress, less physical effort in the test and lower mean post-stress LVEF were associated with perfusion abnormality. These findings corroborate previous studies on obese and non-obese subjects, with or without previous IHD. In the study by Xingping et al.,¹² predictive factors of cardiac mortality and abnormal SPECT-MPI in 2,096 obese subjects without known CAD were age, DM, use of pharmacological stress protocol and reduction of LVEF. Greater ability to exercise reduced mortality risk.¹⁴ Korbee et al.¹⁵ showed that an abnormal

SPECT-MPI, age, and previous heart failure were associated with major cardiovascular events and mortality in obese individuals during up to six years of follow-up following the test. These data have already been included in medical guidelines for appropriate indications of nuclear cardiology in patients with suspected CAD.²⁸

If on the one hand obese individuals are at higher risk for CAD, on the other hand, these patients, particularly severely obese subjects, represent a challenging population concerning eligibility to all kinds of cardiac imaging tests.^{29,30}

Obesity may affect the quality of SPECT-MPI images, reducing the specificity of the method due to diaphragmatic attenuation or increased extracardiac radiotracer activity. The use of higher doses of radiotracers, attenuation correction techniques, acquisition of images in prone position, among other techniques, may reduce the number of false-positive results related to obesity. Male sex and the use of physical stress protocol by the TT are associated with better quality of the images in obese patients undergoing SPECT-MPI.^{27,28}

Positron-emission tomography (PET) with rubidium-82 seems to be the non-invasive method of choice for diagnostic and prognostic assessment of obese individuals with suspected CAD. Sensitivity and specificity of PET with rubidium-82 and SPECT-MPI are estimated to be 91% and 89%, and 87% and 73%, respectively.³¹

Chow et al.,³² in a large multicentric study, evaluated the prognostic value (risk of overall and cardiac mortality) in 6,037 patients, 2,016 of them obese. After a mean follow-up period of 2.2 years, the authors concluded that PET with rubidium-82 improved the prognostic estimates of patients of all weight ranges. A normal PET was associated with a very low annual mortality in normal weight (0.38%), overweight (0.43%) or obese (0.15%) subjects.³²

Although we do not have anatomic information of the patients referred for coronary angiography or coronary angiotomography following SPECT-MPI, we believe that the cases of abnormal SPECT-MPI encompass a wide pathophysiological range, including false-positive cases due to the presence of artifacts, IHD without an obstructive component (associated with endothelial dysfunction or coronary microcirculation impairment), and mostly obstructive CAD.

Limitations

Our data were systematically collected using a standardized questionnaire administered by a nursing technician, nurses or physicians, and hence, some information regarding clinical variables were self-reported.

Most of patients had not undergone attenuation correction techniques, which help to reduce the percentage of abnormal SPECT-MPI associated with artifacts (false-positive results).

Our study was based on physiological variables and detection of ischemia; thus, we do not have anatomical information of patients that were referred for coronary angiography or coronary angiotomography based on SPECT-MPI results. For this reason, the actual percentage of false-positive cases and abnormal SPECT-MPI associated with obstructive CAD or other IHDs caused by endothelial dysfunction or impaired coronary microcirculation could not be determined.

Conclusions

Factors associated with abnormal myocardial perfusion in obese patients without known IHD, after adjustment for relevant variables (multivariate analysis) were – age (2% increased risk per year older), DM (57% increased risk in diabetic patients), typical angina (245% increased risk in patients with typical angina as compared with symptomatic patients), use of pharmacological stress during (61% increased risk as compared with physical stress by TT), less physical exertion (expressed in METs) (10% reduced risk for each additional MET during TT) and post-stress LVEF (1% reduced risk for each 1% increase in LVEF).

Author contributions

Conception and design of the research: Dippe Jr. T, Cunha CLP, Vítola JV; acquisition of data: Cerci RJ, Stier Jr. AL.; analysis and interpretation of the data: Dippe Jr. T, Cunha CLP, Cerci RJ, Vítola JV; statistical analysis: Cerci RJ; writing of the manuscript: Dippe Jr. T; critical revision of the manuscript for intellectual content: Dippe Jr. T, Cunha CLP, Stier Jr. AL., Vítola JV.

Potential Conflict of Interest

There is no potential conflict of interest relevant to this article.

Sources of Funding

There were no sources of funding for this work.

Study Association

This article is part of the thesis of master submitted by Tufi Dippe Júnior, from Universidade Federal do Paraná.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Hospital de Clínicas da UFPR under the protocol number 3026. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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Single Photon Computed Tomography-Myocardial Perfusion Scintigraphy. Diagnostic Tool Anticipating the Disease

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Short Editorial related to the article: Study of Myocardial Perfusion in Obese Individuals without Known Ischemic Heart Disease

Without making any value judgment, a large percentage of physicians who request tests to make diagnoses is observed in the contemporary clinical practice. On the other hand, a considerable percentage of physicians who make the diagnosis and possibly ask for tests to confirm the diagnosis is also observed. Both behaviors are considered valid when common good is achieved: The patients' benefit. However, the request for tests without an appropriate criterion is not only harmful to the patient, but also to the system.

In present-day medicine, there is a large collection of tests considered normal throughout the medical knowledge area, including cardiology. In a publication by Dippe Jr et al.¹ in this issue, the authors, in a retrospective analysis of a database, found this trend. Of 5,526 scans of myocardial perfusion scans performed on obese patients (grade 1), 77% were considered normal. Assuming that the exams were requested for investigation of myocardial ischemia, the authors related the presence of perfusion deficit with myocardial ischemia in only 23%. Based on these data, they found, after applying a "creative statistic", a 245% risk-ratio for typical angina.

Keywords

Obesity; Diabetes Mellitus; Myocardial Reperfusion/radionuclide imaging; Coronary Artery Disease/physiopathology.

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DOI: 10.5935/abc.20180265

It is known, in principle, that perfusion deficit is an expression of intrinsic myocardial abnormality.

The coronary arterial system may or may not contribute to this anomaly. In this scenario, the disproportion between supply and consumption of O₂ by the myocardium has some variables that do not always depend on the coronary arterial system. Thus, to relate the presence of perfusion deficit as a future prediction of clinical variables may be a methodologically dangerous path. Still in this study, the authors mentioned obesity as a predictor of diabetes mellitus in 57% of the people studied. In this case, the subjects had grade 1 obesity at the upper limit of the Body Mass Index (BMI) for overweight.

On the other hand, this classification imposes limitations to its application.

There are some problems with using BMI as a determinant of obesity. Muscular people have high BMIs and are not obese. The elderly need a differentiated classification to determine obesity. Moreover, the World Health Organization concluded that Asian people could be considered obese even with a BMI of 25. Thus, unless better judged, the levels of obesity reported in this research cannot be disseminated as a predictor of the prognosis. Up to a point, this study resembles the study model by Hachamovitch et al.² where the authors, in methodologically biased studies and results subject to discussion, established a percentage of myocardial ischemia close to 12% as a reference for indication of myocardial revascularization. Unfortunately, these results are referenced in the main specialty guidelines. Abstaining from practicing prediction, these results should be placed in the collections of transient truths.

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Outcomes of the Conversion of the Fontan-Kreutzer Operation to a Total Cavopulmonary Connection for the Failing Univentricular Circulation

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Abstract

Background: The Fontan-Kreutzer procedure (FK) was widely performed in the past, but in the long-term generated many complications resulting in univentricular circulation failure. The conversion to total cavopulmonary connection (TCPC) is one of the options for treatment.

Objective: To evaluate the results of conversion from FK to TCPC.

Methods: A retrospective review of medical records for patients who underwent the conversion of FK to TCPC in the period of 1985 to 2016. Significance $p < 0,05$.

Results: Fontan-type operations were performed in 420 patients during this period: TCPC was performed in 320, lateral tunnel technique in 82, and FK in 18. Ten cases from the FK group were elected to conversion to TCPC. All patients submitted to Fontan Conversion were included in this study. In nine patients the indication was due to uncontrolled arrhythmia and in one, due to protein-losing enteropathy. Death was observed in the first two cases. The average intensive care unit (ICU) length of stay (LOS) was 13 days, and the average hospital LOS was 37 days. A functional class by New York Heart Association (NYHA) improvement was observed in 80% of the patients in NYHA I or II. Fifty-seven percent of conversions due to arrhythmias had improvement of arrhythmias; four cases are cured.

Conclusions: The conversion is a complex procedure and requires an experienced tertiary hospital to be performed. The conversion has improved the NYHA functional class despite an unsatisfactory resolution of the arrhythmia. (Arq Bras Cardiol. 2019; 112(2):130-135)

Keywords: Heart Defects Congenital/surgery; Arrhythmias, Cardiac/surgery; Fontan Procedure; Mortality; Fontan-Kreutzer Procedure.

Introduction

The Fontan operation (FO) is an important landmark in the history of congenital heart diseases because it increased the life expectancy of children with single-ventricle hearts.^{1,2} After the development of the superior cavopulmonary connection (Glenn operation), the survival rate in univentricular hearts increased leading to the development of FO. The first description by Fontan and Baudet,³ was depicted as a right-heart bypass in patients with tricuspid atresia to improve the basal saturation and consequently improve their quality of life and life expectancy while avoiding the complications of chronic hypoxia. These and other techniques that use atrial as a conduit are called atrium-pulmonary connections. Many other techniques and strategies for Fontan operation have been developed since its description.

A few years after the first description, in 1973, this technique was modified by Kreutzer,⁴ where the right atrial appendage was connected directly to the trunk of the pulmonary artery with a shorter surgical time than Fontan's previous description. The Fontan-Kreutzer technique (FK) was widely performed and diffused at the beginning, but complications were observed in the long range, such as enlarged atrium, atrial arrhythmias, stasis intracavitary thrombosis and compression of pulmonary veins.⁵⁻⁹ These complications are difficult to treat leading to worsening functional class by New York Heart Association (NYHA) and often evolving to ventricular dysfunction and failure of the univentricular circulation.

The next technique, described by de Leval in 1988,¹⁰ was the cavopulmonary connection using intra-atrial lateral tunnel. In 1990, Marcelletti et al.¹¹ described the total cavopulmonary connection (TCPC) using extra-cardiac tube. In subsequent studies it was observed that the TCPC presented better results than the previous techniques.^{2,12-16}

Nowadays the TCPC is the most used, however, many patients in whom the old techniques, such as FK, were performed survived and it was possible to observe long-term complications. A treatment option for these patients was to perform a conversion of the FK to TCPC. The removal of the atrium from the pulmonary circulation would decrease the volumetric overload reducing atrial dimensions and consequently lessening secondary outcomes.¹⁷⁻²⁶

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Manuscript received March 20, 2018, revised manuscript July 23, 2018, accepted July 23, 2018

DOI: 10.5935/abc.20180256

Objective

The aim of this study is to evaluate the results of the conversion of FK to TCPC in patients with signs of univentricular circulation failure.

Methods

A retrospective review of medical records, in-hospital and outpatient notes, was performed for patients who underwent a Fontan conversion (FC). The inclusive criteria consisted of the conversion of FK to TCPC in the period of 1985 to 2016 regardless of their underlying pathology. This was a single center study performed in the Heart Institute (INCOR – HCFMUSP), São Paulo, Brazil. We reviewed all surgical records comprising age at procedure, ventricle morphology, indications for conversion, mortality, the presence of arrhythmias, functional class and the presence of comorbidities after correction.

We excluded the patients in whom FC was indicated but the death occurred before the surgical procedure or intraoperatively, or in whom the procedure was not accepted by the patient or their surrogate decision maker.

This study has been approved by the ethics committee of this Institution by the number CAAE 56617216.6.0000.0068. As the study is retrospective in nature, there was no need for the elaboration of a consent term.

Statistical analysis

We used the Kolmogorov-Smirnov test to compare and choose the sample of the study. Descriptive analysis was performed, including clinical and surgical characteristics. Continuous numerical variables were presented as median and interquartile range (IQR; 25th-75th percentile). Categorical variables were presented as frequencies, absolute number and percentages. Variables with normal distribution were presented average and standard deviation. Estimated actuarial survival were determined using the Kaplan-Meier method. Statistical analysis was performed with SPSS 23.0 for Windows (IBM Corp. Released 2015, IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY: IBM Corp).

Results

The total number and type of FO performed are shown in Table 1. Of the 18 FK cases, 10 were elected for the conversion to the TCPC due to signs of Fontan circulation failure. All 10 patients previous FK were submitted to a FC procedure and all 10 were included in this study.

The FK were conducted in the beginning of our experience, all were performed before the year 2004, most of them before the year 1999. Only 29 surgeries of lateral tunnels were performed after 2004 and after this year the most performed surgery was the TCPC with extra cardiac tube.

A mortality of 11% (7,9% early deaths and 3,1% of late deaths) was observed for the FO procedure performed in this period. Regarding the ventricle morphology, we observed that 318 cases (75,7%) were classified as left ventricle, 57 (13,6%) as right ventricle, 40 (9,5%) had both ventricles and five (1,2%) had undefined ventricle.

Analyzing the population of the converted, we observed that 40% of the patients were male and 60% female. The youngest patient who underwent conversion was 11 years old and the oldest patient was 42 years old, with the mean average of 23.2 years old.

In nine cases (90%) the surgery was indicated for uncontrolled arrhythmia and one case was indicated by protein-losing enteropathy. In three cases, surgical cryoablation was performed in the same operative time. Before conversion three patients were in functional class I, four in functional class II and three in functional class III.

We observed two deaths in the period, an early death (on the second postoperative day) due to significant bleeding and coagulopathy, and a late death (38th postoperative day) due to multiple sepsis and stroke. Both occurred during hospitalization in a postoperative intensive care unit (ICU). The actuarial survival of 5 and 10 years was 80%, as shown in Figure 1.

After conversion, 80% of the patients who were in functional class II or higher evolved with functional class improvement. Currently, six patients are in functional class I (75%), one patient is in functional class II (12.5%) and one patient is in functional class III (12.5%).

Regarding cardiac arrhythmias, 44% of conversions indicated by arrhythmias had improvements after conversion. Four cases were cured with no need of specialist follow-up and three cases had an arrhythmic condition that needed specialist flow-up.

Before conversion, ventricular dysfunction was present in five patients. One of them evolved to death, and all the others had an improvement in their function in relation to the preoperative period, three of which currently have preserved function and one that had had moderate dysfunction previously, and now presents a slight dysfunction. These variables can be visualized on Table 2.

For three of the cases in which surgical cryoablation was performed, one evolved to death despite of the arrhythmia. The other two cases had episodes of arrhythmia after conversion, one of which evolved to bradyarrhythmia requiring a pacemaker, and currently this patient is being evaluated for heart transplantation.

The mean ICU length of stay (LOS) was 13 days, the shortest time was 2 days and the highest 38 days. The average total hospital LOS was 37 days, the shortest being 17 days and the highest 59 days.

As complications, two patients presented bleeding, one pericarditis, one ischemic stroke, one presented convulsive

Table 1 – Fontan operation performed between years 1995-2016

Fontan Type	Number of patients
Fontan-Kreutzer	18 (4.3%)
Lateral Tunnel	82 (19.5%)
TCPC with extra cardiac tube	320 (76.2%)
Total	420 (100%)

TCPC: total cavo-pulmonary connection.

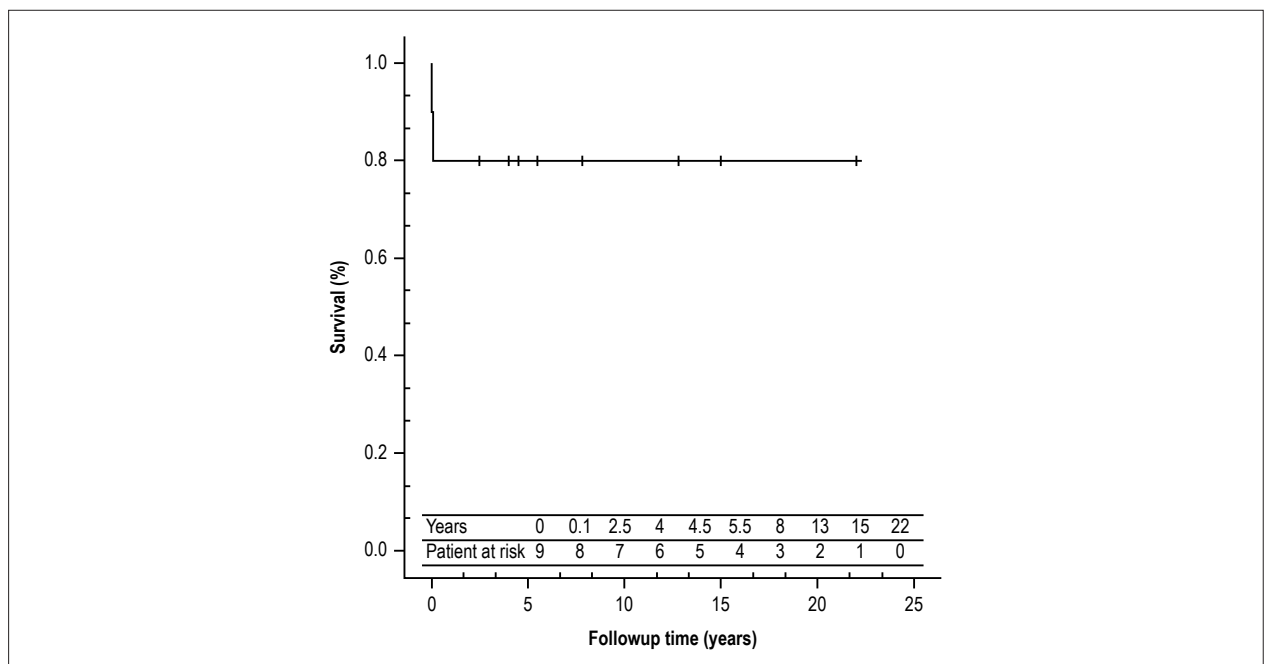


Figure 1 – Survival curve of patients submitted to FK conversion to TCPC.

Table 2 – Clinical improvements after conversion to TCPC

Variables	Before conversion (n = 10)	After conversion (n = 8)
Mildly disfunction	2 (20%)	1 (12.5%)
Moderate disfunction	3 (30%)	0 (0%)
Arrhythmias	9 (90%)	4 (44%)
NYHA Functional class I	3 (30%)	6 (75%)
NYHA Functional class II	4 (40%)	1 (12.5%)
NYHA Functional class III	3 (30%)	1 (12.5%)

TCPC: total cavo-pulmonary connection; NYHA: New York Heart Association.

seizures, one presented ventricular dysfunction and one presented bradyarrhythmia. Currently, eight patients are undergoing an outpatient clinic and one patient is being evaluated for heart transplantation.

Discussion

Fontan-Kreutzer conversion to TCPC is not a simple procedure. Despite a small sample size, we observed a 20% mortality in our experience. The prolonged hospitalization time, average of 37 days, also demonstrates the problems in the management of these patients in the postoperative period. In 25% of the patients evaluated, some types of complications were observed in the postoperative period, where most of them were resolved clinically without the need for new surgical procedures. These facts indicate that ideally this type of surgery should be performed in specialized tertiary centers with the availability of a multidisciplinary team for the best care of the patients.

Caneo et al.² showed a total mortality of 11% for all FO conducted in our Institution, the majority of the death cases were observed in the first period of the study (between years 1984-1994). All atriopulmonary Fontan were performed in the first and second periods (between years 1984-2004), 23,9% of them was elected for conversion years after, and all of these Fontan procedures were performed in the first period. A similar finding was observed in our study, where mortality occurred in the beginning of the experience by the years 1996 and 2000, our first two cases of conversion. It is possible that these two cases have evolved to an unfavorable outcome due to the unavailability of technological resources presented at that time.

Atrial arrhythmias were the main indications of conversion because the modifications performed by Kreutzer resulted in large atrial dilations generating many disorders of the atrial rhythm, which complicated ventricular dysfunction and worsened symptomatology. We obtained an unsatisfactory

rate of resolution of these arrhythmias (only 57% of cases indicated by arrhythmia). In cases in which surgical cryoablation was performed (three cases), the outcomes were not favorable: one case evolved to death in the recent postoperative period (due to bleeding and coagulopathy), one arrhythmia was not resolved, and one case progressed with total atrioventricular block, needing definitive pacemaker implantation. This patient evolved with dysfunctions and is currently in line for cardiac transplantation due to significant worsening of functional class and ventricular function. Although most studies suggest a benefit performing cryoablation,^{24,26-31} our findings suggest that surgical cryoablation should not be performed routinely in conversion to TCPC surgery, despite our small sample size.

Studies from South Korea and Japan^{32,33} have reported security and improvement in clinical outcomes by implanting permanent pacemaker in Fontan conversion. However, our only case with pacemaker implantation had unfavorable outcome, and is now in line for heart transplantation. Takeuchi et al.³⁴ showed favorable outcomes combining FC with resynchronization, but none of our patients were elected for resynchronization.

The presence of ventricular dysfunction before the FC procedure was found in five cases. All cases were elected to conversion by arrhythmia, one of them died and all the survivors had improved ventricular functions. Therefore, we conclude that the procedure presented a satisfactory result in improving the ventricular function. However, we observed no improvement of the arrhythmia in two cases of the survivors who presented preoperative dysfunction.

There was a significant improvement in functional class and quality of life of these patients after conversion, and therefore, our results demonstrate the importance and necessity of converting selected cases. These findings motivated us to perform this surgery in more cases after our first two cases that evolved to death. Currently, we have only a few cases of FK alive being followed in our ambulatory.

A review by Brida et al.³⁵ analyzed 1182 patients from 37 studies and concluded that conversion had substantial mortality risk. However, the results vary between centers

and lower early mortality was associated with earlier age and with treatment being performed at high experienced centers.

Conclusions

The conversion of atrial-pulmonary anastomosis (Fontan-Kreutzer) to TCPC is a complex procedure with high mortality and morbidity justifying a prolonged hospitalization time, so this surgery needs to be performed in experienced tertiary hospitals. The conversion of atrial-pulmonary anastomosis to TCPC has, in our experience, improved the functional class and consequently the patients' quality of life despite an unsatisfactory resolution of the arrhythmia.

Author contributions

Conception and design of the research: Fernandes GC, Silva GVR, Caneo LF; acquisition of data: Fernandes GC, Silva GVR, Caneo LF, Tanamati C, Turquetto AL; analysis and interpretation of the data and critical revision of the manuscript for intellectual content: Fernandes GC, Silva GVR, Caneo LF, Tanamati C, Turquetto AL, Jatene MB; statistical analysis: Fernandes GC, Caneo LF, Turquetto AL; writing of the manuscript: Fernandes GC, Silva GVR, Caneo LF, Turquetto AL, Jatene MB.

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.

Ethics approval and consent to participate

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

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Fontan-Kreutzer Conversion to Total Cavopulmonary Surgery due to Failing Univentricular Circulation. A Feasible Therapeutic Option?

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Short Editorial related to the article: Outcomes of the Conversion of the Fontan-Kreutzer Operation to a Total Cavopulmonary Connection for the Failing Univentricular Circulation

Fontan surgery, a palliative operation for patients with “single-ventricle” heart physiology, has undergone several modifications since the first procedure was performed in 1971 for individuals with diagnosis of tricuspid atresia.¹

The Fontan-Kreutzer (FK) atrial-pulmonary anastomosis technique was widely used in the 1980s. Publications regarding the late follow-up of patients submitted to the technique before 1990 showed a higher frequency of complications such as heart failure, arrhythmias, thromboembolic events, protein-losing enteropathy, plastic bronchitis, sudden death and liver failure.^{2,3}

The study by Fernandes et al.,⁴ published in this issue of the journal, aimed to analyze the results of the conversion of FK to Total Cavopulmonary Connection (TCPC) of patients with signs of failing univentricular circulation submitted to surgery in a single institution.

Of the 420 patients submitted to Fontan surgery between 1995 and 2016, 18 underwent FK, corresponding to 4.3% of the total sample. Of the 18 FK cases, 10 required conversion to TCPC due to signs of failing Fontan circulation, all of which were included in the study analysis. In 9 cases, the main cause of conversion was the presence of uncontrolled arrhythmia and protein-losing enteropathy in 1 case.

A relevant aspect regarding the presented data concerns the New York Heart Association functional class. Before the surgical conversion, 70% of the patients were in functional class II and III, and after the conversion surgery, approximately 80% of the patients showed functional capacity improvement.

Although the main indication for conversion was the presence of difficult-to-control arrhythmias, about 44% of the patients continued to have arrhythmias as a clinical manifestation, showing no satisfactory results with the use of surgical cryoablation, different from studies showing favorable results after its use.⁵ The obtained results also show data that demonstrate the complexity of these patients’ management, such as: prolonged hospitalization time and death rate of 20%. The authors associated the mortality rate found in the study with the period during which the surgeries were performed

between 1996 and 2000, and the learning curve of the service related to the described surgical technique. However, the early mortality rate described by other authors was also high, ranging from 0 to 21%.^{6,7}

Kreutzer et al.,⁸ in a review article, assess the five decades of the FK technique, in which they state that late complications would be strongly associated with surgical strategies and procedures no longer used nowadays, such as prolonged use of pulmonary artery banding, classic Blalock-Taussig shunt, late interventions, late diagnosis of significant hemodynamic changes and the use of surgical techniques currently considered to be inadequate, such as the classic Fontan procedure and the original Kreutzer surgery. Kreutzer et al.⁸ consider that one should be careful when analyzing literature data regarding the surgical technique prior to 1990, as well as when using these results as predictors of long-term outcome in patients submitted to FK surgery today.⁸

Regarding the study by Fernandes et al.,⁴ we do not have this information, which could help us to better understand the late complications observed in this sample.

For the late survivors of the “old-fashioned” FK technique, Kreutzer et al.⁸ consider that the conversion to TCPC would be indicated in cases with arrhythmia, symptomatic ones, and those unresponsive to treatment with amiodarone and in the presence of thrombus in the right atrium.⁸ Heart transplant after Fontan has been considered a therapeutic option in cases of which the main determinant of failure is ventricular dysfunction and some centers have already shown favorable results.^{9,10} In the study by Fernandes et al.,⁴ one patient is awaiting a cardiac transplant after a pacemaker was implanted, with an unfavorable evolution.

Fontan conversion strategy has been described since 1991.⁹ Worldwide, there is limited experience with this procedure, largely restricted to a small number of centers, and even in services with a greater number of surgical procedures, they have usually followed only a few dozen patients.⁶ The long-term evolution and the best time to perform the conversion is still a matter of debate.^{8,9} In a retrospective analysis of ten years of follow-up of patients undergoing Fontan conversion, using Australian and New Zealand registries, Poh CL et al.,⁵ demonstrated that patients submitted to an earlier conversion had more favorable long-term outcomes, with a heart transplant-free 10-year survival of 86%.⁵

A systematic review carried out by Brida et al.,⁷ which analyzed 1,182 patients, concluded that Fontan conversion has a high mortality risk and the combination with arrhythmia surgery seems to be associated with lower early mortality, especially when patients are referred at an early age and treated in centers of expertise.⁷

Keywords

Heart Defects, Congenital; Tricuspid Atresia/surgery; Procedure Fontan-Kreutzer; Heart Bypass, Right.

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DOI: 10.5935/abc.20190021

Short Editorial

Regardless of the sample size analyzed in the article, the results are comparable to the literature data, demonstrating

the complexity of the procedure and the importance of its performance in more experienced centers.¹¹

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Long Term Cardiovascular Outcome Based on Aspirin and Clopidogrel Responsiveness Status in Young ST-Elevated Myocardial Infarction Patients

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Abstract

Background: A subset of patients who take antiplatelet therapy continues to have recurrent cardiovascular events which may be due to antiplatelet resistance. The effect of low response to aspirin or clopidogrel on prognosis was examined in different patient populations.

Objective: We aimed to investigate the prevalence of poor response to dual antiplatelet therapy and its relationship with major adverse cardiovascular events (MACE) in young patients with ST-elevation myocardial infarction (STEMI).

Methods: In our study, we included 123 patients under the age of 45 with STEMI who underwent primary percutaneous intervention. A screening procedure to determine both aspirin and clopidogrel responsiveness was performed on the fifth day of admission. We followed a 2x2 factorial design and patients were allocated to one of four groups, according to the presence of aspirin and/or clopidogrel resistance. Patients were followed for a three-year period. A p-value less than 0.05 was considered statistically significant.

Results: We identified 48% of resistance against one or more antiplatelet in young patients with STEMI. More MACE was observed in patients with poor response to dual platelet therapy or to clopidogrel compared those with adequate response to the dual therapy (OR: 1.875, 1.144-3.073, $p < 0.001$; OR: 1.198, 0.957-1.499, $p = 0.036$, respectively). After adjustment for potential confounders, we found that poor responders to dual therapy had 3.3 times increased odds for three-year MACE than those with adequate response to the dual therapy.

Conclusion: Attention should be paid to dual antiplatelet therapy in terms of increased risk for cardiovascular adverse events especially in young patients with STEMI. (Arq Bras Cardiol. 2019; 112(2):138-146)

Keywords: Acute Coronary Syndrome; Aspirin/adverse effects; Platelet Aggregation; Young Adult; ST Elevation Myocardial Infarction; Mortality.

Introduction

Acute coronary syndrome (ACS) is considered to be the most important cause of death throughout the world, especially in western countries, despite technological improvements, new drugs and an increasing level of awareness.¹ It has been found that aspirin therapy inhibits cardiovascular and cerebrovascular disease in approximately one out of every four patients.² In patients with coronary artery disease, antiplatelet therapy has been included as a Class 1 recommendation in European guidelines.³ ischemic events continue to occur in a significant proportion of patients on antiplatelet therapy. This can be related to increased platelet activity resulting from the use of these drugs, which is called antiplatelet resistance.

Increasing evidence suggests that antiplatelet resistance occurs in varying rates in patients who are at risk for atherothrombotic complications. Moreover, the effect of biochemically detected antiplatelet resistance on cardiovascular adverse events has been found in different studies.⁴⁻⁶ In a meta-analysis with 50-plus studies, the association of aspirin and clopidogrel resistance with cardiovascular events was clearly indicated.⁷

Despite the use of more potent antiplatelets such as ticagrelor and prasugrel, clopidogrel continues to be used in a significant number of patients, sometimes due to financial constraints, and sometimes because of the risk of bleeding. Aspirin and clopidogrel resistance may lead to serious consequences especially in younger myocardial infarction (MI) patients because of the lifelong use. Low response to aspirin and clopidogrel has been studied separately in different groups of patients and its influencing factors have been investigated several times. However, there is insufficient data about both aspirin and clopidogrel response together. In addition, as far as we see, all studies evaluated the prevalence and prognostic effect of the dual antiplatelet resistance on young MI patients. Thus, in our study, the prevalence of aspirin and

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Manuscript received May 07, 2018, revised manuscript July 05, 2018, accepted July 23, 2018

DOI: 10.5935/abc.20180251

clopidogrel resistance and the relationship of low response to dual antiplatelet therapy with major adverse cardiovascular events (MACE) was assessed in young ST-segment elevation myocardial infarction (STEMI) patients who underwent primary percutaneous coronary intervention (PCI). Thus, we aimed to measure the prevalence of dual antiplatelet resistance in younger MI patients and to evaluate the effects of such poor response on their medical condition.

Methods

Patient population

In this prospective observational study, 123 consecutive patients (< 45 years old), who were admitted to a large-volume center with a diagnosis of STEMI and underwent primary PCI were included in the study. The exclusion criteria were: previous treatment with glycoprotein IIb/IIIa inhibitors, anticoagulant or non-steroid anti-inflammatory drugs in the last ten days, active malignancy, chronic inflammatory conditions, hemorrhagic diathesis, thrombolytic treatment within the last month, severe renal or liver disease and platelet counts < 100,000/mL, hematocrit count < 30% and no indication or unsuccessful of PCI. STEMI patients were defined as patients with typical chest pain at rest lasting more than 30 minutes, and ST-segment elevation ≥ 0.2 mV in 2 or more contiguous, precordial leads or adjacent limb leads on the standard 12-lead electrocardiogram (ECG). All primary PCI procedures were performed by operators who perform more than 100 PCIs/year at a single center (> 3000 PCIs/year). The minimum number of patients needed to be included for an effect size of 0.4 and 80% power was 156 for independent samples t-test and Mann-Whitney U test. During the follow-up 33 patients were excluded from the study due to suspected use of medications and finally, 123 patients were included in the study. The power for the final sample size was calculated at 70%. Sample size was calculated using the G-Power 3.9.1.2 package program and was also valid for other statistical tests used in the study. Initially, patients would be allocated into 2 groups – patients with drug resistance ($n = 59$) and drug responders ($n = 64$). However, to in order to make randomization between the groups more precise, 4 groups were formed according to the response to the drugs combined or alone.

The study complied with the Declaration of Helsinki. Written informed consent was obtained from all patients who participated in the study and the study protocol was approved by the ethics committee of our university.

Analysis of patient data

Patients' demographic data, past medical history, and previous medical therapies were collected. Risk factors were categorized as having or not having STEMI. Twelve-lead ECG was recorded for each patient immediately after hospital admission and the MI type was defined from the ECG. At 24–72 h after revascularization, a transthoracic echocardiography (Vivid S5 probe 3 S-RS/GE Healthcare, Wauwatosa, Wisconsin, USA) was performed to calculate left ventricular ejection fraction (LVEF) by using the biplane Simpson method.⁸ Primary angioplasty was

performed only for infarct-related artery (IRA) occlusion (either total or partial). Intervention success was defined as reduction of IRA obstruction or stenosis to 30%, with TIMI 3 flow just after coronary intervention.

Study design

In this prospective observational study, we followed a 2x2 factorial design to create groups according to the presence of aspirin and clopidogrel resistance; poor responders to aspirin ($n = 20$, 39.7 ± 3.7 years old), poor responders to clopidogrel ($n = 23$, 39.6 ± 4.1 years old), dual poor responders ($n = 16$, 40.5 ± 4.1 years old), dual responders ($n = 64$, 38.7 ± 4.0 years old). All patients received dual antiplatelet therapy for 1 year after discharge. After one year, aspirin was prescribed with cardiac therapy. Patients were called for control at the first month after the procedure, and every six months thereafter, and the compliance was checked. Patients who did not use antiplatelet therapy in the follow-up period were excluded from the study. At the end of three years, patients were asked about the occurrence of cardiovascular events and the relationship between these events and the response to antiplatelet agents was evaluated.

Evaluation of antiplatelet resistance

All participants received a chewable 300 mg or 100 mg aspirin (according to previous usage) and clopidogrel (600 mg loading dosage) before coronary angiography. Heparin (100 IU/kg) was administered after the decision to perform coronary intervention. After angioplasty, all patients were admitted to the coronary care unit, where routine antithrombotic therapy was given as daily dose 100 mg of aspirin, 75 mg of clopidogrel and subcutaneous administration of enoxaparin. The timing of platelet aggregation tests to identify hyporesponsiveness is also important. Thus, a screening procedure to determine aspirin and clopidogrel responsiveness was performed on the fifth day of admission to facilitate the steady state of drugs to be sure that platelet aggregation test was performed when maximal inhibition had been achieved. Whole blood aggregation was carried out with an impedance aggregometer, a Multiplate® platelet function analyser that operates on the surface of activated platelets to activate receptors that allow them to bind to artificial surfaces (Multiplate®; Dynabarte GmbH, Munich, Germany). Platelet aggregation was quantified as area under the curve, aggregation degree, and aggregation velocity. Platelet aggregation results were presented as aggregation unit (AU) $\times$ min, and values over 500 AU $\times$ min were defined as resistance to antiplatelet agents (used in combination or separately).⁹

Follow-up

Patients' data during follow-up were obtained from hospital records or by interviewing (in person or by telephone) the patients, their families, or their physicians. Primary clinical outcomes were composed of cardiovascular (CV) mortality, target vessel revascularization (TVR), non-fatal reinfarction, advanced heart failure and stroke. Secondary clinical outcomes were CV mortality, TVR, non-fatal reinfarction, stroke and advanced heart failure one by one.

