ABC Cardiol
Journal of Brazilian Society of Cardiology
Sociedade Brasileira de Cardiologia • ISSN-0066-782X • Volume 110, Nº 6, June 2018

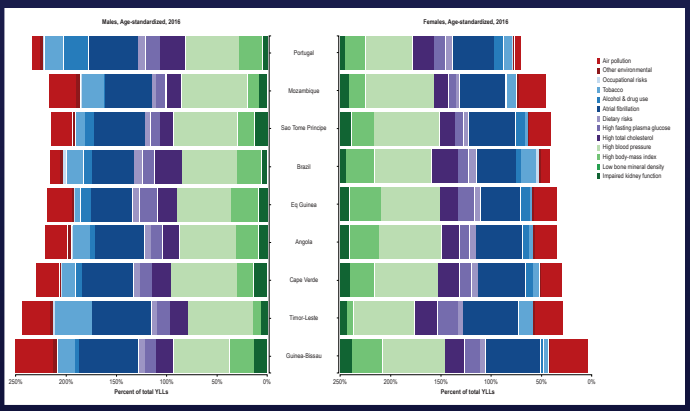


Figure 7 – *Influence of the risk factors on the years of life lost (YLLs) because of cardiovascular diseases, according to sex, in each Portuguese-speaking country in 2016.. Page 507*

ABC Cardiol
Journal of Brazilian Society of Cardiology

Contents

Original Articles

Review Article

Viewpoint

Anatomopathological Correlation

Case Report

Image

Letter to the Editor

ABC Cardiol

Journal of Brazilian Society of Cardiology

A JOURNAL OF SOCIEDADE BRASILEIRA DE CARDIOLOGIA - Published since 1948

Sociedade Brasileira de Cardiologia

President
Oscar Pereira Dutra

Vice-President
José Wanderley Neto

Scientific Director
Dalton Bertolim Précoma

Financial Director
Denilson Campos de Albuquerque

Administrative Director
Wolney de Andrade Martins

Government Liaison Director
José Carlos Quinaglia e Silva

Information Technology Director
Miguel Antônio Moretti

Communication Director
Romeu Sergio Meneghelo

Research Director
Fernando Bacal

Assistance Quality Director
Evandro Tinoco Mesquita

Specialized Departments Director
Audes Diógenes de Magalhães Feitosa

State and Regional Relations Director
Weimar Kunz Sebba Barroso de Souza

Cardiovascular Health Promotion Director - SBC/Funcor
Fernando Augusto Alves da Costa

Chief Editor of the Arquivos Brasileiros de Cardiologia
Carlos Eduardo Rochitte

Chief Editor of the International Journal of Cardiovascular Sciences
Claudio Tinoco Mesquita

Presidents of State and Regional Brazilian Societies of Cardiology:
SBC/AL – Edvaldo Ferreira Xavier Júnior

SBC/AM – João Marcos Bemfica Barbosa Ferreira

SBC/BA – Emerson Costa Porto

SBC/CE – Maria Tereza Sá Leitão Ramos Borges

SBC/DF – Ederaldo Brandão Leite

SBC/ES – Fatima Cristina Monteiro Pedroti

SBC/GO – Gilson Cassem Ramos

SBC/MA – Aldryn Nunes Castro

SBC/MG – Carlos Eduardo de Souza Miranda

SBC/MS – Christiano Henrique Souza Pereira

SBC/MT – Roberto Candia

SBC/NNE – Maria Alayde Mendonca da Silva

SBC/PA – Moacyr Magno Palmeira

SBC/PB – Fátima Elizabeth Fonseca de Oliveira Negri

SBC/PE – Audes Diógenes de Magalhães Feitosa

SBC/PI – Luiza Magna de Sá Cardoso Jung Batista

SBC/PR – João Vicente Vitola

SBC/RN – Sebastião Vieira de Freitas Filho

SBC/SC – Wálmore Pereira de Siqueira Junior

SBC/SE – Sheyla Cristina Tonheiro Ferro da Silva

SBC/TO – Wallace André Pedro da Silva

SOCERGS – Daniel Souto Silveira

SOCERJ – Andréa Araujo Brandão

SOCERON – Fernanda Dettmann

SOCESP – José Francisco Kerr Saraiva

Presidents of the Specialized Departaments and Study Groups

SBC/DA – Maria Cristina de Oliveira Izar

SBC/DCC – João Luiz Fernandes Petriz

SBC/DCC/CP – Andressa Mussi Soares

SBC/DCM – Marildes Luiza de Castro

SBC/DECAGE – Elizabeth da Rosa Duarte

SBC/DEIC – Salvador Rassi

SBC/DERC – Tales de Carvalho

SBC/DFCVR – Antoinette Oliveira Blackman

SBC/DHA – Rui Manuel dos Santos Povoa

SBC/DIC – Marcelo Luiz Campos Vieira

SBCCV – Rui Manuel de Sousa S. Antunes de Almeida

SOBRAC – Jose Carlos Moura Jorge

SBHCI – Viviana de Mello Guzzo Lemke

DCC/GAPO – Pedro Silvio Farsky

DERC/GECESP – Antonio Carlos Avanza Jr

DERC/GECN – Rafael Willain Lopes

DERC/GERCPM – Mauricio Milani

DCC/GECETI – Luiz Bezerra Neto

DCC/GECO – Roberto Kalil Filho

DEIC/GEICPED – Estela Azeka

DCC/GEMCA – Roberto Esporcatte

DEIC/GEMIC – Fabio Fernandes

DCC/GERTC – Juliano de Lara Fernandes

DEIC/GETAC – Silvia Moreira Ayub Ferreira

Arquivos Brasileiros de Cardiologia

Volume 110, Nº 6, June 2018

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

Address: Av. Marechal Câmara, 160 - 3º andar - Sala 330
20020-907 • Centro • Rio de Janeiro, RJ • Brasil
Phone.: (21) 3478-2700
E-mail: arquivos@cardiol.br
www.arquivosonline.com.br
SciELO: www.scielo.br

Commercial Department
Phone: (11) 3411-5500
E-mail: comercialsp@cardiol.br

Editorial Production
SBC - Internal Publication Department

Graphic Design and Diagramming
SBC - Internal Design Department

Affiliated at the Brazilian Medical Association

SUPPORT

Cardiovascular Disease Epidemiology in Portuguese-Speaking Countries: data from the Global Burden of Disease, 1990 to 2016

Bruno Ramos Nascimento,[1] Luisa Campos Caldeira Brant,[1] Gláucia Maria Moraes de Oliveira,[2] Marcus Vinícius Bolívar Malachias,[3] Gabriel Moreira Alves Reis,[1] Renato Azeredo Teixeira,[1] Deborah Carvalho Malta,[1] Elisabeth França,[1] Maria de Fátima Marinho Souza,[4] Gregory A. Roth,[5] Antonio Luiz P. Ribeiro[1]

Universidade Federal de Minas Gerais (UFMG),[1] Belo Horizonte, MG – Brazil
Universidade Federal do Rio de Janeiro,[2] Rio de Janeiro, RJ - Brazil
Faculdade Ciências Médicas de Minas Gerais,[3] Belo Horizonte, MG - Brazil
Ministério da Saúde, Brasília,[4] DF - Brazil
University of Washington,[5] Seattle, WA - USA

Abstract

Background: **Portuguese-speaking countries (PSC) share the influence of the Portuguese culture but have socioeconomic development patterns that differ from that of Portugal.**

Objective: **To describe trends in cardiovascular disease (CVD) morbidity and mortality in the PSC between 1990 and 2016, stratified by sex, and their association with the respective sociodemographic indexes (SDI).**

Methods: **This study used the Global Burden of Disease (GBD) 2016 data and methodology. Data collection followed international standards for death certification, through information systems on vital statistics and mortality surveillance, surveys, and hospital registries. Techniques were used to standardize causes of death by the direct method, as were corrections for underreporting of deaths and garbage codes. To determine the number of deaths due to each cause, the CODEm (Cause of Death Ensemble Model) algorithm was applied. Disability-adjusted life years (DALYs) and SDI (income per capita, educational attainment and total fertility rate) were estimated for each country. A p-value < 0.05 was considered significant.**

Results: **There are 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.**

Conclusion: **Collaboration among PSC may allow successful experiences in combating CVD to be shared between those countries. (Arq Bras Cardiol. 2018; 110(6):500-511)**

Keywords: **Cardiovascular Diseases; Epidemiology; Mortality; Global Burden of Disease / trends.**

Introduction

Cardiovascular diseases (CVD) are a major cause of death worldwide. Although CVD are not the first cause of death in many low- and middle-income countries, they account for 80% of the deaths and 88% of the premature deaths in those countries.[1] The control of infectious, maternal and child diseases, the increase in life expectancy and the growing urbanization have contributed to the trend towards increasing the importance of CVD in low- and middle-income countries. The implementation of health policies, such as promotion of a healthy lifestyle, access to primary and secondary prevention of CVD and treatment of acute cardiovascular events, is, thus, essential for CVD control in all countries.[1]

Portuguese-speaking countries (PSC) were culturally influenced by Portugal at different levels.[2] The study of the trends in morbidity and mortality from CVD in those countries can provide us useful data regarding the similarities and differences between them. Those data can provide an exchange of information between countries regarding well-succeeded actions for fighting CVD, in addition to allowing reflections on the influence of culture on the burden of CVD.

The "Global Burden of Disease Study" (GBD Study) is an important epidemiological observational study that uses metrics of morbidity and mortality related to major diseases, injuries and risk factors at global, at national and regional levels. One of the GBD Study objectives is to understand, by assessing the trends, the changes in the profile of the diseases that affect the 21st century populations.[3]

This study was aimed at describing the trends of CVD morbidity and mortality in the PSC between 1990 and

Mailing Address: Gláucia Maria Moraes de Oliveira •
Universidade Federal do Rio de Janeiro – R. Prof. Rodolpho P. Rocco, 255 – Prédio do HU 8º andar – sala 6, UFRJ. Postal Code 21941-913, Cidade Universitária, RJ – Brazil
E-mail: glauciam@cardiol.br, glauciamoraesoliveira@gmail.com
Manuscript received December 08, 2017, revised manuscript March 21, 2018, accepted March 21, 2018

DOI: 10.5935/abc.20180098

2016, based on the estimates of the GBD 2016 Study, and to assess the association between those trends and the sociodemographic index (SDI) of those countries.

Methods

Portuguese-speaking countries

The PSC are the official members of the Community of Portuguese-Speaking Countries (CPLP), an international organization created in July 17, 1996, aimed at "deepening the mutual friendship and cooperation between its members".[2] The initial list of countries included Angola, Brazil, Cape Verde, Guinea-Bissau, Mozambique, Portugal and Sao Tome and Principe. In 2002, after its independence, Timor-Leste was accepted as a member. In 2014, Equatorial Guinea was accepted as a member, after Portuguese was adopted as the official language of the country. Table 1 and Figure 1 show the location and the demographic, social and economic characteristics of the PSC.

The GBD Study

The GBD Study is a multinational collaborative research project with the goal of producing consistent estimates of health loss due to over 333 diseases and injuries. A wide range of data sources (data of national surveillance, verbal autopsy and vital registration, published and unpublished registries of diseases, and published scientific literature) and methods are applyed to produce specific results with uncertainty intervals for age, sex and country for the years 1990-2016, and such results are annually updated for the entire temporal series. The present study is based on data from the GBD 2016 Study and its previously detailed methodology.[4-6]

Definitions of CVD

The nine most common global causes of death and morbidity related to CVD and an additional category for 'other CVD' were considered, in addition to the estimates of global morbidity and mortality grouped by CVD.[7] The underlying cause of death was defined as CVD according to the International Classification of Diseases (ICD) codes, from the death certificate (DC), the basic document that informs the causes of death in countries with systems of vital statistics, such as Brazil. The following causes were analyzed, with their corresponding ICD-10 codes according to the GBD Study's classification list of causes: 1- rheumatic fever with cardiac involvement (codes I01-I01.9, I02.0, I05-I09.9); 2-ischemic heart disease (codes I20-I25.9); 3- cerebrovascular disease (CbVD) (G45-G46.8, I60-I69.9); 4- hypertensive heart disease (I11); 5- cardiomyopathy and myocarditis (A39.52, B33.2-B33.24, D86.85, I40-I43.9, I51.4-I51.5); 6- atrial fibrillation and flutter (I48); 7- aortic aneurysm (I71); 8- peripheral artery disease (I70.2-I70.7, I73-I73.9); 9-endocarditis (A39.51, I33-I33.9, I38-I39.9). Garbage codes, such as heart failure (I50) and pulmonary embolism (I26), which do not define the pathology that caused the death, were redistributed to those specific causes based on the GBD methodology, according to algorithms defined in that study.

Regarding mortality, those causes were grouped based on the specific sequelae of disease (for example, ischemic heart disease due to acute coronary syndrome, chronic stable angina, chronic ischemic heart disease and ischemic cardiomyopathy). Adjustments were performed for data that did not comply with the specific definition of case (for example, electronic confirmation for the clinical diagnosis).[4,8,9]

Table 1 – Demographic, social and economic characteristics of the Portuguese-speaking countries, 2013

	Angola	Brazil	Cape Verde	Equatorial Guinea	Guinea-Bissau	Mozambique	Portugal	Sao Tome and Principe	Timor-Leste
Population (millions)	19	201	1	1	2	24	10	0.2	1
Population density (inhab/km²)	15	24	127	39	44	31	113	190	79
Area (1,000 km²)	1247	8516	4	28	36	786	92	1.0	15
GDP (USD, billions)	125	2473	2	22	1	16	226	0.3	1
GDP per capita (USD)	4805	12217	3589	20247	611	606	21619	1620	1108
Major religion	Catholicism	Catholicism	Catholicism	Catholicism	Islam	Catholicism	Catholicism	Catholicism	Catholicism
Public expenditure with health (%GDP)	2.6	4.0	2.5	2.6	1.1	2.1	9.1	2.0	10.4
Public expenditure with education (%GDP)	4.9	6.2	5.5	2.2	2.2	5.7	4.2	5.6	16.2
Illiteracy rate (% population)	28.4	8.5	12.8	6.0	40.1	41.2	5.2	9.6	35.9
Sociodemographic index *	0.42	0.66	0.55	0.61	0.29	0.28	0.75	0.45	0.45

**Data from 2015. GDP: gross domestic product. USD: United States dollars.*

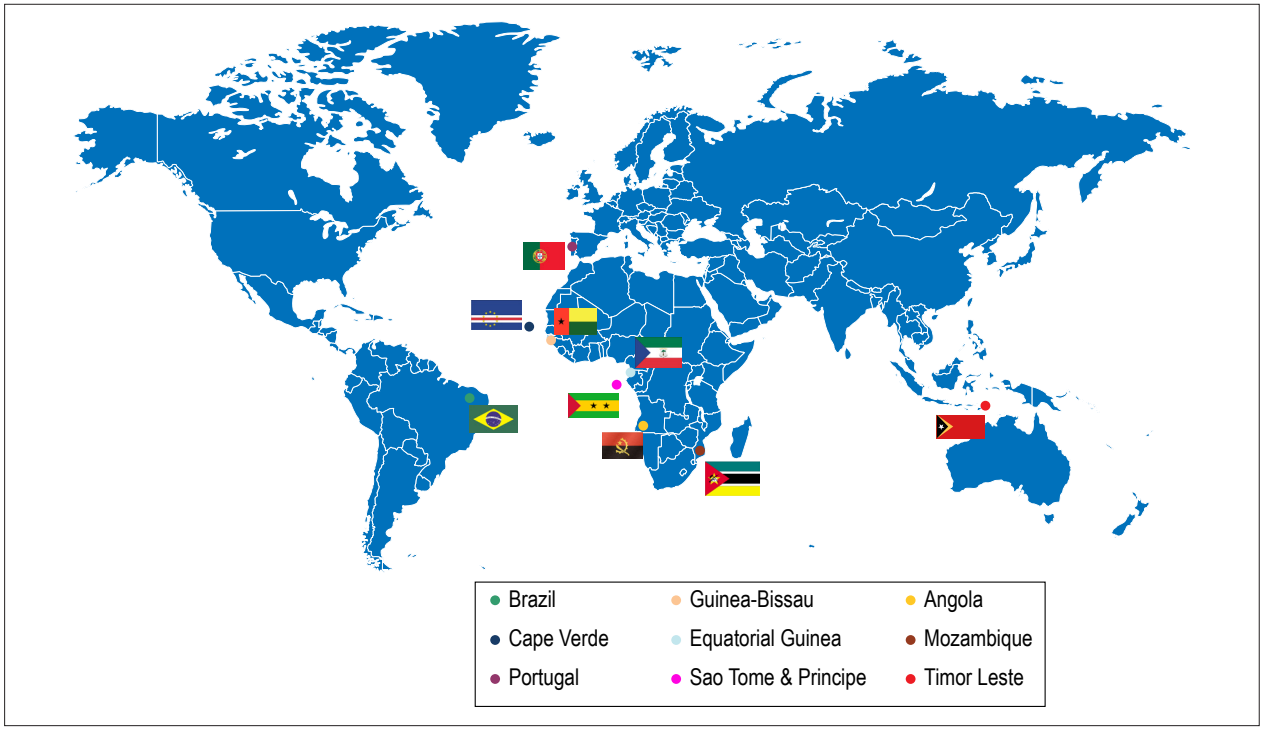


Figure 1 – *Global map showing the location of the Portuguese-speaking countries, 2017.*

Statistical analysis

The statistical models of the GBD 2016 Study previously reported were used.[4-6] The source of data for the models are available on-line at Global Health Data Exchange (http://ghdx.healthdata.org/).

Metrics of mortality and prevalence

The GBD 2016 Study used data available on causes of death in 195 countries. The information was collected according to international standards of death certification, using of information systems on vital statistics, mortality surveillance systems, survey, hospital registries and police registries.[4] The data sources have regional particularities, such as in Brazil, where data were mainly obtained from the Mortality Information System (SIM) of the Brazilian Health Ministry, by using an automated coding system.[10] In Brazil, all deaths require a medical DC provided by the attending physician. For the deaths occurring outside a healthcare facility, the causes are verified by the Death Verification Service, or by a civil agent when a physician is not available, and, in such cases, the causes of death are not registered.[11] Deaths due to external causes are identified by a medical examiner at the Forensic Pathologist Service.

In addition, techniques to correct quality problems regarding the information about the causes of death were used.[9] Corrections were made for underreporting of deaths and for causes considered not useful for the public health analysis, known as Garbage Codes. That term is used to describe causes that cannot or should not be considered as underlying causes of death, or that are unspecified within larger groups of causes. Algorithms of redistribution of Garbage Codes were developed by the GBD Study to increase the validity of the estimates. For this redistribution into specific causes of death, evidence from several sources, such as medical literature, expert opinion and statistical techniques, was considered.[4,8,12]

After treatment of data quality, the GBD 2016 Study used a variety of statistical models to determine the number of deaths per each cause, mainly by use of the Cause of Death Ensemble Model (CODEm) algorithm. To ensure that the number of deaths per cause does not exceed the total estimated number of deaths, a correction technique (CoCorrect) was used. Adjustment by this technique ensures that the sum of the estimated number of deaths per each cause does not exceed 100% of the estimated deaths in a certain year.[13]

The disease prevalence was estimated at a more detailed level of specific sequelae of disease, using as entry data the published systematic reviews of the scientific literature, as well as unpublished data of administrative registries and databases of the health system. Regression equations were used for data adjustment to define the standard case. The data presented were analyzed for the period from 1990 to 2016, and all analyses were stratified by sex and presented as absolute and age-standardized estimates, for the different PSC.

Metrics of burden of disease

The disability-adjusted life years (DALYs) combine information regarding premature death (years of life lost: YLLs) and disability caused by the condition (years lived with disability: YLDs) to provide a brief measure of the healthy years lost due to the condition. The YLLs were calculated by multiplying the deaths observed at each specific age in one year of interest by the reference age-specific life expectancy estimated by use of life table methods.

The YLDs were calculated by multiplying disease prevalence (in number of cases/year) by a health-state-specific disability weight representing a degree of lost functional capacity. The process of estimating the burden of the disability has been previously described in details.[6] Briefly, the burdens of disability were determined via home interviews in several countries, in which the participants were asked to choose between lay descriptions of different health states.[14,15] Adjustment for comorbidity was performed, simulating 40,000 individuals in each age-sex-country-year stratum exposed to the independent likelihood of developing each condition, based on the disease prevalence, with 95% uncertainty intervals (95% UI) reported for each estimate. Age-standardization was obtained via the direct method, applying a global age structure.

Sociodemographic index

The SDI is used as an estimate of the socioeconomic level of each country to assess its association with the CVD burden, as a function of global epidemiological transition.[4,8] Similarly to the method used to calculate the Human Development Index, the SDI was calculated for each country or territory from 1990 to 2016. The SDI is the weighted geometric mean of income per capita, educational attainment and total fertility rate, and allows the comparison of the performance of each country to those of countries with a similar socioeconomic level.

The SPSS software, version 22.0 for Mac OSX (*SPSS Inc., Chicago, Illinois*), was used to perform the correlation (Spearman correlation) between the country's SDI and the variation of the age-standardized mortality rates from CVD between 1990 and 2016. A p-value < 0.05 was considered statistically significant.

Ethical considerations

This study was conducted in a public secondary database, without nominal identification, according to Decree 7.724, of May 16, 2012, and the Resolution 510, of April 7, 2016. The Brazilian GBD 2015 Study was approved by the Committee in Ethics and Research of the Minas Gerais Federal University (Project CAAE 62803316.7.0000.5149).

Results

Causes of mortality from CVD

The importance of CVD as the cause of death has increased in the PSC. In 1990, CVD were the main cause of death only in Brazil and Portugal, while, in the other countries, infectious diseases, such as diarrhea and respiratory infections, were the leading causes. In 2016, however, CVD became the leading cause of death in Cape Verde, Sao Tome and Principe and Timor-Leste, and ranked higher or maintained their ranks in the other countries (Figure 2A). Considering the causes of CVD, there was an increase in the proportional mortality from ischemic heart disease, which, in 2016, was the first cause of death in most countries studied, except for Mozambique and Sao Tome and Principe. In general, there was a reduction in proportional mortality from rheumatic heart disease (Figure 2B).

Trends in the mortality rates from CVD between 1990 and 2016

Figure 3 shows an important reduction in proportional mortality from CVD and in the age-standardized mortality rate from CVD in Portugal, revealing that the decline in mortality occurred at all age groups. In Brazil and in Equatorial Guinea, the proportion of deaths from CVD remained stable, while a consistent reduction was observed over the past 15 years in the age-standardized mortality rate, suggesting there was mainly a reduction in premature mortality from CVD. In the other countries, the proportion of deaths due to CVD increased, and the reduction in age-standardized mortality rate from CVD declined less expressively, suggesting an increasing impact of CVD in those countries.

Although the proportional mortality from CVD decreased in the PSC from 1990 to 2016, the decline was heterogeneous among the countries. Figure 4 shows the age-standardized mortality rates for each PSC in 1990 and 2016.

Figure 5 reveals the positive correlation between the reduction in age-standardized mortality rates from CVD between 1990 and 2016 and the SDI of the country ($r_s = 0.7$; $p = 0.04$), suggesting a reduction in mortality from CVD follows the improvement in the local socioeconomic conditions of the PSC.

Lost of healthy life years (DALY) due to CVD

The trend in DALYs between 1990 and 2015 (Supplementary Figure 1) in the PSC is similar to that reported for the age-standardized mortality rate: there was a heterogeneous reduction in all countries, more expressive in those with better SDI. Regarding the specific causes of CVD, Figure 6 shows the importance of ischemic heart disease and CbVD in all countries, for both sexes. The loss of healthy life years was greater for men in all countries, except for Equatorial Guinea, Sao Tome and Principe and Angola, being mainly due to the other heart diseases. The importance of rheumatic heart disease, which is strictly related to socioeconomic conditions, for the loss of healthy years is evident in the countries with the lowest SDI.

Influence of the risk factors on CVD

Figure 7 reveals the risk factors attributed to the YLLs in each PSC. In general, of the classical risk factors and their determinants, arterial hypertension and dietary factors are the most important. The relevance of obesity is greater among women, being less important in Timor-Leste, despite the importance of metabolic risk factors in that country. The metabolic risk factors (high cholesterol, high blood sugar) have higher influence on the premature mortality from CVD in the countries with higher SDI (Portugal, Brazil and Equatorial Guinea). The relevance of the smoking habit is evenly greater for men, but heterogeneous among the countries. In addition, environmental risk factors, such as air pollution, are heterogeneous among the countries.

Detailed information about the metrics of the disease burden related to CVD and stratified by the PSC is shown in the Supplementary Tables.

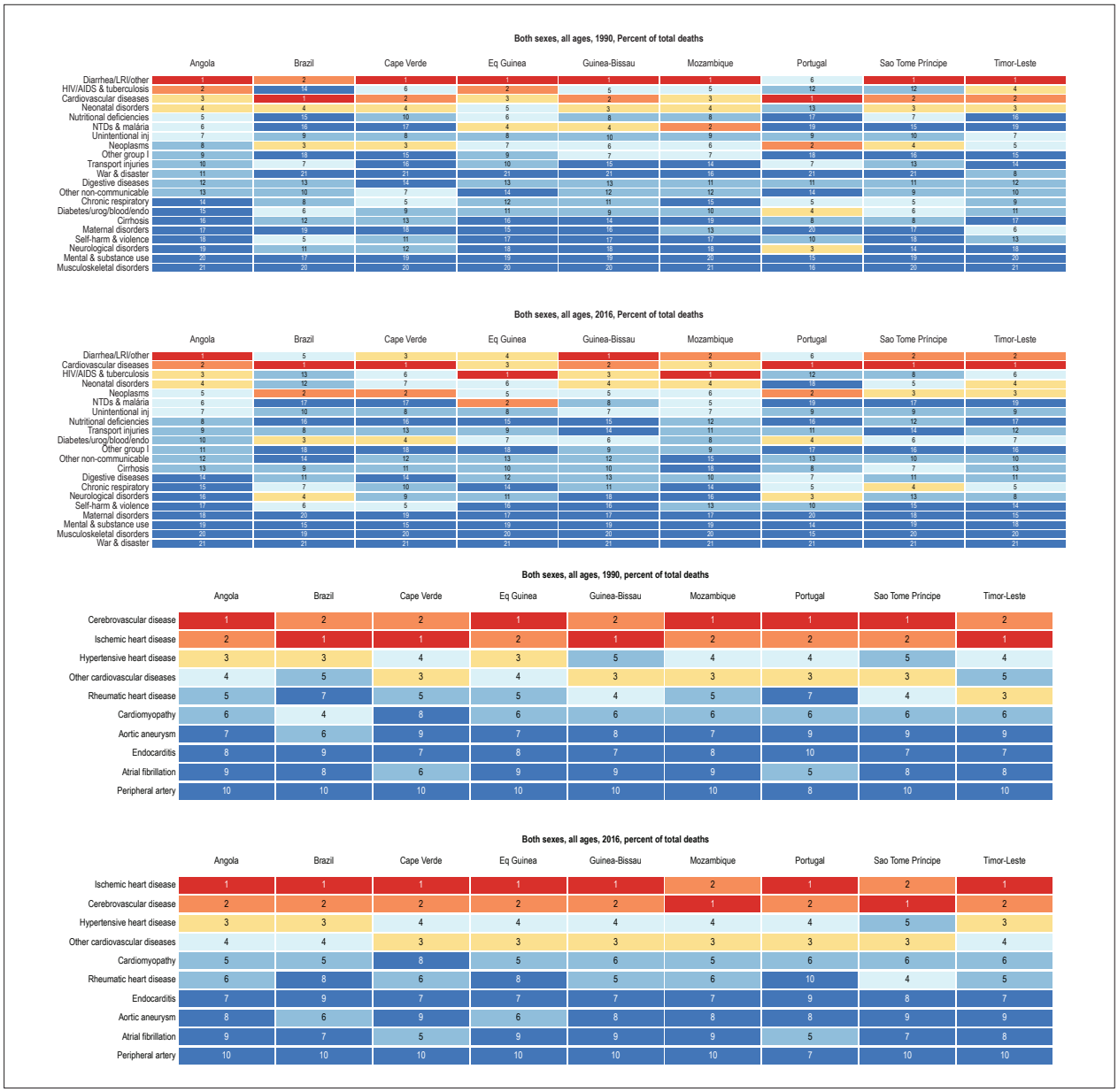


Figure 2 – *Most common causes of death, considering age-standardized mortality, in the Portuguese-speaking countries in 1990 and 2016. A: All causes; B: From cardiovascular diseases. LRI = lower respiratory tract infection; NTD = neglected tropical diseases.*

Discussion

Portuguese is the sixth most spoken language worldwide, by 244 million speakers,[16] the fifth most commonly used language over the Internet, by 82.5 million cybernauts, and the third most commonly used language in the social media *Facebook* and *Twitter*, by 58.5 million people.[17] According to estimates of the Portuguese government, considering the demographic evolution, by 2050, the number of Portuguese speakers will increase to 335 million. Africa should exceed Brazil in the use of Portuguese within 50 years.[16]

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 to the Portuguese from Portugal, from which the PSC were colonies during the expansion of that nation. Independence from the Portuguese domination occurred at different points in time: Equatorial Guinea belonged to the Portuguese Empire only until 1778, being then given to Spain, while Brazil was a Portuguese colony until 1822. The other nations had their independence recognized by Portugal between 1973 and 1975. However, the cultural influence of Portugal on those countries has been striking, an example being Catholicism, the official religion in most of those countries, the predominance of the Portuguese surnames, in addition to the organization of the healthcare systems, which are similar in several aspects.[2,18]

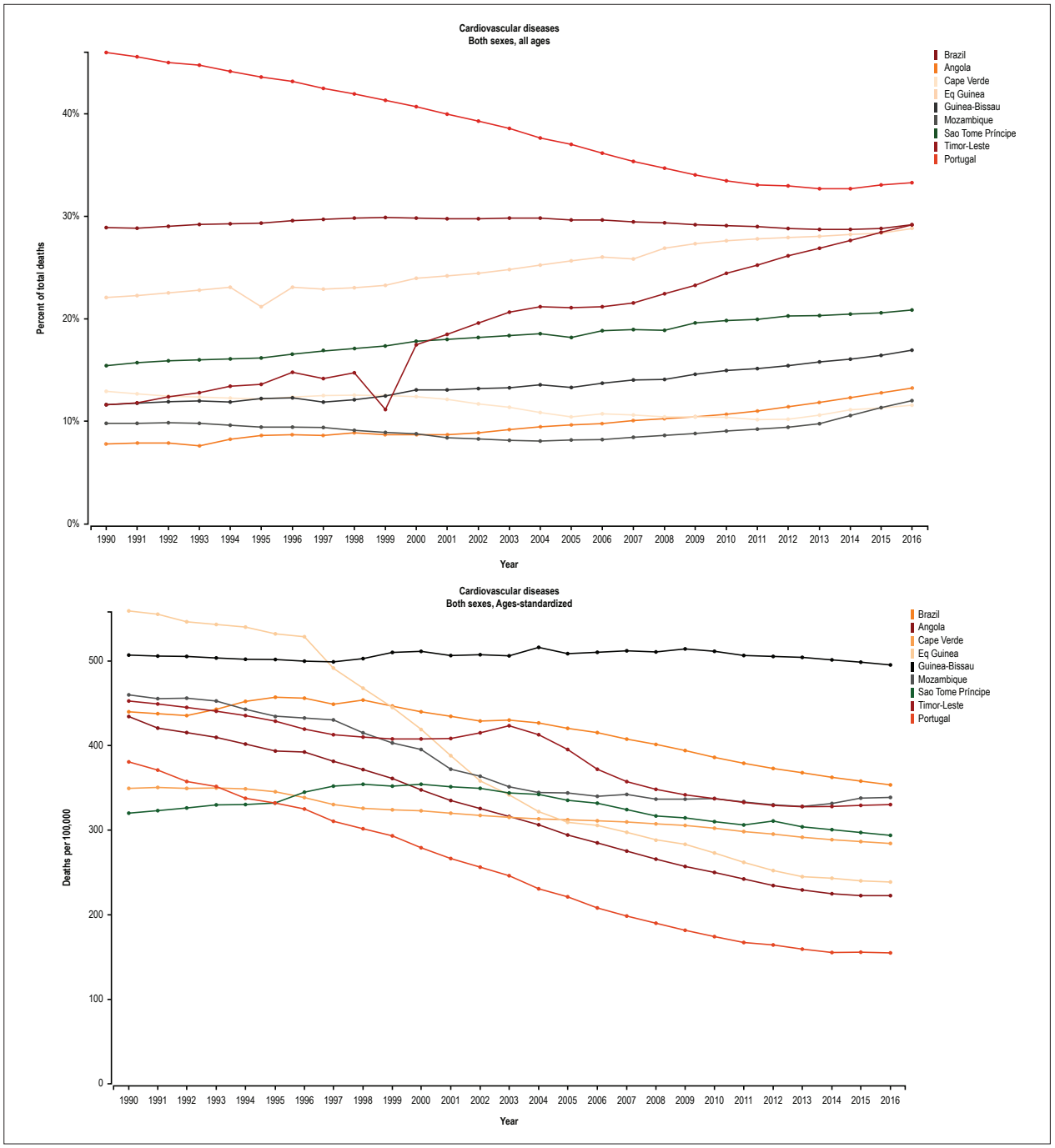


Figure 3 – *Mortality attributed to cardiovascular diseases in the Portuguese-speaking countries from 1990 to 2016. A. Proportional mortality from cardiovascular diseases (%), B. Age-standardized mortality rate from cardiovascular diseases (deaths/100,000).*