Statistical analysis

Statistical analysis was performed using the SPSS software version 18.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov test, Shapiro-Wilk's test) were used to assess the normal distribution of the variables. Descriptive analyses are presented as means and standard deviations for variables with normal distribution, as median and interquartile range for non-normal distribution. The categorical variables are expressed as numbers and percentages. Comparisons between the groups were performed using unpaired Student's *t*-test or one-way ANOVA for continuous variables with normal distribution, and Kruskal-Wallis or Mann-Whitney *U* test for continuous variables without normal distribution. Tukey and Tamhane's *T*2 tests were used based on the equal variance assumption in binary comparisons in groups with normal distribution and more than two independent variables. Mann-Whitney *U* test was used for the binary comparison of multiple groups with non-normal distribution. A Bonferroni correction was employed to adjust for multiple comparisons. Categorical data were compared with the chi-square test. Because of the statistical difference in the total model, the chi-square test was applied in binary groups to compare 3-year MACE results. The cumulative survival curve for 3-year cardiac mortality was executed using the Kaplan-Meier method, with differences assessed by log-rank tests. Multivariate Cox regression backward stepwise, that included variables with $p < 0.01$ on univariate analysis, was carried out to identify independent predictors of 3-year MACE. A *p* value less than 0.05 was considered statistically significant.

Results

Among the 123 patients included in the study, the prevalence of poor responders to aspirin was 16.2%, to clopidogrel 18.6%, and to dual therapy 13.0%. In other words, in young MI patients, 47.8% of resistance against one or more antiplatelet was detected. Among baseline characteristics, hyperlipidemia, presence of family history, platelet counts, and platelet aggregation were different between the groups; no other differences were detected (Table 1).

At the 3-year follow-up, the difference in the primary outcome (composed of CV mortality, non-fatal reinfarction, TVR, advanced heart failure, and stroke) was statistically significant between the groups ($p < 0.001$). When we analyzed secondary outcomes, cardiac mortality and TVR were statistically higher in the group of poor responders to dual therapy ($p = 0.002$, $p = 0.010$ respectively) (Table 2). More MACE was observed in the group of poor responders to dual therapy and clopidogrel poor responders compared to the group of dual responders (OR: 1.875, 1.144-3.073, $p < 0.001$; OR: 1.198, 0.957-1.499, $p = 0.036$, respectively) (Figure 1).

In logistic regression analysis, family history, LVEF and clopidogrel aggregation time were identified as independent predictors of MACE in 3 years. Besides, we found that being a poor responder to dual therapy had 3.3 times increased odds for 3-year major adverse cardiovascular events than being in the dual responder group independent from family history and LVEF (Table 3). Moreover, the Kaplan-Meier survival plot for

three-year CV mortality in dual poor responders and responders to one or both antiplatelet drugs is presented in Figure 2.

Discussion

We can summarize the findings of our study as follows: (a) among STEMI patients under the age of 45 years who underwent PCI, 47.8% have a poor response to aspirin and/or clopidogrel; (b) poor responders to both aspirin and clopidogrel had a significantly higher level of MACE at 3 years follow-up compared with dual responders. Furthermore, secondary outcome analysis showed a significant difference in cardiac mortality and TVR between these two groups; (c) after adjustment for potential confounders, it was found that being a dual poor responder was one of the independent predictors of MACE. Moreover, the Kaplan-Meier survival plot for three-year CV mortality showed poor prognosis of dual poor responder patients (log rank < 0.001).

Antiplatelet resistance is a multifactorial phenomenon that has been studied in many populations with different methods. Therefore, the presence of variable results in the literature makes it difficult to compare our results with those of other studies. However, the lack of previous studies in young STEMI patients and long-term results of the dual antiplatelet resistance in this group make this study unique and valuable.

There is no single way to initiate thrombotic events; therefore, inhibition of a single pathway does not prevent all thrombotic complications. In addition, in some patients, the sensitivity of aspirin and clopidogrel is low, resulting in clinical complications. Therefore, several studies have been conducted to determine the clinical implications of being poor responders to aspirin and/or clopidogrel. In a meta-analysis of 1,813 patients with 12 studies examining the effect of aspirin resistance on prognosis, the mean biochemical aspirin resistance was 27% and the odds ratio for MACE was 3.8 (95% CI: 2.3-6.1) in patients with aspirin resistance.⁴ In another meta-analysis of 2,930 patients, aspirin resistance was detected in 28% of these patients, cardiovascular events in 41% (OR 3.85, 95% CI: 3.08-4.80), mortality in 5.7% (OR 5.99, 95% CI: 2.28-15.72) and acute coronary syndrome in 39.4% (OR 4.06, 95% CI: 2.96-5.56).⁵ In another study with patients with symptomatic peripheral artery disease, aspirin resistance was found as an independent predictor of adverse cardiovascular events with 2.48 hazard ratio.¹⁰ In a study on non-STEMI patients, aspirin resistants were at significantly higher risk of cardiovascular death with hazard ratio of 2.6 (95% CI 1.6-4.3) than aspirin sensitives (23.1% versus 9.6%).¹¹ Although all of the above studies showed an association of aspirin resistance with cardiovascular events, in our study, the increase in MACE in poor responders to aspirin did not reach statistical significance (15% versus 6%, $p = 0.217$). These differences may be explained by several factors. Firstly, the lack of statistical significance may have been caused by our smaller sample size. Secondly, these studies were carried out in different groups of patients using different methods. Besides, in these studies above mentioned, there was no analysis of a subgroup of young patients. We may speculate that aspirin resistance in this group of patients may not affect cardiovascular events due to different pathophysiological mechanisms. However, synergistic contribution to the increase in cardiovascular events with clopidogrel responsiveness was detected in our study. Larger studies need to clarify this conflict.

Table 1 – Baseline characteristics of the study population, mean $\pm$ standard deviation/median-interquartile range or n (%) π

	Adequate response to dual therapy (n = 64)	Poor response to aspirin (n = 20)	Poor response to clopidogrel (n = 23)	Poor response to dual therapy (n = 16)	p
Age, years β	38.7 $\pm$ 4.0	39.7 $\pm$ 3.7	39.6 $\pm$ 4.1	40.5 $\pm$ 4.7	0.372
Male, n (%)	59 (92.2)	18 (90.0)	20 (87)	16 (100.0)	0.520
BMI, kg/m ²	29.9 $\pm$ 4.6	28.6 $\pm$ 3.1	29.8 $\pm$ 4.2	29.3 $\pm$ 4.0	0.668
Hyperlipidemia, n (%)	19 (29.7)	9 (45)	14 (60.9)	10 (62.5)	0.017
Hypertension, n (%)	23 (35.9)	6 (30)	6 (26.1)	6 (37.5)	0.810
Diabetes mellitus, n (%)	7 (10.9)	3 (15)	3 (13)	1 (6.3)	0.861
Smoking, n (%)	46 (71.9)	13 (65.0)	17 (73.9)	11 (68.8)	0.919
Family history, n (%)	4 (6.3)	4 (20)	6 (26.1)	6 (37.5)	0.008
Total Chol. mg/dL β	185.8 $\pm$ 48.7	188.4 $\pm$ 40.0	200.5 $\pm$ 48.7	208.7 $\pm$ 42.3	0.277
HDL, mg/dL β	37.0 $\pm$ 11.8	36.4 $\pm$ 9.4	38.6 $\pm$ 7.9	34.3 $\pm$ 7.9	0.652
LDL, mg/dL β	122.2 $\pm$ 34.1	126.0 $\pm$ 31.9	142.6 $\pm$ 42.1	137.3 $\pm$ 37.6	0.104
Triglycerides, mg/dL β	121.5(69.7-202.2)	111.5(83.0-207.2)	101.0(62.0-194.0)	174.0(142.0-264.0)	0.060
Creatinine, mg/dL β	0.80(0.80-0.90)	0.80(0.70-0.90)	0.80(0.80-1.00)	0.90(0.80-0.97)	0.417
Hematocrit, % β	43.0 $\pm$ 4.0	44.8 $\pm$ 4.8	42.3 $\pm$ 5.2	44.3 $\pm$ 2.6	0.202
Platelet, 10 ³ μ L β	256.5 $\pm$ 45.5	309.4 $\pm$ 71.2	300.2 $\pm$ 81.1	300.3 $\pm$ 77.5	0.001
LVEF, % β	50.0(45.0-56.5)	50.0(42.7-55.0)	55.0(50.0-60.0)	51.5(41.2-58.7)	0.244
Culprit artery, %					0.449
LAD	33 (51.6)	11 (57.9)	11 (47.8)	9 (56.3)	
CX	9 (14.1)	6 (31.6)	5 (21.7)	3 (18.8)	
RCA	22 (34.4)	2 (10.5)	7 (30.4)	4 (25.0)	
Syntax Score	17.6 $\pm$ 9.0	19.4 $\pm$ 10.7	17.6 $\pm$ 7.4	16.3 $\pm$ 7.3	0.766
Aspirin aggregation time (AU x min) β	277.0 $\pm$ 98.9	789.1 $\pm$ 203.0	300.7 $\pm$ 133.7	738.0 $\pm$ 191.2	< 0.001
Clopidogrel aggregation time (AU x min) β	288.5 $\pm$ 234.0-376.0)	347.0(280.2-407.2)	608.0(523.0-728.0)	685.0(607.2-766.0)	< 0.001

BMI: body-mass index; Chol: cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; LVEF: left ventricular ejection fraction; LAD: left anterior descending artery; CX: circumflex artery; RCA: right coronary artery; AU: aggregation unit; min: minute. β p values < 0.05, dual therapy responders vs. other groups; β p value < 0.05, aspirin poor responders vs. adequate response to aspirin; β p values < 0.05, clopidogrel poor responders vs. adequate response to clopidogrel * Kruskal-Wallis test was used for multiple independent variables without normal distribution, and Mann-Whitney U test was used for binary comparisons; π Categorical data were compared with a chi-square test. B One-way ANOVA test was used for multiple independent variables with normal distribution, and for post hoc analysis, Tamhane's T2 and Tukey test were used.

Table 2 – Three-year outcomes of the study population, n (%) $\dagger$

Variable	Dual therapy responders (n = 64)	Aspirin poor responders (n = 20)	Clopidogrel poor responders (n = 23)	Poor responders to dual therapy (n = 16)	p
Primary outcomes *	4 (6.3)	3 (15.0)	5 (21.7)	8 (50.0)	< 0.001
Secondary outcomes$\dagger$					
Cardiac mortality	0 (0)	1 (5.0)	0 (0)	3 (18.8)	0.002
Non-fatal MI	1 (1.6)	1 (5.0)	2 (8.7)	2 (12.5)	0.283
TVR	0 (0)	1 (5.0)	3 (13.0)	3 (18.8)	0.010
Stroke	0 (0)	0 (0)	0 (0)	0 (0)	---
Advanced heart failure	3 (4.7)	1 (5.0)	2 (8.7)	2 (12.5)	0.671

TVR: target vessel revascularization; MI: myocardial infarction. * Primary clinical outcomes were composed of cardiovascular (CV) mortality, non-fatal reinfarction, target vessel revascularization(TVR), advanced heart failure, stroke. $\dagger$ Secondary clinical outcomes were CV mortality, non-fatal reinfarction, TVR, stroke, and advanced heart failure separately; $\dagger$ all data in the table were compared byt the chi-square test and expressed as percentages.

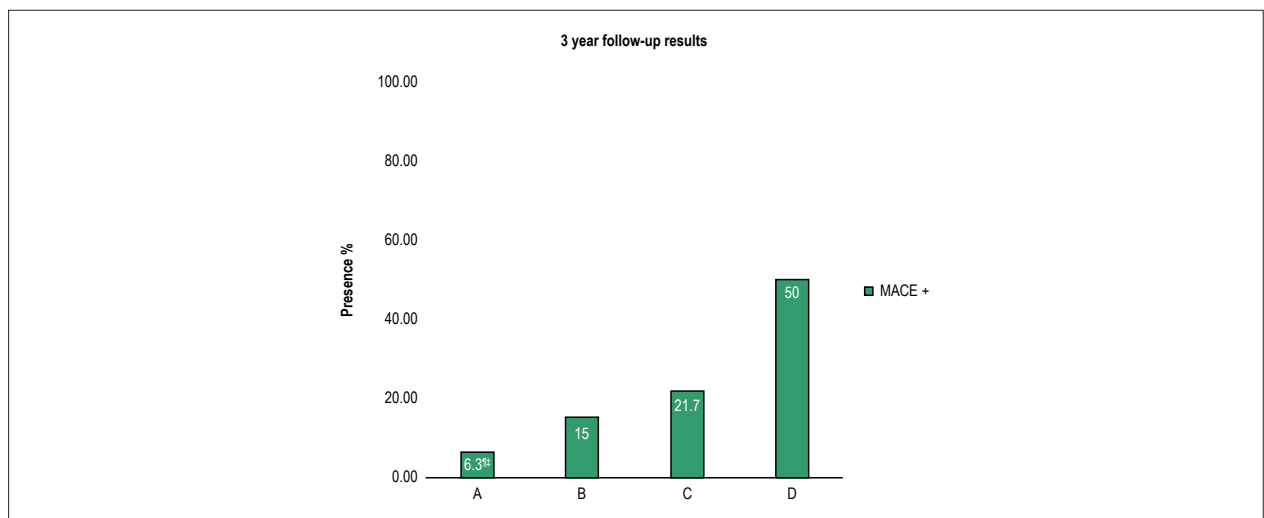


Figure 1 – Bar graph showing major cardiovascular adverse events, in the four groups based on aspirin and clopidogrel response. (A) Patients with adequate response to dual antiplatelet therapy; (B) patients with low response to aspirin; (C) patients with low response to clopidogrel; (D) patients with low response to dual antiplatelet therapy. MACE: major adverse cardiovascular events. * compared with poor responders to dual antiplatelet therapy (OR: 1.875 1.144-3.073, $p < 0.001$); † compared with patients with poor response to clopidogrel (OR: 1.198, 0.957-1.499, $p = 0.036$).

Table 3 – Multivariate logistic regression analysis for potential predictors of major adverse cardiovascular events (MACE) at three-year follow-up

	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p value	OR (95%CI)	p-value
First Model[†]				
Age, years	1.079 (0.951- 1.225)	0.239		
Male	2.420 (0.569-10.292)	0.231		
Family history	5.056 (1.720-14.861)	0.003	5.972 (1.449-24.615)	0.013
Hyperlipidemia	1.142 (0.435-2.994)	0.788		
Diabetes mellitus	3.481 (1.026-11.810)	0.045	5.194 (0.884-30.540)	0.068
Hypertension	2.323 (0.878-6.142)	0.089	3.271 (0.823-12.998)	0.092
Culprit artery*	4.583 (1.434-14.650)	0.010	2.959 (0.604–14.498)	0.181
LVEF, %	0.878 (0.823-0.938)	< 0.001	0.832 (0.761-0.909)	< 0.001
Creatinine, mg/dl	0.828 (0.051-13.450)	0.894		
Asp agg. time (AU x min)	1.002 (1.000-1.003)	0.078	1.000 (0.998-1.003)	0.838
Clop agg time (Au x min)	1.002 (1.000-1.004)	0.041	1.003 (1.000-1.006)	0.022
Second Model[†]				
Responder [‡]	Ref.	Ref.	Ref.	Ref.
Asp res [‡]	2.647 (0.539-12.992)	0.230	2.075 (0.503-8.549)	0.312
Clop res [‡]	4.167 (1.011-17.175)	0.048	4.056 (0.618 -25.612)	0.065
Dual res [‡]	15.000 (3.666-61.366)	<0.001	3.334 (0.484-22.954)	0.002

CI: confidence interval; LVEF: left ventricular ejection fraction; Asp: aspirin; Clop: Clopidogrel; agg: aggregation; min: minute; AU: aggregation unit; res: resistant; MACE: major adverse cardiovascular events; OR: odds ratio. *Culprit artery was divided as left anterior descending artery (LAD) and non-LAD (circumflex artery and right coronary artery); ‡ These groups were included in a second model instead of aspirin and clopidogrel aggregation time; [†] Nagelkerke R square of the first model was 49.2%; [‡] Nagelkerke R square of the second model was 59.4%.

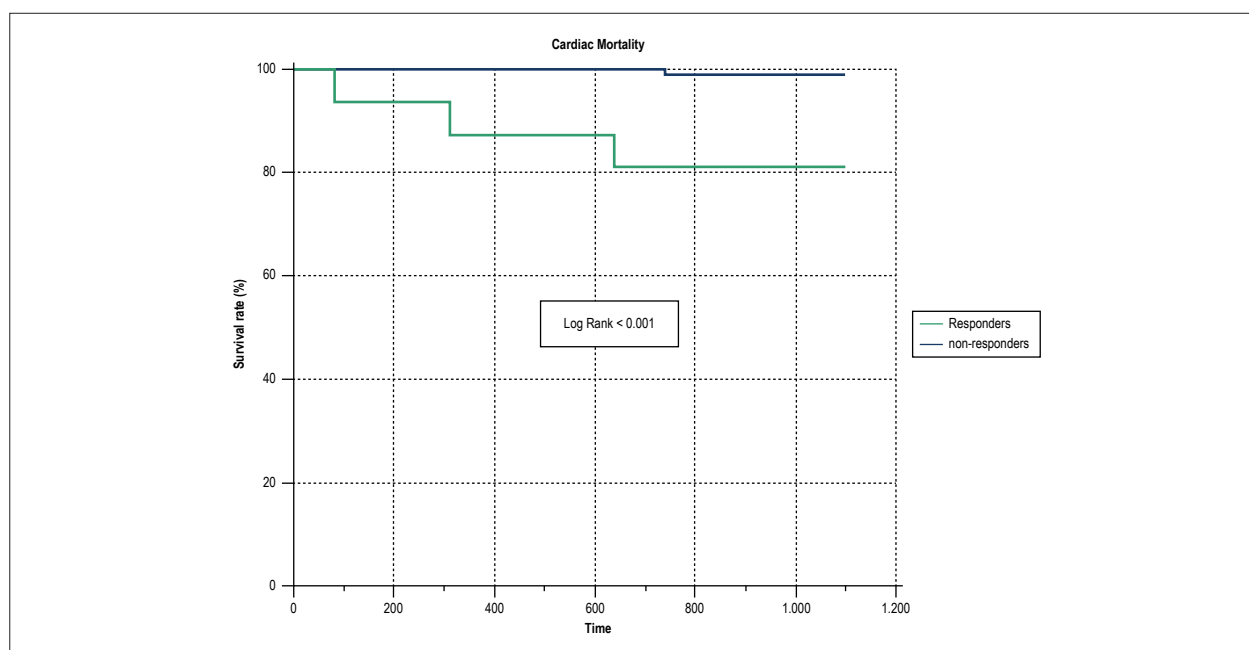


Figure 2 – Kaplan-Meier analysis showing 3-year cardiac mortality rate according to antiplatelet response. Patients with adequate response to aspirin and/or clopidogrel were considered “responders”. Patients with both aspirin and clopidogrel resistance were considered non-responders.

When studies on clopidogrel response are reviewed, it can be seen that clopidogrel resistance is clinically expressed in different patient groups. In a meta-analysis investigating the ability of different platelet-function tests to reliably identify patients at risk of developing secondary cardiovascular events, Wisman et al.⁷ evaluated high on-aspirin and high on-clopidogrel platelet reactivity in 55 studies with 22,441 patients and in 59 studies with 34776 patients respectively. The high on-aspirin platelet reactivity rate was 22.2%, which was associated with an increased risk for cardiovascular events (relative risk [RR] 2.09; 95% confidence interval [CI] 1.77–2.47). They reported a high on-clopidogrel platelet reactivity in 40.4% of patients, which was associated with increased cardiovascular event risk (RR 2.80; 95% CI 2.40–3.27). Moreover, ten studies identified an increased cardiovascular event risk in patients with high-on dual platelet reactivity (RR 2.77; 95% CI 1.87–4.12). In our study, although patients resistant to either aspirin or clopidogrel showed more cardiovascular events, this was not statistically significant. This may be explained by our relatively small sample size. However, similar to the meta-analysis, poor response to dual therapy was found to be an independent predictor of MACE (RR 3.33; 95% CI 0.484–22.954). Furthermore, according to this meta-analysis,⁷ the Multiplate test, the same method used in our study, is one of the most reliable methods to identify cardiovascular events.

The effect of antiplatelet resistance on stent thrombosis as a clinical outcome was examined in some studies. Slottow et al.¹² compared 26 patients who admitted with stent thrombosis under dual antiplatelet therapy with a control group to determine the relationship between stent thrombosis and antiplatelet resistance.¹² In this study, aspirin and clopidogrel reaction units were significantly higher in patients with early drug-eluting stent thrombosis. Similar to these results, in two

other studies evaluating clopidogrel resistance, stent thrombosis was seen more frequently after 6 months of follow-up.^{13,14} In a study comparing clopidogrel response with phenotyping and genotyping, patients with poor response to clopidogrel detected by multiple electrode aggregometry (MEA) had a higher risk of developing MACE or stent thrombosis than clopidogrel responders (12.5% vs. 0.3%, $p < 0.001$, and 18.5% vs. 11.3%, $p = 0.022$, respectively).¹⁵ Although we did not evaluate any stent thrombosis parameter, the frequency of cardiac mortality and TVR was significantly higher in patients with poor response to dual therapy than responders to dual therapy.

In the literature, we identified only one study with a similar grouping design, i.e., considering the response (responders vs. poor responders) to dual platelet therapy. Campo et al.¹⁶ evaluated the responsiveness status of aspirin and clopidogrel in 1,277 patients after elective PCI.¹⁶ In this study, at one-year follow-up they found that poor response to clopidogrel is an independent predictor of periprocedural MI and cardiovascular events whereas poor response to aspirin failed to predict a worse outcome. A distinctive feature of this study was that aspirin and clopidogrel response of 207 patients were evaluated together in subgroups and 25 patients were identified as the dual poor responder. In this subgroup analysis, the one-year composite endpoint of overall mortality, MI, and stroke was higher for dual poor responders compared with responders largely driven by a higher rate of MI (20% vs. 8.6%; $p = 0.007$). It may be expected lower cardiac mortality rates in our study group due to their younger age; however, our study had a longer follow-up than the above-mentioned study, which may have compensated for this. As a result, similar to the above study, we found a significant difference between the groups of nonresponders and the responders in terms of cardiac mortality (18.8% vs. 5.0%, $p = 0.002$).

There are also studies showing that platelet function tests do not have a prognostic significance in contrast to our results. Reny et al.¹⁷ detected that neither specific nor aggregation-based assays of antiplatelet drug responsiveness have additional predictive contribution to the recurrence of ischemic events in stable cardiovascular patients.¹⁷ But in this study, patients who had acute ischemic events less than one month before inclusion were excluded from the study. Consequently, poor antiplatelet drug response may be less critical in a stable cardiovascular patient because of less endothelial thrombogenicity and less platelet activation in the stable patients shown in previous studies.¹⁸⁻²⁰ It may be assumed that platelet function tests may have more impact on clinical outcomes in our study group when considering that platelet activation is related to inflammatory processes, and that inflammation is one of the most important factors in acute coronary syndromes, especially in young STEMI patients.

This study supports the view that standardized maintenance doses of antiplatelet drugs would not prevent MACE in some of the patients. Could it be possible to overcome platelet resistance by increasing the dose of medicine in our patient group? In some trials, increasing the dose of aspirin has allowed some reduction in aspirin resistance rates, but such effect is absent in 5-10% of patients. In addition, gastrointestinal hemorrhage and other side effects may increase when aspirin dose is increased in these patients. In addition, high doses of Aspirin can reduce the production of prostacyclin, an important endogenous vasodilator and antiplatelet agent, by inhibiting cyclooxygenase 2. Also, in our study, patients with only aspirin resistance did not differ in terms of MACE compared with patients with response to dual therapy, whereas patients with only clopidogrel resistance showed a significant difference. Geisler and colleagues have also shown that the response to clopidogrel may be reduced after acute coronary syndrome.²¹ This suggests that high platelet activity following acute coronary syndrome may be present and the standard dose of clopidogrel may not be sufficient to inhibit platelets. In parallel to this, it was found that administration of a 600 mg loading dose of clopidogrel in patients already chronically treated with clopidogrel provide additional inhibition of ADP-induced platelet aggregation.²² This information may be reflected in clinical practice, especially in some risky situations. Thus, in cases of inadequate response to clopidogrel, dose escalation or more potent inhibitors (ticagrelor, prasugrel) may be considered. For these reasons, whether high dose of aspirin or clopidogrel is beneficial to young MI patients with antiplatelet resistance is open to investigation.

There are some limitations in our study. First, this was a single-center study which may result in selection bias. Moreover, since we studied a specific population, the number of patients participating in the study was relatively small. This may have prevented the difference between some groups from reaching statistical significance. Second, antiplatelet sensitivity was only measured once, and some researchers have suggested that it should be measured more than once. Furthermore, when heterogeneous results of different studies are considered, the use of a single laboratory method constitutes one important

limitation of the study. However, the multiple platelet function test reduces the risk of laboratory errors because it is faster, less troublesome, and does not require specific preparation than conventional optical aggregometry. Third, because of the study design, results of platelet sensitivity test cannot be generalized to different age groups with other forms of coronary artery disease. Fourth, clopidogrel was used as the second antiplatelet agent for STEMI, as the use of other P2Y12 inhibitors had not been included in the guidelines during the study period. Therefore, we do not know whether the use of more potent P2Y12 inhibitors would be associated with a lower prevalence of poor aspirin responders. Finally, aspirin and clopidogrel serum levels were not measured. However, the medical history of each patient was taken by one-to-one interview, and patients with irregular drug usage were excluded from the study.

Conclusion

Although there are many studies in the literature on platelet response to different antiplatelet medications, many questions remain unanswered. In summary, we found that poor responsiveness to dual therapy is an essential predictor of MACE, including CV mortality and TVR in a three-year follow-up period in young patients undergoing primary PCI for STEMI. Although more potent P2Y12 inhibitors have been shown to be useful after acute coronary syndrome according to guidelines, there is no clear study of their use after one year. Therefore, aspirin or clopidogrel should be used in the long term after acute coronary syndrome, particularly in young MI patients, who may be more likely to antiplatelet resistance in long-term. For this reason, although routine testing for antiplatelet resistance is not recommended in the general population, it should be considered for young MI patients and, if resistance is detected, more potent antiplatelet therapy may be used one year after acute coronary syndrome. More comprehensive investigations are required to clarify this.

Author contributions

Conception and design of the research: Somuncu MU, Demir AR, Karabag T; acquisition of data: Somuncu MU, Demir AR, Karakurt ST, Karakurt H; analysis and interpretation of the data: Somuncu MU, Karakurt ST, Karakurt H; statistical analysis: Karabag T; writing of the manuscript: Somuncu MU, Demir AR, Karakurt ST; critical revision of the manuscript for intellectual content: Somuncu MU, Karakurt H, Karabag T.

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.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital under the protocol number

07.03.2014. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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Dyslipidemia in Adolescents Seen in a University Hospital in the city of Rio de Janeiro/Brazil: Prevalence and Association

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Abstract

Background: Early exposure to obesity favors greater risks of cardiovascular factors such as dyslipidemia.

Objectives: To establish the prevalence of dyslipidemia, and to evaluate its association with nutritional status of the adolescents attended at the ambulatory of the Adolescent Health Studies Center of the University Hospital Pedro Ernesto.

Methods: This is a cross-sectional, observational study, the sample of which was of convenience, consisting of adolescents from 12 to 18 years old of both genders. The lipid profile was evaluated, along with its association with the anthropometric indicators: body mass index and waist circumference. For statistical analysis, a significance level of 5% was used.

Results: A total of 239 adolescents, 104 boys (43.5%) and 135 girls (56.5%) were evaluated and, of these, 52 (21.8%) were eutrophic, 60 (25.1%) overweight, and 127 (53.1%) obese. Obese adolescents had significantly lower mean values of HDL-cholesterol (44.7 mg/dl vs 53.9 mg/dl; $p < 0.001$) and higher triglycerides (109.6 mg/dl vs 87.3 mg/dl; $p = 0.01$). The changes with higher prevalence were low HDL-cholesterol (50.6%), hypercholesterolemia (35.1%), and hypertriglyceridemia (18.4%). A negative association of HDL-cholesterol with body mass index and a positive association of triglycerides with body mass index could be observed, even after adjustment for gender and skin color.

Conclusion: This study demonstrated high prevalence of dyslipidemia among adolescents. In view of the significant association between lower levels of HDL-cholesterol and increased triglycerides with overweight, the control of these factors should receive attention, with the precocious diagnosis of the dyslipidemia being important, mainly if it is associated with another cardiovascular risk, to develop effective intervention strategies. (Arq Bras Cardiol. 2019; 112(2):147-151)

Keywords: Hyperlipidemias; Adolescent; Obesity; Sedentary Lifestyle; Anthropometry; Cardiovascular Diseases; Risk Factors.

Introduction

Adolescence is a period of intense modification that takes place between childhood and adulthood, and is highlighted by explicit development, growth and body changes. During adolescence there is a physiological increase of the tissues, including adipose tissue, especially in girls, being a critical period to initiate or exacerbate obesity.¹⁻³

In the current scenario, low consumption of fruit and vegetables and high consumption of processed food,^{3,4} along with excessive use of electronic devices and low frequency of regular practice of physical activities were observed among adolescents. It is also observed that the omission of meals and the intake of fast food are also common habits in this age group. Such conditions favor weight gain and risk factors for chronic diseases.²⁻⁶

Data from the Brazilian Institute of Geography and Statistics (IBGE) show a clear increase in the prevalence of overweight and obesity in adolescents in the last 34 years in Brazil, from 1974-1975 to 2008-2009, from 3.7% to 21.7% in boys and 7.6% to 19.4% in girls.⁷ This situation is a concern because obesity is a considerable risk factor for chronic non-communicable diseases, being highlighted among dyslipidemias, which is even more pronounced when associated with a sedentary lifestyle.⁸ Early exposure to obesity favors a higher cardiovascular risk not only in childhood and adolescence, but also a high incidence of premature mortality in adults who were obese in these phases of life.^{9,10} Overweight in childhood and adolescence is considered a more powerful predictor of these risks than overweight in adulthood.⁹⁻¹¹

Dyslipidemia is understood as changes in the lipid profile, which may occur by the elevation in total cholesterol (TC), LDL-cholesterol (LDL-c), triglycerides (TG), or decrease in HDL-cholesterol (HDL-c), with these being primary (genetic factors) or secondary (environmental factors) causes.^{8,12-14} These changes alone and mainly when accompanied of other risk factors may lead to the development of atherosclerosis.¹³

The present study aimed to establish the prevalence of dyslipidemia and to evaluate its association with the nutritional status of adolescents seen at the secondary care clinic of

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Manuscript received March 24, 2018, revised manuscript July 18, 2018, accepted September 05, 2018

DOI: 10.5935/abc.20180254

the University Hospital Pedro Ernesto (HUPE) Center of Adolescent's Health Studies (NESA).

Methods

This is an observational, cross-sectional study, the sample of which was of convenience, consisting of adolescents aged between 12 and 18 years of age, of both genders, referred internally or through the National Regulation System (SISREG) to the Nutrition service with a diagnosis of overweight, dyslipidemia, glucose metabolism changes, or other comorbidity, being attended at the NESA outpatient clinic. Adolescents with a diagnosis of thinness according to body mass index (BMI)/Age; using drugs that may interfere with laboratory tests (statins, steroids, bile acid sequestrants); or who are followed due to genetic syndromes, nephrotic syndrome, familial hypercholesterolemia, rheumatic diseases, type 1 Diabetes Mellitus, hypothyroidism, eating disorder, or disabsorption diseases were excluded.

Demographic data such as age, gender, skin color, and anthropometric data such as weight, height and waist circumference (WC) were collected. The weight (kg) was measured using a Micheliti® electronic digital scale, with an accuracy of 0.1 kg and a maximum of 200 kg, with the adolescent in his/her barefoot, wearing light clothes, and in an orthostatic position.¹⁵ For height (cm), a Sanny® stadiometer fixed to the wall was used, with a precision of 0.1 cm, with the adolescent in his/her barefoot, and the body in anatomical position, head parallel to the ground according to Frankfurt plane.¹⁵ Such measurements were used for the assessment of the adolescent's nutritional status through the BMI for age in z-scores, and the proposal of the World Health Organization for children and adolescents from 5 to 19 years being adopted as reference.¹⁶

WC measurements were performed using an anthropometric inelastic tape with a 0.1 centimeter scale at the midpoint between the last costal arch and the iliac crest at the end of normal expiration. They were classified according to the proposal by Fernández et al.,¹⁷ with the WC being elevated

when ≥ 75 th percentile. The lipid profile evaluation consisted of the following laboratory tests: TC, TG, HDL-c, and LDL-c. To obtain the glucose and lipid profile data, the blood test was always performed with a previous fasting of 12 hours. TG, TC, and HDL-c were measured through the enzymatic colorimetric method, and LDL-c calculated with Friedewald's formula.¹⁸ The reference values used were those recommended by the I Guidelines for the prevention of atherosclerosis in childhood and adolescence.⁸

Statistical analysis

The analyzes were performed using STATA 14 software. The continuous variables were presented as mean and standard deviation and the categorical variables as absolute frequency. The distribution of variables was assessed using the Kolmogorov-Sminov test. The comparisons of the continuous variables with normal distribution were performed with the unpaired Student's t-test and for more than two independent groups, one way variance analysis (ANOVA) and Post Hoc test were used. For the comparisons of categorical variables, the chi-square test or Fisher's exact test was used. For the study of the association, correlation analyzes (Pearson or Spearman) and simple and multiple linear regression were performed. A significance level of 5% was considered in all analyzes. The research project was approved by the Research Ethics Committee of HUPE/UERJ, registry CEP/HUPE: 3051/2011; CAAE: 0193.0.228.000-11.

Results

A total of 239 adolescents with a mean age of 14.4 ± 1.8 years was evaluated, with 104 boys (43.5%), and 135 girls (56.5%). Table 1 describes the anthropometric characteristics and the lipid profile mean of the population evaluated according to gender. The girls had statistically higher BMI mean values, and HDL-c, while THE mean height was higher in boys.

Table 2 describes the anthropometric characteristics and the lipid profile of the population evaluated according to

Table 1 – Mean and standard deviation of the anthropometric and lipid profiles of the total sample, stratified by gender

Variable	Gender						p value
	Total (n = 239)		Female (n = 135)		Male (n = 104)		
	Mean	SD	Mean	SD	Mean	SD	
Weight (kg)	76.2	± 22.4	74.8	± 22.2	77.9	± 22.7	0.14
Height (cm)	162.8	± 0.1	159.0	± 0.1	167.6	± 0.1	< 0.01*
BMI (kg/m²)	28.5	± 7.4	29.4	± 7.8	27.5	± 6.8	0.02*
WC (cm)	89.9	± 15.1	92.3	± 15.9	88.1	± 14.4	0.06
TC (mg/dl)	160.3	± 34.1	163.3	± 34.9	156.5	± 32.9	0.06
LDL-c (mg/dl)	93.9	± 29.2	95.5	± 29.3	92.0	± 29.0	0.18
HDL-c (mg/dl)	47.6	± 14.0	49.4	± 15.4	45.2	± 11.6	0.01*
TG (mg/dl)	99.4	± 53.7	99.1	± 53.8	99.9	± 53.8	0.46

Statistical test: unpaired Student's t test; *Statistically significant difference ($p < 0.05$); SD: standard deviation; BMI: body mass index; WC: waist circumference; TC: total cholesterol; LDL-c: low density lipoprotein; HDL-c: high density lipoprotein; TG: triglycerides.

Table 2 – Mean and standard deviation of anthropometric characteristics and lipid profile according to nutritional status

Variable	Nutritional status according to BMI						p value
	Eutrophy (n = 52)		Overweight (n = 60)		Obesity (n = 127)		
	Mean	SD	Mean	SD	Mean	SD	
Weight (kg)	52.3	± 10.5	66.9	11.7	± 90.3	19.0	< 0.01*
Height (cm)	162.0	± 0.1	162.1	0.1	± 163.3	0.1	0.62
BMI (kg/m²)	19.7	± 2.3	25.3	2.1	± 33.7	5.9	< 0.01*
WC (cm)	77.3	± 10.1	82.6	9.8	± 96.5	14.9	< 0.01*
TC (mg/dl)	158.6	± 34.8	159.1	35.6	± 161.6	33.4	0.82
LDL-c (mg/dl)	87.2	± 26.2	94.8	29.1	± 96.3	30.1	0.16
HDL-c (mg/dl)	53.9	± 16.2	48.2	12.6	± 44.7	12.9	< 0.01*
TG (mg/dl)	87.3	± 45.1	88.5	46.2	± 109.6	58.3	0.01*

Statistical test: ANOVA (One Way) and Post Hoc test; *Statistically significant difference ($p < 0.05$); BMI: body mass index; WC: waist circumference; TC: total cholesterol; LDL-c: low density lipoprotein; HDL-c: high density lipoprotein; TG: triglycerides.

nutritional status. The nutritional status classification revealed that 53.1% of the adolescents were obese, 25.1% overweight, and 21.8% eutrophic. The eutrophic adolescents had mean values of HDL-c significantly higher than the obese ones. Regarding triglycerides, the obese adolescents had values that were significantly higher than the eutrophic ones.

The most prevalent changes were low HDL-c (50.6%), hypercholesterolemia (35.1%), and hypertriglyceridemia (18.4%). Regarding the prevalence of lipid profile changes, according to gender, it was observed that the girls showed higher prevalence of change, but with no statistically significant difference. The prevalence of lipid profile changes in girls and boys were respectively 64.3% and 35.7% ($p = 0.07$) for high TC, 73.1% and 26.9% ($p = 0.07$) in the LDL-c, 50.4% and 49.6% ($p = 0.05$) in HDL, and 59.1% and 40.6% ($p = 0.07$) in TG. Table 3 presents the prevalence of changes in lipid profile according to the nutritional status by BMI. The prevalence of low HDL-c was significantly higher ($p = 0.01$) in obese patients.

In this study, a negative correlation was observed between BMI and HDL-c ($r = -0.23$, $p < 0.01$) and a positive correlation between BMI ($r = 0.25$, $p < 0.01$) and WC ($r = 0.20$, $p = 0.03$) with TG. In the bivariate and multivariate linear regression analysis the negative association of BMI with HDL-c was maintained, as well as the positive association of BMI and WC with TG even after adjustment for gender and skin color (Table 4).

Discussion

This study presented higher mean values of the lipid profile than others in the literature.¹⁹⁻²¹ HDL-c, a lipoprotein that acts as a protective factor against cardiovascular diseases, was the component with the highest change prevalence found among adolescents, as well as in the study by Ribas and da Silva, 2009.²² Another population-based study with more than 30,000 participants also found similar results.¹⁹

This fact is extremely worrying, because dyslipidemia alone and mainly accompanied by other factors, either environmental or genetic, can condition the development of atherosclerosis and, consequently, increase the risk of

cardiovascular events. It is fundamental to always consider the prevention and treatment of dyslipidemias, from childhood to adolescence, to reduce the risks of cardiovascular diseases.^{11,13}

The lipid profile may vary during adolescence, and the female gender usually has higher levels, a fact that may be justified by the menarche.²³ Although no significant difference between the lipid profile means within the genders was observed, it is possible to notice that the girls had higher values for all parameters, and this is commonly observed in the literature.^{19,22,24}

In the study by Garcez MR et al.,²⁰ it was observed that overweight adolescents had higher mean values for TC, LDL-c and TG, as well as low HDL-c, as in this study.²⁰ Similarly, Oliveira et al.²⁴ found such results when they assessed the lipid profile according to the nutritional status.²⁴ The main changes that are usually associated with obesity in this age group, and which have been observed as the standard are changes in HDL-c and TG.^{25,26} This study demonstrated an association between HDL-c/TG and WC/BMI, showing the relationship with adiposity. This same association was seen in other studies, such as in the one by Pavão et al.,²¹ when they evaluated adolescents in a municipality of the state of Paraná and observed a predisposition to dyslipidemia when abdominal obesity, seen through WC, was present. Another study in the city of Recife showed that adolescents with overweight or abdominal obesity had higher values of TG and lower levels of HDL-c.²⁵ This study had as a limitation a convenience sample, which does not allow the generalization of results.

Conclusions

The present study demonstrated a high prevalence of dyslipidemia among adolescents seen at NESA outpatient clinic, mainly low HDL-c in obese adolescents. Considering the significant association between low levels of HDL-c and TG increased with adiposity, the control of these factors should receive attention, with the investigation and early diagnosis of the lipid change being important, especially if it is associated with another cardiovascular risk such as obesity, to develop effective intervention strategies. In addition, data presented

Table 3 – Prevalence of dyslipidemias according to nutritional status by BMI

Lipids	Nutritional diagnosis				p value
	Total (n: 239)	Eutrophy (n: 52)	Overweight (n: 60)	Obesity (n: 127)	
TC (mg/dl)					
Normal	155(64.8%)	35 (22.6%)	39 (25.2%)	81 (52.3%)	0.90
Changed	84(35.2%)	17 (20.2%)	21 (25%)	46 (54.8%)	
LDL-c (mg/dl)					
Normal	213 (89.1%)	43 (23%)	50 (23.5%)	114 (53.5%)	0.18
Changed	26 (10.9%)	3 (11.5%)	10 (38.5%)	13 (50.0%)	
HDL-c (mg/dl)					
Normal	118 (49.4%)	35 (29.7%)	31 (26.3%)	52 (44.0%)	0.01*
Changed	121 (50.6%)	17 (14.0%)	29 (24.0%)	75 (62.0%)	
TG (mg/dl)					
Normal	195 (81.6%)	47 (24.1%)	51 (26.2%)	97 (49.7%)	0.06
Changed	44 (18.4%)	5 (11.4%)	9 (20.4%)	30 (68.2%)	

Statistical test: Chi square; *Statistically significant difference ($p < 0.05$); TC: total cholesterol; LDL-c: low density lipoprotein; HDL-c: high density lipoprotein; TG: triglycerides.

Table 4 – Bivariate and multivariate linear regression analysis between lipid profile and anthropometric variables**

Variables	BMI				WC			
	Gross Coef (95% CI)	p value	Adjusted Coef (95% CI)	p value	Gross Coef (95% CI)	p value	Adjusted Coef (95% CI)	p value
TC (mg/dl)	0.02 (-0.01 – 0.04)	0.19	0.01 (-0.01 – 0.04)	0.33	0.05 (-0.02 – 0.13)	0.17	0.06 (-0.02 – 0.13)	0.13
LDL-c (mg/dl)	0.03 (-0.00 – 0.06)	0.07	0.02 (-0.01 – 0.06)	0.14	0.07 (-0.01 – 0.16)	0.08	0.08 (-0.01 – 0.16)	0.08
HDL-c (mg/dl)	-0.12 (-0.18 – -0.05)	$p < 0.01$ *	-0.13 (-0.20 – -0.07)	$p < 0.01$ *	-0.22 (-0.47 – 0.02)	0.07	-0.23 (-0.47 – 0.02)	0.07
TG (mg/dl)	0.03 (0.02 – 0.06)	$p < 0.01$ *	0.04 (0.02 – 0.05)	$p < 0.01$ *	0.05 (0.00 – 0.10)	0.03*	0.06 (0.01 – 0.10)	0.02*

Statistical test: Bivariate and multivariate linear regression; *Statistically significant difference ($p < 0.05$); **Adjusted for gender and skin color; BMI: body mass index; WC: waist circumference; COEF: coefficient; CI: confidence interval; TC: total cholesterol; LDL-c: low density lipoprotein; HDL-c: high density lipoprotein; TG: triglyceride.

show an alert to the multiprofessional team about the need for a greater incentive to healthy lifestyle measures in the above-mentioned population.

Author contributions

Conception and design of the research, acquisition of data, analysis and interpretation of the data, writing of the manuscript and critical revision of the manuscript for intellectual content: Vizentin NP, Cardoso PMS, Maia CAG, Alves IP, Aranha GL, Giannini DT; statistical analysis: Vizentin NP, Giannini DT.

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.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the CEP-HUPE under the protocol number 0193.0.228.000-11. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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The Importance of Identifying Risk Factors in Childhood and Adolescence

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Short Editorial related to the article: Dyslipidemia in Adolescents Seen in a University Hospital in the city of Rio de Janeiro/Brazil: Prevalence and Association

The study "Dyslipidemia in Adolescents Seen in a University Hospital in the city of Rio de Janeiro/Brazil: Prevalence and Association" showed a high prevalence of obesity (53%) followed by overweight (25.2%) in adolescents. The obese group had a predominance of low HDL-c besides the positive association of body mass index (BMI) and abdominal circumference with triglyceride values.¹ These data are fundamental and warn of the importance of early assessment of risk factors.

Elevated triglycerides and low HDL-c are strongly linked to obesity, especially in youth² and early exposure to that unfavourable metabolic profile will contribute to a higher cardiovascular risk.³

Evidences have shown that atherosclerosis begins in childhood and it is associated with early presence of established risk factors for cardiovascular disease. The progression of atherosclerotic process depends on the time of exposure beyond the interaction between conventional, genetics and environmental risk factors.^{4,5}

Despite the early onset of atherogenesis, children and adolescents do not develop clinic manifestations of coronary heart disease, since cardiovascular outcomes depend on prolonged exposure to risk factors. Even so, few longitudinal studies have linked childhood risk factors to adult cardiovascular disease.

Keywords

Dyslipidemias; Obesity; Overweight; Adolescent; Cholesterol LDL-C; Triglycerides; Risk Factors; Atherosclerosis.

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DOI: 10.5935/abc.20190016

Twig et al.⁶ demonstrated association between higher BMI during adolescence with increased cardiovascular mortality in adulthood throughout 40 years of follow-up.⁶ Increased in BMI and triglyceride level was predictive of cardiovascular event in young adulthood, whereas LDL-c levels did not.⁷

Measurements of carotid intima-media thickness (cIMT) by non-invasive imaging techniques provide a surrogate endpoint to assess early atherosclerosis.⁸ Studies have shown that childhood clustering of risk factors are predictive of adult cIMT.⁹

In the study "International Childhood Cardiovascular Cohort (i3C)", Koskinen et al.¹⁰ demonstrated that obesity, hypertension, and dyslipidemia were predictors of high cIMT in adults.¹⁰ They found that obesity in children was the most prevalent risk factor associated with high cIMT in adult, increasing the risk by 3.7 times.¹⁰ Using risk prediction models, when it added the lipid profile to obesity and hypertension, there was a modest improvement in the risk discrimination for increased cIMT in adulthood (area under the curve increased from 0.698–0.717). It may be due to a weak relationship between LDL-c levels and obesity since obesity interferes minimally with LDL-c levels¹⁰ except where obesity-related metabolic changes unmask an underlying genetic dyslipidemia. In the present cross-sectional study¹ obesity seems to be the driver of the lipid changes as prevalence of low HDL-c and association of abdominal adiposity with triglycerides levels, without changes in LDL-c values.¹ Despite these findings, high LDL-c is a well-established risk factor for atherosclerosis as observed in familial hypercholesterolemia, and early detection allows the initiation of pharmacological therapy even in the children.¹¹

The present study reinforces that current obesity is a growing epidemic.¹ The Universal screening would allow for earlier diagnosis and intervention for children with dyslipidemia secondary to lifestyle or genetic factors.¹²

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The Effect of Garlic and Voluntary Exercise on Cardiac Angiogenesis in Diabetes: The Role of MiR-126 and MiR-210

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Abstract

Background: Diabetes mellitus (DM) is one of the major risk factors for cardiovascular disease, leading to endothelial dysfunction and angiogenesis impairment. MiR-126 and miR-210 support angiogenic response in endothelial cells.

Objective: The present study sought to explore the effect of garlic and voluntary exercise, alone or together, on miR-126 and miR-210 expressions and cardiac angiogenesis in rats with type 1 diabetes.

Methods: Male Wistar rats were divided into five groups (n = 7): Control, Diabetes, Diabetes+Garlic, Diabetes+Exercise, and Diabetes+Garlic+Exercise. Diabetes was induced in the animals by streptozotocin (ip, 50 mg/kg). The rats were then fed raw fresh garlic homogenate (250 mg/kg) or were subjected to voluntary exercise, or to combined garlic and voluntary exercise for 6 weeks. MiR-126 and miR-210 expressions in the myocardium were determined by real time PCR, and the serum lipid profile was measured by enzymatic kits. Angiogenesis was evaluated by immunostaining for PECAM-1/ CD31 in the myocardium.

Results: Diabetes reduced both cardiac miR-126 expression and angiogenesis (p < 0.05). On the other hand, there was a miR-210 expression increase in the myocardium of diabetic animals (p < 0.001). However, those effects reversed either with garlic or voluntary exercise (p < 0.01). Moreover, treating diabetic rats with garlic and voluntary exercise combined had an additional effect on the expressions of miR-126 and miR-210 (p < 0.001). Furthermore, both voluntary exercise and garlic significantly improved serum lipid profiles (p < 0.001).

Conclusion: The induction of diabetes decreased angiogenesis in the myocardium, whereas our treatment using long-term voluntary exercise and garlic improved myocardial angiogenesis. These changes were possibly owing to the enhancement of myocardial miR-126 and miR-210 expressions. (Arq Bras Cardiol. 2019; 112(2):154-162)

Keywords: Rats; Garlic; *Allium Sativum*; Exercise; Diabetes Mellitus; microRNAs ; Angiogenesis Inducing Agents; Neovascularization; Physiologic.

Introduction

Diabetes mellitus (DM) is one of the major risk factors for cardiovascular disease, leading to endothelial dysfunction and angiogenesis impairment.¹ The current trend on research and health care focuses on providing effective therapy with few side effects and low toxicity that can be regularly used to control diabetes complications.²

Exercise is a powerful therapeutic strategy to improve overall cardiovascular health.³ However, exhaustive exercise

may be problematic as it can cause the production of reactive oxygen species (ROS).⁴ Therefore, voluntary exercise, in which the animal has free access to a running wheel, may be a model with more positive effects.⁵ There is evidence that aerobic training can promote cardiac angiogenesis,^{6,7} in which the vascular endothelial growth factor (VEGF) has a critical role.⁵ However, the underlying mechanisms of exercise have yet to be fully elucidated.

One of the most traditional plants in herbal medicine is *Allium sativum* L, which has been reported to have beneficial health effects. It is used as a therapeutic agent in various disorders such as cancer, cardiovascular disease, and diabetes through different mechanisms, including inhibition or stimulation of angiogenesis.^{2,8,9} Considering the effects of garlic in protecting against cardiovascular disease, as well as its effects on angiogenesis in different tissues, it is interesting to examine the effects of garlic on both myocardial angiogenesis and its related mechanisms.

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Manuscript received February 05, 2018, revised manuscript August 02, 2018, accepted August 02, 2018

DOI: 10.5935/abc.20190002

MiRs are small non-coding RNAs that function in RNA silencing and the post-transcriptional regulation of gene expression.¹⁰ MiRs are essential intracellular mediators in many processes such as inflammation, mitochondrial metabolism, apoptosis, and angiogenesis, which can be adjusted through exercise.¹¹ Therefore, miRs can be clinically useful in the treatment of several disorders. Moreover, miRs are released in urine and in the bloodstream following tissue injury, which makes them useful biomarkers for early detection, diagnosis, and prognosis of disorders. Recently, these molecules have been found to be involved in cardiovascular diseases.¹² This includes a high expression of miR-126 in the heart endothelium, as well as its involvement in angiogenesis.^{12,13} Circulating levels of miR-126 are reduced in diabetes,^{14,15} suggesting that its deficiency may impair vascularisation.¹⁶ Moreover, Fasanaro et al.¹⁷ reported that hypoxia-driven miR-210 supports angiogenic response in endothelial cells and that its blockade by anti-miR transfection inhibits the formation of capillary-like structures.¹⁷

Many diabetes complications are well-known to be associated with lipid disorders. Indeed, dyslipidemia impairs numerous organs and is recognized as an important factor of many diabetic complications, including vascular abnormalities.¹⁸

Therefore, the aim of this study was to investigate the effect of voluntary exercise and garlic treatment alone or in combination on miR-126 and miR-210 expressions, serum lipid profile, as well as their relationship with cardiac angiogenesis in diabetes.

Methods

Animals and Experimental Design

The Ethics Committee for Animal Experiments approved the study plan, and all experiments were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. Male Wistar rats (200-250 g) were provided by our university's colony. All animals were housed in a temperature-controlled facility (21-23°C) maintained on a 12:12-h light-dark cycle, with food and water provided ad libitum.

In this study, thirty-five male rats were divided into five groups (n = 7): Control, Diabetes, Diabetes+Garlic, Diabetes+Exercise, and Diabetes+Garlic+Exercise. Control animals received 0.4 mL of sodium citrate buffer, pH 4.5. Diabetes was induced using a single intraperitoneal dose (50 mg/kg) of Streptozotocin (Sigma, St. Louis, Mo, USA). Blood glucose level was measured 72 hours later using a glucometer (Elegance, Model: no: CT-X10 Germany), and induced diabetes was identified if blood glucose level was > 300 mg/dL (16.67 mmol/L).

In this study, sample size was determined based on our similar previous studies.^{8,19}

Voluntary exercise

Rats in the voluntary exercise groups were housed individually in cages with stainless-steel running wheels (1.00 m circumference, TajhizGostar) and were allowed free access to the wheel 24 h per day for 6 weeks. Running distance was monitored daily. If the running distance was below 2000 m/day, that animal was excluded from the study. Sedentary rats were housed in standard holding cages without running wheels for the same period.

Preparing Garlic Homogenate

Garlic (*Allium sativum*) bulbs were purchased from a local market. Cloves were peeled, sliced, ground into a paste and then dissolved in distilled water. The garlic homogenate was freshly prepared each day.

Sampling

At the end of the 6th week, the rats were deeply anesthetized with pentobarbital sodium (35 mg/kg, i.p.), blood samples were collected from the inferior vena cava to measure lipid profile.

Then the heart was quickly removed through midsternal thoracotomy and the left ventricle was excised, frozen in liquid nitrogen, and stored at deep freeze (-70°C) for later measurements. The myocardium was used for miR extraction, real-time PCR study and angiogenesis determination.

MiR Extraction and Real-Time PCR

MiR was extracted from the myocardium using miRCURYTM RNA isolation kit (Exiqon, Vedbaek, Denmark) according to the manufacturer's protocol.^{20,21} The procedure was performed based on the spin column using a proprietary resin as a matrix to separate RNA from other cell components. RNA content and purity were measured using the Nanodrop 1000 spectrophotometer (Thermo scientific, Wilmington, DE 19810 USA). MiR-126 expression profile was obtained for total RNA extracts using universal a cDNA synthesis kit. Briefly, total RNA containing microRNA was polyadenylated and cDNA was synthesized using a poly(T) primer with a 3' degenerate anchor and a 5' universal tag (Exiqon, Vedbaek, Denmark). Each cDNA was used as a template for microRNA quantitative real-time PCR by using the SYBR Green master mix (Exiqon, Vedbaek, Denmark). LNA (Locked Nucleic Acid) forward and reverse primer sets (Exiqon, Vedbaek, Denmark) for microRNA are listed in Table 1. Real-time PCR reactions were performed with a Bio-Rad iQ5 detection System (Bio-Rad, Richmond,

Table 1 – Target sequence list for miRs

Gene name	Accession number	Target sequence*
mo-miR-191	MIMAT0000440	CAACGGAAUCCAAAAGCAGCUG
hsa-miR-126	MIMAT0002957	UCGUACCGUGAGUAAUAUUGC
dme-miR- 210	MIMAT0001233	UUGUGCGUGUGACAGCGGCUA

* Sequences were derived from miRBase (www.mirbase.org).

CA, USA). The amount of PCR products was normalized with housekeeping rno-miR-191 for miR-126 and miR-210.³⁷ We used the $2^{-(\Delta\Delta Ct)}$ method to determine the relative quantitative levels of miR-126 and miR-210. Results were expressed as the fold-difference to the relevant controls.

Immunostaining for PECAM-1/ CD31

To investigate angiogenesis in the myocardium, transversal sections of the ventricles at their midportion were immediately isolated and fixed in 10% buffered-formalin solution, dehydrated in ascending grades of alcohol and embedded in paraffin. Then, serial 3 μ m-thick sections were cut from them and floated onto charged glass slides according to standard histological processing. Tissue pieces were deparaffinised in xylene and dehydrated in a graded series of ethanol. Slides were incubated sequentially in proteinase K and 0.3% hydrogen peroxide to block endogenous peroxidase activity. Sections were overlaid by primary antibody CD31 (Santa Cruz, USA) – an angiogenesis marker – and incubated at +4°C overnight. Afterwards, the sections were washed and incubated with standard avidin–biotin complex (ABC; Santa Cruz) according to the protocol. Then the slides were incubated in DAB (Diamino-benzidine, Santa Cruz) as the chromagen, and counterstained with Mayer's hematoxylin. Finally, the sections were cleared in xylene, mounted with Entellan and analyzed with a light microscope.

Assessment of immunostaining

To evaluate immunostaining, 3 to 5 sections of 1 mm² were randomly selected at a magnification of 400 $\times$, depending on the size of the sample section. Both staining intensity and number of positive cells were evaluated semi-quantitatively. Intensity scoring for CD31 staining was obtained within each area at a 400 $\times$ magnification. Each endothelial cell cluster of immunoreactivity expressing CD31 and forming

lumen or vessels was counted as individual microvessels. Vascular structures positive for CD31 were counted for 5 to 6 slides per animal and 10 fields per slide.

To assess immunostaining, we used the granulation tissue as a positive control, and the intensity of the staining was scored as follows: 0 (<10%); 1 (10% to 25%); 2 (25% to 50%); 3 (50% to 75%) or 4 (75% to 100%).²²

Lipid profile measurement

Blood samples were obtained from the inferior vena cava, then centrifuged at 3500 rpm for 10 min at 4°C, and serum was collected. Triglycerides serum level was determined by enzymatic kits (ZiestChem Diagnostic kits, Iran) using glycerol as the standard. Additionally, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were determined based on enzymatic methods by diagnostic kits, (ZiestChem, Iran) using cholesterol as the standard.

Statistical analysis

All results are expressed as mean $\pm$ SEM for seven animals, and analyses were performed using SPSS statistical software version 16. All parameters were tested for normality using the theone-sample Kolmogorov-Smirnov test. Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. The significant level was set at $p < 0.05$.

Results

Effects of garlic and voluntary exercise on miR-126 in the myocardium

As shown in Figure 1, myocardial miR-126 expression level was significantly lower ($p < 0.05$) in rats with diabetes than in the control group. Treatment with garlic ($p < 0.001$), voluntary exercise ($p < 0.01$), or both combined increased

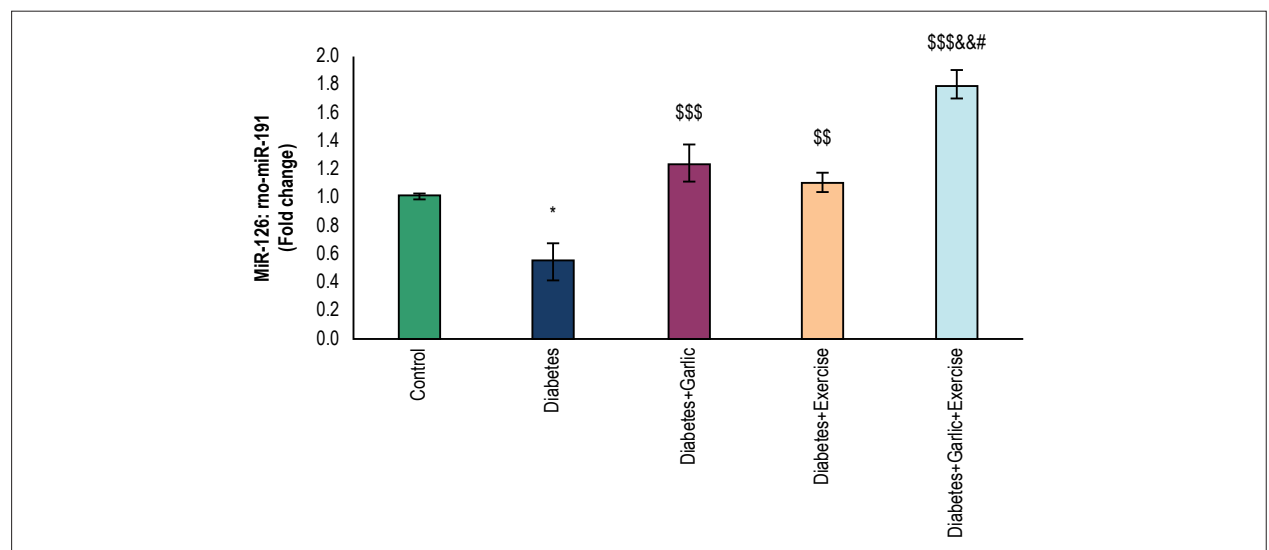


Figure 1 – Real-time quantitative PCR analysis of miR-126 in the heart tissue of experimental groups. The values represent means $\pm$ S.E.M for 7 animals. * $p < 0.05$ vs control group, ss $p < 0.01$ and sss $p < 0.001$ vs diabetes group, s $p < 0.05$ vs Diabetes+Exercise group, and $^{#}$ $p < 0.05$ vs Diabetes+Garlic group.

significantly ($p < 0.001$) the myocardial miR-126 expression in diabetic rats compared to the diabetes group. Moreover, the Diabetes+Garlic+Exercise group had significantly higher level of miR-126 expression compared to the garlic treatment group ($p < 0.05$) and the just voluntary exercise group ($p < 0.01$) in diabetic animals.

Effects of garlic and voluntary exercise on miR-210 in the myocardium

As shown in Figure 2, the expression of miR-210 significantly increased ($p < 0.001$) in animals with diabetes compared with the control group. Treatment with garlic ($p < 0.01$), voluntary exercise ($p < 0.01$), or both combined reduced significantly ($p < 0.001$) the myocardial miR-210 expression in diabetic rats compared to the diabetes group. The combined Garlic+Voluntary Exercise group significantly lowered miR-210 expression compared to the Diabetes+Exercise ($p < 0.05$) and Diabetes+Garlic ($p < 0.01$) groups

Effect of garlic and voluntary exercise on angiogenesis in the myocardium

Immunostaining with CD31 marker was performed for the assessment of angiogenesis in the transversal section of the ventricles at their midportion. Brown stained tissues show CD-31 immunostained endothelial cells. Figure 4 shows the scores for staining intensity, which are as follows: 0 (<10%); 1 (10% to 25%); 2 (25% to 50%); 3 (50% to 75%) or 4 (75% to 100%). As shown in Figures 3 and 4, statistical analysis of our immunohistochemical study revealed that angiogenesis decreased significantly ($p < 0.01$) in the diabetes group compared to the control group. Six weeks of garlic treatment, voluntary exercise, or a combination thereof in the diabetes groups increased significantly ($p < 0.001$) the angiogenesis in their left ventricle compared to the diabetes group (Figure 3 and 4). Combined garlic consumption and exercise in diabetic animals induced more angiogenesis compared to garlic alone and exercise alone, though the difference was not significant.

Effect of garlic and voluntary exercise on serum lipid profile

Lipid profile alterations in different groups are shown in Table 2. The induction of diabetes in the animals increased significantly ($p < 0.001$) the serum TCs and LDL levels while lowering serum HDL and HDL/LDL compared to the control animals. Voluntary exercise reduced significantly ($p < 0.05$) the serum triglycerides levels in the diabetes group compared with the control group. Six weeks of garlic treatment alone or with voluntary exercise decreased significantly ($p < 0.01$) the triglycerides levels in the animals with diabetes. In these, serum LDL levels decreased significantly ($p < 0.001$) after garlic alone and exercise alone or a combination thereof. However, serum HDL level was significantly increased ($p < 0.001$) by garlic treatment, voluntary exercise, or a combination thereof in diabetic rats. Furthermore, the HDL:LDL ratio was significantly higher ($p < 0.001$) in the Diabetes+Garlic, Diabetes+Exercise and Diabetes+Garlic+Exercise groups compared with diabetes group.

Discussion

The present study has shown that the induction of diabetes impaired serum lipid profile, decreased myocardial angiogenesis and miR-126 expression, and increased myocardial expression of miR-210. However, the treatment with garlic alone, voluntary exercise alone or both combined ameliorated these effects in the myocardium of diabetic animals. Interestingly, treating diabetic rats simultaneously with garlic and voluntary exercise had an additional effect on the cardiac expression of miR-126 and miR-210. In line with our study, research has shown that diabetes leads to an impaired function of early endothelial progenitor cells, which results in a reduced capacity of neovascularisation and angiogenesis in the myocardium of diabetic rats.²³ VEGF, as an inducer of angiogenesis, is a highly specific mitogen for endothelial cells.²⁴ It is well-known that the expression of VEGF-A and its receptors decreases in the myocardium of diabetic rats and humans.²⁵ However, the actual process of VEGF and angiogenesis reduction in the diabetic heart has not been fully elucidated.

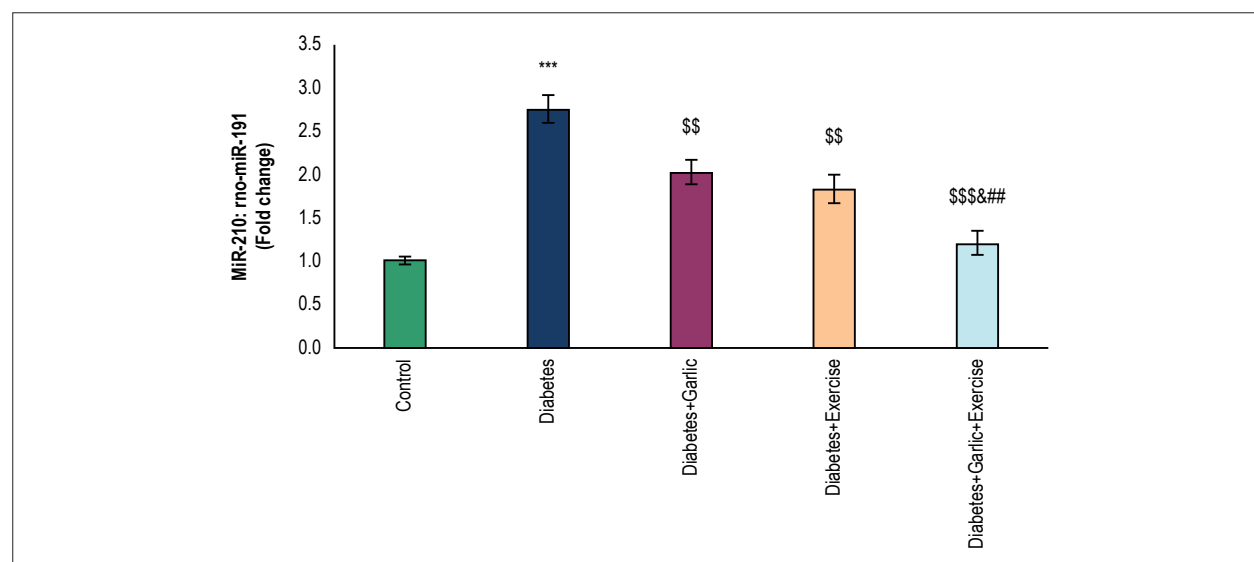


Figure 2 – Real-time quantitative PCR analysis of miR-210 in the heart tissue of experimental groups. The values represent means $\pm$ S.E.M for 7 animals. *** $p < 0.001$ vs control group, \$\$ $p < 0.01$ and \$\$\$ $p < 0.001$ vs diabetes group, \$ $p < 0.05$ vs Diabetes + Exercise group, and ## $p < 0.01$ vs Diabetes + Garlic group.

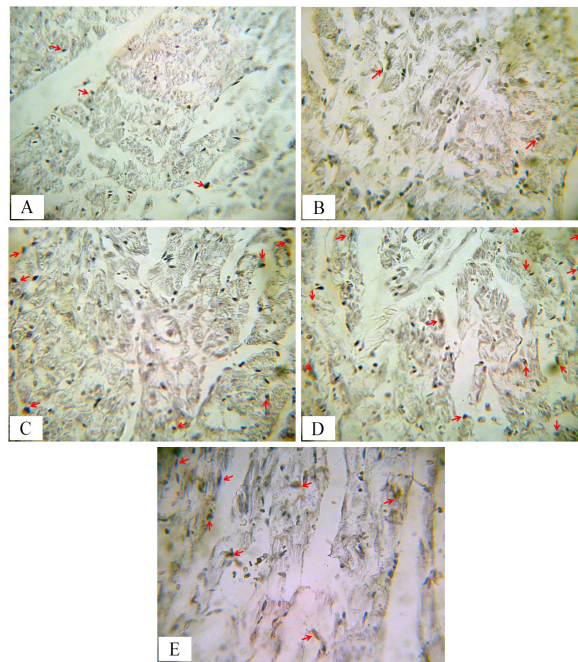


Figure 3 – Immunohistochemical detection of CD31 in myocardial vessels of different groups. Brown stained tissues show CD-31 immunostained endothelial cells in: (A) Control; (B) Diabetes; (C) Diabetes+Garlic; (D) Diabetes+Exercise; and (E) Diabetes+Garlic+Exercise. The intensity of immunostaining for CD31 (arrow head) decreased in the diabetes group compared to the control group. Garlic treatment and exercise alone or combined increased angiogenesis in diabetes compared to the diabetes group (Magnification was 400x).

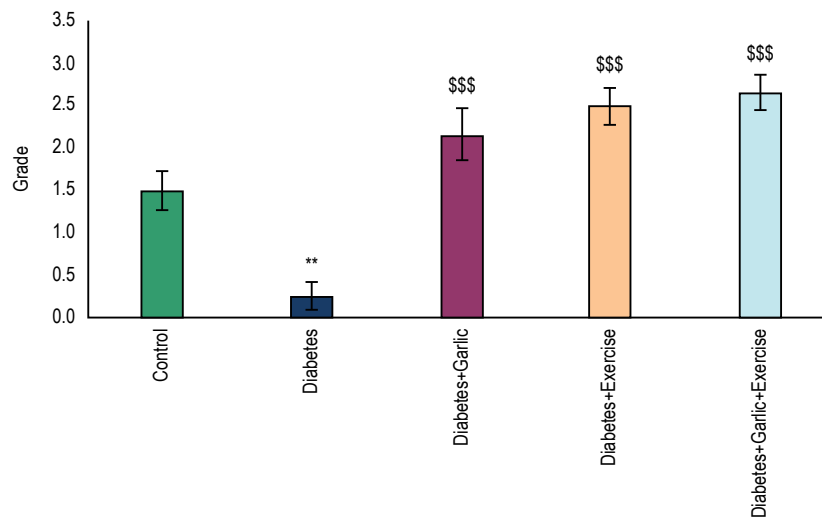


Figure 4 – Effects of garlic treatment and voluntary exercise on angiogenesis in different experimental groups. The intensity of the staining was scored as: 0 (<10%); 1 (10-25%); 2 (25-50%); 3 (50-75%); and 4 (75-100%). The values represent means ± S.E.M for 7 animals. ** $p < 0.01$ vs control group and \$\$\$ $p < 0.001$ vs diabetes group.