Despite their cultural identity, the socioeconomic development was heterogeneous among the PSC. In 2015, the SDI was as low as 0.28 and 0.29 in Guinea-Bissau and Mozambique, respectively, although, in Brazil (0.66) and Equatorial Guinea (0.61), the SDI was closer to that of Portugal (0.75). The same occurred with the Gross Domestic Product per capita (GDP per capita), quantified in United States dollars in that same year, ranging from 606 to 611 in Guinea-Bissau and Mozambique, and from 20,247 to 21,619 in Equatorial Guinea and Portugal, respectively. The percentages of public expenditures on health differed, representing, in 2013, 2% to 3% of the GDP of most PSC, except for Portugal and Timor-Leste, whose percentages were higher, ranging from 9.1% to 10.4%, respectively, while Brazil was at an intermediate level, currently at 4% of the GDP.[2]

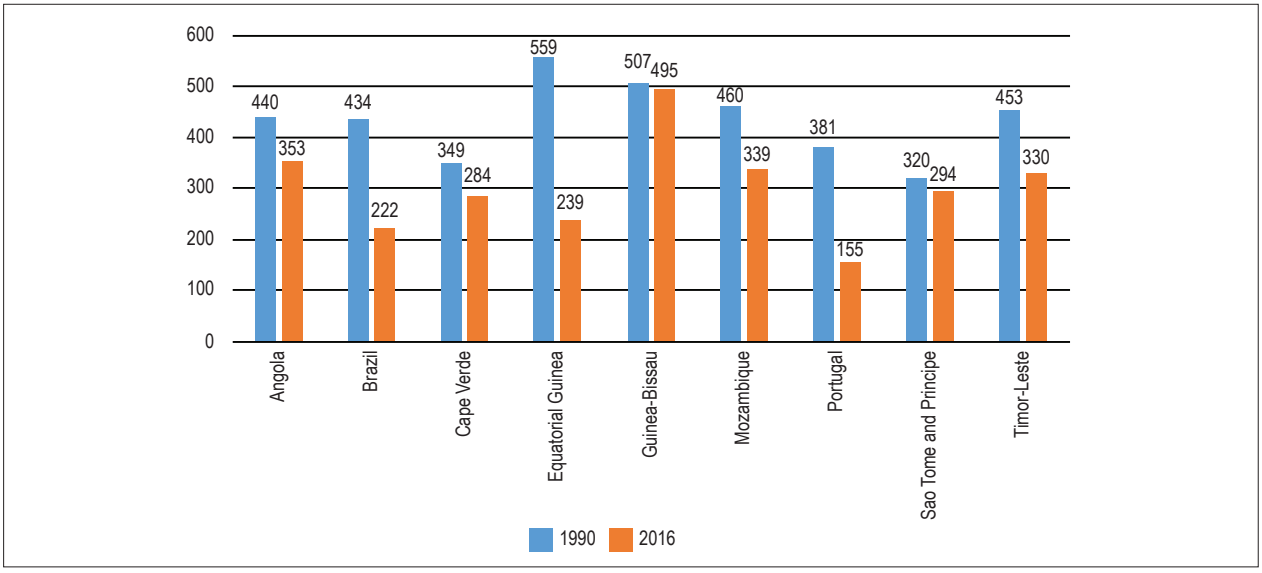


Figure 4 – *Age-standardized mortality rate in the Portuguese-speaking countries in 1990 and 2016.*

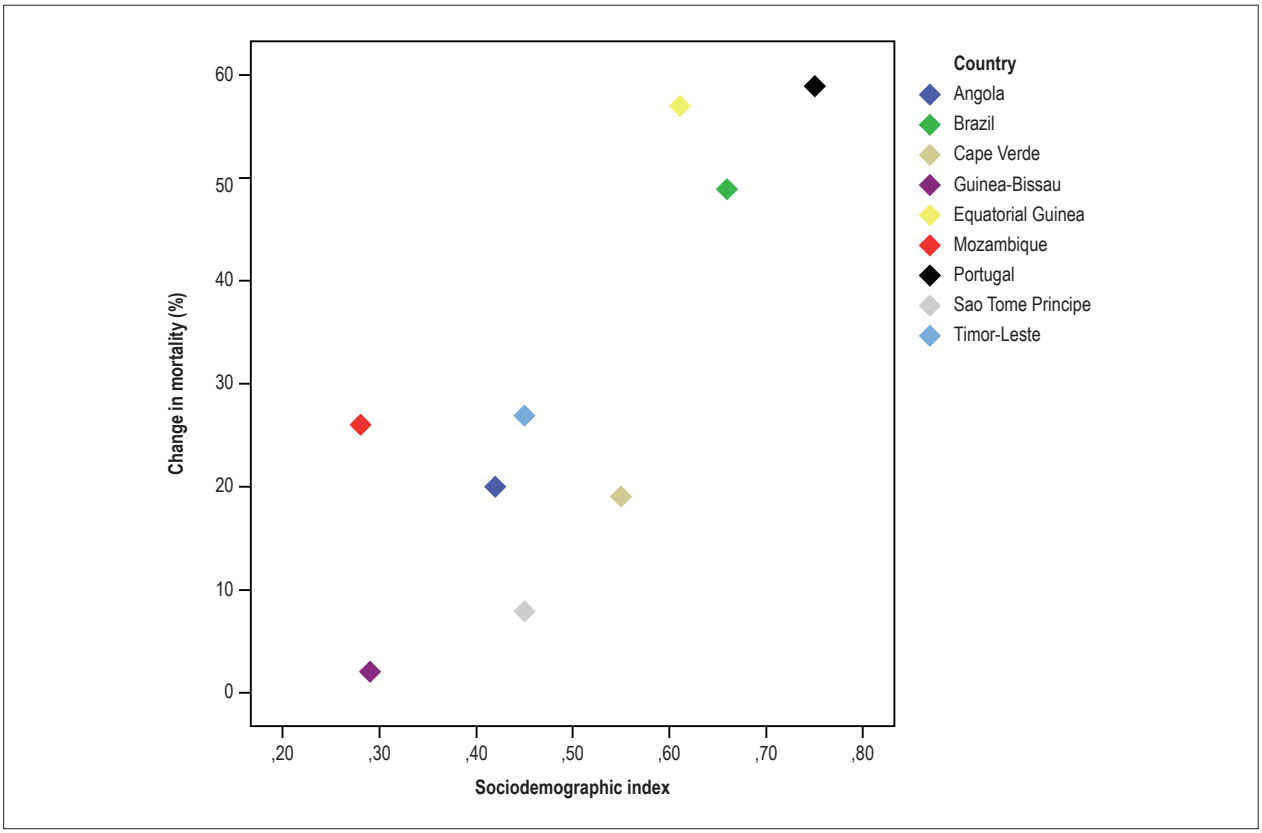


Figure 5 – *Correlation between the change in mortality from cardiovascular diseases between 1990 and 2016 and the sociodemographic index (r_s = 0.7; p = 0.04).*

An important result of this study was to show that in all the PSC, except for Guinea-Bissau, reductions in age-standardized mortality rates from CVD were observed from 1990 to 2016. From a global perspective, the period was marked by a reduction in age-standardized mortality from CVD in all high-income and some middle-income countries, although little change could be observed in most Sub-Saharan countries.[7] In the PSC, a strong positive correlation was observed between the SDI and the reduction in the age-standardized mortality rates from CVD in the past 27 years. However, that pattern was not homogeneous, suggesting that other factors[19] could be associated with the observed mortality reduction.

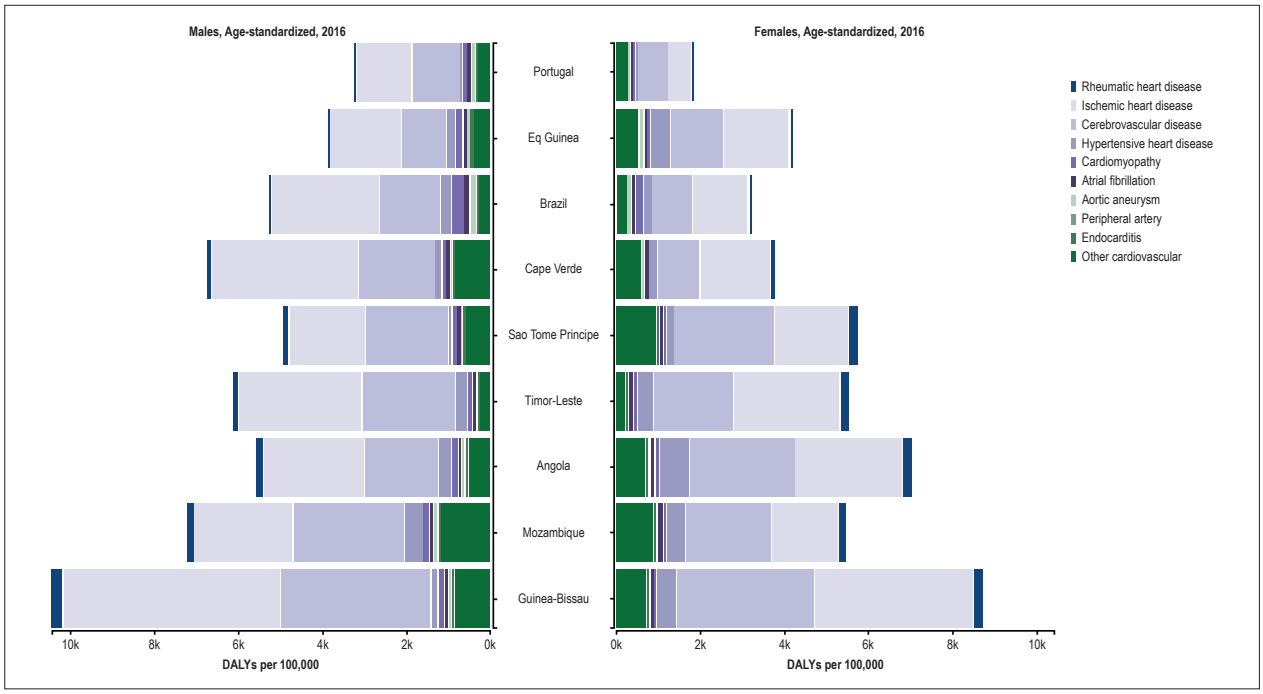


Figure 6 – *Disability-adjusted life years (DALYs) for each cardiovascular disease for each Portuguese-speaking country, 2016.*

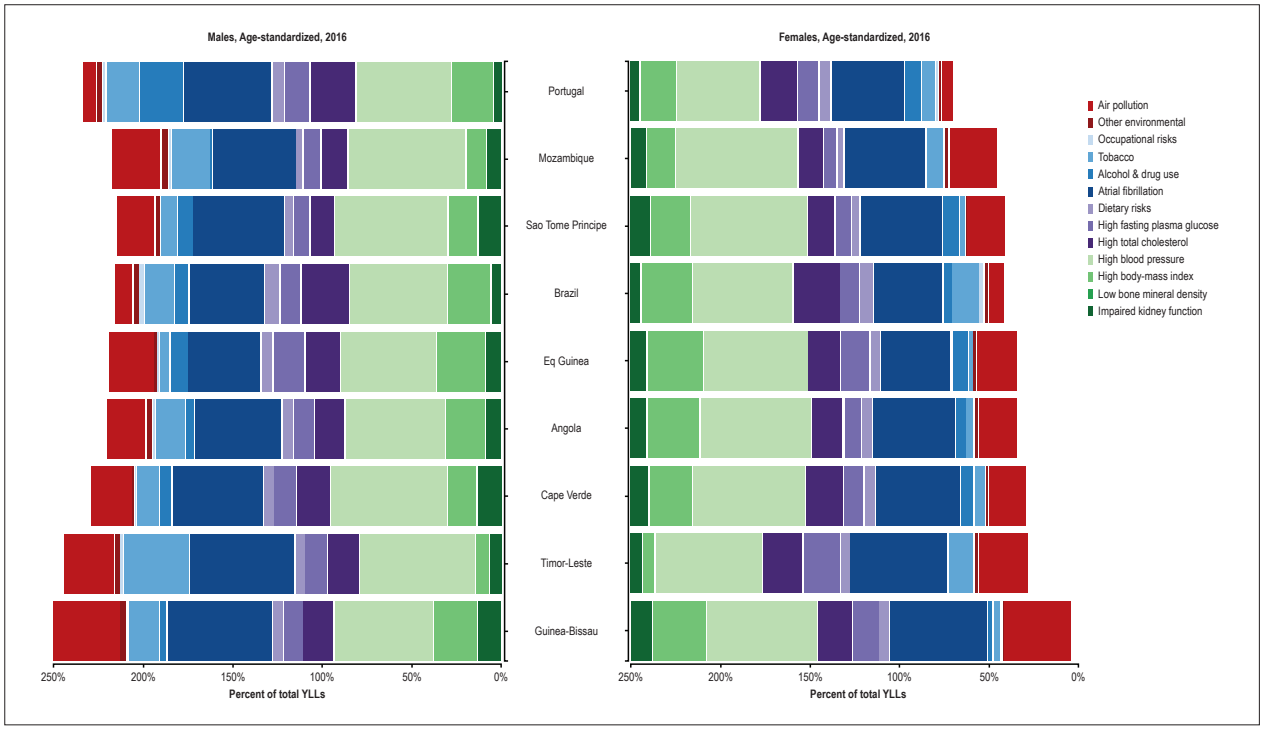


Figure 7 – *Influence of the risk factors on the years of life lost (YLLs) because of cardiovascular diseases, according to sex, in each Portuguese-speaking country in 2016.*

In Brazil and Portugal, that correlation was closer. The decline in age-standardized mortality rates in Brazil, associated with the increase in life expectancy, and the maintenance of a proportional mortality around 30%, from 1990 to 2016, point towards a greater relevance of the reduction in the premature mortality component.[20] The improvement in social inequality observed in Brazil and in some PSC might have contributed to that heterogeneous reduction in mortality as compared to the other Sub-Saharan countries.[21]

It is worth noting that, of the total number of deaths occurring in the Sub-Saharan Africa in 2015, ischemic heart disease was the fifth cause of death, preceded by the infectious causes in both sexes, while hemorrhagic stroke was the seventh, diabetes *mellitus*, the eighth, and ischemic stroke, the fifteenth cause of death in both sexes.[18] In 2016, however, CVD were the first cause of death in almost all PSC, except for Guinea-Bissau, where CVD were the second cause of death, and Mozambique and Equatorial Guinea, where CVD were the third cause of death in both sexes. Ischemic heart diseases predominated in all PSC, except for Mozambique and Sao Tome and Principe, a different pattern from that observed in the other Sub-Saharan countries,[18] suggesting a similarity between the PSC.

The DALYs were reduced in the PSC over the temporal series, from 1990 to 2016, probably reflecting an improvement in the health care provided to those populations.[22] The DALYs were mainly due to ischemic heart disease and CbVD in those PSC. That reduction was greater in the countries with the highest SDI. However, in an analysis of the GBD Study from 1990 to 2013, the SDI did not explain the reduction in the DALYs due to CVD, mainly because of the heterogeneity of the set of countries considered.[22]

The systolic and diastolic blood pressure levels decreased from 1995 to 2015 in most high-income countries.[23] That effect was not observed in most Sub-Saharan countries,[23] and that could explain the predominance of the CbVD as the most important component of the mortality from CVD in Sub-Saharan countries, with the contribution of the increase in body mass and dietary factors.[24] The same has occurred in the PSC, where arterial hypertension and dietary factors had a more relevant influence in both sexes for the DALYs due to CVD. It is worth noting the importance of the dietary risk factors in most PSC under the influence of the global dietary pattern, with ultra-processed foods and excessive amounts of sugars and fats, modifying healthier traditional dietary patterns. Those risk factors can be modified by promoting policies that favor healthy dietary habits, the taxation of ultra-processed foods, and the subsidies to healthy food, such as fruits and vegetables.[25,26]

The African populations are characterized by a great genetic diversity, representing the repository of genetic material of modern humans, who spread around the world over the past 100,000 years, having genetic adaptations in response to different climates, diets, geographic environments and infectious agents to which they have been exposed.[27] The genetic variations of Sub-Saharan Africa have been modelled by geographical and ethnolinguistic similarities.[28] In addition, in the European populations, linguistic similarities have shown to be better predictors of genome differences as compared to geographic differences.[29] The complex genetic interactions with the environmental and sociodemographic factors will be able to help us understand the heterogeneous occurrence of CVD.[30]

Although the quality of the completeness of the data collected and estimates has varied between the different PSC, there was an improvement in the recent years of the temporal series,[4] indicating the importance of the investment in national systems of vital registration and verbal autopsy to understand the burden of CVD in those countries.

Limitations and strengths

The limitations of the analytical models of the GBD Study have been previously discussed in detail.[4-6,8] Despite the improvement in the completeness of the data on prevalence and morbidity in some PSC, the estimates of the GBD 2016 Study indicate that the integrity and quality of those data are heterogeneous. For example, in Brazil and Portugal, the coverage of the data on death exceeds 95%, in contrast to very low or even absent indices in PSC of Sub-Saharan Africa.[4,8] The GBD Study models may have been inadequate for the different countries in some groups of diseases, mainly the non-communicable ones and those under less strict epidemiological surveillance, such as the classification of Latin America as a non-endemic region for rheumatic heart disease, which differs from primary data of prevalence.[31,32]

In addition, the sociocultural, demographic, economic and ethnic differences and particularities between the PSC are not captured by the GBD Study models. Such differences are frequently associated with life habits, health behaviors and risk factors that affect the global burden of CVD. Moreover, despite having the same colonization and cultural similarities, the historical factors and development pattern of the societies differ significantly between the PSC.[2]

Despite those limitations, from the epidemiological point of view, the GBD Study is the most solid and comprehensive initiative to estimate the burden of CVD, being especially useful to enable standardized comparisons between countries, including the PSC, whose primary data are still scarce. Those limitations do not invalidate the importance of this study for the epidemiological evaluation of CVD in the PSC, aiming at the elaboration of educational, preventive and therapeutic strategies more adequate for each country's reality, considering their sociodemographic, economic and cultural differences.

The major strength of this analysis is to consistently demonstrate that the importance of the CVD as a cause of death has grown in the PSC. Although mortality has decreased or remained stable in countries with better SDI – with a significant reduction in age-standardized mortality rates – the same pattern has not been observed in the countries with worse SDI, indicating the important impact of CVD and their association with socioeconomic factors. The GBD Study estimates have great relevance for the continuous reassessment of policies of prevention and health promotion, as well as for the formulation, planning and adequacy of new strategies to be implemented. Regional trends of morbidity and premature mortality from certain groups of CVD, especially in countries with lower SDI and less-structured health systems, should be considered, aiming at the individualization of action plans for countries with similar cultural origin, but very different health realities.

Conclusion

The data presented show great differences in the relative importance of the burden of CVD in the PSC and indicate that such differences relate to the socioeconomic conditions of the countries. Of the CVD, ischemic heart disease is the major cause of death in all PSC, except for Mozambique and Sao Tome and Principe, where CbVD is the major cause. The PSC share the most relevant risk factors for CVD: 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.

Author contributions

Conception and design of the research: Oliveira GMM, Malachias, MVB, Ribeiro AL; Acquisition of data: Reis GMA, Ribeiro AL, Brant LCC, Souza MFM, Malta DC, Teixeira RA; Roth GA; Analysis and interpretation of data: Brant LCC, Reis GMA, Ribeiro AL, Nascimento BR; Statistical analysis: Teixeira RA, Brant LCC, Ribeiro, AL, Roth GA; Obtaining financing: Souza MFM, França E; Writing of the manuscript: Nascimento, BR, Brant, LCC, Oliveira GMM; Critical revision of the manuscript for intellectual content: Todos os autores.

Potential Conflict of Interest

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

Sources of Funding

The Ministry of Health of Brazil was responsible for funding the GBD Brazil Study (Fundo Nacional de Saúde, grant number (17217.9850001 /17-018). Ribeiro was supported in part by CNPq (Bolsa de produtividade em pesquisa, 310679/2016-8 and IATS, 465518/2014-1) and by FAPEMIG (Programa Pesquisador Mineiro, PPM-00428-17).

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 Universidade Federal de Minas Gerais under the protocol number 62803316.7.0000.5149. 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.

References

1. World Health Organization. (WHO). Global action plan for the prevention and control of noncommunicable diseases 2013-2020. Geneva (Switzerland); 2013.
2. Comunidade dos Países de Língua Portuguesa - 2017 (CPLP). [Acesso em 2017 nov 110. Disponível em: https://www.cplp.org/
3. Institute for Health Netrics and Evaluation. Global Burden of Disease (GBD). Seattle (WA): University of Washington; 2017.
4. Naghavi M, Abajobir AA, Abaffati C. GBD Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1151-210.
5. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1211-59.
6. GBD DALYs and HALE Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1260-344.
7. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. J Am Coll Cardiol. 2017;70(1):1-25.
8. GBD Mortality Collaborators. Global, regional, and national under-5 mortality, adult mortality, age-specific mortality, and life expectancy, 1970-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1084-150.
9. Murray CJ, Lopez AD. Measuring the global burden of disease. N Engl J Med. 2013;369(5):448-57.
10. França EB, Passos VM, Malta DC, Duncan BB, Ribeiro AL, Guimarães MD, et al. Cause-specific mortality for 249 causes in Brazil and states during 1990-2015: a systematic analysis for the global burden of disease study 2015. Popul Health Metr. 2017;15(1):39.
11. Franca E, de Abreu DX, Rao C, Lopez AD. Evaluation of cause-of-death statistics for Brazil, 2002-2004. Int J Epidemiol. 2008;37(4):891-901.
12. Foreman KJ, Naghavi M, Ezzati M. Improving the usefulness of US mortality data: new methods for reclassification of underlying cause of death. Popul Health Metr. 2016 Apr 28;14:14.
13. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388(10053):1459-544.
14. Salomon JA, Haagsma JA, Davis A, de Noordhout CM, Prolinder S, Havelaar AH, et al. Disability weights for the Global Burden of Disease 2013 study. Lancet Glob Health. 2015;3(11):e712-23.
15. Haagsma JA, Maertens de Noordhout C, Polinder S, Vos T, Havelaar AH, Cassini A, et al. Assessing disability weights based on the responses of 30,660 people from four European countries. Popul Health Metr. 2015 Apr 3;13:10.
16. Camões - Instituto da Cooperação e da Língua. Ministério dos Negócios Estrangeiros; 2017. [Acesso em 2017 nov 22]. Disponível em: http://www.instituto-camoes.pt/
17. Internet World Stats. Usage and Population Statistics. Miniwatts Marketing Group; 2017. [Accessed in 2017 Nov 22]. Available from: http://www.internetworldstats.com/stats.htm/
18. Agyepong IA, Sewankambo N, Binagwaho A, Coll-Seck AM, Corrah T, Ezeh A, et al. The path to longer and healthier lives for all Africans by 2030: the Lancet Commission on the future of health in sub-Saharan Africa. Lancet. 2018;390(10114):2803-59.

19. Wilkinson RG, Pickett K. Por que uma sociedade mais igualitária é melhor para todos. Rio de Janeiro: Editora Civilização Brasileira; 2015.

20. Ribeiro AL, Duncan BB, Brant LC, Lotufo PA, Mill JG, Barreto SM. Cardiovascular health in Brazil: trends and perspectives. Circulation. 2016;133(4):422-33.

21. Marmot M. Brazil: rapid progress and the challenge of inequality. Int J Equity Health. 2016;15(1):177.

22. GBD Healthcare Access Quality Collaborators. Healthcare Access and Quality Index based on mortality from causes amenable to personal health care in 195 countries and territories, 1990-2015: a novel analysis from the Global Burden of Disease Study 2015. Lancet. 2017;390(10110):231-66.

23. Feigin VL, Roth GA, Naghavi M, Parmar P, Krishnamurthi R, Chugh S, et al; Global Burden of Diseases, Injuries and Risk Factors Study 2013 and Stroke Experts Writing Group. Global burden of stroke and risk factors in 188 countries, during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet Neurol. 2016;15(9):913-24.

24. NCDRF Risk Factor Collaboration. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19.1 million participants. Lancet. 2017;389(10064):37-55.

25. World Health Organization (WHO). Global action plan for the prevention and control of NCDs 2013-2020. [Accessed in 2017 Apr 12]. Available from: http://www.who.int/nmh/events/ncd_action_plan/en/

26. World Health Organization (WHO). Governance: updating appendix 3 of the WHO global NCD action plan 2013-2020. [Internet]. [Accessed in 2017 Apr 12]. Available from: http://who.int/ncds/management/WHO_Appendix_BestBuys.pdf.

27. Lambert CA, Tishkoff SA. Genetic structure in African populations: implications for human demographic history. Cold Spring Harb Symp Quant Biol. 2009;74:395-402.

28. Busby GBJ, Band G, Le AS, Side Q, Jallaw N, Bougama F, et al. Admixture into and within sub-Saharan Africa. Elife. 2016 Jun 21;5. pii:e15266.

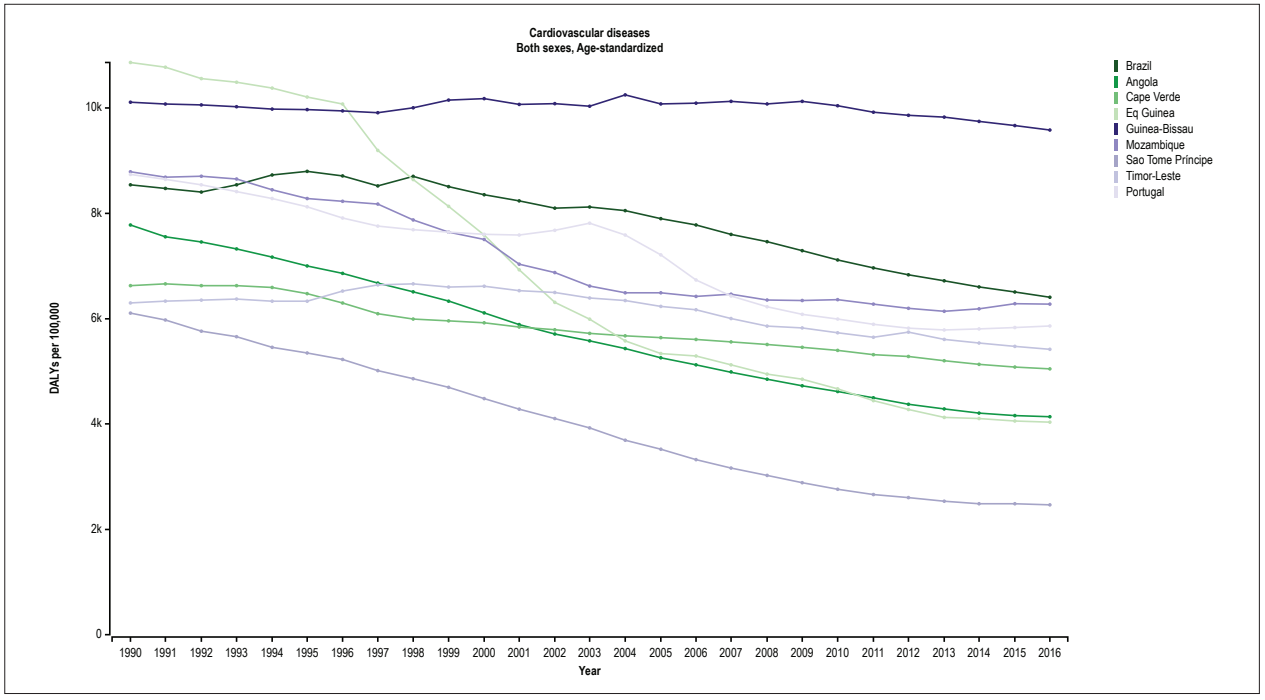
29. Longobardi G, Ghirotto S, Guardiano C, Tassi F, Benazzo A, Ceolin A, et al. Across language families: genome diversity mirrors linguistic variation within Europe. Am J Phys Anthropol. 2015;157(4):630-40.

30. Kullo IJ, Ding K. Patterns of population differentiation of candidate genes for cardiovascular disease. BMC Genet. 2007 Jul 12;8:48.

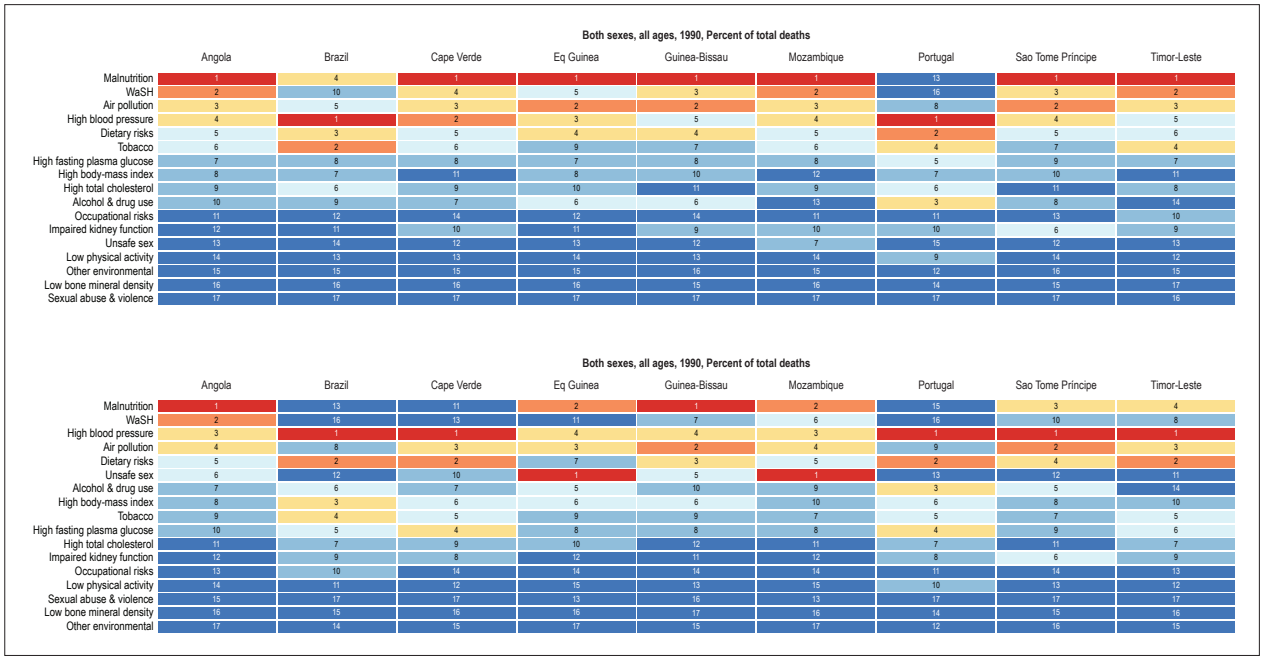
31. Nascimento BR, Beaton AZ, Nunes MC, Lopes EL, Rezende VM, Landay T, et al. Echocardiographic prevalence of rheumatic heart disease in Brazilian schoolchildren: Data from the PROVAR study. Int J Cardiol. 2016 Sep 15;219:439-45.