There is a variety of miRs in the heart tissue, and these tiny regulators are recognized as novel targets/drugs in numerous fields, including cardiology.¹² MiR-126 is known as an endothelial-specific miR that modulates angiogenesis in vivo. Several studies have shown miR-126 to support endothelial homeostasis and angiogenesis,^{12,13,15} which is mediated by SPRED1 and PIK3R2 to

promote VEGF signaling.¹⁵ In addition, miR-126 activates survival kinases such as ERK and Akt by downregulating its targets and promoting the action of VEGF.²⁶ Osipova et al reported in their study that urinary miR-126 levels were reduced in the patients with diabetes; however, circulating miR-126 levels in plasma showed no significant difference.¹

Table 2 – Serum lipid profile in different groups after 6 weeks (Mean ± SEM, n = 7)

Variants	Control	Diabetes	Diabetes+ Garlic	Diabetes+ Exercise	Diabetes+Garlic +Exercise
TG (mg/dl)	21.3 ± 2.9	87.8 ± 14.3 ^{***}	42 ± 2.9 ^{§§}	50.1 ± 9.3 [§]	44.8 ± 3.7 ^{§§}
LDL(mg/dl)	41 ± 1.69	48.87 ± 1.21 ^{***}	38.66 ± 0.61 ^{§§§}	39 ± 0.81 ^{§§§}	38.33 ± 0.76 ^{§§§}
HDL(mg/dl)	28.8 ± 1.07	18.25 ± 0.83 ^{***}	28.16 ± 1.22 ^{§§§}	26.66 ± 1.47 ^{§§§}	27 ± 1.46 ^{§§§}
HDL/LDL	0.7 ± 0.03	0.36 ± 0.01 ^{***}	0.72 ± 0.03 ^{§§§}	0.67 ± 0.03 ^{§§§}	0.7 ± 0.04 ^{§§§}

^{***} $p < 0.001$ vs control group and ^{§§§} $p < 0.001$ vs diabetes group. Triglycerides (TG), High-density lipoprotein (HDL), Low-density lipoprotein (LDL)

Little information is available about the expression of miR in the myocardium of diabetic rats in response to voluntary exercise. Interestingly, in the present study, we observed that garlic, voluntary exercise and a combination thereof increased the levels of miR-126 expression and angiogenesis in the myocardium. Cardioprotective effects of garlic have been reported in some studies related to improvement of antioxidant activities,⁸ AMPK-mediated AKT/GSK-3 β /HIF-1 α activation,²⁷ and Akt-eNOS signaling pathways.²⁸ Moreover, in line with our results, da Silva et al.⁶ showed that aerobic training in healthy rats increased cardiac miR-126 expression, which was possibly related to exercise-induced cardiac angiogenesis.⁶ Furthermore, studies have demonstrated that exercise enhances angiogenesis in the heart both under healthy²⁹ and pathological conditions,^{5,7} which highlights the positive effect of physical activity as a non-pharmacological tool in the treatment of cardiovascular disorders. Considering the increased expression of miR-126 following voluntary exercise, cardiac angiogenesis is possibly related to exercise-induced miR-126 expression and VEGF modulation, which upregulates angiogenic pathways such as MAPK and PI3K/Akt/eNOS.⁶

An important hypoxia-induced miR, miR-210 is stimulated following hypoxia and HIF activation.³⁰ The elevation of miR-210 gene expression is evidence of hypoxic conditions in the cardiac muscle, in which hypoxia stimulates a number of physiological responses such as angiogenesis through HIF-1 α -induced miR-210 expression.³¹ MiR-210 upregulation is a major element of endothelial cell response to hypoxia, which leads to angiogenesis via its target gene Ephrin-A3.¹⁷ The upregulation of miR-210 and VEGF has been shown to enhance myocardium angiogenesis in acute myocardial infarction in response to Huoxue Anxin Recipe.³² Greco et al.³³ described that, in addition to hypoxia, hyperglycemia is another stimulator that upregulates miR-210 expression, which is observed in diabetes.³³ Osipova et al.¹ showed that miR-210 level was upregulated in plasma and urine of type 1 diabetic children,¹ as well as in cardiomyocytes and endothelial cells in diabetic patients.³³ In line with these studies, we showed that the induction of diabetes increased myocardial miR-210 level, which was reduced by both garlic, voluntary exercise and a combination of both. Similarly, a recent study demonstrated that plasma miR-210 levels decreased in chronic kidney disease after acute exercise.³⁴ On the contrary, some studies have shown that miR-210 was not responsive during acute, exhaustive exercise, sustained aerobic exercise¹¹ and swimming³⁵ in the heart tissue. Furthermore, both garlic and exercise have been shown to be involved in providing good glycemic control and prevention against long-term

diabetic complications.^{3,8,19} Therefore, in the present study, the decrease of miR-210 expression back to normal levels seems to stem from glycemic control. Additionally, garlic extract-mediated angiogenesis probably occurs through the upregulation of the neovasculogenic c-kit protein expression and the activation of the PI3-K/Akt/NF- κ B signaling pathways,³⁶ which regulates e-NOS activation and NO production.¹¹

Hyperglycemia is currently considered to be primarily responsible for the alteration of lipid profile. In general, dyslipidemia is well confirmed in diabetes mellitus; it is known as a criterion for the diagnosis of type I diabetes and potential beta-cell lipotoxin.³⁷ It is worth noting that dyslipidemia is related to atherosclerosis and a risk of heart disease.³⁷ Dyslipidemia is possibly mediated by the alteration of LXR α expression in the liver and intestine, the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase pathways, and the consequent inhibition of eNOS activity, causing impaired angiogenesis.^{15,38} In addition, dyslipidemia is related to decreased levels of circulating miR-126.¹³ Riedel et al.³⁹ showed that exercise in patients with chronic heart failure significantly improved HDL-induced miR-126 expression.³⁹ In this study, treatment with garlic and voluntary exercise alone and together ameliorated lipid profile in the serum of diabetic rats, which is in agreement with previous studies.^{6,9,40} Therefore, garlic and exercise have possibly modulated angiogenesis in the myocardium of the diabetic animals by modulating serum lipid profile and the expression of pro-angiogenic miRs. With regard to the limitations of this study, we did not measure other factors involved in angiogenesis. Further studies are necessary to clarify the pathophysiological mechanisms of garlic and voluntary exercise in the treatment of diabetic complications.

Conclusion

This study showed that garlic and voluntary exercise modulated serum lipid profile and the expression of miR-126, miR-210, thus increasing angiogenesis in myocardium of diabetic rats. These findings suggest that garlic and voluntary exercise alone and combined may hold benefits in the treatment of diabetes.

Author contributions

Conception and design of the research, analysis and interpretation of the data and statistical analysis: Naderi R, Mohaddes G, Mohammadi M; acquisition of data: Naderi R, Ghaznavi R, Ghyasi R; obtaining funding, writing of the manuscript and critical revision of the manuscript for intellectual content: Naderi R; Histological finding and

interpretation of the data: Alihemmati A; Contribute to real time PCR protocol: Khamaneh A.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by a grant from Drug Applied Research Center, Tabriz University of Medical Sciences.

Study Association

This article is part of the thesis of Doctoral submitted by Roya Naderi, from Drug Applied Research Center, Tabriz University of Medical Sciences.

Ethics approval and consent to participate

This study was approved by the Ethics Committee on Animal Experiments of the Tabriz University of Medical Sciences under the protocol number 91.4-2.4.

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Exercise and Garlic Modulate microRNAs Involved in Diabetic Cardiopathy

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Short Editorial related to the article: *The Effect of Garlic and Voluntary Exercise on Cardiac Angiogenesis in Diabetes: The Role of MiR-126 and MiR-210*

Diabetes mellitus (DM) is a major risk factor for cardiovascular disorders and stroke development and is associated with increased morbidity and mortality.¹ The prevalence of diabetes is increasing at an alarming rate worldwide. Indeed, according to estimates of the International Diabetes Federation, 552 million people are expected to be diabetic in 2030.² Although a definitive cure is not on the horizon, with proper management, diabetic patients can attenuate the development of serious complications that reduce life quality and expectancy. Facing a considerable rate of occurrence and prognosis complications, studies focusing on high efficiency and low toxicity treatments are of great importance.³

MicroRNAs are small non-coding RNAs controlling gene expression and participating in many physiopathological processes. These small molecules are getting a lot of attention nowadays since they are universally recognized as major regulators of gene expression and as key controllers of several biological and pathological processes.⁴ They are essential intracellular mediators in a variety of cellular processes, such as inflammation, mitochondrial metabolism, apoptosis, among others. Therefore, miRNAs could be potential targets to treat some chronic diseases. Besides, these molecules can also be used as early biomarkers, once they are released in urine and blood when in presence of tissue lesion.⁵ Recently, it was verified that miRNAs are also involved in cardiovascular disorders, especially those which impaired angiogenesis is observed.⁶

Considering this scenario, Mostafa et al.⁷ evaluated the effects of garlic consumption and voluntary exercise, alone and together, on microRNAs 126 and 210, involved in cardiac angiogenesis, in diabetic rats.

Garlic, *Allium sativum* L, is commonly used in traditional phytotherapy and there are many studies showing its beneficial effects in several disorders, such as cancer, cardiovascular diseases and diabetes. Also, some authors already showed its effects in angiogenesis.⁸ Indeed, Mostafa et al.,⁷ found that diabetes reduced cardiac angiogenesis and garlic consumption increased this angiogenesis in diabetic rats.

Aerobic exercise is a non-pharmacological therapeutic approachable to improve cardiovascular health in general. Regular practice of exercises results in several health benefits, such as improvement in body composition, physical capacity, insulin resistance, endothelial function, arterial hypertension, and quality of life.⁹ Besides these benefits, exhaustive exercise practice can contribute to oxidative stress, producing reactive oxygen species (ROS). In animal models, some authors believe that voluntary exercises could show more positive effects.¹⁰ In fact, Mostafa et al.⁷ observed that voluntary exercises reduced triglycerides and LDL cholesterol serum levels and enhanced HDL serum levels and HDL/LDL ratio in comparison to the diabetic control group.

In Mostafa study,⁷ miRNAs 126 expression is reduced in diabetic rats. Both treatments, physical exercise or garlic ingestion, were able to increase its expression. Interestingly, when taken together, exercise and garlic, there was an additional increase in miRNA 126 expression. MicroRNA 126 is endothelium-specific, modulating angiogenesis and contributing to endothelium homeostasis. Possibly, miRNA 126 acts through inhibition of negative regulators of VEGF pathway.¹¹

In response to hypoxia conditions, endothelium cells increase miRNA 210 expression to promote angiogenesis. In the same way, other authors have described the high expression of this miRNA in hyperglycemia contexts, such as diabetes.¹² These studies corroborate Mostafa et al. results, that showed increased miRNA 210 in diabetic rats. This expression was reduced with both treatments, voluntary exercise or garlic consumption, and there was a bigger reduction when taken together.

It is well known that physical exercise has positive effects in controlling glycemia levels. Moreover, practice of physical exercise is recommended to good health maintenance and quality of life.¹³

A systematic review of garlic effects on lipidic and glucose parameters in diabetic patients was recently published. The authors concluded that garlic can reduce lipid profile as well as glucose parameters and be therapeutically effective in patients with cardiovascular diseases and diabetes.^{14,15}

Some of those positive effects obtained by physical exercise and garlic ingestion may be to modulation of specific microRNAs, according to Mostafa and collaborators. It is interesting to observe that the response to those treatments was amplified when they were combined, almost like an adjuvant effect.

Although these promising and interesting results, more studies on what mechanisms and which intracellular pathways modulate microRNAs expression involved in the cardiac angiogenesis and lipidic profile improvement provided by voluntary physical exercise and garlic consumption in diabetes mellitus are necessary.

Keywords

Diabetes Mellitus/complications; Exercise/prevention & control; MicroRNAs; Heart Diseases; Angiogenesis Inducing Agents.

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DOI: 10.5935/abc.20180259

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Cut-Point for Satisfactory Adherence of the Dietary Sodium Restriction Questionnaire for Patients with Heart Failure

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Abstract

Background: The low or non-adherence to reduction of sodium intake has been identified as one of the main precipitating factors of heart failure (HF). The Dietary Sodium Restriction Questionnaire (DSRQ) identifies factors that can interfere with adherence to this recommendation. However, there is still no cut-point to define adherence for this questionnaire.

Objectives: To identify the cut-point for satisfactory adherence to the Brazilian version of the DSRQ, (the Questionário de Restrição de Sódio na Dieta, QRSD).

Methods: Multicenter study. Patients with HF in outpatient treatment (compensated) and those treated in emergency departments due to acute HF (decompensated) were included. For the cut-point definition, the DSRQ scores were compared between groups. A ROC curve was constructed for each subscale to determine the best point of sensitivity and specificity regarding adherence. A 5% significance level was adopted.

Results: A total of 206 compensated patients and 225 decompensated were included. Compensated patients exhibited scores that showed higher adhesion in all subscales (all $p < 0.05$). Scores ≥ 40 points of a total of 45 for the subscale of Attitude and Subjective Norm; scores $\leq$ eight of a total of 20 for Perceived Behavioral Control; and $\leq$ three of a total of 15 for Dependent Behavior Control were indicative of satisfactory adherence.

Conclusions: Based on the evaluation of patients in these two scenarios, it was possible to determine the cut-point for satisfactory adherence to the reduction of sodium in the diet of patients with HF. Countries with similar culture could use this cut-point, as other researchers could also use the results as a reference for further studies. (Arq Bras Cardiol. 2019; 112(2):165-170)

Keywords: Heart Failure/physiopathology; Sodium,Dietary; Surveys and Questionnaires; Behavior Control; Decision Making; Multicenter Study.

Introduction

Reduction of sodium intake is usually part of the non-pharmacological treatment for patients with heart failure (HF), since the excessive consumption is associated with fluid retention and congestive situations.^{1,2} A poor or non-adherence to this recommendation has been identified among the main precipitating factors of HF decompensation³⁻⁵ and has been linked to the need for hospitalization and worse outcomes.^{6,7}

To understand what factors could potentially interfere with the adherence to the reduction of sodium intake, researchers from

the United States of America developed the Dietary Sodium Restriction Questionnaire (DSRQ).⁸ This instrument is based on the Planned Behavior Theory and considers three constructs: attitude, subjective norm and perceived behavioral control. Recently, the DSRQ was adapted (transculturally) and validated for the Portuguese language in Brazil, with the name *Questionário de Restrição de Sódio na Dieta* (QRSD).^{9,10} Although the DSRQ has already been the object of other studies,¹¹⁻¹³ there is still no cut-point to define satisfactory adherence for the interviewed patients. Seeking to fill this gap, this study was designed to identify a cut-point for satisfactory adherence to sodium restriction when using the QRSD, both for stable patients on outpatient care, and for decompensated patients.

Methods

Design and sample

This is a case-control study, conducted in two institutions in southern Brazil from March 2010 to October 2014.

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Manuscript received April 06, 2018, revised manuscript July 21, 2018, accepted August 02, 2018

DOI: 10.5935/abc.20190011

Adult patients, with a diagnosis of HF – reduced or preserved left ventricular ejection fraction (LVEF)⁹ – were included. Patients in outpatient treatment (compensated) and those admitted to emergency rooms due to acute HF (decompensated) participated in this study. It was used a convenience sample, with a total of 431 HF patients (206 compensated and 225 decompensated).

Patients with cognitive impairment or barrier (e.g., decreased hearing acuity, neurological sequelae) were excluded since these impairments could make it difficult for patients to fill out the questionnaire.

Data collection

Clinical and sociodemographic data were collected from medical records. The QRSDs were administered by the researchers in a private room, with a mean duration of 40 minutes.

The Brazilian version of the DSRQ comprises 27 items, 11 descriptive questions and 16 questions divided into three subscales, which are scored using the 5-point Likert scale:¹⁰

- a) Attitude and subjective norm (nine items, with scores ranging from nine to 45) – assesses the patient's beliefs regarding the results of performing a diet with reduced sodium and the importance of other people's approval or disapproval of this practice;
- b) Perceived behavioral control (four items with scores ranging from four to 20) – assesses the patient's ability to identify facilitators and barriers related to the reduction of sodium in their diet;
- c) Dependent behavior (three items with scores ranging from three to 15) – assesses the presence or absence of resources and constraints for a patient to follow a sodium-reduced diet.

In the first subscale – attitude and subjective norm – the lowest score indicates a “strong disagreement” and the highest, a “strong agreement”. In the second and third subscales – perceived behavioral control and dependent behavior – the minimum score indicates “not at all”, while the maximum indicates “a lot”.⁸

This study was approved by the Ethics Committee of the institutions involved and all participants signed a written informed consent form before taking part in this study.

Data analysis

Data were analyzed using the Statistical Package for Social Sciences version 18.0. Continuous variables with normal distribution were expressed as mean and standard deviation and without normal distribution, as median and interquartile range. Categorical variables were expressed as absolute numbers and relative frequency. To compare continuous variables, unpaired Student's t-test or Mann-Whitney test were used, according to data distribution. Associations between categorical variables were analyzed using the chi-square test or Fisher's exact test. A 5% significance level was adopted.

To define the cut-points, the QRSD scores were compared between compensated and decompensated patients. A ROC curve was constructed for each subscale, and an additional comparison of patients by functional class (I - II) and (III - IV)

was performed to determine the best point of sensitivity and specificity regarding adherence to the diet. determine the best point of sensitivity and specificity regarding adherence to the diet.

Results

A total of 431 HF patients participate in the study. Of the total, 206 were in outpatient treatment (compensated) and 225 patients sought emergency care (decompensated). Sociodemographic and clinical characteristics of the studied population are shown in Table 1. Mean age was 63 ± 13 years, and 59.2% of the participants were male; mean LVEF was $36.8 \pm 14.0\%$.

Regarding the QRSD scores, compared with decompensated patients, compensated patients had better scores, showing greater adherence in all subscales. Mean scores for compensated and decompensated groups, and for categories of functional classes are shown in Table 2.

According to the ROC curve analysis, the area under the curve was 0.725 (95%CI; 0.677 to 0.772) for the attitude and subjective norm subscales; 0.670 (95%CI; 0.620 to 0.721) for the perceived behavioral control subscale; and 0.544 (95%CI; 0.489 to 0.598) for the dependent behavior subscale (Figure 1).

The results of the functional class analysis were 0.631 (95%CI; 0.578 to 0.685) for the attitude and subjective norm subscales; 0.628 (95%CI; 0.574 to 0.682) for the perceived behavioral control subscale; and 0.561 (95%CI; 0.506 to 0.617) for the dependent behavior subscale.

Sensitivity and specificity were, respectively, 53.8 and 83.5 for the attitude and subjective norm subscales; 68.0 and 58.3 for perceived behavioral control subscale; and 60.9 and 51.0 for dependent behavioral subscale. Cut-off points for adherence were scores greater than or equal to 40 points in the attitude and subjective norm subscale; lower than or equal to eight points for perceived behavioral control; and lower than or equal to three points for Dependent Behavior (Table 3).

Discussion

This is the first study conducted in a clinical scenario that tried to establish cut-points for the DSRQ/QRSD regarding adherence. This instrument considers the knowledge, barriers and attitudes of patients with HF regarding sodium restriction in the diet. Adherence can be defined as the degree to which individuals comply with recommendations (related to pharmacological treatment of changes in lifestyle) from the health team.¹⁴ In the context of HF, treatment adherence is considered an essential component to the success of self-care and prevention of complications, including hospitalizations.¹⁵

The sample was predominantly male patients older than 60 years, poorly educated, and with predominantly reduced LVEF, similar to other studies that addressed adherence in patients with HF.^{8,16,17}

Compared with compensated patients, in decompensated patients' group, there were fewer men, fewer people with white ethnicity and a greater number of people living alone. These characteristics have already been related to lower adherence in previous studies. Lennie et al.¹¹ investigated

Table 1 – Characteristics of the participants

Characteristics	Compensated (n = 206)	Decompensated (n = 225)	p
Sociodemographic			
Age (years)*	60 ± 12	66 ± 12	< 0.001
Male (%)†	65.0	53.8	0.023
Ethnicity (%)†			< 0.001
White	85.4	57.8	
Black	9.7	16.4	
Mixed-race	4.9	25.8	
Years of study (%)†			0.083
Until 8 years	75.7	83.0	
9 to 11 years	19.9	12.1	
12 years or more	4.4	4.9	
Marital status (%)†			< 0.001
Lives with a companion	69.4	49.3	
Lives alone	30.6	50.7	
Clinical			
LVEF (%)*	31.3 ± 9.1	42.0 ± 15.7	< 0.001
Functional class NYHA (%)†			< 0.001
I	42.0	1.4	
II	34.2	20.7	
III	23.3	63.1	
IV	0.5	14.9	
Etiology (%)†			0.002
Ischemic	33.0	43.2	
Hypertensive	18.0	10.9	
Others	49.0	45.9	
Medications prior to admission (%)†			
Beta-blockers	85.4	69.2	< 0.001
Anti-hypertensives	96.6	87.5	0.001
Diuretics	82.5	83.9	0.795

LVEF: left ventricular ejection fraction; NYHA: New York Heart Association. *Continuous variables described as mean ± standard deviation, unpaired Student's *t* test;

† categorical variables expressed as %, chi-square test.

the relationship between knowledge, attitudes, and barriers to adherence of a low-sodium diet in patients with HF, and also found similar sociodemographic characteristics, with mean age of 65 years and 32% of participants living alone. In fact, advanced age is among the main factors that contribute to high rehospitalization rates due to decompensation of HF patients.¹⁸ In addition to advanced age, many patients with HF have cognitive deficits, including memory loss.¹⁹ Regarding ethnicity, a recent study demonstrated an association between non-white race and non-adherence in patients with HF after hospital discharge.²⁰ The fact of living alone can interfere with adherence, since this behavior is largely influenced by the opinion of people whom patients consider important, including spouses and family members.¹² Lack of family support can make the patient feel alone. The inclusion of family members

in the treatment of HF – mainly in relation to adherence to non-pharmacological measures – seems to be a crucial point and has been used as a strategy for self-care.^{21,22}

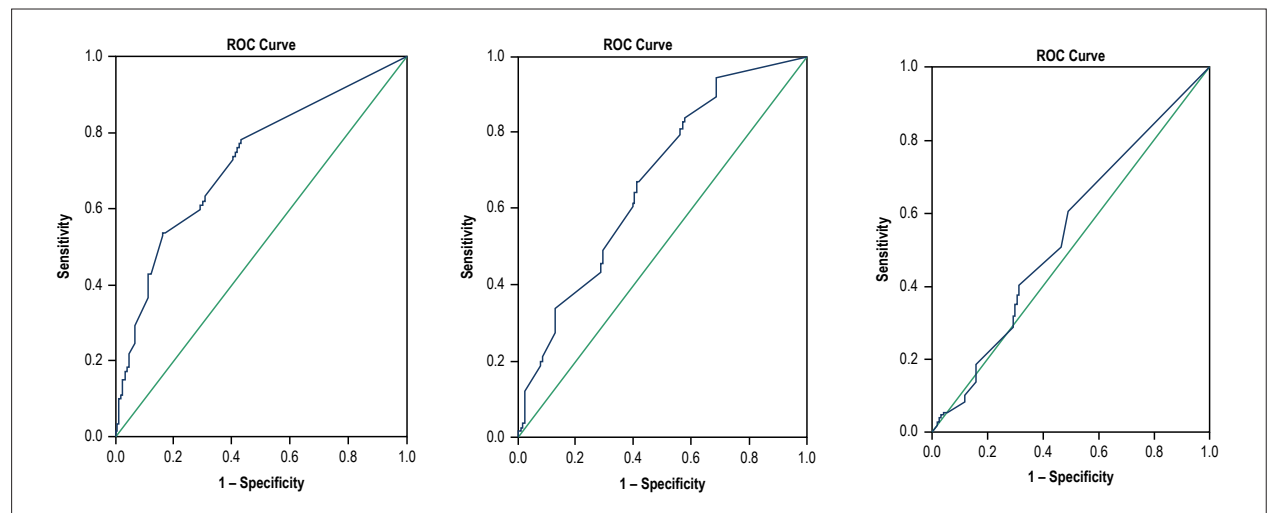
The multifactorial causation and subjectivity related to adherence could explain the difficulty encountered by health professionals to measure patients' commitment to a particular behavior. In this context, instruments that can provide more reliable information on patient outcomes in terms of knowledge, barriers and attitudes, with cut-points for adequate and poor adherence could help to identify factors that potentially influence this outcome.²³

According to the researchers responsible for developing the QRSD, the instrument was built with the goal of being a self-administered tool.⁸ However, considering cultural

Table 2 – Scores of the Dietary Sodium Restriction Questionnaire subscales for compensated and decompensated patients and for categories of functional class

	Attitude and subjective norm	Perceived behavioral control	Dependent behavior
Situation			
Compensated	42.6 ± 4.0	8.4 ± 4.1	5.2 ± 3.0
Decompensated	38.5 ± 6.3	10.9 ± 4.2	5.5 ± 3.0
p value	< 0.001	< 0.001	0.399
Functional class			
I x II	41.6 ± 5.1	8.8 ± 4.4	5.0 ± 2.8
III x III	39.3 ± 6.1	10.6 ± 4.2	5.6 ± 3.2
p value	< 0.001	< 0.001	0.038

*Continuous variables described as mean ± standard deviation

**Figure 1 – ROC curves for all three DSRQ subscales.****Table 3 – Cut-point for adherence established to the Dietary Sodium Restriction Questionnaire subscales**

Subscales	Scores (min - max)	Cut-point (adherence)	Sensitivity (%)	Specificity (%)
Attitude and subjective norm	9 - 45	≥40	53.8	83.5
Perceived behavior control	4 - 20	≤8	68.0	58.3
Dependent behavior control	3 - 15	≤3	60.9	51.0

differences between the studied populations, it is recommended that, in the Brazilian population, the QRSD be applied by means of interviews, by trained investigators. In addition, because each subscale relates to a particular construct, we sought to identify different cut-points for each of them.

High scores observed in the subscale of attitudes and subjective norm contributed to raising the cut-point (≥ 40 , a total of 45 points) and indicated that patients are aware of the importance of adhering to sodium reduction, and can identify signs and symptoms associated with excessive intake, as well as benefits related to the reduction. However, as described in the literature,^{7,24,25} knowledge alone does not seem to be

sufficient to ensure compliance, to which other skills are required, such as motivation and willpower.²¹ Accordingly, incorporating this measure into the routine remains a major challenge for patients.

On the same subscale, the last three questions that denote adherence are influenced by the opinion of people considered important by patients (spouse, family members, physicians and other health professionals). The inclusion of family members in the treatment of patients with HF appears to be a crucial point and is gaining more space as a strategy for self-care, with positive results in the reduction of sodium intake by these patients.^{21,22}

Regarding the scores and the cut-point identified for the subscale perceived behavioral control (≤ 8 , a total of 20 points), the main barriers – for both compensated and decompensated patients – are the palatability of foods with little salt, food preferences of patients, and less significantly, the willpower to change their diets, factors already described previously. Palatability of foods with low sodium content has been referred as one of the main barriers to adherence.^{26,27} Furthermore, when compared to healthy individuals, patients with HF have a preference for highly salted foods.²⁸ This can be explained largely by changes in the renin-angiotensin-aldosterone system, which promotes a higher desire for salt.²⁹

The low scores observed in the dependent behavior subscale influenced the determination of a low cut-point (≤ 3 , of a total of 15 points). In a study conducted with a sample of 225 patients with decompensated HF,¹² decision-making situations that occur outside the home – going to restaurants and the supermarket – did not influence significantly adherence in this population, possibly due to the limitations imposed by the severity of the disease. In addition, the trip to the supermarket and the choice of food is often performed by a family member or the person responsible for their care, which may explain the small impact caused by this factor.⁶

In the comparative analysis of patients by functional class to determine the cutoff point for satisfactory adherence, it was observed that both sensitivity and specificity values were lower than those obtained in the comparison between compensated and decompensated patients. Thus, our findings indicated that adherence was higher in outpatients compared with patients hospitalized for decompensated HF.

Limitations

Other factors other than sodium restriction may affect HF decompensation, which can lead to a small bias in the determination of the cut-point.

Although it was a case-control study, matching was not sufficient to minimize discrepancies between the two groups (compensated and decompensated). Other studies with the same design may contribute to elucidate the findings of this study.

Another limitation refers to the inexistence of national and international studies on specific cut-points in the evaluation of adherence using the QRSD, which makes comparisons with other investigations impossible.

Conclusions

Assessment of knowledge, barriers and attitudes towards dietary sodium among patients with HF in two different scenarios – outpatient and emergency services – allowed the determination of cut-points for satisfactory adherence to dietary sodium reduction. Countries with similar cultures may use this cut-point, as other researchers could also use it as reference in further studies.

We suggest this cut-point to identify facilitators and barriers related to reduction of dietary sodium intake in HF patients in Brazil, and be used to guide strategies, seeking better results.

Author contributions

Conception and design of the research, analysis and interpretation of the data, obtaining funding and critical revision of the manuscript for intellectual content: d'Almeida KSM, Barilli SLS, Souza GC, Silva ERR; acquisition of data, statistical analysis and writing of the manuscript: d'Almeida KSM, Barilli SLS.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by Fundo de incentivo à pesquisa e eventos do Hospital de Clínicas de Porto Alegre.

Study Association

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

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre under the protocol number 130343 and Hospital Nossa Senhora da Conceição/Grupo Hospitalar Conceição under the protocol number 13-049. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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Evaluating Sodium Restriction in Heart Failure

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Short Editorial related to the article: Cut-Point for Satisfactory Adherence of the Dietary Sodium Restriction Questionnaire for Patients with Heart Failure

Although salt and water retention plays a crucial role in heart failure (HF) pathophysiology, controversy still exists about dietary salt restriction in the treatment of HF patients.¹ Small clinical studies have suggested that excessive sodium restriction (< 5 g of salt per day), as compared with normal-sodium diet (approximately 7 g of salt per day), may be associated with deleterious effects in patients with chronic HF, including increased neurohormonal activation, and higher hospitalization and mortality rates.^{2,3}

A recent meta-analysis⁴ of nine studies involving 479 HF patients undergoing dietary sodium restriction was inconclusive for the recommendation of this strategy in hospitalized patients. None of the studies analyzed in the meta-analysis included hard endpoints such as all-cause death or cardiovascular mortality. However, a modest tendency for improvement of functional class was observed in outpatients undergoing sodium restriction intake. The author reinforces the need for randomized, prospective studies including large sample sizes, evaluating the effect of different regimens of sodium intake on relevant outcomes to build evidence base for detailed recommendations.

Restriction of sodium intake – < 3 g/day or < 7 g/sodium chloride (table salt) – is one of the non-pharmacological measures recommended by the Brazilian Guidelines on Heart Failure¹ and

by the American Heart Association⁵ (AHA) guidelines. The AHA also recommends evaluating patient understanding and the level of water and sodium intake restriction, as well as educating patients to reduce sodium intake.

However, compliance with this recommendation remains challenging. In 2009 Bentley et al.⁶ proposed the adoption of a new instrument, the Dietary Sodium Restriction Questionnaire (DSRQ), aimed at measuring attitude, beliefs and barriers of symptomatic HF patients (NYHA II/III) in following a low-sodium diet. Based on the Theory of Planned Behavior, the questionnaire assesses adherence through three subscales: attitude, subjective norm, and perceived behavioral control.

D'Almeida et al.⁷ adapted the DSRQ to the Brazilian population in 2012,⁷ and showed its validity and reliability in 2013.⁸ The Brazilian version of the DSRQ is composed of 27 items, 11 descriptive questions and 16 questions divided into three subscales: attitude and subjective norm, perceived behavioral control, and dependent behavior.

In this issue of Arquivos Brasileiros de Cardiologia, the same authors proposed the determination of a cut-off point to evaluate adherence to a low-sodium diet in Brazilian patients with HF. This was a case-control study that compared the scores of each subscale between 206 outpatients with compensated HF and 255 patients with uncompensated HF. Mean application time of the instrument was 40 minutes. The best area under the ROC curve was observed for the attitude and subjective norm scale (0.725). The cut-off for this subscale was 40 out of 45 points, with a 53.8% sensitivity and 82.5% specificity.

Previous studies had already shown an association between subjective norm subscale and an increased sodium urinary excretion⁹ and that the attitude subscale is the only associated with long-term adherence (six months),¹⁰ which corroborate the validity of their results. The proposed cut-off points to measure adherence to a low-sodium diet can be useful for future longitudinal studies aiming at elucidating the role of sodium restriction in the treatment of patients with HF.

Keywords

Heart Failure/complications; Diet-Sodium- Restricted/ methods; Diet Therapy; Treatment Adherence and Compliance; Patient Compliance; Survey and Questionnaires.

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DOI: 10.5935/abc.20190017

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The Effects of Trimetazidine on QT-interval Prolongation and Cardiac Hypertrophy in Diabetic Rats

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Abstract

Background: Trimetazidine (TMZ) is an anti-ischemic drug. In spite of its protective effects on cardiovascular system, there is no scientific study on the usefulness of TMZ treatment for prolonged QT interval and cardiac hypertrophy induced by diabetes.

Objectives: To evaluate the effects of TMZ on QT interval prolongation and cardiac hypertrophy in the diabetic rats.

Methods: Twenty-four male Sprague-Dawley rats (200-250 g) were randomly assigned into three groups (n = 8) by simple random sampling method. Control (C), diabetic (D), and diabetic administrated with TMZ at 10 mg/kg (T10). TMZ was administrated for 8 weeks. The echocardiogram was recorded before isolating the hearts and transfer to a Langendorff apparatus. Hemodynamic parameters, QT and corrected QT interval (QTc) intervals, heart rate and antioxidant enzymes were measured. The hypertrophy index was calculated. The results were evaluated by one-way ANOVA and paired t-test using SPSS (version 16) and $p < 0.05$ was regarded as significant.

Results: The diabetic rats significantly indicated increased hypertrophy, QT and QTc intervals and decreased Left ventricular systolic pressure (LVSP), Left ventricular diastolic pressure (LVDP), rate pressure product (RPP), Max dp/dt, and min dp/dt ($\pm$ dp/dt max), heart rate, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase in the heart. Treatment with TMZ in the diabetic animals was significantly improved these parameters in comparison to the untreated diabetic group.

Conclusions: TMZ improves QTc interval prolongation and cardiac hypertrophy in diabetes. (Arq Bras Cardiol. 2019; 112(2):173-178)

Keywords: Diabetes Mellitus; Trimetazidine; Cardiomegaly; Electrocardiology; Oxidative Stress; Rats.

Introduction

Diabetes is associated with cardiovascular disorders and increased mortality rate in diabetic patients.¹ The statistic reveals that 30 million people were suffered from diabetes worldwide in 1985 and recently, it is predicted by WHO, there will be 300 million by the year 2025.²

Diabetic cardiomyopathy is known as the structural and functional alterations in the heart induced by diabetes that are associated with cardiac hypertrophy, diastolic and/or systolic dysfunction in the absence of hypertension, valvular and ischemic heart diseases and other cardiac disorders.^{3,4}

QT and QTc intervals are electrocardiographic parameters that regarded as critical predictors of mortality and stroke in diabetic patients.^{5,6} The pathological QT prolongation is known as a risk factor that increases ventricular arrhythmias and other

heart diseases. Moreover, ventricular hypertrophy plays an important role in developing prolonged QT interval-related diabetes.⁷ A previous study has confirmed the negative effects of hypertrophy and QT interval prolongation on the function of heart in diabetes.⁸ The homeostasis of energy is effective in decreasing the hypertrophy in the heart.⁹

Trimetazidine (TMZ) is an anti-angina agent that is known to improve metabolism of energy in the heart subjected to ischemia.^{10,11} Previous studies have indicated reduced fatty acid oxidation via reducing mitochondrial 3-ketoacyl CoA thiolase (3-KAT) activity in beta-oxidation by TMZ treatment.¹² Others also indicated that TMZ has protective effects on cardiac fibrosis resulted from pressure overload.¹³ In addition, there are some other investigations showing that the treatment with TMZ has positive effects on cardiac function in diabetic individuals with cardiovascular disorders.¹⁴ Taken together, these results from related studies make evidence that TMZ has beneficial effects on cardiovascular system. However, the role of TMZ in QT interval prolongation and cardiac hypertrophy improvement in diabetes was still unknown. Therefore, the present study was undertaken to evaluate the effects of TMZ on QT interval prolongation and cardiac hypertrophy in the diabetic animals.

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Manuscript received April 24, 2018, revised manuscript July 23, 2018, accepted July 23, 2018

DOI: 10.5935/abc.20180248

Methods

Chemical

Trimetazidine (TMZ), heparin and alloxan were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Ketamine and xylazine purchased from Alfasan Co (Woderen- Holland).

Animal

Twenty-four adult male Sprague-Dawley rats (250 ± 20 g) were housed under standard conditions ($20 \pm 5^\circ\text{C}$, 12-hour light/dark cycle, and free available to water and food) during the study period. All the experimental protocols followed the Consensus Author Guidelines on Animal Ethics and Welfare and the national guidelines for conducting animal studies (Ethics Committee permission No. APRC-94-25 Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran).¹⁵

The sample size of each group was computed to be eight by the formula:¹⁶

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \times (S_1^2 + S_2^2)}{d^2} =$$

$$\frac{(1.96 \times 1.29)^2 \times (13.52^2 + 9.07^2)}{(89-70)^2} = 7.75 \sim 8$$

where S_1^2 and S_2^2 are means.

The animals were randomly divided into three groups ($n = 8$) by simple random sampling method. Control (C), diabetic (D) and diabetic administrated with TMZ at 10 mg/kg (T10).¹⁷ TMZ was treated orally by gavage once daily for 8 weeks.

Diabetic model

Diabetes was induced by intraperitoneal administration of alloxan at 120 mg/kg. After 6 h, the animals were orally treated with 10% glucose solution (10 mL). They were further kept for 24 h on 5% glucose solution to reduce fatal hypoglycemic resulted from alloxan. The rats, indicating fasting blood glucose ≥ 250 mg/dL, reduced body weight, dyslipidemia, increased hepatic enzymes and clear signs of polyuria, polyphagia and polydipsia after 4 days were regarded as diabetic animals and used for the experiment.¹⁸

Electrocardiography

The animals were anesthetized by heparin, ketamine and xylazine (1000 U/kg, 50, and 5 mg/kg, respectively), lead II was recorded by Bio Amp and controlled using a Power Lab system (AD Instruments, Australia). QT interval and heart rate were measured. Corrected QT interval (QTc) was calculated by Bazett formula normalized as $QTc = QT/(RR/f)^{1/2}$, where RR is R-R interval and $f = 150$ ms.^{19,20}

Isolation of hearts

After echocardiogram (ECG) recording, the cannulation and ventilation of trachea were performed using an animal ventilator (UGO BASILE, model: 7025). The cannulation of aorta was carried out by a central incision in the aorta.