32. Ribeiro GS, Tartof SY, Oliveira DW, Guedes AC, Reis MG, Riley LW, et al. Surgery for valvular heart disease: a population-based study in a Brazilian urban center. PloS One. 2012;7(5):e37855.

Original Article



Supplementary Figure 1 – *Age-standardized Disability Adjusted Life Years (DALY) attributable to cardiovascular disease (CVD) in Portuguese-speaking countries, from 1990 to 2016.*



Supplementary Figure 2 – *Mortality attibutable to risk factors in Portuguese-speaking countries, in 1990 and 2016.*

Cardiovascular Diseases in Portuguese: The Importance of Preventive Medicine

Fausto J. Pinto

Clínica Universitária de Cardiologia, CAML, CCUL, Universidade de Lisboa, Lisboa - Portugal

Short Editorial regarding the article: Cardiovascular Disease Epidemiology in Portuguese-Speaking Countries: data from the Global Burden of Disease, 1990 to 2016

Cardiovascular diseases (CVD) are the leading cause of mortality and morbidity worldwide.[1] Because several CVD have sequelae that significantly impact the life of affected individuals, knowing the importance of those diseases, as well as their associated factors, is essential to develop preventive measures to reduce that impact.[2-4]

The study published in this issue of the *Arquivos Brasileiros de Cardiologia*[5] conducts an epidemiological assessment of CVD in Portuguese-speaking countries (PSC) from 1996 to 2016, being, in that context, unprecedented and relevant. Despite some limitations, always present in that type of study, the analysis has considerable merit and allows us to draw very important conclusions. That study assesses, from an innovative perspective, CVD in a set of countries scattered around the world, which share a common language and cultural base, but have totally distinct geographic locations. In that type of analysis, the impact of local aspects, such as sanitation structures, health policies, economic and political conditions, on the parameters assessed must be properly considered, and that study does it in a very elegant way. The authors clearly indicate that the relative importance of the burden of CVD differs in the different PSC, and they directly relate those differences to the socioeconomic conditions of the countries. Of the CVD, ischemic heart disease is the major cause of death in all PSC, except for Mozambique and Sao Tome and Principe. In addition, the authors report that the most relevant risk factors for CVD, arterial hypertension and dietary factors, are common in the PSC. Furthermore, they conclude that "Genetic factors, implicit in the cultural identity, the factors inherent in the host, as well as the huge social inequality might have contributed to explain the mortality rates observed." It is worth noting that the authors report the general reduction in mortality from CVD as a common denominator among all the PSC, although the intensity of that reduction differs in the countries.

The introduction of several therapeutic strategies, such as drugs and medical devices, has determined a substantial reduction in mortality from CVD in general. In fact, the therapeutic and diagnostic advances in the cardiovascular field have contributed to an 80% increase in the life expectancy of the world population. That is an exceptional accomplishment. However, it is currently known that concomitantly with the decrease in mortality, several risk factors account for the increase in the prevalence of CVD. Arterial hypertension, diabetes, dyslipidemia, obesity and smoking habit have contributed to a general increase in the prevalence of CVD. It is worth noting that, despite the significant therapeutic advances, preventive measures, mainly the control of risk factors and promotion of healthy lifestyles, must be taken. Currently there is scientific evidence of the relationship between the implementation of preventive strategies and the corresponding reduction in cardiovascular events and mortality.[6,7] An example is the immediate impact of the enforcement of the smoke-free environment legislation on the incidence of acute myocardial infarction.[8-10] The reduction in hospitalization rates has been accompanied by a significant reduction in mortality rates[5] in the acute phase and during follow-up, reflecting the disseminated use of evidence-based treatments, such as reperfusion therapies and drugs to prevent the progression of ischemic heart disease. Some of those interventions protect against other manifestations of CVD, such as stroke.

The study this editorial refers to confirms those aspects and emphasizes the need to develop preventive medicine policies, which have clearly shown great efficacy when properly implemented. In addition, it portrays, for the first time, a vast and robust set of data from countries that share several similarities, despite their specific features. The study should be properly disclosed to the sanitary authorities of the PSC to reinforce the need for measures to reduce the impact of CVD on those countries. Above all, it is an excellent example of cooperation that should be duly emphasized and replicated. I congratulate the authors and the Portuguese-speaking cardiology community.

Keywords

Cardiovascular Diseases / mortality; Cardiovascular Diseases / prevention & control; Morbidity; Risk Factors; Myocardial Ischemia; Terapeutics / trends; Community of Portuguese- Speaking Countries.

Mailing Addres: Fausto J. Pinto •
Clínica Universitária de Cardiologia, CAML, CCUL, Universidade de Lisboa, Lisbon - Portugal
email: faustopinto@medicina.ulisboa.pt

DOI: 10.5935/abc.20180103

References

1. Timmis A, Townsend N, Gale C, Grobbee R, Maniadakis N, Flather M, et al; Atlas Writing Group. European Society of Cardiology: cardiovascular disease statistics 2017. Eur Heart J. 2018;39(7):508-79.
2. Lopez-Sendon J, Gonzalez-Juanatey JR, Pinto FJ, Castillo JC, Badimon L, Dalmau R, et al. Quality markers in cardiology: measures of outcomes and clinical practice--a perspective of the Spanish Society of Cardiology and of Thoracic and Cardiovascular Surgery. Eur Heart J. 2016;37(1):12-23.
3. Arena R, Guazzi M, Lianov L, Whitsel L, Berra K, Lavie CJ, et al. Healthy lifestyle interventions to combat noncommunicable disease-a novel nonhierarchical connectivity model for key stakeholders: a policy statement from the American Heart Association, European Society of Cardiology, European Association for Cardiovascular Prevention and Rehabilitation, and American College of Preventive Medicine. Eur Heart J. 2015;36(31):2097-109.
4. Andrade JP, Pinto FJ, Arnett DK. (editors). Prevention of cardiovascular diseases - from current evidence to clinical practice. New York: Springer; 2015.
5. Nascimento BR, Brant LCC, Oliveira GMM, Malachias MVB, Reis GMA, Teixeira RA, et al. Epidemiologia das doenças cardiovasculares em países de Língua Portuguesa: dados do "Global Burden of Disease", 1990 a 2016. Arq Bras Cardiol. 2018; 110(6):500-511.
6. Smolina K, Wright FL, Rayner M, Goldacre MJ. Determinants of the decline in mortality from acute myocardial infarction in England between 2002 and 2010: linked national database study. BMJ. 2012;344:d8059. Erratum in: BMJ. 2013;347:f7379.
7. O'Flaherty M, Buchan I, Capewell S. Contributions of treatment and lifestyle to declining CVD mortality: why have CVD mortality rates declined so much since the 1960s? Heart. 2013;99(3):159-62.
8. Abreu D, Sousa P, Matias-Dias C, Pinto FJ. Longitudinal Impact of the Smoking Ban Legislation in Acute Coronary Syndrome Admissions. Biomed Res Int. 2017;2017:6956941.
9. Mackay DF, Irfan MO, Haw S, Pell JP. Meta-analysis of the effect of comprehensive smoke-free legislation on acute coronary events. Heart. 2010;96(19):1525-30.
10. Cox B, Vangronsveld J, Nawrot TS. Impact of stepwise introduction of smoke free legislation on population rates of acute myocardial infarction deaths in Flanders, Belgium. Heart. 2014;100(18):1430-5.

Metabolic Syndrome-Related Features in Controlled and Resistant Hypertensive Subjects

Arthur Santa Catharina,[1] Rodrigo Modolo,[1] Alessandra Mileni Versuti Ritter,[1] Andréa Rodrigues Sabbatini,[1] Heno Ferreira Lopes,[2] Heitor Moreno Junior,[1] Ana Paula de Faria[1]

Faculdade de Ciências Médicas - Universidade Estadual de Campinas (UNICAMP),[1] Campinas, SP – Brazil

Instituto do Coração (InCor) - Faculdade de Medicina da Universidade de São Paulo,[2] São Paulo, SP – Brazil

Abstract

Background: Metabolic syndrome (MetS) is widespread among hypertensive patients. Clinical features and potential biomarkers of MetS in the presence of hypertension and resistant hypertension (RHTN) represent a great area of interest for investigation.

Objective: The purpose of this study was to evaluate the prevalence of MetS and the clinical features associated with it in resistant and mild to moderate hypertensives.

Methods: This cross-sectional study included 236 patients, (i) 129 mild to moderate hypertensive patients and (ii) 107 patients with RHTN. We measured blood pressure (BP) and adipokines levels, and performed bioelectrical impedance analysis. Microalbuminuria (MA), cardiac hypertrophy and arterial stiffness were also assessed. The significance level of alpha = 0.05 was adopted.

Results: We found a MetS prevalence of 73% in resistant and 60% in mild-to-moderate hypertensive patients. In a multiple regression analysis, MA (odds ratio = 8.51; p = 0.01), leptin/adiponectin ratio (LAR) (odds ratio = 4.13; p = 0.01) and RHTN (odds ratio = 3.75; p = 0.03) were independently associated with the presence of MetS apart from potential confounders.

Conclusions: Our findings suggest that both resistant and controlled hypertensive subjects have a high prevalence of MetS. In addition, MetS-related metabolic derangements may cause early renal and hormonal changes. Finally, LAR may be useful as a reliable biomarker for identifying those hypertensive subjects who are at risk for developing MetS. (Arq Bras Cardiol. 2018; 110(6):514-521)

Keywords: Metabolic Syndrome / diagnosis; Cardiovascular Diseases / mortality; Cholesterol; Waist Circumference; Triglycerides.

Introduction

Metabolic syndrome (MetS) is a cluster of metabolic abnormalities that affects approximately a quarter of worldwide adult population, which makes it a serious public health challenge.[1] Ever since the MetS was described in 1988,[2] several scientific organizations have attempted to formulate a general definition for the syndrome. The National Cholesterol Education Program - Adult Treatment Panel III (NCEP-ATPIII) definition[3] has become the most widely used definition, probably because it provides a relatively simple approach for diagnosing MetS with easily measurable risk factors.

The relationship between MetS and cardiovascular diseases (CVDs) is noteworthy.[4] In the largest meta-analysis on the theme comprising nearly one million patients, MetS was associated with a 2-fold increase in risk of CVD, cardiovascular mortality, myocardial infarction and stroke, and a 1.5-fold increase in the risk of all-cause mortality.[4]

The negative prognostic impact of MetS was also observed in patients with hypertension.[5] Studies have shown a high prevalence of hypertension-related asymptomatic organ damage in hypertensive patients with MetS, such as left ventricular hypertrophy (LVH), elevated urinary albumin excretion rate and arterial stiffness.[5] The majority of these patients have shown a deregulated production of adipokines.[6] Adiponectin, an adipokine with anti-atherogenic, insulin sensitization, lipid oxidation, and vasodilatation activities[7] showed to be decreased in obese and subjects with essential and resistant[8] hypertension (RHTN). In contrast, elevated leptin levels are associated with MetS, hypertension and atherosclerosis. On the other hand, there are few data regarding MetS, resistant hypertension and mild to moderate hypertension. Thus, this study aimed to evaluate the prevalence of MetS and the clinical features associated with MetS in resistant and mild to moderate hypertensive patients.

Mailing Address: Arthur Santa Catharina •
Rua Manoel Avelino, 09. Postal Code 13450-445, Vila Linópolis, Santa Bárbara d´Oeste, Campinas, SP – Brazil
E-mail: arthurscatharina@gmail.com
Manuscript received August 03, 2017, revised manuscript November 15, 2017, accepted December 01, 2017

DOI: 10.5935/abc.20180076

Methods

Study population

In this cross-sectional study, a convenience sample of 107 resistant and 129 mild to moderate hypertensive patients regularly followed at the Resistant Hypertension Outpatient Clinic and Hypertension Outpatient Clinic of the University of Campinas (Campinas, Brazil) were enrolled, and classified into those with MetS (n = 157) and without MetS (n = 79). Suitable subjects who agreed to participate in the study were screened for a 6-month period of clinical follow-up to exclude (i) secondary hypertension (pheochromocytoma, aortic coarctation, Cushing's or Conn's syndrome, renal artery stenosis and obstructive sleep apnea) and (ii) pseudoresistance hypertension, including poor medication adherence (verified by pill counts) and white coat hypertension (verified by ambulatory blood pressure monitoring-ABPM).

The diagnosis of "true" RHTN was done according to the 2008 American Heart Association Scientific Statement,[9] the last guideline published which properly defines a condition as (1) high blood pressure (BP) levels despite the use of at least three antihypertensive agents of different classes or (2) controlled BP after the use of four or more drugs. Ideally, one of the three agents should be a diuretic and all agents should be prescribed at optimal doses. Mild to moderate hypertensive subjects (grade I and II hypertension) were defined in accordance to the 2013 European Society of Hypertension (ESH) guidelines,[10] the last guideline on essential hypertension. Exclusion criteria were clinically-evident coronary artery disease or cerebrovascular disease, significant impaired renal or liver function, myocardial infarction and peripheral vascular disease.

Diagnosis of MetS

Diagnosis of MetS was determined according to the criteria proposed by the NCEP-ATPIII revised in 2005,[3] as the presence of at least three of the following criteria: (i) waist circumference (WC) ≥ 88 cm for women or ≥ 102 cm for men, (ii) HDL-cholesterol < 50 mg/dL for women or 40 mg/dL for men, (iii) triglycerides ≥ 150 mg/dL (or in current use of fibrate), (iv) cutoff BP values of ≥ 130/85 mmHg (or current antihypertensive treatment), and (v) fasting glucose ≥ 100 mg/dL (or current treatment for type 2 diabetes).

Bioelectrical impedance analysis (BIA)

Fat-free mass (FFM), fat mass (FM), total body water (TBW) and basal metabolic rate (BMR) were determined by BIA using the Bioimpedance Analyser 450 (Biodynamics Corporation, Seattle, USA). The measurements were performed after 4-hour period of fasting. Also, patients were instructed to avoid physical activity and smoking prior to the examination.

Office and Ambulatory BP measurements

Office systolic BP (SBP) and diastolic BP (DBP) were evaluated at approximately 08:00 a.m. in the right arm using a validated digital sphygmomanometer (HEM-907XL, OMRON Healthcare Inc., Bannockburn, IL, USA).

The 24-h ABPM measurements were performed with a validated automatic device (Spacelabs 90217, Spacelabs Inc, Redmon, WA, USA), and measurements were taken every 20min. Patients were instructed to maintain their usual daily activities and inform them in a personal diary. Both office and ambulatory BP measurements were performed according to 2013 ESH guidelines.[10]

Biochemical measurements

The laboratory exams analyzed were: fasting blood glucose (FBG), insulin, glycated hemoglobin (HbA1c), serum sodium and potassium, plasma cortisol, total cholesterol, low and high-density lipoprotein-cholesterol (LDLc and HDLc, respectively), triglycerides, urea, creatinine and renin. The values between 30 and 300 mg/g of urine albumin/creatinine ratio grouped the patients as having microalbuminuria (MA) for comparisons of early renal damage. Plasma concentrations of adiponectin and leptin (R&D Systems, Minneapolis, USA) were determined by ELISA and aldosterone (Immunotech SAS, Marseille, France) by chemiluminescence, according to the manufacturer's instructions.

Pulse wave velocity

Arterial stiffness was determined by pulse wave velocity (PWV), in meters per second (m/s), dividing the distance between the right carotid and femoral arteries by the pulse transit time through these two sites of interest. We used the Sphygmocor device (AtCor Medical, USA), synchronized with the electrocardiogram. We used the mean of two PWV values in the analyses, or the median of three consecutive readings if the difference between the two measurements was greater than 0.5 m/s. The patients were considered as having arterial rigidity if PWV ≥ 10 m/s, for comparisons of vascular damage.[11]

Echocardiography

Left ventricular (LV) measurements were performed according to the recommendations of the American Society of Echocardiography using two-dimensional M-mode echocardiography.[12] Examinations were performed by an echocardiography expert and reviewed by two blinded investigators, following standard technique, using a cardiovascular ultrasound machine (Siemens Acuson CV70, Munich, Bavaria, Germany) with a multi-frequency sector transducer (2-4 MHz). We calculated LV mass index (LVMI), and considered those with LVMI > 95 g/m^2 (females) and > 115 g/m^2 (males) as having left ventricular hypertrophy (LVH). The intraobserver and interobserver coefficients of variation were less than 9.5% for the LVMI.