The hearts were conveyed to the Langendorff system. The perfusion of heart was carried out by Krebs-Henseleit solution (5% carbon dioxide and 95% oxygen, 37°C , pH = 7.4, 8 ml/min). A latex balloon was inserted in the left ventricle for the measurement of left ventricular pressure (LVP) by Power Lab system (AD Instruments, Australia). Left ventricular end diastolic pressure (LVEDP) was approximately regulated 5-10 mmHg by the alteration of balloon volume. Left ventricular systolic pressure (LVSP), Max dp/dt, and min dp/dt ($\pm$ dp/dt max) were measure.²¹ Left ventricular diastolic pressure (LVDP) and rate pressure product (RPP) were calculated by following formula:

$$LVDP = LVSP - LVEDP$$

$$RPP = LVDP \times \text{heart rate}$$

Measurement of hypertrophy

After assessment of hemodynamic parameters using the Langendorff system, the hearts were removed and put in saline, then on a paper for assessment of the heart weight. Cardiac hypertrophy index (mg/g) was calculated from the total heart weight (mg) relative to total body weight (g) of the rat.²²

Measurement of antioxidant enzymes

After measurement of hypertrophy, 100 mg of heart tissue was frozen in liquid nitrogen and stored at -70°C . The tissue samples were homogenized in phosphate buffered saline (PBS; 50 mM at pH of 7.4) using a Homogenizer (Heidolph Silenterosher M, Germany), and centrifuged at 14000 g for 15 minutes. The assessment of enzyme levels including glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) was performed on supernatant. GPx and SOD were measured using Randox kits (Randox Lab, UK) and CAT activity was evaluated using Zellbio kit (Zellbio Lab, Ulm, Germany).

Statistical analysis

The results were indicated as mean and standard deviation (SD). In the present study, the normal distribution of the results was carried out by Kolmogorov-Smirnov analysis. One-way ANOVA and Least Significant Difference (LSD) test were used for comparison between the various groups. The comparison of pre and post metabolic in each group was performed by paired t-test using SPSS (version 16). A $p < 0.05$ was regarded statistically significant.

Results

Electrocardiographic parameters

The QT and QTc intervals significantly increased in the diabetic animals in comparison with the control group (100 ± 13.80 vs. 70 ± 8.34 , 82.52 ± 13.03 vs. 58.4 ± 7.33 , $p = 0.007$ and $p = 0.009$, respectively). TMZ treatment was associated with a significant reduction in the QT and QTc intervals in comparison with the untreated diabetic rats (80 ± 10.69 vs. 100 ± 13.80 , 63.11 ± 7.05 vs. 82.52 ± 13.03 , $p = 0.043$ and $p = 0.040$, respectively, Figure 1). As shown in

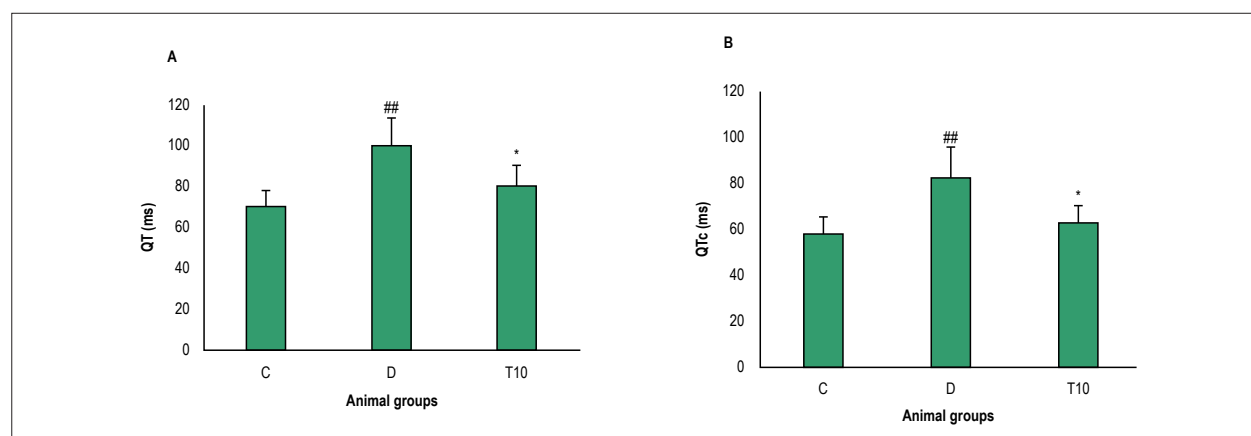


Figure 1 – QT interval (a), QTc interval (b) values in control (C), diabetic (D) and diabetic treated with TMZ (10 mg/kg, T10) groups eight weeks after treatment in the rats. The results were presented as mean $\pm$ SD. ^{##} $p < 0.01$ compared to the control group, ^{*} $p < 0.05$ compared to the diabetic group.

Table 1, the diabetic rats indicated a decrease in the heart rate compared to the control rats (198 ± 41.21 vs. 268 ± 27.99 , $p = 0.002$). Obviously, the administration of diabetic group with TMZ significantly increased the heart rate compared to the untreated diabetic rats (263 ± 35.02 vs. 198 ± 41.21 , $p = 0.006$).

Markers of cardiac function

At the end of the experiment, LVSP, LVDP, $\pm dp/dt$ max and RPP were observed significantly lower in the diabetic group than control group. However, TMZ administration for 8 weeks was associated with a significant increase in these parameters in comparison with the untreated diabetic rats (Table 1).

Effect of TMZ on myocardial hypertrophy

As indicated, the hypertrophy index increased significantly in the diabetic rats on 8 weeks compared to the control group (56.62 ± 6.50 vs. 48.62 ± 7.90 , $p = 0.039$). According to our findings, in the diabetic rats, administration with TMZ remarkably decreased the hypertrophy index when compared to the diabetic rats (41.87 ± 7.50 vs. 56.62 ± 6.50 , $p < 0.001$, Figure 2).

Effect of TMZ on antioxidant enzymes

As indicated in Table 2, antioxidant enzymes, GPx, CAT and SOD significantly decreased in the heart of diabetic animals as compared to the control group ($p < 0.001$, $p = 0.002$, respectively). However, oral administration with TMZ was significantly improved GPx, CAT and SOD ($p < 0.001$, $p < 0.049$, respectively).

Discussion

Our results indicated that alloxan injection significantly increased QT and QTc intervals and decreased heart rate, LVSP, LVDP, RPP, $\pm dp/dt$ max, and cardiac hypertrophy, SOD, GPx and CAT in the heart of the diabetic rats when compared with control group. However, treatment with TMZ was able to improve QT and QTc intervals, heart rate, hemodynamic parameters, SOD, CAT and hypertrophy significantly.

Previous studies have demonstrated that diabetes is associated with the alterations of electromechanical and prolonged QTc interval in the heart.²³

Diastolic and systolic dysfunctions are the earliest manifestations in the development of diabetic cardiomyopathy.²⁴ The $\pm dp/dt$ max, LVSP, LVDP, RPP, cardiac diastolic and systolic indexes, are widely used to evaluate cardiac function. The alloxan-induced diabetic rats progressed cardiac dysfunction as demonstrated by a significant decrease in $\pm dp/dt$ LVSP, LVDP, RPP. TMZ treatment in turn improved each of these parameters.

In our model of type 1 diabetes, ECG indicated prolonged QTc, a finding that is consistent with previous studies. Treatment with TMZ significantly decreased these QT and QTc dispersions. This result is in agreement with previous reports which indicated that TMZ treatment improved QT prolongation in individuals with kidney disorders.^{25,26}

In the present study, we also observed that diabetes led to bradycardia in the diabetic animals. It is revealed that in the diabetic rats heart rate tends to decrease after eight weeks.²⁷ On the other hand, diabetes increases vagal tone and decreases sympathetic tone in diabetic rats.²⁸ In addition, treatment with TMZ improves autonomic tone in individuals with acute coronary syndrome.²⁹ Improved sympathetic and parasympathetic tone can partly explain the increased heart rate in the diabetic rats treated with TMZ.

Diabetic cardiomyopathy is associated with cardiac hypertrophy and dysfunction. High blood glucose and oxidative stress maybe considered to be critical factors that involved in hypertrophy and dysfunction of the heart.³⁰ In the present study, the diabetic rats showed cardiac hypertrophy demonstrated by the increased heart weight/body weight ratio. Similar results have been indicated in previous studies.³¹ It is well established that, increased VLDL-c and decreased HDL-c levels can result in reduction in anti-oxidant defense system.²⁷ In a previous study, it was indicated that the impairment of lipid profile levels in diabetic animals could be attributed to increased lipid breakdown and release of a large amount of free fatty acids.¹⁷ The released free fatty acids are susceptible to oxidation

Table 1 – Hemodynamic parameters in the heart

Groups	C	D	T10	P value D VS. C	P value T10 VS. D
Heart rate (beats/min)	268 ± 27.99	198 ± 41.21	263 ± 35.02	0.002 ^{##}	0.006 [”]
LVSP (mmHg)	75 ± 20.91	60.78 ± 16.76	79.75 ± 10.16	0.041 [#]	0.028 [’]
LVDP (mmHg)	74.37 ± 18.76	56 ± 18.37	74.25 ± 9.93	0.030 [#]	0.031 [’]
RPP (mmHg)	14965 ± 5582	10184 ± 4589	14099 ± 3859	0.041 [#]	0.049 [’]
Max +dp/dt(mmHg)	2294 ± 255.27	1035 ± 370.33	1727 ± 410.60	< 0.001 ^{###}	0.001 [”]
Min -dp/dt (mmHg)	-1220 ± 229.09	-594.77 ± 210	-962 ± 194	< 0.001 ^{###}	0.002 [”]

(Mean ± SD, n = 8) in control (C), diabetic (D) and diabetic treated with TMZ (T10), (one-way ANOVA followed by LSD post hoc test). LVSP: left ventricular systolic pressure; LVDP: left ventricular diastolic pressure; RPP: rate pressure product.

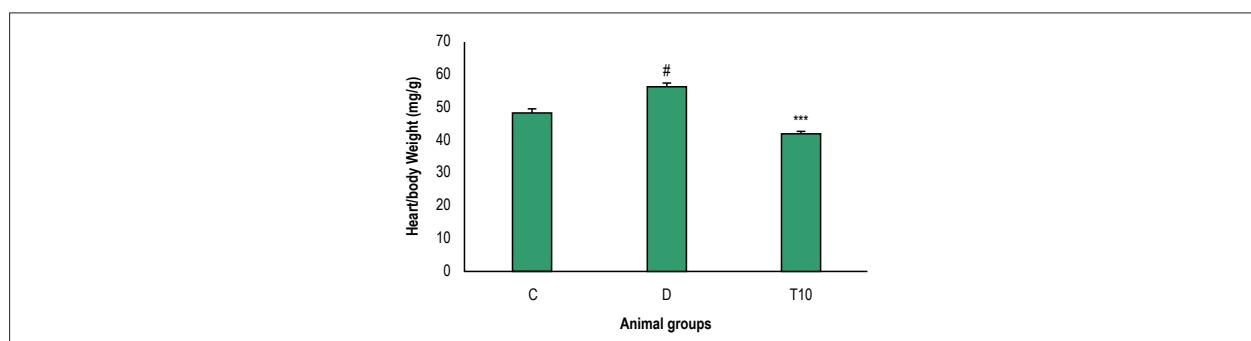


Figure 2 – Hypertrophy value in control (C), diabetic (D) and diabetic treated with TMZ (T10) groups eight weeks after treatment in the rats. The results were presented as mean ± SD. # p < 0.05 compared with control group, *p < 0.001 compared to the untreated diabetic group.**

Table 2 – Antioxidant enzymes activities

Groups	C	D	T10	P value D vs. C	P value T10 vs. D
SOD (U/dl)	8.46 ± 1.51	5.86 ± 0.69	7 ± 1.54	0.002 ^{##}	0.049 [’]
CAT (U/ dl)	10.52 ± 0.60	1.90 ± 4.08	10.71 ± 0.50	0.002 ^{##}	< 0.001 ^{***}
GPx (U/ dl)	28.50 ± 2.67	13.22 ± 0.95	24.03 ± 1.73	< 0.001 ^{###}	< 0.001 ^{***}

(Mean ± SD, n = 8) in control (C), diabetic (D) and diabetic treated with TMZ (T10), (one-way ANOVA followed by LSD post hoc test). SOD: superoxide dismutase. CAT: catalase; GPx: glutathione peroxidase.

which result in decreased anti-oxidant level and anti-oxidant defense system.³² Increased level of fatty acid oxidation in the diabetic heart leads to lipid accumulation and cardiac hypertrophy.³³ Reduction in fatty acid oxidation and oxidative stress by TMZ treatment can partly attribute to improvement of cardiac hypertrophy.

Previous studies have indicated that SOD level reduced in type 1 diabetes and it is mostly demonstrated that increased reactive oxygen species (ROS) negatively associated with the enzyme antioxidant values such as SOD and GPx.³⁴ SOD quickly alters O₂ to H₂O₂, which is further destroyed via GPx and CAT. The antioxidant enzyme levels are sensitive to the oxidative stress, and enhanced or reduced values have been indicated in various pathologies in which an increase of ROS is a cause or result of the disorder such as diabetes.^{35,36} In addition, superoxide anions and ROS have also been indicated to be contributed to cardiac hypertrophy resulted from various stimuli; therefore, SOD is a primary defense against oxidative stress that involves in the hypertrophy of the heart.³⁷ Our findings indicated that SOD and CAT levels in

hearts from TMZ treated diabetic rats was significantly higher than that in the untreated diabetic animals. GPx values was slightly but not significantly more in the hearts from TMZ treated diabetic animals compared to the diabetic rats.

Taken together, these findings indicated that the diabetic rats showed hypertrophy and dysfunction in the heart as well as increased cardiac oxidative damage in comparison with the control animals, showing that these undesirable factors are connected. TMZ probably improved these factors by antioxidant effects. Based on the results of present study, more studies require to be carried out to assessment mechanisms involved in the improvement of hypertrophy and cardiovascular disorders resulted from diabetes using TMZ treatment.

Conclusions

All these observations show that TMZ treatment contributes to the improvement of impaired function and electrical activity as well as hypertrophy of the heart in diabetic cardiomyopathy in rats. Improvements observed in TMZ treatment is associated with decrease oxidative stress.

Author contributions

Design and conception of the study: Ramezani-Aliakbari F, Badavi M; Acquisition of data: Dianat M, Mard SA, Ahangarpour A; Analysis and interpretation of the data: Dianat M, Mard SA, Ahangarpour A; Statistical analysis: Ramezani-Aliakbari F, Badavi M; Obtaining financing: Badavi M; Writing of the manuscript: Ramezani-Aliakbari F, Badavi M; Critical revision of the manuscript for intellectual content: Badavi M.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by Ahvaz Jundishapur University of Medical Sciences (grant No. APRC-94-25).

Study Association

This article is part of the thesis of Doctoral submitted by Fatemeh Ramezani-Aliakbari, from Physiology Research Center of Ahvaz Jundishapur University of Medical Sciences.

Ethics approval and consent to participate

This study was approved by the Ethics Committee on Animal Experiments of the Ahvaz Jundishapur University of Medical Sciences under the protocol number APRC-94-25.

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Cardiac Effects of Trimetazidine in Diabetic Rats

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Short Editorial related to the article: *The Effects of Trimetazidine on QT-interval Prolongation and Cardiac Hypertrophy in Diabetic Rats*

Diabetes mellitus may be associated with a specific form of cardiomyopathy independent of other comorbidities. Left ventricular hypertrophy is the main pathological change described in cardiomyopathy of patients with diabetes mellitus. Different mechanisms may be operative in the pathogenesis of the hypertrophy: metabolic derangements, inflammation and among other factors, oxidative stress.¹ Oxidative stress may lead to cellular damage by free radical-induced oxidation of DNA, proteins and lipids.²

Reactive oxygen species may lead to consequences in cardiomyocytes including hypertrophy, apoptosis and fibrosis; in this regard a cellular detoxification mechanism may be missing for attenuating severity of damage induced by some

reactive oxygen species.³ Hence, interventions that might be protective would be worth to be investigated, including drug therapy.

Trimetazidine (1-[2,3,4-trimethoxybenzyl] piperazine dihydrochloride)⁴ is one of the drugs that may be used in combination with other drugs for the treatment of patients with angina pectoris.⁵ It is a piperazine derivative⁶ characterized as a metabolic modulator⁵ that reduces long-chain fatty acid (3-ketoacyl CoA thiolase) oxidation.⁴

In this issue,⁷ an experimental study of alloxan induced diabetes in Sprague-Dawley rats tested the hypothesis that the administration of trimetazidine might prevent pathologic changes induced in the heart of the studied animals, including QT interval, heart weight relative to body weight, myocardial contractility indices and antioxidant enzyme activities (superoxide dismutase, catalase and glutathione peroxidase). The authors found that the administration of trimetazidine reduced the modifications in the studied variables induced by diabetes in the rats. Thus, additional experimental findings were added to current knowledge about the interaction between diabetes, cardiomyopathy and drug therapy in experimental animals, rats in this specific study. In the event of progress in accumulating knowledge together with other studies, evidences may evolve to deserving clinical studies.

Keywords

Diabetes Mellitus; Diabetic Cardiomyopathies; Hypertrophic, Left Ventricular/metabolismo; Oxidative Stress; Angina Pectoris/metabolismo; Trimetazidine/therapeutic use.

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DOI: 10.5935/abc.20190012

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Evaluation of Collagen Fibers, MMP2, MMP9, 8-OHdG and Apoptosis in the Aorta of Ovariectomized LDL Knockout Mice Submitted to Aerobic Exercise

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Abstract

Background: In menopause, there is greater cellular exposure to oxidative stress, related to the decreased antioxidative effects of estrogen. These metabolic changes favor the progression of cardiovascular diseases, such as atherosclerosis. Abnormal function of the aorta - the most important artery - is associated with many cardiovascular diseases. Collagen, especially types I and III, is one of the most important aortic wall components and it can be affected by many factors, including menopause. The 8-OHdG is one of the main markers of DNA oxidative damage induced by reactive oxygen species (ROS).

Objective: We aimed to investigate effects of moderate aerobic training on the ascending aorta of LDL-knockout (LDL-KO) and ovariectomized female mice.

Methods: A total of 15 C57BL/6 mice and 15 LDL-KO mice were divided into experimental groups. The thickness and volume density of types I and III collagen fibers were performed by morphoquantitative analysis, whereas the MMP-2 and MMP-9 and 8-OHdG were detected by immunohistochemistry and apoptosis was detected by the TUNEL assay. The significance level for all tests was $p < 0.05$.

Results: Exercise causes an increase in the thickness of the aorta in LDL-KO groups, particularly accentuated in the ovariectomized groups. The type I collagen fibers showed an increase in volume density influenced by training in both Control groups and in the LDL-KO group. Type III collagen density decreased in both groups. The MMP-2 showed moderate immunostaining in the tunica media in LDL-KO groups, which did not occur in the control groups and the MMP-9 stained irregularly in all tissues. The marker 8-OHdG was stronger in the exercise training groups. Additionally, the ovariectomy, the exercise training and the LDL-KO treatments increased apoptosis.

Conclusion: These results suggest that moderate-intensity aerobic exercise in ovariectomized mice associated to an increase in LDL rate possibly increases oxidative stress and apoptosis induction. (Arq Bras Cardiol. 2019; 112(2):180-188)

Keywords: Rats; Cardiovascular Diseases; Menopause; Fibrillar Collagens/analysis; Ovariectomy; Exercise; Cholesterol, LDL.

Introduction

Menopause is a period during which women suffer changes in metabolic profile due to decreased production of hormones such as estrogen.¹⁻³ These metabolic changes favor the progression of cardiovascular diseases, such as atherosclerosis.⁴ Abnormal function of the aorta - the most important artery - is associated with many cardiovascular diseases. Collagen, especially types I and III, is one of the most

important aortic wall components and it can be affected by many factors, including menopause.⁵

Physical exercises are recommended for preventing cardiovascular diseases during menopause.^{6,7} However, moderate-to-high intensity physical activity causes increased oxidative stress in cells and tissues, raising the risk of cardiovascular disease.⁸⁻¹⁰ The adaptation of the body to oxidative stress may be impaired in individuals with low levels of estrogen, which binds to specific cellular receptors and accelerate the production of various antioxidants by cells.

Little is known about the effects of physical activity on the development of atherosclerosis and metabolic changes that are characteristic of menopause. Relevant data for the elucidation of these effects have been obtained with the use of markers such as 8-hydroxydeoxyguanosine (8-OHdG), metalloproteinases (MMPs), apoptosis detection and quantification of collagen types III and I.

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Manuscript received December 09, 2017, revised manuscript May 04, 2018, accepted July 23, 2018

DOI: 10.5935/abc.20180263

8-OHdG is one of the main markers of DNA oxidative damage induced by reactive oxygen species (ROS).^{11,12} MMPs play key roles in the function of various tissues during growth, development and aging of the organism.¹³⁻¹⁷ The excessive or unbalanced MMP activity is associated with the pathogenesis of many diseases.^{18,19} among them cardiovascular diseases, such as atherosclerosis.²⁰

The detection of apoptosis in tissues is a marker related to mitochondrial injury, reactive oxygen species production, and oxidative stress. In apoptosis, DNA breakage results in several fragments with free 3'-OH ends. The identification of cells undergoing the process of apoptosis consists in detecting enzymatically the free 3'-OH ends with the addition of nucleotides modified by the TdT enzyme (terminal deoxynucleotidyl transferase).

Thus, we aimed to verify the effects of moderate aerobic training on the ascending aorta of, low-density lipoprotein receptor LDL knockout and ovariectomized female mice.

Methods

Animals and group formation

The experiments were performed in 15 female mice C57BL/6 and 15 of low-density lipoprotein receptor knockout female mice (LDL-KO) weighing 20-25g, from the Animal House of the São Judas Tadeu University, São Paulo, Brazil. The mice received the standard laboratory chow and water *ad libitum*. The animals were placed in cages in a room with controlled temperature (22°C) and a 12-h light-dark cycle. All surgical procedures and protocols were approved by the Experimental Animal Use Committee of Universidade São Judas Tadeu (058/2007). After a simple randomization, the mice were divided into six groups (n = 5): sedentary control (S-C), ovariectomized sedentary control (OS-C), ovariectomized trained control (OT-C), sedentary LDL KO (S-LDL KO), ovariectomized sedentary LDL KO (OS-LDL KO) and ovariectomized trained LDL KO (OT-LDL KO). The animals were separated physically and randomly between the groups / boxes.

The sample size definition was performed according to previous data from other authors,²¹⁻²³ which were based on the instructions of CONCEA (Conselho Nacional de Controle de Experimentação Animal) Normative Instruction N°. 27 and determined by the formula $n = (2\alpha/2\delta) 2/E^{24}$ was used, where n stands for sample size; $(2\alpha)^2$ stands for a critical value that corresponds to the desired degree of confidence, δ stands for the population standard deviation and E stands for the margin of error (difference between the sample mean and the mean of the true population).

Ovariectomy

At nine months of age, the animals were anesthetized (ketamine 120 mg/kg + xylazine 20 mg/kg), and a small abdominal incision was performed where the ovaries and oviducts were found, sectioned and removed. Then, the skin and muscle wall were sutured.^{25,26} The efficacy of the ovariectomy was determined by observation of vaginal secretions during four consecutive days.

Training protocol

Seven days after the ovariectomy, all animals were adapted on the treadmill for ten minutes during three days before initiating the training. The maximal exercise test was performed in all groups at the beginning and at the ending of the training program, providing the basis for the prescription of physical training, and with the purpose to evaluate the physical capacity of the trained animals.

The trained groups were submitted to a moderate physical training protocol on a treadmill, with progressive speed and load (1 hour a day/5 days a week at 50-60% of maximum effort speed) during 4 weeks, as previously described.²⁷

Histological procedures

At the end of the experiment, the animals were weighed and subsequently euthanized by decapitation. Thoracotomy was performed by cutting the ascending aorta at the heart base. The aorta samples were washed (PBS - phosphate buffered saline 0.1M, pH 7.4) and fixed in 10% formaldehyde for 24 hours. Then, they were dehydrated, cleared and embedded in paraffin. The aorta was sectioned transversely (5 μ m thick), and the samples were stained in H&E for histomorphometric analysis.⁷ In order to analyze the effects of aerobic training on the whole aorta, the tunica intima was not separated from the tunica media. Picrosirius staining was used for classification of collagen fibers I and III. The images were captured at 4 points, at 0°, 90°, 180° and 270°, with x10 magnification to measure the aorta thickness, and x40 for other evaluations, and transferred to an image analysis program (Axion Visio Software, Zeiss®). To analyze the volume density of types I and III collagen fibers, the images were captured by light microscope with polarized light, analyzed using a test system of 252 points, and the values were expressed as percentages.

Immunohistochemical analysis: 8-OHdG, MMP-2 and MMP-9

Five 4- μ m cross-sections, mounted on previously silanized slides, were used to show the expression of 8-OHdG, MMP-2 and MMP-9. The slides were then deparaffinized, cleared, hydrated and washed in running water. Then, endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide, protein blocking was performed with 0.3% skim milk diluted in PBS and the slides were incubated overnight with anti-8-OHdG (SC66036 Santa Cruz® Biotechnology, CA, USA) primary antibody, titrated 1:100, and MMP2/72KDa and MMP9/KDa (SC-10436 Santa Cruz® Biotechnology, CA, USA; SC-6840 Santa Cruz® Biotechnology, CA, USA), titrated 1:150 in PBS-BSA 0.1%. All slides were then placed in a humid chamber at 4°C overnight. The material was washed with PBS buffer and incubated with biotinylated secondary antibody. For revelation, the 3-3'-diaminobenzidine chromogenic substrate was used at a ratio of 0.06 g per 100 mL of PBS, and 1 mL of 20-volume H₂O₂ for five minutes at 37°C and counter-stained with Mayer's hematoxylin for 3 minutes. Finally, the slides were mounted with cover slips and entellan® for analysis under light microscopy.

Investigation of the apoptotic cell death by TUNEL immunocytochemistry

TUNEL staining was performed using the ApopTag Peroxidase In Situ Apoptosis Detection Kit (Millipore®, Germany) according to the manufacturer's instructions.

Quantification of apoptotic cells

For the quantification of immunostained cells for apoptosis, 30 images of the intima-media layer were captured (10 images/animal; $n = 3$ animals/group; with $\times 10$ magnification to measure the aorta thickness, and $\times 40$ for other evaluations, and transferred to an image analysis program (Axion Visio Software, Zeiss®) for each experimental group. For each image, the total number of immunostained cells was obtained as a relative frequency (%) in relation to the total number of cells. The light microscope coupled to a digital camera (Zeiss, Germany) was used to obtain the images, and the photomicrographs were scanned with the AxioVision software (Zeiss®, Germany).

Statistical analysis

The results were presented as mean and standard deviation. Analysis of variance (ANOVA) and Tukey's post-hoc tests were properly applied in data analysis. The significance level for all tests was $p < 0.05$. The data were evaluated using the software Stata 7.0. All continuous variables were normally distributed (Shapiro-Wilk test). To evaluate the normality of the data, the Shapiro-Wilk calculation was used, which found that the data were allocated within the Gaussian curve. Considering a level of significance of 5%, the test assumed the normality hypothesis for the variable with normal distribution.

Results

Histopathological and histomorphometric analysis

The histopathological analysis showed that the animals from the control groups (S-C, OS-C and OT-C) did not exhibit changes in the elastic fiber arrangement and thickness pattern. However, the dyslipidemic groups (S-LDL KO, OS-LDL KO and OT-LDL KO), showed greater spacing between elastic fibers (Figure 1).

Thickness of the tunica media - intima (μm)

We observed a significant increase in the thickness of the tunica media and intima in dyslipidemic animals, when compared to the control group animals. The ovariectomy and the exercise in the LDL KO groups were a determining factor for the increase of this variable. In both control and LDL KO groups the training exercise did not reverse this process (Figures 1 and 2).

Volume density of types I and III collagen fibers in the intima-media and adventitia tunica

Similar behavior of the type III collagen fiber was observed between the intima-media and adventitia tunica. We observed a significant decrease in the volume density of the type III collagen fiber in the LDL KO groups, when compared to the S-C (Figure 3).

The type I collagen fibers of the tunica adventitia and media-intima showed increase in volume density, influenced by training in the Control groups. The dyslipidemia induces an increase in type I collagen fibers in the LDL KO groups when compared to the Control groups and did not undergo any change by ovariectomy, or by training (Figure 3).

Immunohistochemical analysis

Figure 4 shows the tissue staining caused by the oxidative stress marker 8-OHdG. Note that the immunoexpression of the marker occurred in all groups. The staining was moderate for LDL KO and Control groups, both the Sedentary and the Ovariectomized Sedentary groups. However, for the Ovariectomized and trained groups, the observed staining was intense.

In all control groups (S-C, OS-C and OT-C) the immunoexpression of MMP-2 occurred both in the tunica intima (arrow) and in the tunica adventitia (arrowhead) of

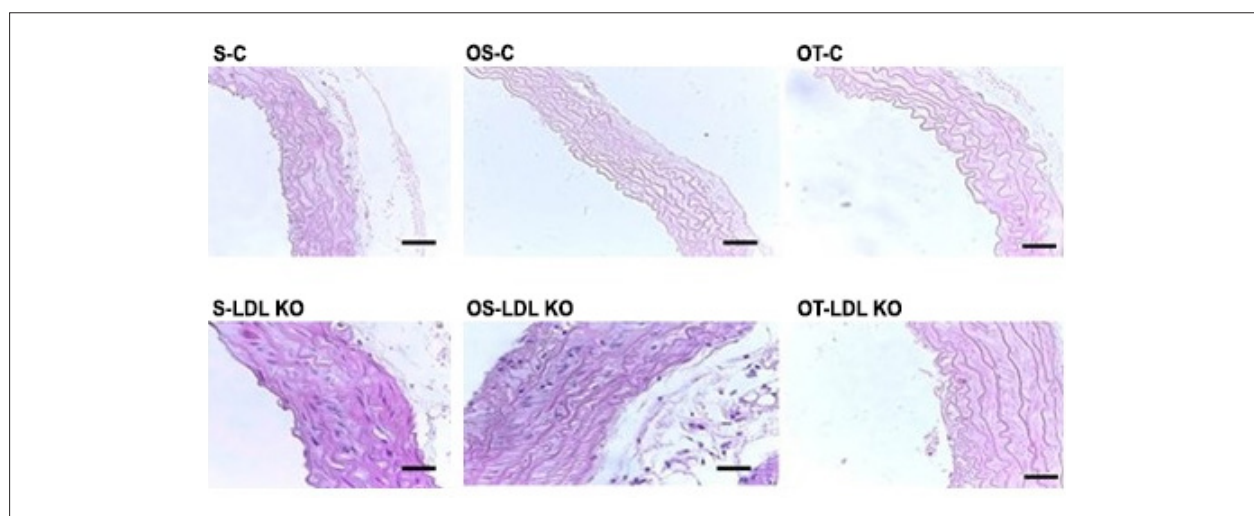


Figure 1 – Mouse aorta cross-sections showing the arrangement of elastic fibers. The control groups showed a similar pattern of arrangement and thickness of the elastic fibers. The LDL KO groups showed more spaced fibers and thicker vessel walls compared to the controls. Photomicrographs, H&E. Calibration Bar = 100 μm . Sedentary control (S-C), ovariectomized sedentary control (OS-C), ovariectomized trained control (OT-C), sedentary LDL KO (S-LDL KO), ovariectomized sedentary LDL KO (OS-LDL KO) and ovariectomized trained LDL KO (OT-LDL KO).

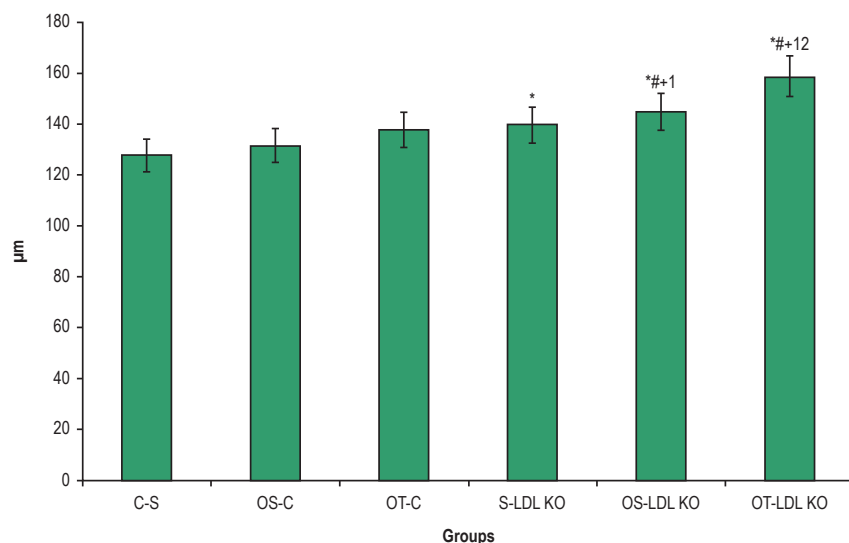


Figure 2 – Thickness of the tunica media-intima (μm) in the studied groups. Values are expressed as $M \pm SD$. * $p < 0.05$ vs. S-C; # $p < 0.05$ vs. OS-C; + $p < 0.05$ vs. OT-C; $^1p < 0.05$ vs S-LDL KO; $^2p < 0.05$ vs OS-LDL KO.

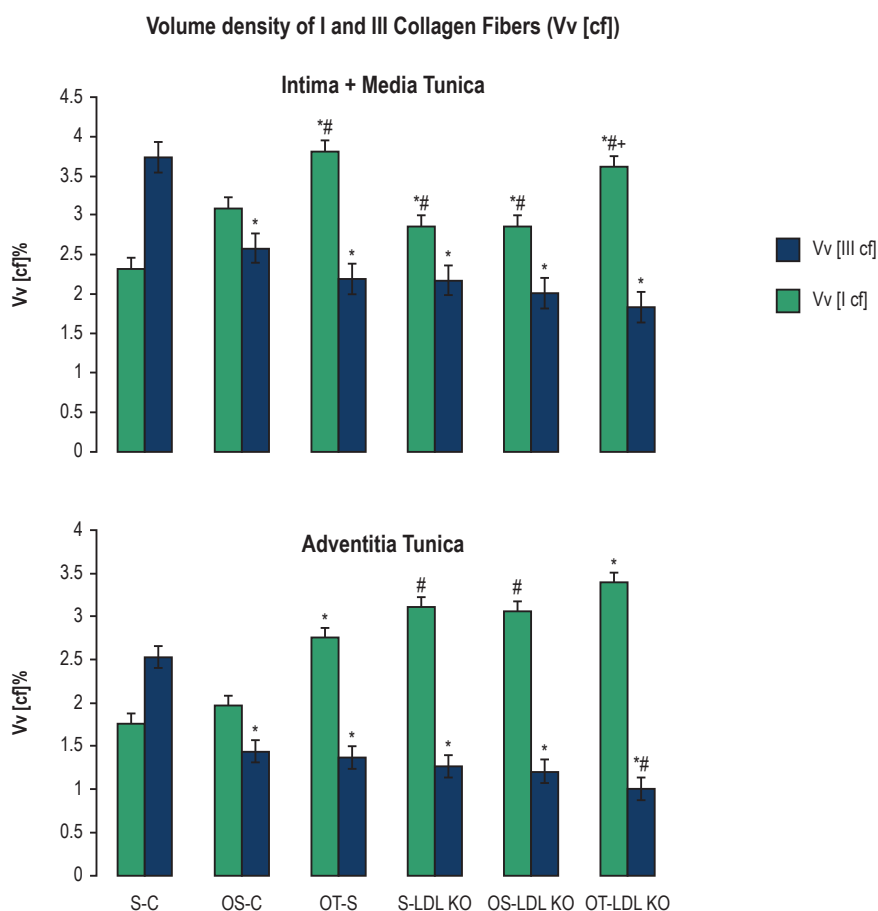


Figure 3 – Volume density of types I and III collagen fibers (Vv[cf]) in Intima-Media and Adventitia Tunica of ascending aorta. Values are expressed as $M \pm SD$. * $p < 0.05$ vs. S-C; # $p < 0.05$ vs. OS-C; + $p < 0.05$ vs. OT-S.

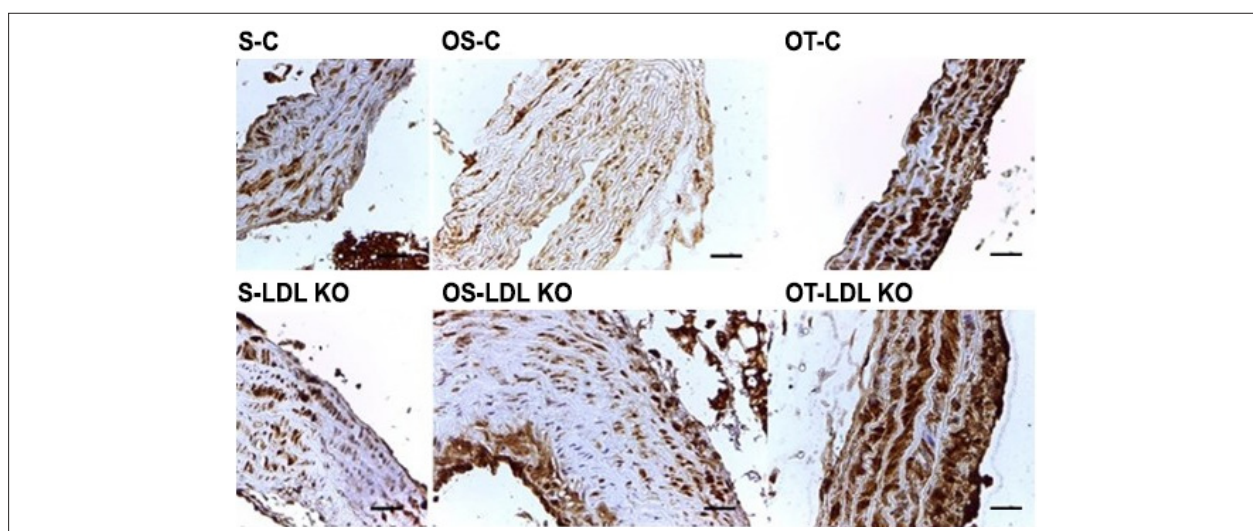


Figure 4 – Photomicrographs of cross-sections of the aorta of mice submitted to immunohistochemical reaction for 8-OHdG. The immunoexpression of 8-OHdG was observed in all investigated groups. The staining was moderate for LDL KO and Control groups, both the S and the OS. However, for the O and T groups, the observed staining was intense. Calibration bar = 100 μ m.

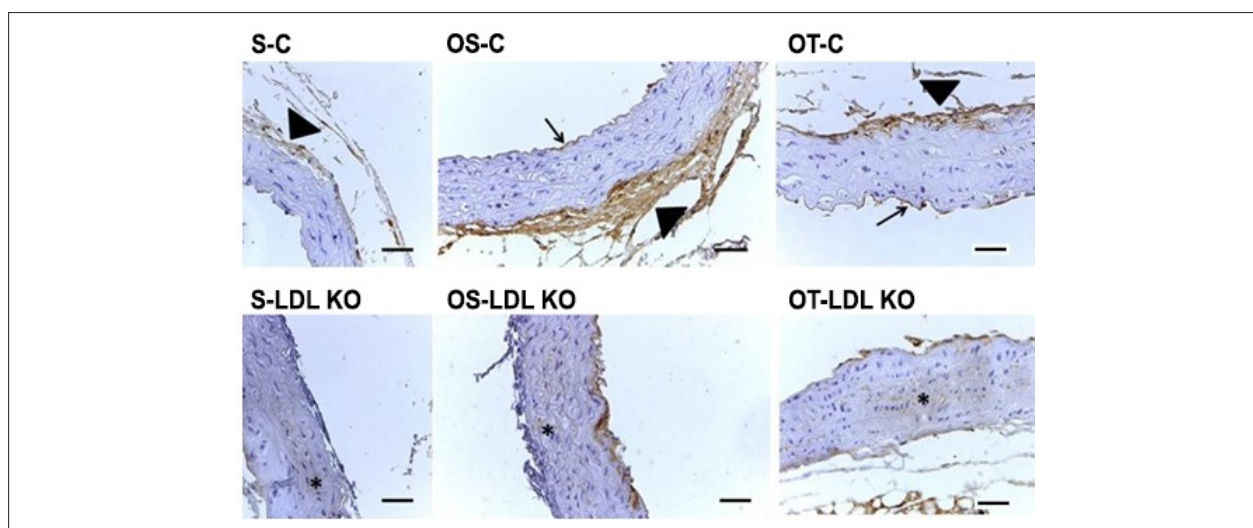


Figure 5 – Photomicrographs of cross-sections of the ascending aorta of mice submitted to immunohistochemical reaction for MMP-2. Note the presence of MMP-2 immunoexpression in the tunica intima (arrow) and adventitia (arrowhead) in all control groups (S-C, OS-C and OT-C). In general, the MMP-2 immunoexpression in the LDL KO groups was observed in the intima and adventitia layers, as well as in the middle layer of the aorta (*), which did not occur in any animal of the control groups. Calibration bar: 100 μ m.

the ascending aorta. The LDL groups, in general, showed MMP-2 immunoexpression beyond the tunica intima and adventitia, but also in the tunica media of the aorta (although very slightly), which was not observed in any groups of control animals (Figure 5). The MMP-9 was expressed in all layers of the ascending aorta of all groups; however, the distribution was heterogeneous (Figure 6).

Apoptotic cells were distributed in the media-intima layer of the ascending aorta in all studied groups. The comparison of relative frequency showed that Ovariectomy statistically increased apoptosis in +12,6% in the sedentary group (OS-C) and +19% in physical training group (OT-C) when compared to the apoptosis of the sedentary control group (S-C) (Figure 7).

Among the knockout groups, apoptosis rates were higher than the respective controls, regardless if the mice were ovariectomized or not. Thus, the knockout sedentary group increased apoptosis frequency in +28,8% when compared with the control group. Ovariectomy and knockout statistically increased the apoptosis in +24,5% in the sedentary (OS-LDL KO) and +32,3% in the physical training (OT-LDL KO) groups, when compared to the sedentary control group (S-C). No significant change was observed when comparing the knockout sedentary group (S-LDL-KO) to knockout ovariectomy sedentary (OS-LDL KO) or physical training (OT-LDL KO) groups, with decreased apoptosis in -4,3% and improved apoptosis in +3,5%, respectively. However, the relative frequency of ovariectomy, sedentary and

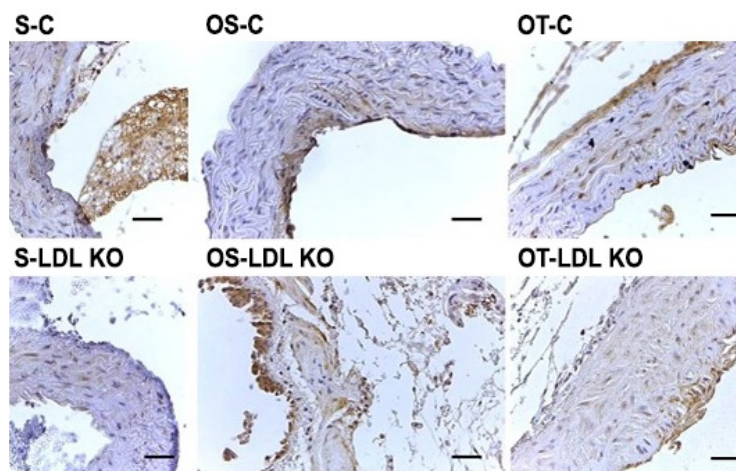


Figure 6 – Photomicrographs of cross-sections of the ascending aorta of mice submitted to immunohistochemical reaction for MMP-9. Note the presence of immunorexpression in all layers, heterogeneously, in all groups. Calibration bar: 100 μ m.

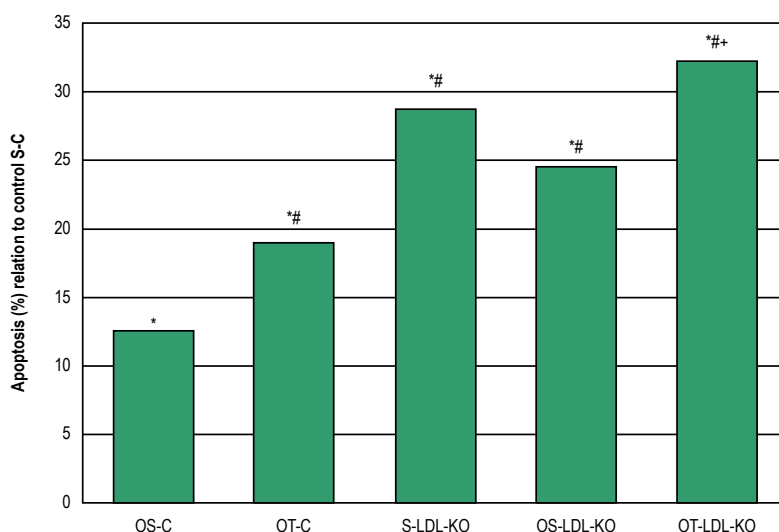


Figure 7 – Relative frequency (%) of apoptotic cells in the ascending aorta of the control sedentary group (S-C) vs. the different evaluated groups as evidenced by the TUNEL assay. * $p < 0,05$ vs. S-C, # $p < 0,05$ vs. OS-C, + $p < 0,05$ vs. OS-LDL-KO. Values are expressed as mean $\pm$ SD ($p < 0,05$).

physical training knockout groups showed to be statistically increased in +7,8% (Figure 7).

Discussion

The main objective of this study was to verify the effects of moderate aerobic training on the ascending aorta of ovariectomized female mice, knockout for the low-density lipoprotein receptor LDL through the analyses of types I and III collagen fibers, the expression of 8-OHdG oxidative stress markers of MMP-2 and MMP-9 metalloproteinases.

Collagen and elastin are major structural and functional components of the arterial wall. These components actively participate in arterial wall remodeling in response to hemodynamic alterations and during atherogenesis.

The histopathological analysis did not show any morphological changes in the control group. However, the LDL KO group showed increased thickness and larger spacing between the elastic fibers. Some studies have demonstrated an association between an increase in intima-media thickness and the occurrence of cardiac events,²⁸ which confirmed the existence of the association between increased intima-media thickness of the carotid and the presence of cardiovascular risk factors, including infection and inflammation markers. Collagen I is mostly a structural collagen; collagen III, in turn, is more frequent in pathological processes. Our results have shown that the number of cells in apoptosis were significantly lower in the control group and remained constant in the Control groups (OS-C and OT-C) and the LDL knockout groups (S-LDL KO, OS-LDL KO and OT-LDL KO), without significant

differences in the percentage of apoptotic cells between the Control or LDL Knockout groups for each parameter used. However, the apoptosis process was greater in animals of LDL knockout groups, when compared with animals of the control groups, regardless if they were ovariectomized or not. This suggests that ovariectomy was not a major factor in the processes of apoptosis induction in the aorta.

The high levels of LDL in the bloodstream may have been the apoptosis-inducing factor in the endothelium and the tunica media of the ascending aorta in LDL knockout groups. The endothelial dysfunction induced by LDL oxidation (ox-LDL) has been associated to the pathogenesis of atherosclerosis and other vascular disorders. It is known that the ox-LDL activates ROS release and has been associated to apoptosis and endothelium damage.²⁹ The apoptosis of vascular smooth muscle cells (VSMC) is associated with the occurrence of vascular diseases. In atherosclerosis, cell apoptosis induction has been associated with atherosclerotic plaque rupture, clotting, vessel remodeling, tunica media atrophy, aneurysm formation and calcification.³⁰ Furthermore, in various human diseases such as Marfan syndrome and cystic necrosis of the tunica media (CMN), the apoptotic processes results in higher breakage of the elastic fibers, abnormal extracellular matrix deposition and tunica media expansion.³¹ In this environment, the release of interleukin IL1 α and IL8, as well as the chemoattractant protein expression of monocytes (MCP-1) occurs during the VSMC apoptosis, which have causes infiltrating macrophages *in vivo*, increasing the observed tissue damage.³²

The animals of the OT-C group showed a higher number of apoptotic cells compared to the OS-C and S-C groups ($p < 0,05$). Physical activity has been associated with increased apoptosis levels in rats' thymocyte, mice skeletal muscle and lymphocytes.^{33,34} Oxidative stress resulting from metabolism in physical activities has been largely associated to apoptosis. In patients with cardiovascular diseases, the deficiency in nitric oxide (NO) production, associated with oxidative stress, results in a decline of NO bioavailability, inducing apoptosis of endothelial cells and therefore, resulting in endothelial dysfunction.³⁵ The ovariectomized animals showed a higher percentage of apoptotic cells than the trained and sedentary control groups (OT-C and OS-C). This suggests that decreased hormone production may be related to a reduction in antioxidative effects on the body.

8-OHdG is one of the main oxidative products of DNA, which is considered a reliable marker of oxidative DNA damage. Thus, 8-OHdG has been widely used as a sensitive biomarker of oxidative stress.³⁶ The immunohistochemical analysis of 8-OHdG showed staining in all groups; however, the trained groups showed higher intensity staining. Goto et al.,⁹ found that high-intensity exercise increases 8-OHdG levels in the plasma, which explains the higher degree of staining in the trained groups.

There were differences in MMP-2 expression, and the control group showed staining in the intima and adventitia layers, while the LDL KO group showed staining in the tunica media. According to Sakalihasan et al.,³⁷ this occurs because the atherosclerotic lesion causes the migration of MMP-2 at the ends and at a lower quantity in normal tissue, for the tunica

media, i.e., the formation of atherosclerotic plaques activates a set of chain reactions, which can increase the amount of MMP-2 present in the tissue. With regard to the MMP-9 expression, all groups showed tissue staining, but without a pattern. No evidence explaining this heterogeneous staining was found.

Conclusion

The experimental model analyzed shows histomorphometric changes with increased expression of 8-OHdG in trained groups. An increase in the apoptosis rate was observed in the trained groups and the LDL KO ovariectomized group. The groups stained with MMP-2 showed migration and its increased expression in the tunica media of LDL KO groups. However, the MMP-9 staining appeared in all groups, but did not follow a homogeneous pattern. Finally, studies on the expression of metalloproteinases in cardiac muscle tissues with atherosclerosis are very scarce, suggesting the need for further studies to investigate the issue. Thus, the results described herein suggest that moderate-intensity aerobic exercise in ovariectomized mice associated to an increase in LDL rates possibly increases oxidative stress and apoptosis induction.

The evaluation of the parameters under study (MMPs, apoptosis and 8-OHdG) was performed by immunohistochemistry. However, other more sensitive technologies (such as molecular biology ones) could be used in these assessments, leading to more precise results and interpretations.

Author contributions

Conception and design of the research: Maifrino LBM, Souza LB, Quintana HT, Fonseca FLA; acquisition of data: Maifrino LBM, Lima NEA, Cardoso CG, Souza LB, Oliveira F, Fonseca FLA; analysis and interpretation of the data: Lima NEA, Cardoso CG, Souza LB, Tomé TC, Oliveira F, Reis BCAA; statistical analysis: Marques MR, Quintana HT, Oliveira F; obtaining funding: Maifrino LBM; writing of the manuscript: Maifrino LBM, Lima NEA, Marques MR, Souza LB, Oliveira F, Reis BCAA; critical revision of the manuscript for intellectual content: Maifrino LBM, Marques MR, Cardoso CG, Tomé TC, Reis BCAA, Fonseca FLA.

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.

Ethics approval and consent to participate

This study was approved by the Ethics Committee on Animal Experiments of the Universidade de São Judas Tadeu under the protocol number 058/2007.

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Analysis of Iron Metabolism in Chronic Chagasic Cardiomyopathy

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Abstract

Changes in iron metabolism in heart failure (HF) have been described as an important prognostic marker.

To check if the markers of iron kinetics are related to the morbidity and etiology of chagasic cardiomyopathy.

Patients with Chronic Chagasic Cardiomyopathy (CCC, n = 40), with indeterminate form (IND, n = 40), besides non-chagasic cardiomyopathy (NCh, n = 40).

The mean age was 50.98 ± 5.88 in CCC, 50% were male, 49.68 ± 5.28 in IND, 52.2% were male, and 49.20 ± 10.09 in NCh, 12.5% were male. Lower levels of iron (FeSe) were observed in the CCC groups (93.15 ± 36.53), when compared to IND (125.30 ± 22.79) and NCh (114.77 ± 18.90) ($p = 0.0004$), lower IST transferrin saturation index in CCC (29.48 ± 6.59), when compared to IND (30.95 ± 7.06) and in the NCh group (39.70 ± 7.54) ($p = 0.0001$), total binding capacity of the lower CTIF iron in the CCC group (297.30 ± 36.46), when compared to the IND group (196.52 ± 56.95) and the NCh group ($275.18 \pm 33, 48$) ($p = 0.0001$), lower ferritin in the CCC group ($134.55, 1.56-42.36$), when compared to the IND group ($156.25, 1.72-42.20$) and the NCh group ($112.95, 2.88-42.66$) ($p = 0.0004$). It was also observed that FeSe (95% CI 1.00-1.04, $p = 0.0014$), IST (95% CI 1.02-1.22) ($p = 0.0012$) and gender (95% CI 1.07-14.43 $p = 0.0038$) were independently associated with the degree of ventricular dysfunction in chagasic cardiomyopathy.

CCC patients showed greater change in iron metabolism regarding the indeterminate form and other forms of cardiomyopathies.

Introduction

Functional iron deficiency (Fe) can be defined as the imbalance between the required amount of Fe for hemoglobin synthesis and its supply.¹ It occurs in the absence of Fe stock, a characteristic of iron deficiency anemia (FA), and in the presence of blockade of Fe homeostasis, as in anemia of inflammation. In AI, cytokines and reticuloendothelial system cells induce alterations that interfere in different pathways of erythropoiesis leading to anemia.²

Keywords

Iron Metabolism Disorders; Chagas Disease; Chagas Cardiomyopathy; Inflammation; Anemia.

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Manuscript received March 20, 2018, revised manuscript August 13, 2018, accepted September 05, 2018

DOI: 10.5935/abc.20190006

Pro- and anti-inflammatory cytokines, derived from macrophages or T cells, as well as acute phase proteins, are believed to be involved in AI Fe homeostasis disorders. The demonstration of the importance of IL-1 and TNF- α in Fe homeostasis occurred from experiments with rats, where the administration of these cytokines was associated with hypoferrremia, and induction of ferritin synthesis by SRW. It is now known that IL-1 and IL-6 are able to modulate the translation of ferritin acting on the 5'-untranslated portion of the ferritin messenger RNA.^{3,4}

IL-6 appears to play a key role in the stimulation of HAMP transcription, although IL-1 α and IL-1 β also play a role in the transcription of this gene. IL-6 has the ability to bind to the cell membrane through specific receptors, and to activate the signal transducer and transcriptional activator 3 (Stat3), the JAK/STAT signaling pathway, which acts on the gene promoter region by stimulating hepcidin transcription. Excessive production of hepcidin occurs in individuals with inflammatory and infectious diseases, particularly Chagas disease, and this excess explains the sequestration of Fe in macrophages, and the inhibition of intestinal absorption of Fe, two hallmarks of AI.⁵

The expression of ferroportin is decreased in SRE cells due to inflammation; it is not only due to the internalization and degradation of ferroportin by the action of hepcidin, but also by a negative regulation of its expression. Alterations in the differentiation and proliferation of erythroid precursors (BFU-E and CFU-E): a blockade is observed due to the inhibitory effect of several cytokines, in particular: interferon- α , β e γ , α tumoral necrosis factor (TNF- α), and interleukin 1 (IL-1). The related mechanism appears to be the induction of apoptosis; however, cytokines also exert a direct toxic effect on progenitor cells by inducing the formation of free radicals.⁶

Chronic chagasic cardiomyopathy (CCC) is the most severe manifestation of Chagas disease (Chd), in which intense and extensive inflammatory and fibrotic action is observed on the myocardium,¹ causing structural and autonomic alterations that affect approximately 20% to 30% of the infected people.^{7,8,9} In addition, CCC presents, as a fundamental morphological substrate, a chronic, progressive and fibrosing and, consequently, clinical myocarditis, ranging from silent to more severe forms, such as refractory heart failure (HF), complex arrhythmias, ventricular aneurysms, and sudden death.¹⁰

The impairment of cardiac function, as well as the progression of neurohormonal and inflammatory compensation mechanisms, can either alter iron metabolism by simply reducing its intestinal absorption, or dynamically change its distribution in the reticuloendothelial and hematopoietic system.^{7,8,9,6,11,12}

Anemia is known to be the last compensatory stage when there is iron bioavailability impairment for the erythropoietic processes from complex pathophysiological mechanisms. The objective of this study was to check if the iron kinetics

markers correlate with the degree of ventricular dysfunction of Chagas cardiomyopathy compared to non-chagasic cardiomyopathy (NCh).

Methods

Study population

Forty patients with CCC, 40 chagasic patients with undetermined form (IND), and 40 patients with NCh were consecutively selected according to the inclusion and exclusion criteria. Patients with CCC and with the indeterminate form of Chagas disease showed confirmatory serology for *T. cruzi*. This study was approved by the Research Ethics Committee of UFMG-COEP with identification number ETIC 359/04.

Study design and procedure

At the time of inclusion, all patients underwent clinical examination, laboratory examination, 12-lead ECG. Functional capacity was assessed by the New York Heart Association (NYHA) scales. The severity of cardiac involvement was determined by echocardiographic indices (ejection fraction [EF], and left ventricular end-diastolic volume, LV).

Hematologic evaluation

Serum iron dosage (FeSe), transferrin saturation index (TSI), total iron-binding capacity (TIBC), and ferritin were sent to the Central Laboratory of Hospital das Clínicas. Their quantifications were done through the following methods:

- serum iron (two-point kinetics)
- transferrin saturation index, ferritin (immunoturbidimetry), were quantified by the calculation (serum iron - total iron-binding capacity).
- total iron-binding fixation capacity (enzymatic kinetics).
- transferrin dosage (immunoturbidimetry).

Sample size calculation

The work by Jankowska et al. (2012), in which functional classes III and IV HF patients were studied, was the basis for the sampling calculation, and showed alterations in iron metabolism and association with the degree of ventricular systolic dysfunction, and morbidity. Sample size was calculated by the BioStat 5.3 Software, for which the mean and standard deviation [$\pm$] of each iron kinetic variable with minimum test power of 0.80 were used, assuming a significance of 0.05% ($> 5\%$) and beta error lower than 20% (test power); we decided to select 40 patients in each IND, CCC and NCh group.

Statistical analysis

Statistical analysis was performed using SPSS Software version 22.0 (SPSS Inc., Chicago, Illinois, United States). A descriptive analysis of continuous and categorical variables was performed. For the presence or absence of normal distribution of variables, the Shapiro-Wilk test was performed. For the multivariate analysis presented in Table 2, the Cox regression model was used, and the association of variables

that were related to death, hazard ratio (HR) and 95% CI was evaluated, assuming a statistical significance of 0.05%.

Selection criteria applied to the multivariate model

Presence of left ventricular systolic dysfunction (LVEF $\leq 35\%$). Left ventricular diameter (LVD) $< 55\text{mm}$. FeSe serum iron $< 31 \mu\text{g/dL}$. When the IST transferrin saturation index is less than 20%. TIBC $< 250 \mu\text{g/dL}$. Ferritin $< 200 \text{mg/dL}$.

Results

The demographic, clinical, laboratory and echocardiographic characteristics of the groups are presented in Table 1. There was a predominance of male patients, and most of the patients were NYHA I functional class, with mean left ventricular (LV) FE below 45%.

In the univariate analysis, it was found that the variables that were associated with left ventricular systolic dysfunction below 35% were TSI, (OR = 0.89, $p = 0.05$), iron (OR = 0.97, ferritin (OR = 1.27, $p = 0.017$), gender (OR = 0.26, $p = 0.05$), HF etiology (OR = 2.40, $p = 0.011$), and anemia (OR = 8.97, $p = 0.04$). In the multivariate analysis, there was an independent association between low left ventricular dysfunction of 35% and TSI (OR = 1.12, $p = 0.012$), FeSe (OR = 1.02, $p = 0.014$), gender (OR = 3.94, $p = 0.038$), and the etiology of HF (OR = 2.6, $p = 0.036$); 35 individuals with left ventricular dysfunction (87.5%) were identified, who presented the outcome in the sample.

Discussion

Iron kinetic markers were found to correlate with the degree of ventricular dysfunction of chagasic cardiomyopathy in relation to NCh; the following observations were obtained as main results: (a) CCC patients, when compared with IND and NCh patients, had lower serum levels of iron, ferritin, TSI and TIBC; (b) patients with CCC have lower serum levels of iron, TSI, TIBC, and ferritin than patients with Chagasic and non chagasic cardiomyopathy; (c) lower serum levels of iron, TSI, TIBC, and ferritin are associated with the level of systolic ventricular dysfunction; (d) low serum levels of iron, TSI, TIBC, and ferritin are associated with the degree of cardiac morbidity.

As demonstrated by the results presented, we observed that patients with CCC, when compared with chagasic patients in the indeterminate form (IND) and those with NCh, present lower serum levels of iron, TIBC, TSI and ferritin. Currently, there are no studies specifically related to CCC and iron metabolism; thus, we will base our pathophysiological hypotheses on studies performed with other causes of cardiomyopathy. Despite the peculiarities of CCC, there seems to be similarity in the genesis of changes in iron metabolism observed in other pathologies and CCC. As possible pathophysiological mechanisms for iron metabolism alterations in HF, some theories have been described, such as chronic inflammation, intestinal loop edema, and hypoperfusion of the gastrointestinal tract (GIT).^{5,6,7} A prospective case control study with 499 patients with chagasic cardiomyopathy reported the persistence of the parasitic element (DNA) through PCR analysis.⁷ There was an association between parasite load and disease severity measured from clinical parameters.⁷

Brief Communication

Table 1 – Demographic, clinical, laboratory and echocardiographic characteristics of the IND, CCC and NCh groups

Characteristics	IND (n = 40)	CCC (n = 40)	NCh (n = 40)	p
Age*	49.68 ± 5.28	50.98 ± 5.88	49.20 ± 10.09	0.929
Height (cm)	1.69 ± 0.065	1.97 ± 0.158	1.94 ± 0.123	0.889
Weight (kg)*	79.100 ± 8.58	73.75 ± 10.15	71.88 ± 11.47	0.621
Male [n(%)]	21/40 (52.2%)	20/40 (50%)	5/40 (12.5%)	0.979
NYHA functional class	-	-	-	-
I	40/40 (100%)	12/40 (30%)	32/40 (80%)	0.410
II	-	15/40 (37.5%)	6/40 (15%)	0.510
III	-	8/40 (2.5%)	0.312	
IV	-	5/40 (12.5%)	1/40(2.5%)	0.112
FeSe (µg/dL)*	125.30 ± 22.79	93.15 ± 36.53	114.77 ± 18.90	0.004
Hb (g/dL)*	14.84 ± 1.56	13.62 ± 1.23	14.02 ± 1.25	0.010
TSI (%)*	30.95 ± 7.06	29.48 ± 6.59	39.70 ± 8.54	0.001
TIBC (µg/dL)*	196.52 ± 56.95	297.30 ± 36.46	275.18 ± 33.48	0.001
Ferritin (ng/mL)**	156.25 (1.7-42.20)	134.5 (1.56-42.36)	112.95 (2.8-42.66)	0.004
LVD (mm)*	46.38 ± 7.34	65.43 ± 7.70	46.38 ± 7.34	0.002
E/e' ratio*	6.6 ± 2.82	14.9 ± 4.58	12.15 ± 12.06	0.001
LVEF**	65.85 ± 5.9	35.92 ± 8.59	34.95 ± 8.12	0.001

NYHA: New York Heart Association functional class; FeSe: serum iron; Hb: hemoglobin; TSI: transferrin saturation index; TIBC: total iron binding capacity; LVD: diameter of the left ventricle in diastole; E/e': diastolic velocity; LVEF: ejection fraction of the left ventricle.

Table 2 – Variables of iron kinetics markers independently associated with left ventricular systolic dysfunction below 35% (multivariate Cox Hazard proportional hazard model)

Variables	Univariate model				Multivariate model			
	OR	95% CI	X ²	p	OR	95% CI	X ²	p
TSI%	0.89	0.816-0.979	5.86	0.015	1.12	1.02-1.22	6.3	0.012
FeSe	0.97	0.95-0.99	5.34	0.021	1.23	1.00-1.04	5.9	0.014
FERRITIN	1.27	1.044-1.56	5.65	0.017	-	-	-	-
Creatinine	1.01	7.54 ± 13.7	1.36	0.243	-	-	-	-
GFR, mL/min/1.73 m	1.00	0.95-1.05	1.41	0.786	-	-	-	-
Hb	0.99	0.56-1.74	0.001	0.99	-	-	-	-
Anemia	8.97	1.01-7.90	3.90	0.04	1.22	0.126-11.83	0.030	0.862

Univariate and multivariate logistic regression analysis of laboratory parameters. FeSe: serum iron; Hb: hemoglobin; TSI: transferrin saturation index; GFR: glomerular filtration rate.

Regarding the analysis of the iron kinetics markers and the echocardiographic variables, we found that the higher the systolic ventricular dysfunction, the lower the serum FeSe, TSI, TIBC, and ferritin levels. This finding is interesting because it reinforces our hypothesis that low levels of iron kinetics markers correlate with the degree of ventricular dysfunction.

In the multivariate analysis, we found low levels of FeSe ($p = 0.014$) and TSI ($p = 0.012$), as well as statistically significant results for gender ($p = 0.038$) as independent markers for left ventricular systolic dysfunction. The fall of ten units of iron is associated with a 23% higher chance of occurrence of systolic ventricular dysfunction. The fall of

ten units of TSI is associated with a 12% higher chance of occurrence of systolic ventricular dysfunction.

Conclusion

In the population studied, analyzes of iron metabolism in patients with CCC showed that there is an association with the degree of myocardial impairment, and the lower the iron serum levels, the total iron binding capacity, the transferrin saturation index and ferritin, the greater the degree of ventricular dysfunction. It is concluded that in chagasic cardiopathy there is a change in the iron metabolism, and it is more pronounced than in non-chagasic cardiopathies, thus evidencing its infectious nature.

Author contributions

Conception and design of the research, analysis and interpretation of the data, statistical analysis, writing of the manuscript and critical revision of the manuscript for intellectual content: Miranda CP, Botoni FA, Nunes MCP, Rocha MOC; acquisition of data: Miranda CP, Botoni FA, Rocha MOC; obtaining funding: Miranda CP.

Potential Conflict of Interest

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

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of master submitted by Carla Paixão Miranda, from Universidade Federal de Minas Gerais.

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The Year in Cardiology 2018: ABC Cardiol and RPC at a glance

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Portuguese is the sixth most spoken language worldwide, by 244 million speakers, the fifth most commonly used language over the Internet, by almost 83 million cybernauts, and the third most commonly used language in the social media *Facebook* and *Twitter*. Portuguese is the official language of eight countries (Portugal, Brazil, Angola, Mozambique, Guinea-Bissau, Cape Verde, Sao Tome and Principe, and Timor-Leste). Despite the incorporation of native words, and the grammatical and pronunciation changes characteristic of each country, their languages remain united and they share many important health problems such as cardiovascular diseases (CVD).¹

Currently, two journals are published in the Portuguese language all over the world, *Revista Portuguesa de Cardiologia* (Rev Port Cardiol) and *Arquivos Brasileiros de Cardiologia* (currently nicknamed ABC Cardiol), and both published the best papers in the Portuguese language.

Rev Port Cardiol, also known as Portuguese Journal of Cardiology, is the official scientific journal of the Portuguese Society of Cardiology. With more than 35 years of uninterrupted scientific activity, it is now a prestigious international journal with global visibility.²

The histories of the Brazilian Society of Cardiology and ABC Cardiol have been completely interlaced since the beginning, and in 2018 ABC Cardiol completed 70 years of existence. ABC Cardiol is an open access publication, scientific home, reading for all the 14,000 cardiologists and members of the Brazilian Society of Cardiology, with almost one third of its articles coming from international authors. ABC Cardiol is indexed in the main databases and has the best Impact Factor for journals in the area of Cardiology and Cardiovascular Sciences in Latin America.³

Every year both journals publish dozens of high-quality scientific articles. In the year 2018, *Revista Portuguesa*

de Cardiologia has published a total of 194 papers, with 62 original articles, and ABC Cardiol published a total of 240 papers, with 96 being original articles. The selection of the 10 best research papers (Tables 1 and 2) from both journals is always a difficult endeavor, given their overall high scientific quality. Moreover, in the absence of specific metrics, this selection is always imperfect and influenced by some degree of subjectivity. Nonetheless, a judging committee composed by highly selected scientists in the field brings us probably the fairest results for the top ten articles in these Journals. Both journals have also published several important review papers, which were out of the scope of this selection.

Coronary artery disease

In 2018, Rev Port Cardiol reported the 15-year results of the Portuguese Registry of Acute Coronary Syndromes (PorACS).⁴ This is a multi-center, continuous, and ongoing registry which has already involved more than 45,000 events, from 45 centers. It is one of the largest national registries in this field, which is only exceeded by the SWEDEHEART⁵ and the MINAP⁶ registries. This article provides very important information about the epidemiology and evolution of ACS treatment. First, it demonstrates that the clinical profile of ACS patients changed little over the years, and the proportion of ST-elevation myocardial infarction (STEMI) remained stable (45%). More importantly, over the years, there has been a major improvement in the overall quality of ACS care. For example, more than 85% of STEMI patients in Portugal now receive reperfusion therapy, which is mainly performed by primary PCI (only 5.2% underwent thrombolysis). This improvement in ACS care led to a remarkable reduction of in-hospital mortality, which decreased from 6.7% in 2002 to 2.5% in 2016. Therefore, the cardiovascular community must be acknowledged for this striking achievement. Nevertheless, the work is not finished, and this study also shows important gaps in ACS care that should be addressed. Unfortunately, time-to-reperfusion has not improved sufficiently, and there is an urgent need to improve both the “patient-delay” and “system-delay” times.

In another interesting article published in 2018, Pereira H. et al.⁷ evaluated the determinants of this “patient-delay” time in the Portuguese health system in 994 patients with suspected STEMI. Although most healthcare systems focus their performance measures in the evaluation of “door-to-balloon times”,⁸ it is important to understand and address the reasons for this “patient-delay time”, which means the time from symptom onset to the first medical contact.⁹ The investigators observed that patient-delay time was too long (about 120 minutes) and

Keywords

Periodicals/trends; Scientific and Technical Activities/trends; Cardiovascular Diseases/prevention and control; Cardiovascular Diseases/epidemiology; Cardiomyopathies; Heart Valve Diseases.