Statistical analyses

For continuous variables we calculated the mean and standard deviation or median (1st, 3rd quartiles), according to normal distribution, measured by the Kolmogorov-Smirnov test. We compared them using either unpaired Student´s t-test or Mann-Whitney test, according to distribution of data. Categorical variables were presented in absolute

numbers and/or percentages and compared by chi-square test. A logistic regression model was applied to determine association of clinical variables with the presence of MetS, apart from potential confounders. All statistical tests were performed using SigmaPlot 12.5 version (Systat software, Inc.). A significance level of alpha = 0.05 was adopted.

Results

Baseline characteristics of hypertensive subjects with and without MetS are shown in Table 1. We found a MetS prevalence of 66% in all hypertensive population. No differences were found between groups regarding age, race and gender. As expected, BMI, WC, FM and TBW were higher in hypertensive patients with MetS. Office heart rate (HR) was significantly higher in patients with MetS. Neither office and ambulatory BP levels nor the proportion of patients with uncontrolled office BP (≥ 140/90 mmHg) were different between groups. The patients with MetS showed a higher prevalence of MA compared to their counterparts. The medication use was similar between groups, except for the calcium channel blockers and antidiabetics that were higher in MetS group (Table 1).

As expected, the evaluation of biochemical parameters showed increased triglycerides, as well as fasting glucose and HbA1c in subjects with MetS (Table 2). Additionally, adiponectin levels were significantly lower in patients with MetS, while leptin demonstrated to be increased in those patients, compared to the subjects without MetS (Table 2).

Finally, the multiple logistic regression revealed that MA, leptin/adiponectin ratio (LAR) and resistance to antihypertensive treatment were independently associated with the presence of MetS (Table 3).

Discussion

Our main findings suggest that MA and increased LAR are associated with the presence of MetS in hypertensive population, apart from potential confounders. Also, resistance to antihypertensive treatment is strongly associated with MetS. The high prevalence of these coexisting conditions – hypertension and MetS – may explain the increased prevalence of hypertension-related target organ damage (TOD), such as elevated urinary albumin excretion.[5] Additionally, this early renal organ damage may in part explain the increased cardiovascular risk conferred by MetS in hypertensive patients, since this marker of TOD is a well-known predictor of CV events.[13] In this sense, the identification and treatment of risk factors for cardiovascular and renal diseases, as well as an early detection of hypertension-related TOD may directly affect the prognosis of hypertensive patients with MetS.[14]

Our finding of increased MA in hypertensive patients with MetS is supported by previous studies.[13] The common underlying mechanisms that may explain increased MA in patients with MetS include factors such as: (i) overactivation of the renin-angiotensin system; (ii) increase in oxidative stress and (iii) inflammation.[15] In addition, the presence of MA may affect reflect progressive endothelial and vascular dysfunction.[16] It is worth to mention that we found no difference in BP levels between the groups. Thus, in our cross-sectional study MA is probably associated with other components that comprise MetS. Another hypothesis is that the greater use of calcium channel blockers by hypertensive patients with MetS could have resulted in BP control, but not in avoiding early renal damage, in agreement with several studies.[17] Another point to be mentioned is that despite of the greater use of antidiabetic drugs by patients with MetS, HbA1c remained higher in this group. On the other hand, studies[18] have consistently shown that levels of HbA1c < 7% are associated with a reduced risk of structural and clinical manifestations of diabetic nephropathy in patients with diabetes type 1 and type 2. For instance, the U.K. Prospective Diabetes Study (UKPDS)[18] demonstrated a nearly 30% risk reduction for the development of MA in the group intensively treated for hyperglycemia (HbA1c of 7%).[18]

Hypoadiponectinemia and hyperleptinemia are commonly found in hypertensive and obese patients. Previous studies have shown an inverse association between adiponectin levels and low-grade albuminuria in essential[19] and resistant hypertensive patients.[20,21] Similarly in experimental studies, adiponectin knockout rats have higher levels of albuminuria (twice above normal values), and after replacement of the protein, albuminuria returned to its normal levels.[22] Hyperleptinemia is also an independent risk factor for coronary artery disease [23] and strong predictor of acute myocardial infarction. Besides that, leptin acts as a powerful sympathostimulator, associated with increased BP and tachycardia, which consequently contributes to obesity-related hypertension and kidney damage.[24] Furthermore, a study has supported that the LAR is more beneficial than either alone for the diagnosis of MetS.[25] The use of LAR has the potential to assess insulin sensitivity and MetS in the non-fasting state, since the difference between adiponectin and leptin tends to be small in the fasting *versus* postprandial state.[26] Our study showed that LAR was independently associated with the presence of MetS. There are several studies that relate MetS to various cytokines and adipokines, but no biomarker is currently used in clinical practice to help in predicting and establishing MetS in individuals. Therefore, the deregulated adipokine levels (LAR) might be a valuable tool for diagnosis, prognosis or even early detection of MetS in the high-risk hypertensive population, although these associations should be tested. This may also guide a rational therapeutic approach and risk management, since adipokines are altered after lifestyle modifications and medications.[27,28]

The prevalence of MetS has been increasing worldwide,[29] and it is higher in hypertensive patients than in general population.[5] In our study, we found a considerable prevalence of MetS in all hypertensive subjects (66%) – 73% in resistant and 60% in mild-to-moderate hypertensive patients. Similar data have been reported in the Global Cardiometabolic Risk Profile in Patients with hypertension disease (GOOD) study,[30] in which 58% of essential hypertensive patients had MetS. Indeed, other similar study also indicated a high proportion of RHTN among patients with MetS.[31] This high prevalence may be explained by the older age of the population in the studies, since prevalence of MetS is highly age-dependent.[1] In our study, RHTN was associated with MetS independently of potential

Table 1 – General characteristics of hypertensive patients with and without metabolic syndrome

	Patients with MetS (n = 157)	Patients without MetS (n = 79)	p-value
Clinical data			
Age (years)	63 (56 – 70)	65 (56 – 71)	0.39
White race (%)	122 (77)	52 (65)	0.05
Female gender (%)	106 (67)	47 (59)	0.23
BMI (kg/m^2)	31 (27 – 34)	26 (23 – 28)	< 0.01
WC (cm)	100 ± 13	89 ± 12	< 0.01
FFM (Kg)	54 (46 – 62)	52 (44 – 63)	0.13
FM (Kg)	24 (19 – 31)	17 (13 – 23)	< 0.01
TBW (%)	74 (72 – 75)	73 (72 – 75)	0.03
BMR (cal/day)	1672 (1436 – 1947)	1616 (1369 – 1954)	0.23
Office SBP(mmHg)	142 (134 – 150)	146 (132 – 154)	0.39
Office DBP(mmHg)	82 (75 – 89)	82 (80 – 88)	0.44
Office HR (bpm)	67 (61 – 76)	64 (58 – 72)	0.01
24h-ABPM SBP(mmHg)	128 (118 – 139)	129 (118 – 136)	0.78
24h-ABPM DBP(mmHg)	77(70 – 81)	78 (70 – 86)	0.28
ABPM HR (bpm)	64 ± 14	64 ± 13	0.94
Uncontrolled office BP (%)	96 (61)	48 (60)	0.97
TODs			
MA ≥ 30 (mg.g^{-1}), n (%)	31 (20)	3 (4)	< 0.01
PWV ≥ 10 (m.s^{-1}), n (%)	68 (43)	35 (44)	0.94
LVH, n (%)	83 (53)	44 (55)	0.96
Medication			
Total anti-HA drugs	3 (2 – 4)	3 (2 – 4)	0.27
Diuretics, n (%)	123 (78)	64 (80)	0.75
CCBs, n (%)	112 (71)	42 (52)	< 0.01
ACEIs, n (%)	36 (22)	26 (32)	0.13
ARAs, n (%)	108 (69)	48 (60)	0.27
Beta-blockers, n (%)	67 (43)	28 (35)	0.39
Spironolactone, n (%)	33 (21)	8 (10)	0.06
Central α-agonists, n (%)	24 (15)	8 (10)	0.37
Oral antidiabetics, n (%)	90 (57)	16 (20)	< 0.01
Statins, n (%)	111 (70)	51 (63)	0.41
Antiplatelet drugs, n (%)	67 (43)	23 (29)	0.06

Values are expressed as mean ± standard deviation or median (1st, 3rd quartiles), according to data distribution. Continuous variables were compared using unpaired Student´s t-test or Mann-Whitney test, according to data distribution. Categorical variables were compared by chi-square test. BMI: body mass index; WC: waist circumference; FFM: fat free mass; FM: fat mass; TBW: total body water; BMR: basal metabolic rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; ABPM: ambulatory blood pressure monitoring; LVH: left ventricular hypertrophy; MA: microalbuminuria; PWV: pulse wave velocity; CCBs: calcium channel blockers; ACEIs: angiotensin converting enzyme inhibitors; ARAs: angiotensin II receptor antagonist; TODs: target organ damages.

confounders. Although our study does not affirm causality between this association, it seems reasonable to say that the metabolic derangements associated with MetS promote alterations in the vasculature and the kidney that might lead to RHTN and chronic kidney disease.[32] Furthermore, the increased renal impairment in patients with MetS is probably linked to the underlying condition of prior hypertension in these patients[33] (Figure 1). In this context, our findings highlighted the importance of improving strategies to prevent cardiovascular and renal outcomes. Still, it points out that not only RHTN patients require a close clinical attention, but also mild to moderate hypertensive subjects, who demonstrated a high prevalence of MetS comparable to RHTN patients.

Table 2 – Biochemical parameters of hypertensive patients with and without metabolic syndrome

	Patients with MetS (n = 157)	Patients without MetS (n = 79)	p-value
Cholesterol (mg.dL^{-1})	166 (139 – 192)	179 (150 – 200)	0.06
LDL-c (mg.dL^{-1})	88 (70 – 111)	98 (73 – 118)	0.19
HDL-c (mg.dL^{-1})	43 (37 – 49)	57 (51 – 65)	< 0.01
Triglycerides (mg.dL^{-1})	142 (97 – 199)	81 (68 – 115)	< 0.01
FBG (mg.dL^{-1})	107 (95 – 130)	91 (86 – 97)	< 0.01
HbA1c (%)	6.30 (6– 7.40)	5.90 (5.50 – 6)	< 0.01
hs-CRP (mg.dL^{-1})	0.39 (0.17 – 0.65)	0.25 (0.11 – 0.48)	0.02
Na (mEq.dL^{-1})	141 (140 – 143)	142 (138 – 143)	0.61
K (mEq.dL^{-1})	4.40 (4.10 – 4.70)	4.30 (4.20 – 4.60)	0.82
PAC (ng.dL^{-1})	83 (48 – 162)	65 (41 – 125)	0.10
CC (ml.min^{-1}.(1,73m^2)$^{-1}$)	80 (55 – 97)	71 (53 – 94)	0.53
Creatinine (mg.dL^{-1})	0.93 (0.80 – 1.12)	0.95 (0.77 – 1.20)	0.97
Renin (pg.ml^{-1})	23 (12 – 64)	30 (11 – 80)	0.78
Urea (mg.mL^{-1})	35 (26 – 44)	36 (28 – 44)	0.81
Cortisol (ug.dL^{-1})	14 (10 – 20)	14 (10 – 16)	0.44
Leptin (ng.mL^{-1})	21.0 (14.40–41.60)	15.70 (6.30–33.20)	< 0.01
Adiponectin (µg.dL^{-1})	5.30 (2.60– 7.80)	7.50 (3.80 – 11.90)	< 0.01
LAR	4.81 (2.14 – 10.80)	2.22 (1.10 – 5.20)	< 0.01
LAR > 3.72, n (%)	85 (54)	24 (30)	< 0.01

Values are expressed as mean ± standard deviation or median (1st, 3rd quartiles), according to data distribution. Continuous variables were compared using unpaired Student´s t-test or Mann-Whitney test, according to data distribution. Categorical variables were compared by chi-square test. MetS: metabolic syndrome; LDL-c: low density lipoprotein-c; HDL-c: high density lipoprotein-c; FBG: fasting blood glucose; HbA1C: glycated hemoglobin; hs-CRP: high-sensitivity c-reactive protein; Na: serum sodium; K: serum potassium; PAC: plasma aldosterone concentration; CC: creatinine clearance; LAR > 3.7: leptin adiponectin ratio > 3.7 (the cutoff value was determined by median value).

Finally, pharmacological approaches should be carried out in order to improve obesity, dyslipidemia, hyperglycemia and hypertension[33] for renal protection. However, the cornerstone of treating MetS remains lifestyle modification,[3] which mainly involves healthy diet, aerobic exercise, and behavioral counseling. To date, current guidelines do not specifically address the management of mild to moderate hypertension and RHTN in the patient with MetS. However, considering the increased risk of developing diabetes in these patients, it seems reasonable that the first consideration in antihypertensive treatment is to be focused on the inhibition of the renin-angiotensin system with either angiotensin converting enzyme or angiotensin II receptor inhibitors.[34] There has been increasing interest in combination strategies of antihypertensive agents in RHTN patients with MetS to reduce the pill burden. Future works are still needed to define the best antihypertensive therapy in this group of high-risk patients.

The limitations of this study include: (i) the cross-sectional design with no cause-effect inference; (ii) a small sample size and (iii) inclusion of patients from one outpatient clinic only. Although studies have shown significant differences between patients with mild to moderate hypertension and RHTN,[35,36] we did not dichotomize the hypertensive population because they both had a high prevalence of SMet with similar metabolic profile, then contributing to the objective of evaluating the influence of SMet on all these subjects together.

Conclusion

In summary, our study showed that MetS is significantly associated with MA, RHTN and adipokines levels. These findings suggest that hypertensive patients with MetS tend to develop early manifestations of end-organ damage with metabolic/hormonal changes, culminating in increased cardiovascular risk and renal impairment. However, as we mentioned earlier, we cannot infer from this cross-sectional study the exact nature of the association between MetS, MA, RHTN and adipokines levels. Early diagnosis of MetS in hypertensive patients may enable more accurate prediction of adverse cardiovascular events and renal impairment, as well as the implementation of more efficient strategies in terms of primary prevention. Besides that, prompt identification of MetS in resistant hypertensive patients allows modification of multiple risk factors that promote resistance to antihypertensive therapy, as well as guide the treatment to individual components of the syndrome. Thus, targeted treatment to individual components of the syndrome along with weight loss and lifestyle modifications can prevent resistance to antihypertensive treatment, as well as contribute to effective therapy in resistant hypertensive patients

Table 3 – Multiple logistic regression for the presence of metabolic syndrome*

	Odds ratio	95% CI	p-value
LAR > 3.7	4.13	1.38 – 12.34	0.01
HR (bpm)	0.97	0.92 – 1.03	0.39
MA > 30 (mg.g^{-1})	8.51	1.53 – 47.14	0.01
hs-CRP (mg.dL^{-1})	2.92	0.83 – 10.19	0.09
RHTN	3.75	1.09 – 12.92	0.03

** The variables in this model were also adjusted for age, gender and race. MetS: metabolic syndrome; hs-CRP: high-sensitivity c-reactive protein; HR: heart rate; MA: microalbuminuria; RHTN: resistant hypertension; LAR > 3.7: leptin adiponectin ratio > 3.7 (the cutoff value was determined by median value).*

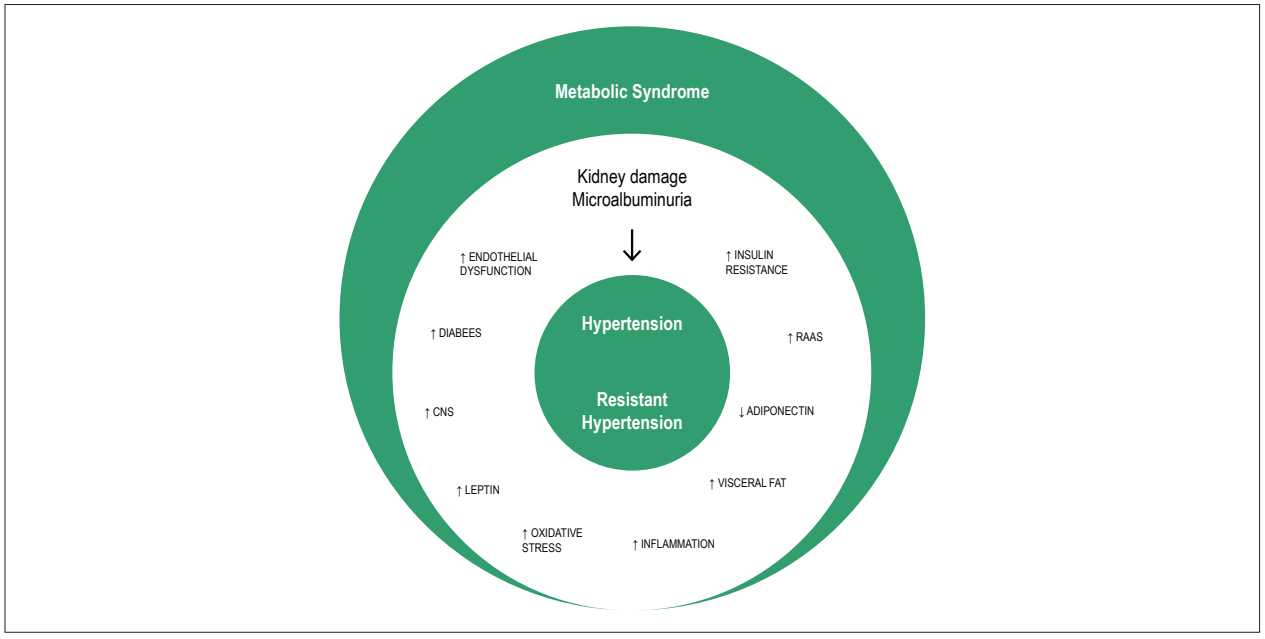


Figure 1 – *Diagrammatic representation of the metabolic syndrome effects on hypertension and resistant hypertension (RHTN). Abbreviations: renin-angiotensin-aldosterone system (RAAS); central nervous system (CNS).*

with MetS. Given the alterations that MetS confers on RHTN, future clinical trials can begin to address this important topic. Once the syndrome is identified, lifestyle changes and a different therapeutic approach can enhance the prognosis of the disease. Indeed, further studies on LAR in a larger hypertensive population with MetS is needed to assess whether this marker is sensitive and specific for identifying those who are at risk for developing MetS. The LAR could be used as a relatively easy, minimally-invasive tool for early MetS diagnosis and, consequently, decrease the chance of maladaptive effects caused by this syndrome.