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Manuscript received December 11, 2018, revised manuscript December 14, 2018, accepted December 18, 2018

DOI: 10.5935/abc.20190015

Table 1 – List of the 10 best original articles published in 2018 in Revista Portuguesa de Cardiologia

Author	Title _link
Timóteo A et al.	Portuguese Registry of Acute Coronary Syndromes (ProACS): 15 years of a continuous and prospective registry https://www.sciencedirect.com/science/article/pii/S2174204918301983
Monteiro P et al.	The SAFIRA study: A reflection on the prevalence and treatment patterns of atrial fibrillation and cardiovascular risk factors in 7500 elderly subjects https://www.sciencedirect.com/science/article/pii/S2174204918300849
Pereira H et al.	Factors influencing the patient delay to primary angioplasty in myocardial infarction with ST-segment elevation (STEMI): the Stent for life initiative in Portugal https://www.sciencedirect.com/science/article/pii/S0870255117300811
Menezes MN et al.	Comparative analysis of fractional flow reserve and instantaneous wave-free ratio: Results of a five-year registry https://www.sciencedirect.com/science/article/pii/S217420491830134X
Cardim N et al.	The Portuguese Registry of Hypertrophic Cardiomyopathy: Overall results https://www.sciencedirect.com/science/article/pii/S0870255117305425
Andrade N et al.	Knowledge about cardiovascular disease in Portugal https://www.sciencedirect.com/science/article/pii/S0870255117306832
Timóteo A et al.	What is the role of beta-blockers in a contemporary treatment cohort of patients with acute coronary syndrome? A propensity-score matching analysis https://www.sciencedirect.com/science/article/pii/S217420491830388X
Fontes-Carvalho R et al.	Left atrial deformation analysis by speckle tracking echocardiography to predict exercise capacity after myocardial infarction https://www.sciencedirect.com/science/article/pii/S2174204918303520
Rodrigues PM et al.	Body adiposity is associated with risk of high blood pressure in Portuguese schoolchildren https://www.sciencedirect.com/science/article/pii/S2174204918301259
Pereira-da-Silva T et al.	Optimizing risk stratification in heart failure and the selection of candidates for heart transplantation https://www.sciencedirect.com/science/article/pii/S0870255117300641

Table 2 – List of the 10 best original articles published in 2018 in ABC Cardiol

Author	Title - link
Nascimento BR et al.	Cardiovascular Disease Epidemiology in Portuguese-Speaking Countries: data from the Global Burden of Disease, 1990 to 2016 http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0066-782X2018000600500&lng=en&nrm=iso&tng=en&ORIGINALLANG=en
Farsky PS et al.	Persistent Inflammatory Activity in Blood Cells and Artery Tissue from Patients with Previous Bare Metal Stent http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0066-782X2018001400134&lng=pt&nrm=iso&tng=en&ORIGINALLANG=en
Borges JMDM et al.	Factors Associated with Inadequate Management of Antiplatelet Agents in Perioperative Period of Non-Cardiac Surgeries http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0066-782X2018001600596&lng=es&nrm=i&tng=en&ORIGINALLANG=en
de Souza e Silva CG et al.	Up to 15-Year Survival of Men and Women after Percutaneous Coronary Intervention Paid by the Brazilian Public Healthcare System in the State of Rio de Janeiro, 1999-2010 http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0066-782X2018001600553&lng=pt&nrm=iso&tng=en&ORIGINALLANG=en
Stephan LS et al.	Oral Anticoagulation in Atrial Fibrillation: Development and Evaluation of a Mobile Health Application to Support Shared Decision-Making http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0066-782X2018000100007&lng=en&nrm=iso&tng=en&ORIGINALLANG=en
Gripp EA et al.	Global Longitudinal Strain Accuracy for Cardiotoxicity Prediction in a Cohort of Breast Cancer Patients During Anthracycline and/or Trastuzumab Treatment http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0066-782X2018000200140&lng=en&nrm=iso&tng=en&ORIGINALLANG=en
Miyazaki Y et al.	The Role of Quantitative Aortographic Assessment of Aortic Regurgitation by Videodensitometry in the Guidance of Transcatheter Aortic Valve Implantation http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0066-782X2018001400193&lng=pt&nrm=iso&tng=en&ORIGINALLANG=en
Martins CN et al.	Mid- and Longterm Neo-Aortic Valve Regurgitation after Jatene Surgery: Prevalence and Risk Factors http://www.scielo.br/scielo.php?pid=S0066-782X2018005008104&script=sci_arttext
Silva DV et al.	Comparison of Cardiac and Vascular Parameters in Powerlifters and Long-Distance Runners: Comparative Cross-Sectional Study http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0066-782X2018001800772
Rodrigues JA et al.	Physical Exercise and Regulation of Intracellular Calcium in Cardiomyocytes of Hypertensive Rats http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0066-782X2018001400172

Review Article

identified five predictors of increased patient-delay: 1) age > 75 years; 2) symptom onset between 0:00 and 8:00 a.m.; 3) attending a primary care unit before first medical contact; 4) not calling the national medical emergency number; and 5) self-transport to the emergency department. Therefore, this article provides important information to plan more effective patient-directed campaigns that can decrease patient-delay time and improve STEMI management and prognosis.¹⁰

The latest 2018 myocardial revascularization guidelines have focused on the importance of hemodynamic assessment of intermediate-grade coronary artery lesions, which can be done either by FFR or iFR.¹¹ iFR is a new technique to access the severity of coronary stenosis, which has the advantage of not requiring the administration of a vasodilator, such as adenosine. Two recently published randomized trials have shown comparable clinical results between these two techniques in patients with intermediate-grade stenosis.^{12,13} However, some studies have shown that there can be some inconsistencies between the two measurements.¹⁴ In a provocative article published in *Rev Port Cardiol*, Menezes et al.¹⁵ report their experience directly comparing FFR and iFR information in 150 patients. They have demonstrated that, in general, FFR and iFR are concordant, but in a significant proportion of cases (13%) the results differed between the two techniques. Therefore, this article is another important contributor for the ongoing discussion about the underlying mechanisms responsible for this discordance and their clinical implications.¹⁶⁻¹⁸

An issue that remains open in coronary artery disease care is higher mortality after coronary artery bypass surgery (CABG) in patients with stent. Farsky et al.¹⁷ evaluated inflammatory markers (LIGHT, IL-6, ICAM, VCAM, CD40, NFkB, TNF α , IFN γ) in peripheral blood cells and in coronary artery tissue obtained during CABG in patients with stent ($n = 41$) compared to controls ($n = 26$). They observed that patients with stent showed higher TNF α ($p = 0.03$) and lower CD40 gene expression ($p = 0.01$) in peripheral blood cells than controls without stent. In coronary artery samples, the TNF α protein staining was higher in patients with stent, not only in the intima-media layer (5.16 ± 5.05 vs 1.90 ± 2.27 ; $p = 0.02$), but also in the adipose tissue (6.69 ± 3.87 vs 2.27 ± 4.00 ; $p < 0.001$), which had a higher interleukin-6 protein ($p = 0.04$). They concluded that higher systemic levels of inflammatory markers in patients with stents may contribute to a worse clinical outcome, contributing to our better understanding of pathophysiological changes that occur in patients with coronary stents who underwent coronary artery bypass grafting.

Another challenge in coronary artery management is cardiac complications and deaths in the post-operation period of non-cardiac surgeries, mainly due to acute myocardial infarction (AMI). Antiplatelet agents are the cornerstone for primary and secondary prevention of cardiovascular events. Borges et al.¹⁸ conducted a cross-sectional study to assess factors associated with inadequate management of antiplatelet agents in the perioperative period of non-cardiac surgeries. The sample consisted of adult patients undergoing non-cardiac surgeries and who would use acetylsalicylic acid (aspirin) or clopidogrel ($n = 161$). The management failed to comply with the recommendations in the guidelines in 80.75%

of the sample. After multivariate analysis it was observed that patients with a higher level of education (OR = 0.24; CI95% 0.07-0.78), and those with a previous episode of AMI (OR = 0.18; CI95% 0.04-0.95) had a higher probability of using a therapy complying with the guidelines. These findings emphasized the importance of a Heart Team to develop a patient-directed educational tool to improve adherence to the treatment of coronary artery disease to patients.

In medical science, it is important to keep questioning established dogmas. For decades, the use of beta-blockers has been considered a cornerstone of medical therapy after ACS, having a class I or a class IIa indication for patients after STEMI and non-STEMI, respectively.^{19,20} However, in the era of reperfusion therapy, several studies have questioned this indication, especially in patients without left ventricular dysfunction.^{21,22} In the November issue of *Rev Port Cardiol*, Timoteo et al.²³ published a new article about this topic which “adds more fuel to the fire” to the ongoing discussion. Using a single center registry, they have used propensity score analysis to evaluate the one-year outcome of patients treated with beta-blockers in a sample of 1520 post-ACS patients. They observed that beta-blocker use was an independent predictor of total mortality, including in patients with normal or mildly reduced ejection fraction. This analysis had some limitations. Although they have used propensity score matching, some caution is advised in the interpretation of these results because of residual confounding. Moreover, compliance with treatment and, more importantly, the reasons for not prescribing a beta-blocker could not be assessed in this study. Therefore, this study is important because it reinforces the urgent need to design a pragmatic clinical trial to reassess the effectiveness and safety of beta-blockers in the modern era of reperfusion therapy.

Also, in medical science, it is important to keep questioning the treatment effectiveness delivered to our patients. De Souza e Silva et al.²⁴ studied the survival rate of ischemic heart disease adult patients treated with percutaneous coronary intervention (PCI), in the state of Rio de Janeiro (RJ), from 1999 to 2014, paid by the Brazilian public healthcare system (SUS). They showed data of 19,263 patients (61 ± 11 years old, 63.6% men), and survival rates of men vs. women in 30 days, one year and 15 years were: 97.3% (97.0-97.6%) vs. 97.1% (96.6-97.4%), 93.6% (93.2-94.1%) vs. 93.4% (92.8-94.0%), and 55.7% (54.0-57.4%) vs. 58.1% (55.8-60.3%), respectively. They also observed that the oldest age group was associated with lower survival rates in all periods; PCI with stent placement had higher survival rates than those without stent placement during a two-year follow-up, and women had a higher survival rate than men within 15 years after PCI. These findings performed in a real-world population may help physicians to make decisions regarding the indication of PCI, considering the benefits and risks observed with this procedure.

Arrhythmias

Atrial fibrillation (AF) is the most common sustained arrhythmia, and a significant risk factor for stroke, heart failure and mortality.^{25,26} The SAFIRA study,²⁷ recently published in the *RPC* journal, aimed to determine the prevalence and epidemiology of AF in a large sample of 7500 elderly

Portuguese individuals. The study included a significant subset of 400 individuals that underwent 24-hour Holter monitoring and another subset of 200 individuals which had a 2-week event loop recorder to identify paroxysmal AF. Several interesting data came from this study. First, they observed a very high prevalence (9%) of AF in this elderly population, which was higher than previously reported.^{28,29} Second, more than one-third (35.9%) of AF patients were not aware of having the disease, and 18.6% had paroxysmal AF, which reinforces the need for more systematic AF screening.³⁰ More importantly, in this “real-world” study the rates of anticoagulation were very disappointing. Although the mean CHADSVASC score was high (3.5 ± 1.2), most AF patients (56.3%) did not receive anticoagulation and only 25.8% were considered to be adequately anticoagulated. Therefore, this study highlights the enormous challenges in the diagnosis and management of AF in elderly patients and the urgent need to implement specific healthcare policies (involving patients, caregivers, doctors and health authorities) that can tackle these important problems.

As previously mentioned, the treatment of atrial fibrillation is a challenge in clinical practice especially with regard to the use of oral anticoagulants, which are fundamental for the prevention of stroke. Considering the challenges imposed by this sort of treatment, Stephan et al.³¹ hypothesized that mobile health support for shared decision-making may improve patients’ knowledge and optimize the decisional process. The authors developed an application (App aFib) to be used during the clinical visit, including a video about atrial fibrillation, risk calculators, explanatory graphics and information on the drugs available for treatment. In the pilot phase, 30 patients interacted with the application, which was evaluated qualitatively, and through a disease knowledge questionnaire and a decisional conflict scale. The number of correct answers in the questionnaire about the disease was significantly higher after the interaction with the application (from 4.7 ± 1.8 to 7.2 ± 1.0 , $p < 0.001$), and the decisional conflict scale, administered after selecting the therapy with the app support, resulted in an average of $11 \pm 16/100$ points, indicating a low decisional conflict. Although these were initial findings, the App aFib improves patients’ disease knowledge, and in the future newer studies may confirm if this finding could be translated into clinical benefit.

Cardiovascular disease prevention and Epidemiology

The presence of cardiovascular risk factors in childhood creates a life-long burden which increases the risk of cardiovascular disease in adulthood.^{32,33} Therefore, several studies have showed the importance of evaluating risk factors and promoting healthy lifestyle across all lifespan, starting as early as in pre-school children.^{34,35} In an interesting study published in 2018, Melo Rodrigues et al.³⁶ analyzed the prevalence and interrelation of cardiovascular risk factors in a sample of 1555 schoolchildren (6-9 years). First, they have found an enormous prevalence (29.1%) of overweight and obesity in this population, showing the magnitude of the childhood obesity epidemic.³⁷ The prevalence of high-normal blood pressure (4.5%) and hypertension (3.7%) was also much

higher than expected. There was a strong association between anthropometric body fat indicators and blood pressure, which reinforces the need for blood pressure measurement, in obese children. However, the most important take-home message from this study is to remember that our lifestyle behaviors as adults are linked to our previous exposures during childhood³¹ and, therefore, cardiovascular health promotion should involve all ages, starting from pre-school children, and the entire family.³⁸

It is also known that lifestyle behaviors associated with an increased risk of cardiovascular disease are influenced by the individual’s health-related knowledge (health literacy) and by their perception of the risk of disease.³⁹ Therefore, improving health literacy should be viewed as an essential tool to reduce the global burden of cardiovascular disease and improve risk factor control. In an innovative article published in 2018 in *Rev Port Cardiol*, Andrade et al.⁴⁰ evaluated, in a large sample of 1624 portuguese individuals, the specific knowledge on cardiovascular disease, and its relationship with sociodemographic factors, health literacy and clinical history. It was striking to observe a major deficit in cardiovascular health-related knowledge. Only around one-third of the population was able to estimate their risk of myocardial infarction or stroke. Interestingly, participants identified non-smoking and a healthy diet as the main behaviors for cardiovascular disease prevention and attributed a lower importance to blood pressure control. It was also observed that only a very low percentage of individuals would call the national emergency number when faced with symptoms suggestive of a possible stroke or myocardial infarction, as also demonstrated in other studies.⁴¹ Therefore, this study clearly shows that there are important gaps in cardiovascular health-related knowledge in the general population. All of us, both as doctors, scientific community and society, need to create increasing awareness for the importance of improving health literacy in the community. This is a new and important strategy to help prevent cardiovascular disease.

It is fundamental to know about gaps in cardiovascular healthcare, and the knowledge about common problems and solutions shared by Portuguese-speaking countries (PSC) can provide us useful data regarding the similarities and differences between them, emphasizing well-succeeded actions for fighting CVD. Nascimento et al.¹ described trends in cardiovascular disease morbidity and mortality in the PSC between 1990 and 2016, stratified by sex, and their association with the respective sociodemographic indexes (SDI) using the Global Burden of Disease (GBD) 2016 data and methodology. They observed large differences, mainly related to socioeconomic conditions, in the relative impact of CVD burden in PSC. Among CVD, ischemic heart disease was the leading cause of death in all PSC in 2016, except for Mozambique and Sao Tome and Principe, where cerebrovascular diseases have supplanted it. The most relevant attributable risk factors for CVD among all PSC are hypertension and dietary factors. Genetic factors, implicit in the cultural identity, factors inherent in the host, as well as the huge social inequality might have contributed to explain the mortality rates observed. Collaboration between the PSC might allow sharing successful experiences to confront CVD between those countries.

Review Article

Cardiomyopathies and Valvular heart disease

In the January 2018 issue of *Rev Port Cardiol*, Cardim et al.⁴² report the overall results of the Portuguese national Registry of Hypertrophic Cardiomyopathy (PRO-HCM), which included 1042 patients from 29 centers. This is one the largest and most significant worldwide registries of HCM, and provides a detailed contemporary assessment of the clinical profile, management strategies and outcomes of HCM in Portugal. The main conclusions were that HCM is characterized by relatively advanced age at diagnosis, with more than one fourth of patients diagnosed over the age of 65 years. There was a limited use of CMR for HCM assessment but, on the contrary, more than 50% performed genetic testing. The long-term mortality (0.65%/year) and the risk of sudden-cardiac death (0.22%/year) was low, but morbidity remained considerable. This registry shows that there are important differences in HCM management between guidelines and clinical practice, which was also demonstrated in other registries.^{43,44} This can be the result of different HCM clinical courses representing the heterogeneous spectrum of HCM. Finally, these data reinforce the importance of using clinical registries as an important source of information that should be used to inform practice but also to influence the writing of the guidelines.⁴⁵

Advances in non-invasive cardiac imaging have provided important new insights in the pathophysiology of valvular heart disease and cardiomyopathies, and diagnosis of implanted device or bioprosthesis related complications.⁴⁶ Gripp et al.⁴⁷ used global longitudinal strain to assess the incidence of cardiotoxicity in 49 patients treated for breast cancer, and the independent factors associated with that event. Cardiotoxicity was identified in 5 (10%) on the third (n = 2) and sixth (n = 3) months of follow-up. Strain was independently associated with the event (p = 0.004; HR = 2.77; 95%CI: 1.39-5.54), with a cutoff point for absolute value of -16.6 (AUC = 0.95; 95%CI: 0.87-1.0) or a cutoff point for percentage reduction of 14% (AUC = 0.97; 95%CI: 0.9-1.0). They concluded that the 14% reduction in strain (absolute value of -16.6) allowed the early identification of patients who could develop anthracycline and/or trastuzumab-induced cardiotoxicity.

The role of incremental diagnostic and prognostic value of combination of imaging techniques or fusion imaging is growing exponentially.⁴⁸ In the Valve Academic Research Consortium-2 (VARC-2) consensus document, quantitative and semi-quantitative hemodynamic assessments are recommended to assess aortic regurgitation (AR) severity by echocardiogram, and moderate-to-severe AR is defined as valve failure⁴⁸ that is associated with poor outcome and mortality. Miyazaki et al.⁴⁹ investigated a quantitative angiographic assessment of AR by videodensitometry before and after Balloon post-dilatation (BPD) since this technique provides an accurate assessment of the severity of paravalvular leak (PVL) and correlates with increased mortality and impaired reverse cardiac remodeling by echocardiography after transcatheter aortic valve implantation (TAVI). The authors showed that videodensitometry AR (VD-AR) decreased significantly from 24.0[18.0-30.5]% to 12.0[5.5-19.0]%, and the relative delta of VD-AR after BPD ranged from -100% (improvement) to +40% (deterioration). Significant AR (VD-AR > 17%) was observed in 47 patients (77%) before, and in 19 patients (31%) after BPD.

They concluded that VD-AR after transcatheter heart valve implantation provides a quantitative assessment of post-TAVI regurgitation and can help in the decision-making process on performing BPD and in determining its efficacy.

The increasing number of children with evolving congenital heart diseases who had lower mortality, especially in recent years, demands greater preparation of professionals and institutions that handle them. Jatene surgery became the surgical procedure of choice to repair transposition of the great arteries (TGA) in neonates and infants, and nowadays the behavior of the neo-aortic valve is a cause of concern because of its potential for requiring late reoperation. Martins et al.⁵⁰ assessed the prevalence and risk factors of neo-aortic valve regurgitation in 127 patients in the late postoperative period and observed 29% of mild and 18% of moderate neo-aortic valve regurgitation, in a long follow-up. Those patients had a higher aortic annulus Z-score, although reoperation rate due to neo-aortic regurgitation associated with aortic dilation was only 1.5%, all in patients with complex TGA group. So, this study shows that, despite the low incidence of reoperation after Jatene surgery, these patients require strict vigilance due to the time-dependent phenomenon, and one of the major risk factors for neo-aortic valve regurgitation was the preoperative pulmonary artery diameter.

Cardiac function, exercise capacity and heart failure

Several studies have shown that left atrium (LA) size and function are important predictors of cardiovascular events in several clinical settings and can be involved in the progression to heart failure.⁵¹⁻⁵² In another interesting article published in *Rev Port Cardiol*, Fontes-Carvalho et al.⁵³ evaluated in 94 patients after AMI the role of different indices of LA function (assessed by speckle tracking) as determinants of exercise capacity by cardiopulmonary exercise testing. They found a significant correlation between exercise capacity and LA conduit function, but not with contractile function. LA longitudinal strain was also associated with worse exercise capacity parameters, suggesting that this echocardiographic parameter can be used to predict reduced exercise capacity. Finally, it was shown that LA functional parameters were interdependent with LV diastolic function, highlighting the pathophysiologic importance of correct atrioventricular coupling. Therefore, this study highlights that although the LA was frequently viewed as a bystander in the regulation of cardiac function, the availability of new echocardiographic parameters for LA assessment (such as speckle tracking assessment) has shown its clinical utility as an important functional and prognostic marker in several clinical settings, especially in heart failure (HF).⁵⁴

Heart failure patients have a significant risk of cardiovascular events. Therefore, several studies have tried to improve risk stratification tools to predict HF hospitalizations or the need for heart transplantation. The most commonly used score is the Seattle Heart Failure Model (SHFM), which is based on 24 clinical variables.⁵⁵ Other scores are also available,⁵⁶ but there is an ongoing need to improve risk stratification in HF. In the February issue of *Rev Port Cardiol*, Pereira-da-Silva demonstrate that VE/VCO₂ slope, obtained from cardiopulmonary exercise testing (CPET), can be a good predictor of events in patients with HF with reduced EF

(<40%). Although most previous studies have assessed the role of peak VO₂ as a prognostic marker, it is known that the VE/VCO₂ slope is a particularly interesting parameter, because it reflects ventilatory efficiency and is independent on the level of patient effort.⁵⁷ The authors identified a threshold of VE/VCO₂ slope > 39 as an excellent marker of worse outcome, with a c-statistic value of 0.79. Nevertheless, it is commonly said “in Medicine there are no magical numbers”. This is especially true in the selection of HF patients for heart transplantation, where individual clinical decision requires a team-based approach, with extensive clinical experience and a multiparametric approach. However, this interesting study highlights the importance of integrating the information provided by CPET, especially of VE/VCO₂, as another important clinical parameter to better stratify these patients.

Exercise training induces cardiovascular adaptations secondary to changes in blood pressure, as well as other hemodynamic and metabolic changes in response to physical exertion that are most of the time desired by the cardiologist. Rodrigues et al.⁵⁸ checked the effects of aerobic exercise training on contractility and intracellular calcium (Ca²⁺) transients of cardiomyocytes, and on the expression of microRNA 214 (miR-214) in the left ventricle of spontaneously hypertensive rats (SHR). They demonstrated that exercise training reduced systolic arterial pressure in hypertensive rats and increased the availability of intracellular Ca²⁺ by accelerating the sequestration of these ions in left ventricular myocytes of hypertensive rats, despite increased expression of miR-214 and maintenance of cell contractility. This study confirmed the anti-hypertensive effects of aerobic exercise, as already reported previously.

But will any level of exercise be beneficial to all? Silva et al.⁵⁹ hypothesized that athletes engaging in high-intensity strength training for long periods of time show changes in cardiac structure associated with reduced cardiac function when compared to long-distance runners, and long-time exposure to high-intensity strength training could lead to a reduction of endothelial function caused by pressure overload. They evaluated 40 high-performance athletes (powerlifters [PG], n = 16; runners [RG], n = 24) and assessed heart structure and function performing echocardiography and checking systolic and diastolic blood pressure (SBP/DBP), flow-mediated dilation (FMD), peripheral vascular resistance (PVR), maximum force (squat, bench press, and deadlift), and

maximal oxygen uptake (spirometry). The authors concluded that cardiovascular adaptations are dependent on training modality, and the borderline structural cardiac changes are not accompanied by impaired function in powerlifters. However, a mild increase in blood pressure seems to be related to PVR rather than endothelial function.

Conclusions

We hope this review of the best in Cardiology and Cardiovascular Science published in the Portuguese language by 2 major journals can help our readers to update their knowledge in an easy and pleasant format and yet, get excited and interested in going deeper on the articles published last year on their field of expertise. The specific areas covered by this review included coronary artery disease, arrhythmias, cardiovascular disease prevention and epidemiology, cardiomyopathy and valvular heart disease, and finally cardiac function, exercise and heart failure. Articles published in all these fields demonstrated important innovation, new and original information with direct effect on clinical routine patient management, and also new insights on better understanding of disease process and treatment. Population and epidemiological data of particular importance for Portuguese speaking countries were also presented.

Author contributions

Conception and design of the research, writing of the manuscript and critical revision of the manuscript for intellectual content: Fontes-Carvalho R, Oliveira GMM, Oliveira GMM, Rochitte CE.

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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Early Diagnosis and Treatment in Infective Endocarditis: Challenges for a Better Prognosis

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Infective endocarditis (IE), a microbial infection of the cardiac or adjacent vascular endothelium, remains a feared disease, although the modern diagnosis systematizations date back to 1885 by Osler.¹ Although relatively uncommon,² with approximately 3-10 cases per 100,000 individuals/year,³ the mortality remains high: more than one-third of patients die in the first year after the diagnosis.^{1,4} Only early diagnosis and treatment, whether exclusively clinical or associated with cardiac surgery, may interfere to reduce this high mortality rate.

IE used to be more frequent in young and middle-aged adults with rheumatic or congenital heart disease.³ However, recent studies have shown a significant reduction in the incidence of IE in these groups, especially in more developed countries.²

IE can be increasingly seen in patients with valve prostheses, vascular catheters, implantable electronic devices such as pacemakers and implantable cardiac defibrillators^{5,6} and new surgical devices, such as transcatheter valve implantation.² Moreover, due to the population aging, even in Brazil, an increased incidence has been observed in the elderly, especially when associated with comorbidities such as diabetes (20%), chronic kidney disease (14%) and anemia (10%),⁵ with a 4.6-fold increase in IE, when compared to the general population.^{5,6} At the same time, reflecting the change in the epidemiology, the incidence of endocardial infection by staphylococci has been steadily increasing, even predominating in relation to streptococci in many centers.^{3,7}

The diagnosis of IE is based on the modified Duke Criteria for Infective Endocarditis: the association of clinical signs (such as fever and presence of murmur in patients with risk of heart disease), positive blood culture for frequent etiological agents and typical echocardiographic findings (vegetation, periannular abscess)⁴ show high sensitivity (> 80%), mainly in native valve infections.^{4,6} However, the criteria show lower diagnostic

accuracy for an early diagnosis in clinical practice, mainly in the previously mentioned group of patients, in which the incidence has been increasing. The diagnosis is challenging, especially if the echocardiography is normal or inconclusive, as it occurs in up to 30% of cases,⁸ or when blood cultures are negative.^{4,6}

In fact, negative blood cultures occur in approximately 2% to 20% of cases of endocarditis. Common causes are: concomitant or prior use of antibiotics and presence of slow-growing or difficult-to-detect microorganisms in routine cultures. The following microorganisms stand out: *Coxiella burnetii*, *Bartonella* species and fungi.⁴

The incidence of negative blood cultures has been reduced³ with automated blood culture techniques, specific serologies (*Coxiella* sp) and polymerase chain reaction (PCR). These methods² allow the direct identification of bacterial species, especially in difficult-to-recognize cases, helping to attain an early diagnosis in relation to routine culture methods.³ (Figure 1)

Imaging methods, mainly echocardiography, play a key role in the diagnosis and management of IE.⁶ Being the technique of choice for the initial investigation, it should be rapidly performed, and if the clinical suspicion persists in the transthoracic modality, the transesophageal assessment should be carried out, with an evident increase in the method accuracy.

Individuals with prostheses and catheters or devices often require assessment by transesophageal echocardiography (TEE), considering that the sensitivity and specificity rates are between 40-70% for transthoracic echocardiography (TTE) and 85% for TEE in prosthetic valves.⁸ A negative result in the TEE does not exclude IE in patients with strong clinical suspicion. Therefore, the examination should be repeated within seven days for diagnostic clarification, whenever there is the possibility of IE.

The echocardiographic diagnosis may be limited by acoustic shadowing, confusing images, especially in the postoperative period, very small vegetation or absence of vegetation.¹ These limitations led to a growing interest in the use of other imaging modalities that would complement the echocardiography.^{9,10}

The use of transesophageal three-dimensional echocardiography has improved the evaluation of cardiac volumes and structures, mainly for better identification of paraprosthetic regurgitation. This technique has improved and will certainly be even more useful in the near future.⁸

Keywords

Endocarditis; Bacterial/mortality; Prosthesis Implantation; Catheters; Pacemaker, Artificial; Diagnostic Imaging; Echocardiography.

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Manuscript received May 15, 2018, revised manuscript August 08, 2018, accepted September 05, 2018

DOI: 10.5935/abc.20180270

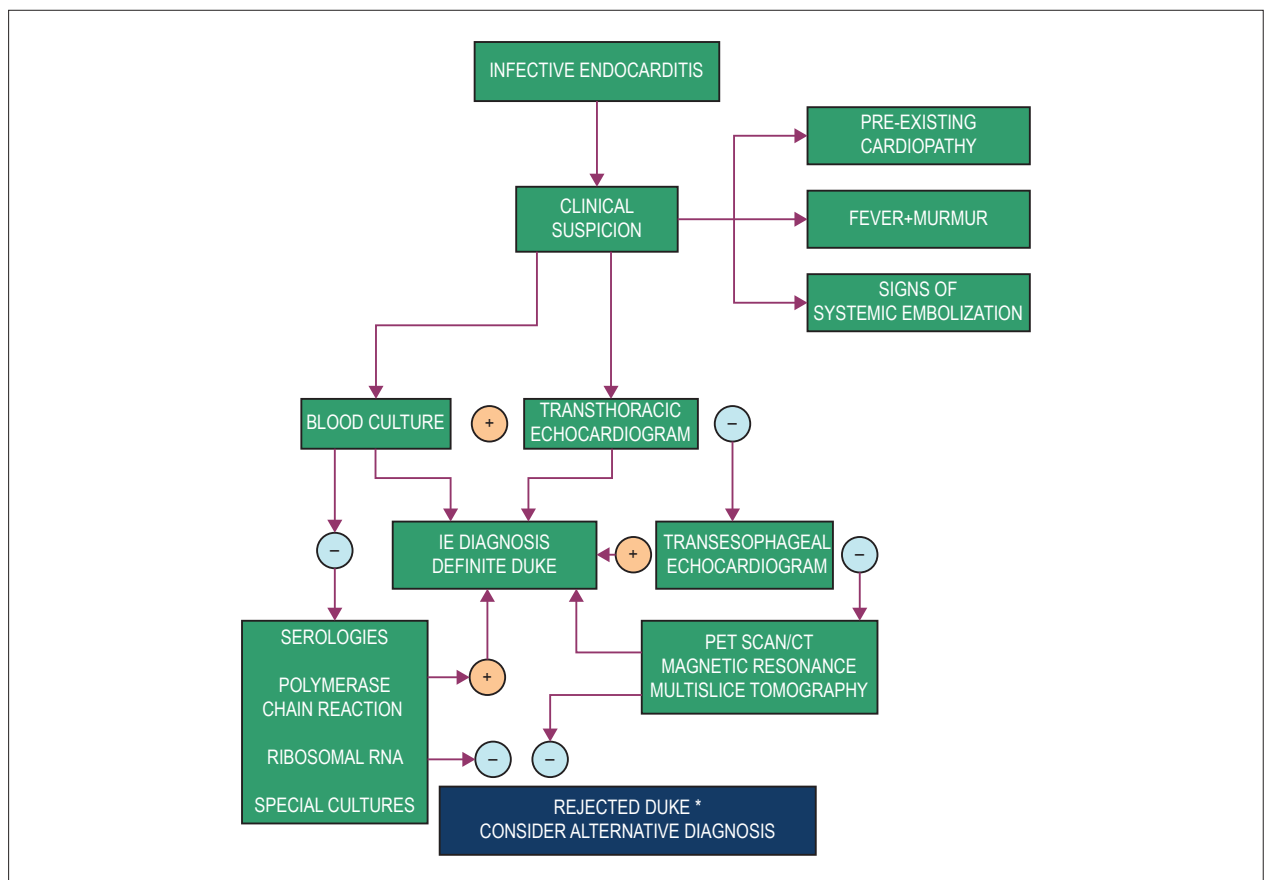


Figure 1 – IE diagnosis flowchart. *Possible cases according to Duke's criteria are all those that do not fit as definite or rejected cases.

Other imaging methods have also shown to be promising in the early diagnosis of patients with suspected IE that is difficult to be confirmed, such as multislice computed tomography (MSCT), magnetic resonance imaging (MRI) and positron-emission computed tomography (PET/CT).¹

PET/CT has been shown to be particularly important in cases of patients with valve prostheses or cardiac devices with more than three months of implantation (Figure 1), in addition to the relevant potential in detecting extracardiac infectious foci, malignancy, and other types of inflammation.^{7,9}

When assessing prosthetic valve dysfunction, a recent study⁶ suggested that MSCT may be equivalent or superior to the echocardiography to identify prosthesis-related vegetation, abscesses, pseudoaneurysms and dehiscence. However, there have been few studies comparing the two techniques and, therefore, the echocardiogram persists as the first-choice method in the investigation.⁶ Thus, it is worth emphasizing that even the most modern imaging techniques are not always conclusive or unquestionably clarify the presence of endocarditis, particularly in these difficult-to-diagnose subgroups, such as the elderly and patients with implantable devices/catheters.

In conclusion, the trinomial high clinical suspicion, microbiological and imaging methods remain essential for the early diagnosis in IE. The inclusion of new imaging

and microbiological identification methods, associated to a multidisciplinary team consisting of cardiologists, infectologists, imaging specialists, microbiologists and other specialties, for specific cases, such as neurologists are crucial in this scenario.⁶

We emphasize that the change in the course of IE prognosis depends on the rapid establishment of targeted therapy, which in turn is only possible when an early diagnosis is attained.³ High-risk subgroups, such as the elderly and patients with implanted prosthetic material deserve special attention, as a delayed diagnosis has led to increased mortality. Thus, future guidelines should consider the inclusion of these new techniques in the diagnosis of IE.²

Author contributions

Conception and design of the research: Sampaio RO; acquisition of data: Sobreiro DI, Brazil CVA; analysis and interpretation of the data: Sobreiro DI, Brazil CVA; writing of the manuscript: Sobreiro DI, Sampaio RO, Lopes ASSA, Branco CEB; critical revision of the manuscript for intellectual content: Sampaio RO, Tarasoutchi F, Strabelli TMV.

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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Case 1/2019 – A 51-year-old Man with Arterial Hypertension, Aortic Dissection and Aortic Valve Regurgitation, in Addition to Heart Failure with Unchanged Clinical Course After Surgical Intervention

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A 51-year-old male, hypertensive patient, a former smoker, was transferred for treatment of thoracic aortic dissection and heart failure.

After a three-week period with progressively more intense chest pain, accompanied by dyspnea, sweating and vomiting, he was admitted to the Hospital in the city where he lived.

At hospital admission, he had high blood pressure and the diagnosis of thoracic aortic dissection was made. He received antihypertensive and beta-blocker medications. On the fifth day, he was transferred to Instituto do Coração for treatment. At that moment, he was asymptomatic.

The physical examination (June 14, 2012) showed good general health status, paleness, ++ / 4+, increased jugular venous pressure, heart rate of 80 bpm, blood pressure 80 x 60 mmHg; clear lungs; cardiac auscultation disclosed rhythmic heart sounds and ++++ diastolic murmur on the left sternal border, no abdominal alterations, lower limbs without edema, besides palpable and symmetrical pulses.

Laboratory tests (June 14, 2012) showed hemoglobin 15.9 g/dL; hematocrit, 49%; leukocytes, 10,080/mm³ (neutrophils 64%, eosinophils 7%, lymphocytes 21%, and monocytes 8%); platelets 232,000/mm³; CKMB, 1.33 ng/mL; troponin I, 0.106 ng/mL; urea, 38 mg/dL; creatinine, 0.94 mg/dL; sodium, 137 mEq/L; potassium, 4.2 mEq/L; prothrombin time (PT) (INR), 1.1; APTT time ratio, 0.78; AST, 30 U/L; ALT, 61 U/L; gamma-GT, 116 IU/L; alkaline phosphatase, 81 U/L; and negative serology for hepatitis B, C, and HIV.

The electrocardiogram (ECG) performed on June 16, 2012 showed sinus rhythm, left atrial and left ventricular overload with strain pattern (Figure 1).

The echocardiogram performed on June 17, 2012, disclosed the following measurements: aorta, 37 mm; left atrium, 48 mm; septal thickness and posterior wall, 9 mm; left ventricle, 87/78 mm; ejection fraction, 22%. The patient showed

eccentric hypertrophy with diffuse hypokinesis; moderate mitral regurgitation; marked aortic regurgitation; ascending aortic dissection was observed, with the original intimal tear 25 mm from the valve plane. The aortic measurements at the different levels were: aortic sinus, 37 mm, sinotubular junction, 46 mm, ascending aorta, 67 mm and aortic arch, 34 mm.

The posteroanterior chest X-ray performed on June 18, 2012 showed normal lung fields, aorta with an image suggestive of aneurysm, and enlarged cardiac area (Figure 2)

The coronary angiography did not show any coronary lesions. The pressures were: aorta: (syst/diast/mean) 100/50/67 mmHg and left ventricular: (Syst/initial diast/end diast) 100/10/20 mmHg. The left ventricle showed diffuse hypokinesis. There was marked aortic regurgitation and an ascending aortic aneurysm, with an image suggestive of dissection (Figure 3).

The patient underwent surgery for repair of the ascending aortic dissection, with the interposition of a Dacron tube and aortic valve repair (June 19, 2012). The postoperative period was uneventful, and the patient was discharged on the ninth postoperative day.

Almost a month after hospital discharge (July 11, 2012), he sought emergency medical attention for worsening of dyspnea, attributed to non-adherence to the prescribed medication.

The physical examination (July 11, 2012) disclosed a heart rate of 60 bpm, blood pressure 80 x 60 mmHg; clear lungs; normal cardiac auscultation, no abdominal alterations; lower limbs without edema and no signs of deep vein thrombosis, with normal pulses.

The laboratory tests (July 18, 2012) showed hemoglobin 10.7 g/dL; hematocrit, 32%; leukocytes, 9,750/mm³ (band cells 1%, segmented 69%, eosinophils 8%, basophils 3%, lymphocytes 14%, monocytes 5%); platelets, 443,000/mm³; C-reactive protein, 65.05 mg/L; urea 29 mg/dL; creatinine, 0.90 mg/dL; sodium, 130 mEq/L; potassium, 4.8 mEq/L; magnesium, 1.70 mEq/L; BNP, 1280 pp / mL; venous lactate 21 mg/dL; venous gasometry: pH 7.38, pCO₂, 48 mmHg; pO₂, 34.9 mmHg, O₂ saturation, 53.8%; bicarbonate, 26.5 mEq/L, base excess, 2 mEq/L. Blood, urine and catheter tip cultures were negative.

One month after this episode he was again brought to emergency care (August 11, 2012) with dyspnea on minimal exertion, orthopnea and oliguria for two days. He denied coughing, fever, coryza or diarrhea; chest pain or palpitation. He reported correct use of medications and hydrosaline restriction.

The physical examination on admission showed blood pressure of 80x60 mmHg, heart rate of 102 bpm, fine pulses and decreased peripheral perfusion; jugular venous pulse was present; rhythmic heart sounds, presence of third heart

Keywords

Hypertension; Aortic Valve Insufficiency; Aortic Aneurysm/surgery; Aneurysm, Dissecting/surgery; Heart Failure.

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DOI: 10.5935/abc.20190013

Anatomopathological Correlation

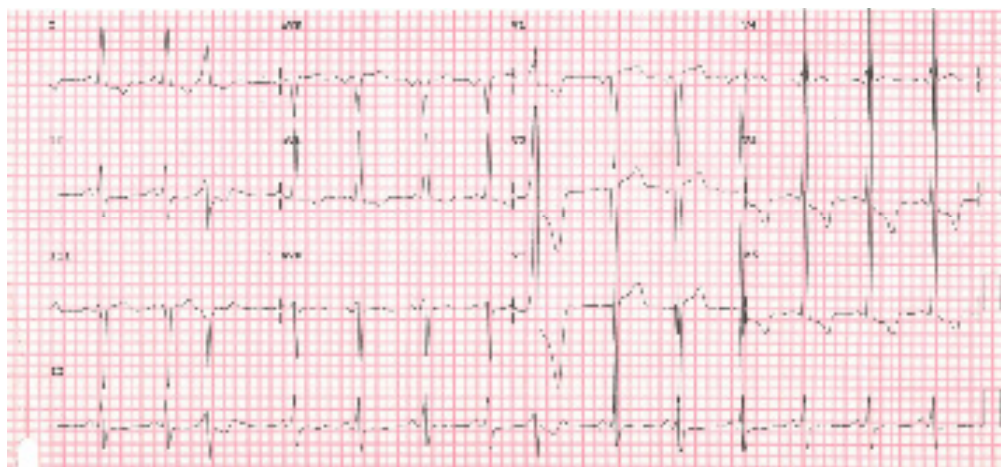


Figure 1 – ECG showing atrial and left ventricular overload, the latter with a strain pattern.



Figure 2 – Chest x-ray: mediastinal enlargement, suggestive of aortic aneurysm and cardiomegaly.

sound, mitral systolic murmur +++ / 6+, tricuspid systolic murmur +++ / 6+ and aortic systolic murmur ++ / 6+; abdominal assessment - liver palpable at 5 cm from the right costal border; palpable and symmetrical lower-limb pulses.

He was started on intravenous dobutamine, vasodilator and diuretic drugs, in addition to the antibiotics vancomycin and meropenem.

Laboratory tests (August 11, 2012) showed hemoglobin: 9.2 g/dL, hematocrit: 31%, leukocytes: 12,980/mm³ (neutrophils 74%, eosinophils 1%, basophils 1%, lymphocytes 19%, monocytes 5%), platelets: 395,000/mm³, C-reactive protein: 79.58 mg/dL, urea: 55 mg/dL, creatinine: 1.26 mg/dL, sodium: 135 mEq/L, potassium: 5.1 mEq/L, prothrombin time: (INR) = 1.2, APTT:(rel) = 0.97, venous lactate: 55 mg/dL.

The chest x-ray showed pulmonary congestion and cardiomegaly.

The echocardiogram (August 13, 2012) showed the following measurements: aorta, 41 mm; left atrium, 50 mm; right ventricle, 35 mm; septum, 9 mm; posterior wall, 10 mm; left ventricle, 86 mm; ejection fraction, 20%; hypertrophic left ventricle with diffuse hypokinesis; right ventricle with moderate hypokinesis; severe mitral regurgitation; normal aortic valve; pulmonary valve with indirect signs of pulmonary hypertension; estimated pressure of 43 mmHg and presence of Dacron tube in the aorta.

The transesophageal echocardiogram (August 16, 2012) also showed the presence of marked tricuspid regurgitation and no images suggestive of thrombi or vegetations were observed.

The laboratory tests performed on August 12 showed hemoglobin decrease to 7.7 g/dL and no evidence of bleeding and, therefore, an upper digestive endoscopy was requested, which did not show any alterations.

Anatomopathological Correlation

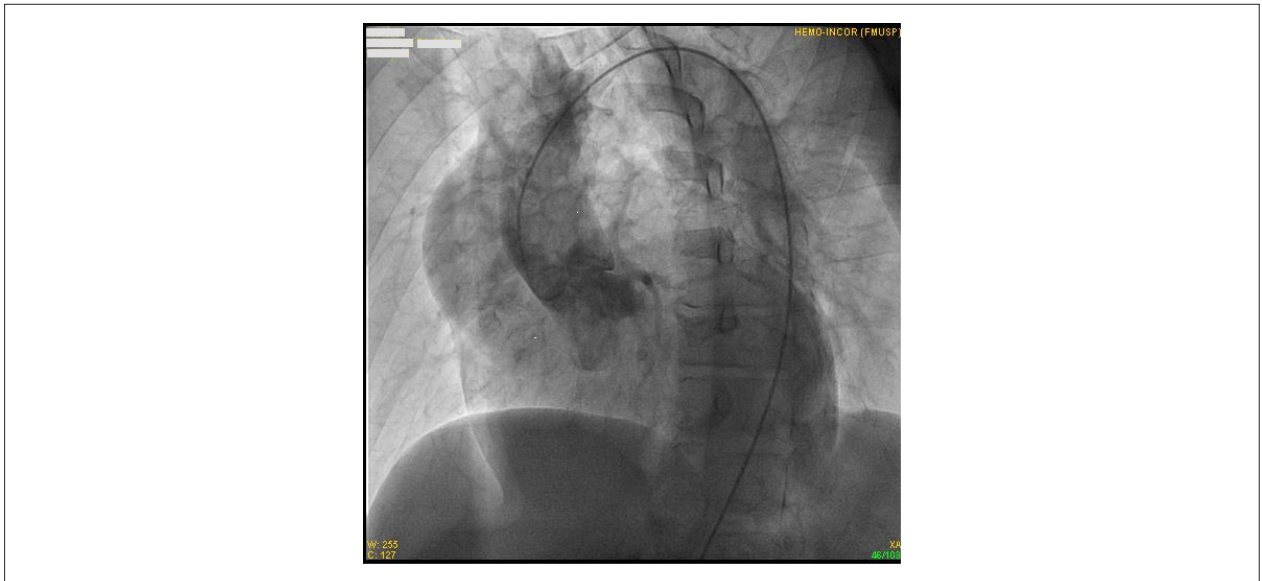


Figure 3 – Aortography. Ascending aortic aneurysm with dissection and catheter in the true lumen.

A Doppler ultrasonography of the lower limbs carried out due to suspicion of pulmonary thromboembolism (recent RV dysfunction) showed no signs of thrombosis. The patient then underwent a chest tomography, which identified a small consolidation on the right side, with right pleural effusion.

He developed refractory shock, despite the use of antibiotics. The right pleural effusion was punctured, and the presence of transudate was identified.

All blood cultures and urine cultures were negative. He required orotracheal intubation for ventilatory support on August 29, 2012, developing an increasing need for noradrenaline, and the antibiotics were replaced by daptomycin, micafungin, and rifampicin.

Laboratory reassessment (September 4, 2012) showed: hemoglobin: 10 g/dL; hematocrit: 33%; leukocytes: 5,450/mm³ (neutrophils 85%, lymphocytes 10% and monocytes 5%); platelets: 313,000/mm³; C-reactive protein: 221.38 mg/L; urea: 44 mg/dL; creatinine: 2.21 mg/dL; phosphorus: 2.2 mg/dL; magnesium: 1.6 mEq/L.

The patient remained in shock and died (September 4, 2012).

Clinical aspects

This was a male patient, who presented with ascending aortic dissection, and who, even after surgery for dissection repair, developed a picture of severe heart failure and died.

The International Registry of Acute Aortic Dissection (IRAD) showed that patients with aortic dissection were older than our patient, 61 years; and a majority of males (63%). Regarding the diseases related to the dissection, they were: Marfan syndrome (6.7%), hypertension (69.3%), atherosclerosis (24.4%), previously known aortic aneurysm (12.4%), previous aortic dissection 3.9% and diabetes mellitus (4.3%). Also, 15.9% had a history of previous cardiac surgery and iatrogenic cause in 4.8% (1.7% coronary angiography and 3.1% after cardiac surgery).¹ The 2015 update of the same registry, with

a ten-fold higher number of patients, showed an increase in hypertension (75.5%) and a decrease in the presence of Marfan syndrome (4.5%), (75.5%), atherosclerosis (19.6%) and previous cardiac surgery (10.6%).²

Genetic tests can be performed in the presence of aortic aneurysm in younger patients. The syndromes related to the presence of aortic aneurysms are Marfan, Loeys-Dietz, Ehler-Danlos syndromes, the cutis laxa or elastolysis and that related to a defect in the transforming growth factor beta (TGFβ).

Classically, the genetic alterations found in Marfan syndrome are related to the fibrillin-1 gene.³ Changes in the physical examination involve ocular (myopia, ectopia lentis, and risk of retinal detachment), skeletal (exaggerated growth and joint laxity, exaggerated growth of the extremities) and cardiovascular alterations (dilation of the aorta at the level of the sinuses of Valsalva, predisposing to dissection). In the present case, we do not have a description of these phenotypic changes that might suggest such diagnosis.

Loeys-Dietz syndrome includes several manifestations similar to those of Marfan Syndrome, but they also include hypertelorism, broad or bifid uvula, cleft palate and generalized arterial tortuosity, aneurysms, and arterial dissection. Generally no increase of extremities and ocular alterations are observed.⁴

As well as for Marfan syndrome, we do not have evidence in the present case to suggest such a diagnosis.

The Ehler-Danlos syndrome is characterized by fragility of the connective tissue and the manifestations occur in the skin (hyperelasticity, atrophic scars and easy ecchymosis), joints (hypermotility, frequent dislocations and arthralgias) and vessels (aneurysms and spontaneous vessel ruptures). The vascular form, in which dissection occurs most frequently, occurs through a mutation in the alpha-1 gene of type III collagen, with a silent substitution that leads to the replacement of glycine in the collagen chain.⁵

Anatomopathological Correlation

We also have no clinical evidence of such changes in the present case. A more recent study showed gene panel alterations in 25% of patients with aortic aneurysms.⁶

What should be taken into account is that even today the criterion for the indication of surgical treatment for rupture prevention remains the diameter of the aneurysm, 50 mm.

In the present case, the patient had arterial hypertension and it must have had an important role in the development of the thoracic aneurysm and its rupture. Biomechanical studies show that for hypertension to lead an aneurysm development, there must be concomitant failure in the composition and maintenance of the extracellular matrix and membrane receptors. Consequently, there is damage to the mechanical stress transduction in the cell-signaling response.^{7,8}

Regarding the patient's unfavorable evolution after the surgery, it is probably due to the long evolution of the aortic aneurysm with aortic valve regurgitation, leading to left ventricular dilation and severe dysfunction, which in the very late states do not undergo regression or relief despite valve replacement surgery and progress to progressive heart failure. The ECG disclosed left ventricular overload with strain and the echocardiogram showed large left ventricular dilation and marked dysfunction.

The European Guidelines of Cardiology and Thoracic Surgery recommends valve replacement surgery in patients with aortic dilation or aortic regurgitation accentuated with symptoms. In asymptomatic patients, this recommendation appears if there is reduction in ejection fraction (<50%)

or ventricular dilation (diastolic diameter >70 mm or diastolic >50 mm).⁹

The patient had degrees of dilation (diastolic diameter, 87 mm and systolic diameter, 78 mm) and ventricular dysfunction (ejection fraction of 22%) well beyond those recommended for the indication of valve replacement surgery.

This is the most plausible explanation for the poor evolution. **(Dr. Desiderio Favaratto)**

Diagnostic hypotheses: Aneurysm in the thoracic aorta, chronic aortic valve regurgitation, aortic dissection. Etiology: arterial hypertension and extracellular matrix disease of the aorta. Final clinical picture: cardiogenic shock due to valvular heart disease. **(Dr. Desiderio Favaratto)**

Necropsy

The heart weighed 890g, with marked increase in volume and dilation of all chambers, predominantly the ventricles (Figure 4). The atrioventricular valves showed no abnormalities. The aortic valve showed thickening of the free margins of the semilunar leaflets, with a central non-coaptation aspect, compatible with valve regurgitation (Figure 5). A corrugated tube replaced the ascending aorta and was sutured just above the aortic sinotubular junction (Figure 6). Around the junction area between the ascending aorta and the ventricular mass, we noticed cavitation with irregular margins, partially filled by liquefied material, of a yellowish-brown color (Figure 5). There was also a concave circular lesion of the aortic intima, with 1.5 cm in diameter, just below the emergence of the renal arteries. The lungs showed

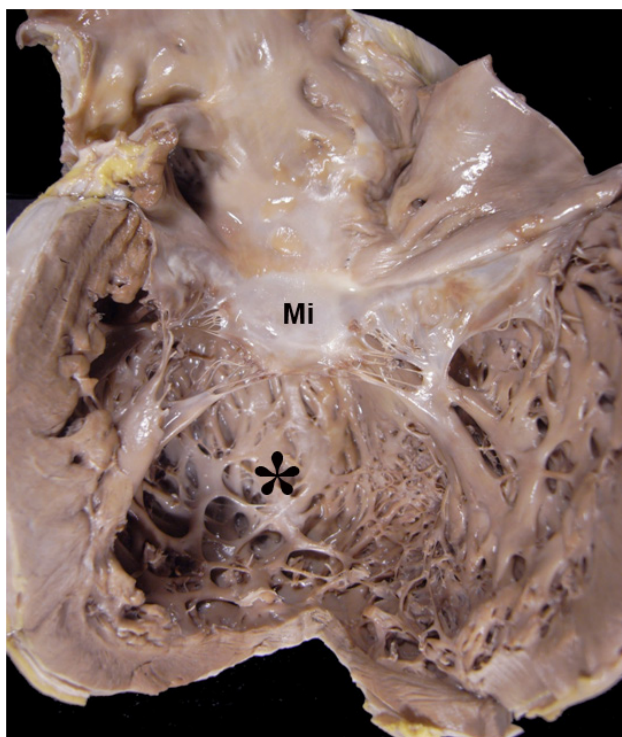


Figure 4 – Macroscopic aspect of the open left heart chambers, with marked left ventricular dilation (asterisk); Mi: mitral valve.

Anatomopathological Correlation

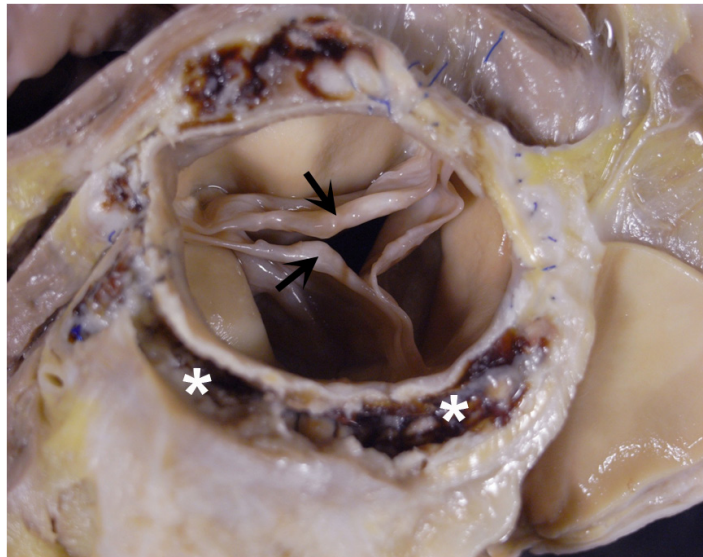


Figure 5 – Aortic valve seen from its arterial aspect. There is thickening of the free edge of the semilunar leaflets (arrows) and lack of central coaptation. A multiloculated cavitory lesion (asterisks) is seen around the aorta, from which a pasty material emerged.

areas of wine-colored parenchyma condensation, triangular in shape when sectioned. There were signs of generalized visceral congestion, as well as ascites (700 mL) and bilateral pleural effusion (200mL in each hemithorax).

Histological examination showed accumulation of mucoid material in the tunica media throughout the aorta, as well as focal rupture of elastic fibers in the cavitory lesion described in the abdominal aorta (Figure 7). The anatomopathological study of the peri-aortic cavitory lesion showed mixed inflammation, with necrotic cellular and polymorphonuclear neutrophil debris, among sutures and other synthetic materials (Figure 8). Bacterial and fungal tests were negative at this site. The aortic valve showed fibrous thickening of the margins. There was chronic passive visceral congestion, with hepatic centrolobular necrosis; the wine-colored lesions in the lungs corresponded to recent infarctions. (**Dr. Vera Demarchi Aiello**)

Anatomopathological diagnoses

- Post-surgical correction of acute dissection of the ascending aorta
- Peri-aortic cavitory lesion, with mixed inflammatory reaction, without the identification of infectious agents
- Intramural dissection located in the abdominal aorta
- Aortic valve regurgitation
- Recent pulmonary infarctions

Cause of death: Congestive heart failure with terminal shock (**Dr. Vera Demarchi Aiello**)

Comments

Aortic dissection is a serious disease, which is usually associated with systemic arterial hypertension and has as

morphological finding the delamination of the vessel wall, with an intimal orifice called the "intimal tear" usually located in the ascending aorta, and the creation of a false lumen. This can extend to the tunica adventitia and undergo rupture, with massive bleeding into a cavity (pericardial, pleural or abdominal cavity).

When the dissection does not rupture, there is usually an orifice called a re-entry, located more distally in the aortic lumen, usually in the descending aorta.

Histologically, the presence of glycosaminoglycan accumulation in the tunica media, sometimes in the shape of the so-called "mucoïd lakes",¹⁰ in addition to the rarefaction and fragmentation of elastic fibers and decrease of collagen in the external third of the aortic wall can be observed, leading to the weakness of this part of the wall.

In addition to the rupture, multiple organ ischemia due to the flow steal in the false lumen and aortic valve regurgitation are complications, due to collapse of its insertion when the dissection orifice is nearby.

In our case, the dissection was limited to the ascending aorta, which was replaced by a synthetic tube. Although there was a reference to aortic valvuloplasty in the surgery, the patient developed congestive heart failure, probably as a result of the remaining valvular regurgitation, which was not detected on the echocardiogram, possibly due to hemodynamic changes (patient in shock). This situation was responsible for the poor postoperative evolution.

The finding of a cavitory lesion in the aortic root, associated with the sutures, containing a purulent-like fluid, could mean local infection, but histological analysis did not detect the presence of microorganisms. (**Dr. Vera Demarchi Aiello**)

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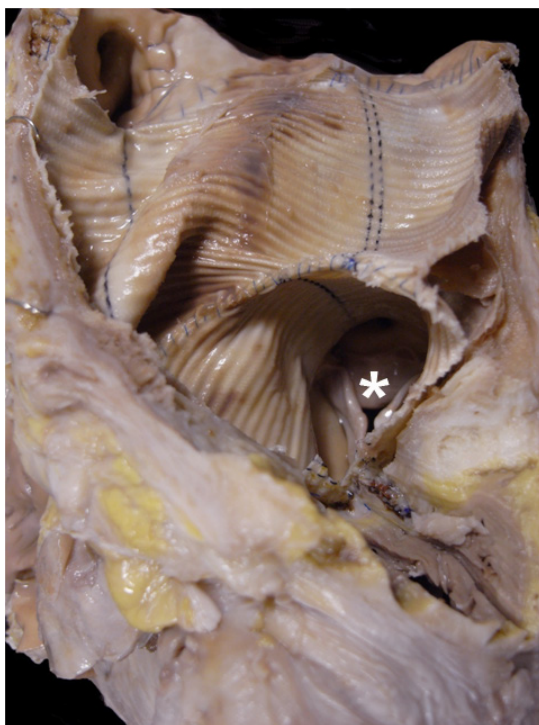


Figure 6 – Macroscopic aspect of the corrugated synthetic tube that replaced the ascending aorta. The aortic valve can be seen in the background (asterisk).

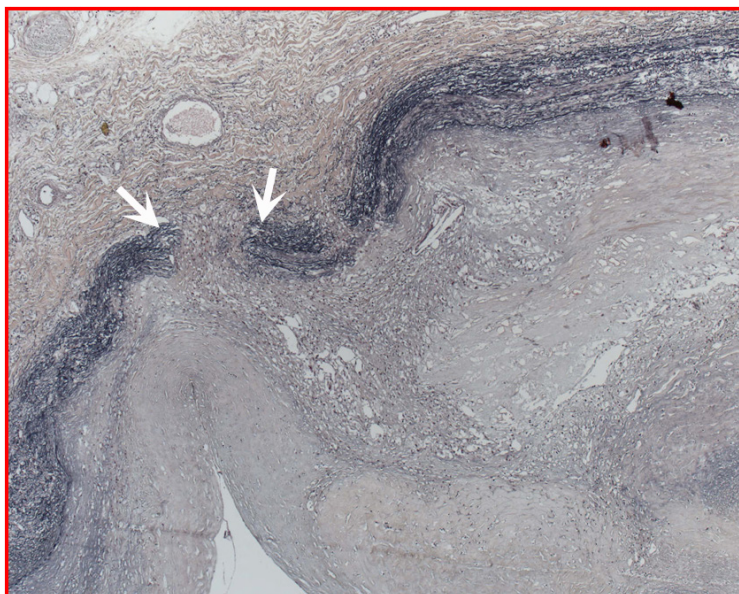


Figure 7 – Photomicrography of the aortic wall at the level of the cavitory lesion described in the abdominal aorta. One can observe the rupture (between the arrows) of the elastic fibers (black bundles) of the tunica media, characterizing localized intramural dissection. Verhoeff staining for elastic fibers, objective magnification =5x.

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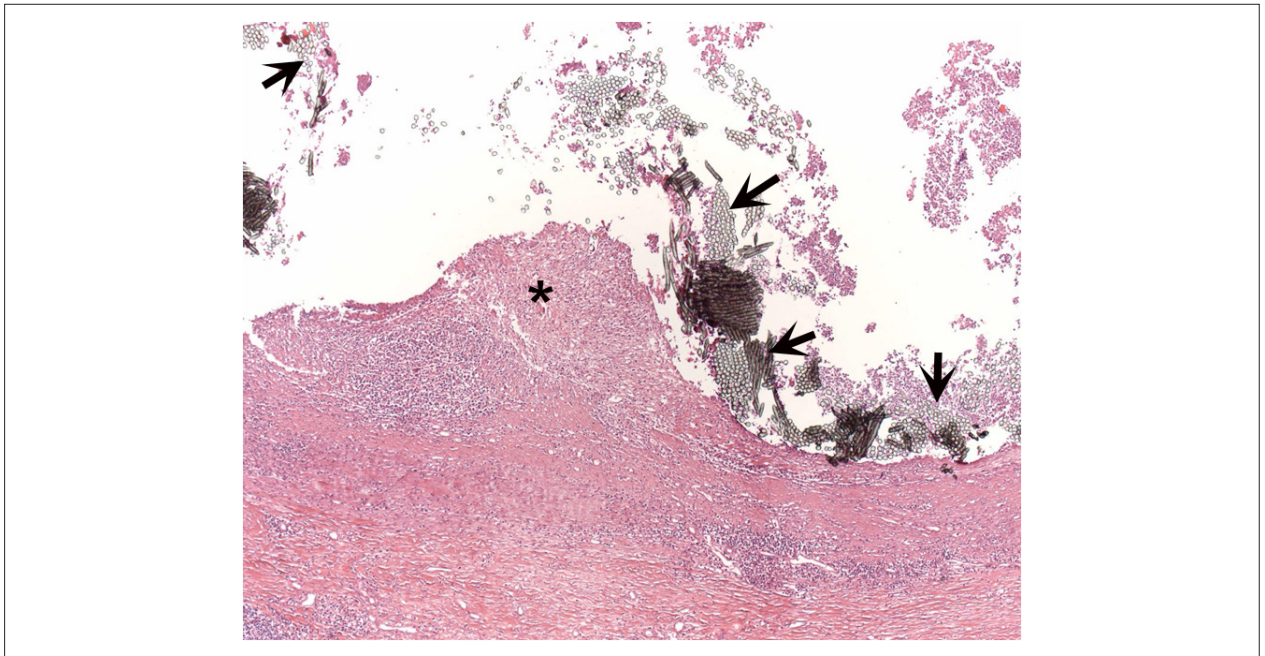


Figure 8 – Photomicrography of the cavitory lesion wall described in the aortic root. We can see accumulations of polymorphonuclear neutrophils (asterisks) amid the synthetic tissue and sutures (arrows). Hematoxylin-eosin staining, objective magnification =10x.

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Large Bilateral Coronary Artery Fistula: 10-year Follow-up in Clinical Treatment

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We report on the 10-year evolution of an asymptomatic patient with a large bilateral coronary artery-pulmonary artery fistula for whom clinical treatment was chosen. Published previously,¹ the report reinforces the need for treatment individualization in patients with moderate coronary fistulas.

Case Report

A 59-year-old asymptomatic female patient, with a diagnosis of a large bilateral coronary-pulmonary artery fistula made in 2007 was investigated after a cardiac murmur was identified on a routine examination. At the time, conservative treatment was chosen. Cardiac auscultation showed a more audible systolic-diastolic murmur in the upper left sternal border, with a more audible component in systole. There were no other findings in the cardiological physical examination or even the overall segmental examination. The patient had no comorbidities at the time, except for a prior history of smoking (10-pack-years). During the evolution, at the annual outpatient follow-up, she had diagnoses of dyslipidemia, glucose intolerance and depression. At the last consultation, in 2017, the patient was asymptomatic. She used atenolol 25 mg/ day, metformin 850 mg/day, atorvastatin 20 mg/day and sertraline 50 mg/day.

The examinations performed after 10 years of follow-up were compared with those at the time of diagnosis. The current echocardiogram showed right coronary (RC) with 4 mm of diameter at the origin and 7 mm in the middle third; the left main coronary artery (LMCA) with 8 mm. The patient had a fistulous trajectory with tortuous flow communicating both coronaries with the pulmonary trunk, without the presence of pulmonary hyperflow. Additionally, the evolution of mitral regurgitation showed to be of an important degree. Table 1 shows the echocardiographic parameters during follow-up.

Keywords

Arterio-Arterial Fistula/diagnosis; Coronary Angiography; Diagnostic, Imaging; Radionuclide Imaging; Coronary Vessel Anomalies; Mitral Valve Insufficiency; Myocardial Ischemia

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Manuscript received March 19, 2018, revised manuscript July 02, 2018, accepted July 02, 2018

DOI: 10.5935/abc.20180267

Myocardial scintigraphy with dipyridamole and 99m-technetium-sestamibi showed no changes in perfusion, as well as the previous examinations performed in 2007 and 2011. The ergospirometry treadmill test (modified Balke protocol, 3.4 mph), lasting 7 minutes and 38 seconds, was maximal (109% of maximal HR), with VO₂ peak of 22.4 mL/kg/min (87% of predicted VO₂).

The angiotomography of the coronary arteries was performed in 2017 and the comparison with the 2007 examination can be seen in Figure 1. The finding of a systemic-pulmonary fistula persists, in the RC + ADA with the LMCA, described as the presence of a high-caliber branch emerging from the right coronary artery origin, with a tortuous trajectory, surrounding the pulmonary trunk anteriorly and communicating with the proximal third of the anterior descending artery. It shows communication with the pulmonary trunk, associated with two aneurysms along its trajectory, measuring 19x16 mm and 14x13 mm. There is no pulmonary dilation or other signs suggesting hemodynamic repercussion. Total coronary calcium score of 246 (Agatston), corresponding to the 99th percentile for the age group and gender, and absence of significant coronary luminal reduction were also observed.

Discussion

Coronary fistulas (CFs), abnormal communications between one or more coronary arteries with some cardiac or thoracic structure, usually congenital in origin,² have a prevalence of 0.05% to 0.88%, depending on the diagnostic method used.³ They originate from one or more branches of the coronary arteries, and the pulmonary trunk is the most frequent termination of bilateral CFs.^{2,4} They may be associated with mitral regurgitation/mitral valve disease – a finding present in this case – atrial and/or ventricular septal defect, pulmonary stenosis and atresia.⁵ In the adult population, 75% are symptomatic, with chest pain and dyspnea being the most frequently complaints. Heart murmur is observed in 37% of patients at clinical examination.⁵

Patient evolution seems to be quite variable and depends on the size and hemodynamic repercussion of the CF, in addition to associated malformations. Long-term follow-up^{2,4} shows that patients can progress from being asymptomatic to symptoms of heart failure due to decreased ejection fraction, left atrial enlargement and pulmonary hypertension, and a few with coronary aneurysm, which is associated mainly with unilateral fistulas. Coronary aneurysms may favor coronary rupture and may also generate ischemia through the flow steal mechanism.^{5,6}

The ideal treatment of CFs remains uncertain, especially regarding the moderate and asymptomatic cases.

Table 1 – Evolution of echocardiographic parameters along the years

	2007	2013	2016	2017
Left Atrium (mm)	30	37	40	38
Interventricular Septum (mm)	7	9	9	8
LV Posterior Wall (mm)	7	8	8	8
LV Diastolic Diameter (mm)	54	56	58	57
LV Systolic Diameter (mm)	37	38	39	41
LVEF (%)	59	60	60	59
Aortic Sinus (mm)	31	32	33	32
RV Systolic Function	Normal	Normal	Normal	Normal
Additional findings	Mild MR	Mild MR. Mild TR. Minimal AR.	Minimal systolic displacement of the posterior cusp towards the left atrium. Moderate MR Mild degree TR. Mild PF.	Posterior cusp prolapse towards the left atrium. Important MR (eccentric jet directed to the interatrial septum). Qp/Qs ratio of 0.8.

LV: left ventricle; LVEF: left ventricular ejection fraction; RV: right ventricle; MR: mitral regurgitation; TR: tricuspid regurgitation; AR: aortic regurgitation; PF: pulmonary failure; Qp/Qs: pulmonary artery and aortic flow ratio.

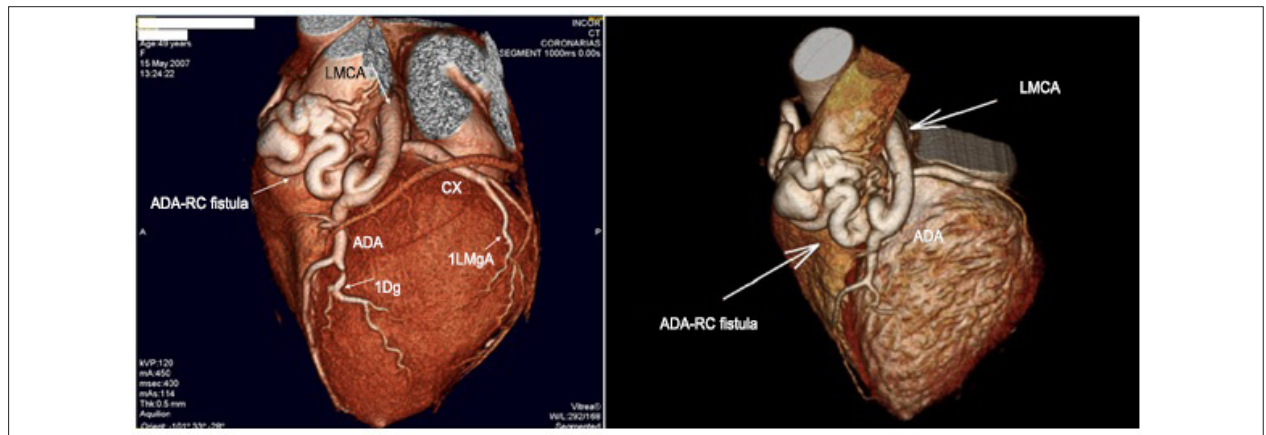


Figure 1 – Comparative image of the coronary fistula (to the left in 2007 and to the right in 2017) - ADA: Anterior Descending Artery; LMCA: Left Main Coronary Artery; Cx: Circumflex Artery; 1Dg: First Diagonal Artery; 1LMgA: First Left Marginal Artery.

The conservative treatment should be considered in small, asymptomatic fistulas. The fistula spontaneous closure is rare and occurs in only 1-2% of cases.

The interventional treatment for CF closure, whether surgical or percutaneous, should be considered in large CFs and in more proximal locations, presence of symptoms, presence of other cardiovascular diseases / associated cardiac malformations, and hemodynamic repercussion (high-flow fistulas).^{5,8} However, these are not complication-free procedures.

The surgical treatment can show a high rate of periprocedural myocardial infarction and occurrence of residual tricuspid reflux.⁹ Percutaneous treatment with occlusion devices (coils used in small fistulae and Amplatzer used in large CFs)^{8,10} may also be complicated by aneurysmal dilatation and thrombosis leading to embolization and myocardial ischemia, as well as device migration (mainly coils in large, high-flow

fistulas). Situations in which occlusion is incomplete favor the occurrence of infective endocarditis and hemolysis.^{5,8}

In the present case, initially described 10 years ago, of an asymptomatic moderate CF without clinical or hemodynamic repercussions, where we chose to carry out a clinical follow-up, we observed a very favorable evolution, with the patient remaining asymptomatic and with good aerobic (cardiovascular) fitness throughout the period, in the absence of myocardial ischemia and pulmonary hyperflow, with preserved ventricular function, and showing a slight increase in the RC (6 to 7 mm) and the LMCA (7 to 8 mm) diameters, in addition to a slight left chamber dilatation, the latter justified by mitral valve prolapse that developed into significant regurgitation, an association found in some cases.

As previously discussed,¹ we emphasize that the conservative treatment is safe and should be carried out in

Case Report

asymptomatic patients and / or those without complications, as the one described in this case report. In symptomatic or complicated patients, however, percutaneous or surgical interventions are indicated.

This report shows, once again, the need for the individualization of management in the presence of the diagnosis of asymptomatic coronary artery fistula.

Author contributions

Conception and design of the research and analysis and interpretation of the data: Kulchetscki RM, Lechinewski LD, Dourado L; acquisition of data: Kulchetscki RM, Lechinewski LD, Hueb WA, César LAM; writing of the manuscript: Kulchetscki RM, Lechinewski LD, Dourado L, César LAM;

critical revision of the manuscript for intellectual content: Dourado L, César LAM.

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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January issue of 2019, vol. 112(1), pages 67-75

In "Sex-Related Effects of Prenatal Stress on Region-Specific Expression of Monoamine Oxidase A and B Adrenergic Receptors in Rat Hearts", consider Tanja Jevdjovic as the correct form for the name of the author Tanja Jevdjovic.

January issue of 2019, vol. 112(1), pages 91-103

In "Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (ARVC/D) - What We Have Learned after 40 Years of the Diagnosis of This Clinical Entity", table 1 of the Portuguese version, item "5. Arritmias", consider correct for the phrase "TV não sustentada ou sustentada com morfologia tipo BRD e eixo superior" BRE in place of BRD.