Author contributions

Conception and design of the research, Obtaining financing and Writing of the manuscript: Catharina AS, Faria AP; Acquisition of data: Sabbatini AR, Catharina AS, Ritter AMV, Faria AP; Analysis and interpretation of the data and Statistical analysis: Catharina AS, Modolo R, Ritter AMV, Faria AP; Critical revision of the manuscript for intellectual content: Sabbatini AR, Catharina AS, Modolo R, Ritter AMV, Lopes HF, Moreno Junior H.

Potential Conflict of Interest

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

Sources of Funding

This study was partially funded by FAPESP, CNPq and CAPES.

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 Universidade Estadual de Campinas under the protocol number 188.161 (CAAE: 11189712.8.0000.5404). 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.

References

1. Grundy SM. Metabolic syndrome pandemic. Arterioscler Thromb Vasc Biol. 2008;28(4):629-36.
2. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes. 1988;37(12):1595-607.
3. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al; American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005;112(17):2735-52. Erratum in: Circulation. 2005;112(17):e298. Circulation. 2005;112(17):e297.
4. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol. 2010;56(14):1113-32.
5. Mule G, Cottone S, Nardi E, Andronico G, Cerasola G. Metabolic syndrome in subjects with essential hypertension: relationships with subclinical cardiovascular and renal damage. Minerva Cardioangiol. 2006;54(2):173-94.
6. Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, et al. Adiponectin as a biomarker of the metabolic syndrome. Circ J. 2004;68(11):975-81.
7. Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. Adiponectin and metabolic syndrome. Arterioscler Thromb Vasc Biol. 2004;24(1):29-33.
8. Ghantous CM, Azrak Z, Hanache S, Abou-Kheir W, Zeidan A. Differential Role of Leptin and Adiponectin in Cardiovascular System. Int J Endocrinol. 2015;2015:534320.
9. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, et al. Resistant hypertension: diagnosis, evaluation, and treatment. A scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. Hypertension. 2008;51(6):1403-19.
10. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens. 2013;31(7):1281-357.
11. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, et al; Artery Society; European Society of Hypertension Working Group on Vascular Structure and Function; European Network for Noninvasive Investigation of Large Arteries. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens. 2012;30(3):445-8.
12. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation. 1978;58(6):1072-83.
13. Ingelsson E, Sullivan LM, Murabito JM, Fox CS, Benjamin EJ, Polak JF, et al. Prevalence and prognostic impact of subclinical cardiovascular disease in individuals with the metabolic syndrome and diabetes. Diabetes. 2007;56(6):1718-26.
14. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr, et al; National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA. 2003;289(19):2560-72. Erratum in: JAMA. 2003 Jul 9;290(2):197.
15. Gobal F, Deshmukh A, Shah S, Mehta JL. Triad of metabolic syndrome, chronic kidney disease, and coronary heart disease with a focus on microalbuminuria death by overeating. J Am Coll Cardiol. 2011;57(23):2303-8.
16. Ochodnicky P, Henning RH, van Dokkum RP, de Zeeuw D. Microalbuminuria and endothelial dysfunction: emerging targets for primary prevention of end-organ damage. J Cardiovasc Pharmacol. 2006;47 Suppl 2:S151-62.
17. Zhao HJ, Li Y, Liu SM, Sun XG, Li M, Hao Y, et al. Effect of calcium channels blockers and inhibitors of the renin-angiotensin system on renal outcomes and mortality in patients suffering from chronic kidney disease: systematic review and meta-analysis. Ren Fail. 2016;38(6):849-56.
18. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet. 1998;352(9131):837-53. Erratum in: Lancet. 1999;354(9178):602.
19. Tsioufis C, Dimitriadis K, Chatzis D, Vasiliadou C, Tousoulis D, Papademetriou V, et al. Relation of microalbuminuria to adiponectin and augmented C-reactive protein levels in men with essential hypertension. Am J Cardiol. 2005;96(7):946-51.
20. de Faria AP, Modolo R, Sabbatini AR, Barbaro NR, Correa NB, Brunelli V, et al. Adiponectin -11377C/G and +276G/T polymorphisms affect adiponectin levels but do not modify responsiveness to therapy in resistant hypertension. Basic Clin Pharmacol Toxicol. 2015;117(1):65-72.
21. Sabbatini AR, Faria AP, Barbaro NR, Gordo WM, Modolo RG, Pinho C, et al. Deregulation of adipokines related to target organ damage on resistant hypertension. J Hum Hypertens. 2014;28(6):388-92.
22. Sharma K, Ramachandrarao S, Qiu G, Usui HK, Zhu Y, Dunn SR, et al. Adiponectin regulates albuminuria and podocyte function in mice. J Clin Invest. 2008;118(5):1645-56.
23. Ren J. Leptin and hyperleptinemia - from friend to foe for cardiovascular function. J Endocrinol. 2004;181(1):1-10.
24. Adelman RD. Obesity and renal disease. Curr Opin Nephrol Hypertens. 2002;11(3):331-5.
25. Falahi E, Khalkhali Rad AH, Roosta S. What is the best biomarker for metabolic syndrome diagnosis? Diabetes Metab Syndr. 2015;9(4):366-72.
26. Finucane FM, Luan J, Wareham NJ, Sharp SJ, O'Rahilly S, Balkau B, et al. Correlation of the leptin: adiponectin ratio with measures of insulin resistance in non-diabetic individuals. Diabetologia. 2009;52(11):2345-9.
27. Lee JM, Kim JH, Son HS, Hong EG, Yu JM, Han KA, et al. Valsartan increases circulating adiponectin levels without changing HOMA-IR in patients with type 2 diabetes mellitus and hypertension. J Int Med Res. 2010;38(1):234-41.
28. Kok P, Roelfsema F, Frolich M, van Pelt J, Meinders AE, Pijl H. Activation of dopamine D2 receptors lowers circadian leptin concentrations in obese women. J Clin Endocrinol Metab. 2006;91(8):3236-40.
29. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al; International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5.
30. Kjeldsen SE, Naditch-Brule L, Perlini S, Zidek W, Farsang C. Increased prevalence of metabolic syndrome in uncontrolled hypertension across Europe: the Global Cardiometabolic Risk Profile in Patients with hypertension disease survey. J Hypertens. 2008;26(10):2064-70.

31. Chaudhary K, Buddineni JP, Nistala R, Whaley-Connell A. Resistant hypertension in the high-risk metabolic patient. Curr Diab Rep. 2011;11(1):41-6.

32. Hall ME, do Carmo JM, da Silva AA, Juncos LA, Wang Z, Hall JE. Obesity, hypertension, and chronic kidney disease. Int J Nephrol Renovasc Dis. 2014 Feb 18;7:75-88.

33. Mule G, Calcaterra I, Nardi E, Cerasola G, Cottone S. Metabolic syndrome in hypertensive patients: an unholy alliance. World J Cardiol. 2014;6(9):890-907.

34. Vejakama P, Thakkinstian A, Lertrattananon D, Ingsathit A, Ngarmukos C, Attia J. Reno-protective effects of renin-angiotensin system blockade in type 2 diabetic patients: a systematic review and network meta-analysis. Diabetologia. 2012;55(3):566-78.

35. Sim JJ, Bhandari SK, Shi J, Reynolds K, Calhoun DA, Kalantar-Zadeh K, et al. Comparative risk of renal, cardiovascular, and mortality outcomes in controlled, uncontrolled resistant, and nonresistant hypertension. Kidney Int. 2015;88(3):622-32.

36. Figueiredo VN, Yugar-Toledo JC, Martins LC, Martins LB, de Faria AP, de Haro Moraes C, et al. Vascular stiffness and endothelial dysfunction: Correlations at different levels of blood pressure. Blood Press. 2012;21(1):31-8.

Identifying the Impact of Metabolic Syndrome in Hypertensive Patients

Luiz Aparecido Bortolotto

Instituto do Coração (Incor), São Paulo, SP - Brazil

Short Editorial regarding the article: Metabolic Syndrome-Related Features in Controlled and Resistant Hypertensive Subjects

Recent definitions of systemic arterial hypertension (AH) include metabolic changes and sustained hypertension, based on the association of AH with dyslipidemia, glucose intolerance and obesity.[1] The mechanisms of this association are involved in the pathophysiology of AH and target-organ damage, including activation of the sympathetic nervous and the renin- angiotensin- aldosterone systems, endothelial dysfunction and inflammation.[2] These metabolic changes, in addition to hypertension, constitute the so-called metabolic syndrome (MS), a clinical condition associated with higher risk for cardiovascular disease[3] and chronic kidney disease.[4] The study by Catharina et al.,[5] published in this issue, add interesting data on the relationship between MS, repercussions of AH and resistant hypertension (RH). The study included hypertensive patients at different stages and evaluated various biomarkers, such as adipokines, as well as cardiovascular properties. The first notable finding was the high prevalence of MS in patients with AH in both RH and control groups, and the prevalence was slightly higher in the former. This result is in accordance with what has been observed in the clinical practice in the last years – a high prevalence of obesity and metabolic abnormalities associated with hypertension and its consequences.[2] This should be considered in therapeutic approaches of these patients, and changes in life style should be encouraged aiming at better controlling blood pressure and preventing related cardiovascular diseases.[5] The study also reported an association of MS with early kidney injury (microalbuminuria), the leptin/adiponectin ratio (L/A) and RH. No association was found between MS and increased arterial stiffness or left ventricular hypertrophy. The authors draw attention to the early detection of renal injuries in MS, regardless of the hypertension stage, emphasizing the role of metabolic changes on the development of microalbuminuria in hypertensive patients,[6] and the need for controlling these changes to prevent kidney injury prevention. On the other hand, the lack of differences in vascular and cardiac lesions according to the presence of MS suggests that blood pressure is the component of greatest impact on these target-organ lesions, and that an adequate blood pressure control is essential for their prevention, as previously described.[7,8] As the authors pointed out, the main finding of the study was the association between the L/A ratio and MS in hypertensive patients, reinforcing the role of increased leptin and reduced adiponectines in the pathophysiology of MS, showing as a potential therapeutic target in these patients. Besides, the L/A ratio, as suggested by the authors, may be a key tool for the screening of patients at higher risk for MS and provide them with early intervention. This, in turn, would delay the development of renal lesions and increase the likelihood of better control of blood pressure. Nevertheless, further prospective, long-term studies involving a higher number of patients are needed, as the study by Catharina et al.[5] shows the association of the L/A ratio and MS in hypertensive patients in a cross-sectional design only. In summary, metabolic changes and obesity negatively affect blood pressure control and its repercussions in hypertensive patients and should be the target of therapeutic interventions in these individuals.

Keywords

Metabolic Syndrome / diagnosis; Cardiovascular Diseases / mortality; Cholesterol; Triglycerides, Waist Circumference.

Mailing Address: Luiz Aparecido Bortolotto •
Av. Dr. Enéas de Carvalho Aguiar, 44. Postal Code 05403-000, São Paulo, SP – Brazil
E-mail: hipbortolotto@gmail.com

DOI: 10.5935/abc.20180102

References

1. Malachias M, Plavnik FL, Machado CA, Malta D, Scala LC, Fuchs S. 7th Brazilian Guideline of Arterial Hypertension: Chapter 1 - Concept, Epidemiology and Primary Prevention. Arq Bras Cardiol. 2016;107(3 Suppl 3):1-6.
2. Kotsis V, Jordan J, Micic D, Finer N, Leitner DR, Toplak H, et al. Obesity and cardiovascular risk: a call for action from the European Society of Hypertension Working Group of Obesity, Diabetes and the High-risk Patient and European Association for the Study of Obesity: part A: mechanisms of obesity induced hypertension, diabetes and dyslipidemia and practice guidelines for treatment. J Hypertens. 2018 Apr 9. [Epub ahead of print].
3. Kachur S, Morera R, De Schutter A, Lavie CJ. Cardiovascular risk in patients with prehypertension and the metabolic syndrome. Curr Hypertens Rep. 2018;20(2):15.
4. Prasad GV. Metabolic syndrome and chronic kidney disease: current status and future directions. World J Nephrol. 2014;3(4):210-9.
5. Catharina AS, Modolo R, Sabbatini A, Lopes HF, Moreno Junior H, Faria AP. Características relacionadas à síndrome metabólica em indivíduos com hipertensão controlada e hipertensão resistente. Arq Bras Cardiol. 2018; 110(6):514-521.
6. DeBoer MD, Filipp SL, Musani SK, Sims M, Okusa MD, Gurka M. Metabolic Syndrome Severity and risk of CKD and Worsened GFR: the Jackson Heart Study. Kidney Blood Press Res. 2018;43(2):555-67.
7. Tedla YG, Gepner AD, Vaidya D, Colangelo L, Stein JH, Liu K, et al. Association between long-term blood pressure control and ten-year progression in carotid arterial stiffness among hypertensive individuals: the multiethnic study of atherosclerosis. J Hypertens. 2017;35(4):862-9.
8. Lønnebakken MT, Izzo R, Mancusi C, Gerdts E, Losi MA, Canciello G, et al. Left ventricular hypertrophy regression during antihypertensive treatment in an outpatient clinic (the Campania Salute Network). J Am Heart Assoc. 2017 Mar 8;6(3). pii: e004152.

Prevention of Sudden Cardiac Death in Hypertrophic Cardiomyopathy: What has Changed in The Guidelines?

Liliana Reis, Rogerio Teixeira, Andreia Fernandes, Inês Almeida, Marta Madeira, Joana Silva, Ana Botelho, João Pais, José Nascimento, Lino Gonçalves

Centro Hospitalar e Universitário de Coimbra, Serviço de Cardiologia, Coimbra – Portugal

Abstract

Background: The new European Society of Cardiology guidelines for hypertrophic cardiomyopathy (HCM) define the estimation of sudden cardiac death (SCD) risk as an integral part of clinical management. An implantable cardioverter defibrillator (ICD) is recommended (class IIa) when the risk is ≥ 6%.

Objectives: To compare the SCD risk stratification according to the 2011 and 2014 recommendations for ICD implantation in patients with HCM.

Methods: Retrospective study including 105 patients diagnosed with HCM. The indication for ICD was assessed using the 2011 and 2014 guidelines. Statistical analysis was performed using SPSS software version 19.0.0.2®. The tests performed were bilateral, considering the significance level of 5% ($p < 0.05$).

Results: Regarding primary prevention, according to the 2011 ACCF/AHA recommendations, 39.0% of the patients had indication for ICD implantation (level of evidence IIa). Using the 2014 guidelines, only 12.4% of the patients had an indication for ICD implantation. Comparing the two risk stratification models for patients with HCM, we detected a significant reduction in the number of indications for ICD implantation ($p < 0.001$). Of the 41 patients classified as IIa according to the 2011 recommendations, 68.3% received a different classification according to the 2014 guidelines.

Conclusion: Significant differences were found when comparing the SCD risk stratification for ICD implantation in the two guidelines. The current SCD risk score seems to identify many low-risk patients who are not candidates for ICD implantation. The use of this new score results in a significant reduction in the number of ICD implanted. (Arq Bras Cardiol. 2018; 110(6):524-531)

Keywords: Death, Sudden Cardiac / prevention & control; Cardiomyopathy, Hypertrophic / complications; Defibrillators, Implantable / trends; Syncope; Diagnostic Imaging.

Introduction

Hypertrophic cardiomyopathy (HCM) is characterized by left ventricular hypertrophy (LVH) not explained only by ventricular overload conditions.[1] It is the most common cardiovascular genetic pathology, with an estimated prevalence in the general population of 1:500 individuals.[2,3] Hypertrophic cardiomyopathy is a complex disease, regarding genetic diversity (for which, more than 1400 mutations have been identified in 11 different genes), phenotypic expression, histological characteristics and manifested symptoms.[4,5]

Sudden cardiac death (SCD) is the most unpredictable and devastating consequence of HCM, occurring mainly in young or asymptomatic individuals or those with frustrated symptomatology.[4-6] Recent data have pointed to a 0.7%/year incidence of SCD, the total incidence of cardiovascular death being 1.4%/year.[7] The exclusive efficacy of implantable cardioverter defibrillator (ICD) in the prevention of SCD is well known.[1,8,9] When approaching patients with HCM and their families, the correct assessment of the SCD risk and potential benefit of implanting that device for primary prevention is fundamental.[1-3]

According to the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) recommendations for the diagnosis and treatment of HCM published in 2011, the presence of at least one risk factor for SCD [maximal left ventricular (LV) wall thickness ≥ 30 mm, unexplained syncope, nonsustained ventricular tachycardia (NSVT), family history of sudden death and abnormal blood pressure response during exercise] is a class IIa recommendation for the implantation of ICD in primary prevention.[10]

However, a recent study by O'Mahony et al. has suggested that the use of those criteria overestimates the risk for SCD, resulting in the excessive and unnecessary implantation of ICD in a substantial percentage of patients, exposing them to unnecessary iatrogenic complications.[11] In addition,

Mailing Address: Liliana Reis •
Serviço de Cardiologia - Centro Hospitalar e Universitário de Coimbra - Hospital Geral Quinta do Vales, São Martinho do Bispo. 3041-801, Coimbra - Portugal
E-mail: liliana.teles@hotmail.com
Manuscript received July 20, 2017, revised manuscript Ouctober 17, 2017, accepted November 11, 2017

DOI: 10.5935/abc.20180099

those authors have concluded that the limited power in risk stratification results from the fact that the algorithm is based on a dichotomous classification of the risk variables.[11] Thus, the risk factors are recognized to be non-static and to have a cumulative evolutionary potential, with corresponding increase in the likelihood of SCD.[12]

In 2013, a new mathematical model was proposed to estimate the individual risk of SCD at 5 years.[13,14] That model, based on a retrospective study of a population of 3675 patients from six centers, comprises some classical risk factors combined with LV outflow tract gradient, left atrial diameter, and age, which are considered continuous variables.[13] The following formula is used:

Probability of SCD at 5 years = 1 – 0.998 exp(prognostic index)

Prognostic index = [0.15939858 x maximal wall thickness (mm)] - 0.00294271 x maximal wall thickness^2 (mm^2)] + [0.0259082 x left atrial diameter (mm)] + [0.00446131 x maximal LV outflow tract gradient (rest/Valsalva - mm Hg)] + [0.4583082 x family history of SCD] + [0.82639195 x NSVT] + [0.71650361 x unexplained syncope] - [0.01799934 x age on clinical assessment (years)].

According to the literature, that score is more accurate to differentiate patients at low risk from those at high risk,[13] and was incorporated into the most recent European Society of Cardiology (ESC) recommendations published in 2014 as a valid and independent method for risk stratification.[1]

The direct comparison of the discriminative value of the two risk score systems to identify patients requiring an ICD in a non-selected population with HCM has not been performed in Portugal.

This study aimed at comparing the risk stratification of SCD in a population of patients with HCM, according to the 2011 and 2014 recommendations, and at characterizing the clinical performance of the risk model of SCD due to HCM individually in a Portuguese population with HCM.

Methods

Population

Retrospective single-center analysis of patients diagnosed with HCM and regularly followed up at a cardiology outpatient clinic of one single tertiary center for 6 years. The definition of HCM was based on a wall thickness ≥ 15 mm in one or more LV myocardial segments, which was not explained only by LV overload, and measured by use of any imaging technique [echocardiography, cardiac magnetic resonance imaging (CMRI) or computerized tomography (CT)]. The clinical diagnosis of HCM in a first-degree relative of a patient with unequivocal disease (LVH ≥ 15 mm) is based on the presence of unexplained LV wall thickening ≥ 13 mm in one or more myocardial segments, measured by use of cardiac imaging techniques.[1-3,15,16]

This study included 109 patients with LVH. Those whose complementary study revealed hereditary metabolic and neuromuscular causes (2 patients with cardiac amyloidosis, 1 patient with Noonan syndrome and 1 patient with Anderson-Fabry disease) were excluded. The total sample of this study comprised 105 index patients diagnosed with HCM.

The indication for an ICD implantation was based on the 2011 ACCF/AHA recommendations, and the patients received an ICD when they had at least one risk factor for SCD, according to the 2011 guidelines.

Later, a new analysis was performed based on the current recommendations (2014 ESC), using the data of the patients at the time of the diagnosis. The current model of risk for SCD due to HCM is part of a predefined set of 7 potentially prognostic variables.[1] By using an online calculator, a predictive risk score of SCD due to HCM at 5 years was generated. According to that value, patients were stratified into three risk categories for ICD implantation: < 4%/5 years (ICD usually not considered); 4% to 6%/5 years (ICD can be considered); > 6%/5 years (ICD should be considered).[1]

Characteristics of the population base and complementary study

The following baseline characteristics were collected at the time of diagnosis: age, sex, arterial hypertension, diabetes *mellitus*, atrial fibrillation, unexplained syncope, history of SCD in a first-degree relative (< 40 years), New York Heart Association (NYHA) functional class.

All patients underwent initial 12-lead electrocardiography, with assessment of LVH voltage criteria, Q waves, left axis deviation and atrioventricular conduction disorders.

All patients underwent transthoracic echocardiography. The following parameters were recorded: LV diastolic diameter, LV wall thickness from base to apex, presence of LV outflow tract gradient at rest and after the Valsalva maneuver, left atrial diameter, classification of LV systolic (LV ejection fraction) and diastolic function. The LV outflow tract obstruction caused by the systolic anterior motion (SAM) of the mitral valve leaflets was defined as a peak pressure gradient at the LV outflow tract ≥ 30 mm Hg at rest or during physiological challenge.[1] Twenty-five patients (23.8%) with no gradient at rest underwent exercise echocardiography to assess the presence of gradient during exercise.

All patients underwent 24-hour Holter at the initial assessment or during clinical follow-up, allowing the identification of ventricular extrasystoles and/or NSVT episodes, defined as the presence of at least three consecutive ventricular complexes, lasting less than 30 seconds and without hemodynamic impairment.

All patients underwent exercise test according to the Bruce protocol to assess blood pressure response during exercise. Anomalous response was defined as the lack of blood pressure increase by 20 mmHg or a decrease of at least 20 mmHg during exertion.

Cardiac magnetic resonance imaging was performed in 85 (80.2%) patients who had access to a magnetic resonance scanner 1.5 Tesla (Phillips®). The following parameters were recorded for analysis: left atrial area, greater LV wall thickness, LV ejection fraction and presence of late enhancement after intravenous gadolinium administration.

Screening for sarcomere protein gene mutation (*MYL2* and *MYL3* = myosin light chain 2 and 3; *MYBPC3* = myosin-binding protein C; *MYH7* = myosin heavy chain 7; *TNNI3* = cardiac troponin I; *TNNT2* = cardiac troponin T; *TPM1* = tropomyosin alpha-1 chain) was conducted in 83 patients (79.0%), and screening for Anderson-Fabry disease, in 76 patients (72.4%). The screening for Anderson-Fabry disease in men was based on dried blood spot (DBS) testing to assess galactosidase A (GLA) activity. When GLA activity was reduced (< 5%), a 10-mL blood sample was collected in an EDTA tube for further GLA gene sequencing at a medical genetic center. In women, GLA gene sequencing analysis was performed in an external laboratory to identify mutations.[17] One patient was diagnosed with that disease, being excluded from the study.

Statistical analysis

The numeric variables were expressed as means and standard deviations, and the categorical variables, as absolute and relative frequencies. Regarding the recommendations for ICD implantation in primary prevention, the comparison between the two guidelines was performed by use of the McNemar test. On the first analysis, we assumed that the 2014 ESC classification IIb does not usually recommend ICD implantation, therefore, that classification was grouped together with the recommendation level III. The potency of that test is 99.9%, considering: the significance level of 5%; sample size of 105; the 0.001 proportion of patients classified as III according to the 2011 guideline and as IIa according to the 2014 guideline; and the 0.28 proportion of patients classified as IIa according to the 2011 guideline and as IIb/III according to the 2014 guideline.

Later, four groups of patients were defined as follows: patients classified as III according to both 2011 and 2014 guidelines; patients classified as IIa according to the 2011 guideline and as III according to the 2014 guideline; patients classified as IIa according to the 2011 guideline and as IIb according to the 2014 guideline; and patients classified as IIa according to both 2011 and 2014 guidelines. Because one of the assumptions to apply the chi-square test with asymptotic distribution was not met, those groups were compared regarding the percentage of ICD implantation by use of the exact chi-square test.

It is worth noting that, given the size of the sample, its power was calculated, ensuring that the number of patients was sufficient to draw conclusions.

The statistical analysis was performed by using the SPSS software, version 19.0.0.2®. The tests performed were bilateral, and the significance level of 5% ($p < 0.05$) was adopted.

Results

The study sample comprised 105 patients, 53% of whom were of the female sex, the mean age at the time of diagnosis being 58 ± 18 years. Table 1 shows the major characteristics of the population. The functional capacity on the initial assessment was as follows: 45 (42.8%) patients were asymptomatic (NYHA class I), 40 (38.1%) had mild symptoms (NYHA class II), and 9 (8.6%) had severe symptoms (NYHA classes III and IV).

Table 1 – Major characteristics of the population

Personal antecedents	
Arterial hypertension	74 (70.5%)
Atrial fibrillation	34 (32.4%)
Family history of sudden cardiac death	18 (17.1%)
Type 2 diabetes *mellitus*	16 (15.2%)
Previous syncope	14 (13.3%)
Previous coronary artery disease	10 (9.4%)
12-lead electrocardiogram	
Criteria of LVH	69 (65.7%)
Left anterior hemiblock	25 (23.8%)
First-degree AVB	16 (15.2%)
Complete right bundle-branch block	7 (6.7%)
Complete left bundle-branch block	5 (4.8%)
Transthoracic echocardiogram	
Septal HCM	72 (68.5%)
Concentric HCM	17 (16.1%)
Apical HCM	15 (14.3%)
Obstructive HCM	43 (40.9%)
LVEF ≤ 40%	4 (3.8%)
Mitral regurgitation	
- Mild	55 (52.4%)
- Moderate	16 (15.2%)
- Severe	8 (7.6%)
Exercise test	
Hypotensive response to exertion	4 (3.8%)
Cardiac magnetic resonance	
LA area, cm^2	43.6 ± 69.2
LV mass, g	168.2 ± 58.9
Maximal thickness measured, mm	18.2 ± 5.7
LVEF, %	64.8 ± 11.8
Late enhancement	34 (32.1%)

LVH: left ventricular hypertrophy; AVB: atrioventricular block; HCM: hypertrophic cardiomyopathy; LVEF: left ventricular ejection fraction; LA: left atrial; LV: left ventricular.

Obstruction of the LV outflow tract was present in approximately 40.9% of the patients, resulting in a gradient of 36 ± 36 mmHg. The echocardiographic measures were as follows: interventricular septum thickness, 17 ± 5 mm; posterior wall thickness, 11 ± 3 mm; left atrial diameter, 43 ± 7 mm. Table 1 shows the results of the exercise test and major continuous variables assessed on CMRI.

The screening for mutations for HCM was performed in 83 (79.0%) patients, 28 of whom (26.7%) had one mutation as follows: the *MYBPC3* gene mutation in 20 patients (71.4%); the *TNNT2* gene mutation in 3 (10.7%); the *MYH7* gene mutation in 3 (10.7%); and the *TPM1* gene mutation in 2 (7.1%) patients.

Complex ventricular dysrhythmia episodes were identified in 25 (23.8%) patients on 24-hour Holter.

Regarding primary prevention, according to the 2011 ACCF/AHA recommendations, 38.1% of the patients had indication for ICD implantation (level of evidence class IIa). The device was implanted in 24 (22.9%) patients. It is worth noting that 6 patients refused the device implantation, and 10 patients did not undergo implantation because of their comorbidities.

During the 6-year clinical follow-up, 1 patient received appropriate shock due to ventricular fibrillation (risk score for SCD due to HCM 1.71% - ICD usually not considered). In 25 (23.8%) patients, the ICD recorded ventricular tachycardia (VT) episodes and 3 inappropriate shocks. Ten (9.5%) patients died (6 patients due to heart failure, 1 patient due to ventricular fibrillation, and 3 patients due to neoplasm).

According to the 2011 ACCF/AHA recommendations, 38.1% of the patients had indication for ICD implantation (level of evidence class IIa), while 61.9% did not (level of evidence class III) – Figure 1.

According to the 2014 recommendations, the mean risk score for SCD due to HCM in the study population was 3.1±2.7%. Based on that value, the patients were stratified into three risk categories for ICD implantation: 81 (77.1%) patients had a score < 4% (ICD usually not considered – recommendation level III); 11 (10.5%) had a score between 4% and 6% (ICD can be considered – recommendation level IIb); and 13 (12.4%) had a score >6% (ICD should be considered – recommendation level IIa) – Figure 1.

Grouping together the patients classified as 2014 ESC classes IIb and III, 13 (12.4%) patients had recommendation for ICD implantation for primary prevention, while 64 (61.0%) patients did not have that recommendation according to the 2011 and 2014 guidelines. According to the 2011, but not the 2014, guideline, 28 (26.7%) patients had recommendation for ICD implantation. Thus, in 77 (73.3%) patients, the classifications were concordant, but not in 26.7%. The discordant patients were in the same circumstance, that is, according to the 2011 guideline they had indication for ICD implantation for primary prevention, while, according to the 2014 guidelines, ICD implantation would not usually be considered. This is not random, because, of the 28 discordant patients, there were significantly more patients for implantation in 2011 and not in 2014, than vice-versa ($p < 0.001$ McNemar test).

After that analysis, four groups of patients were defined, and, by using the exact chi-square test, the occurrence of dysrhythmic events during clinical follow-up was compared between groups – Figure 2.

Regarding the patients classified as recommendation level III according to both guidelines, that is, no indication for ICD implantation, the device was implanted in 3 out of 64 patients. We observed that of the 61 patients who did not undergo ICD implantation, 3 (4.9%) had VT during follow-up. The 3 patients who underwent ICD implantation for primary prevention had no dysrhythmic event. The groups with and without ICD were compared regarding the percentages of events, but no statistical difference was found between them ($p = 1.00$) – Table 2.

Regarding the group classified as level IIa in 2011 but level III in 2014, of 17 patients, 10 did not undergo ICD implantation, while 7 underwent ICD implantation for primary prevention. Of the 10 who did not undergo ICD implantation, 2 (20.0%) had VT. Of those who had an ICD implanted, 3 (42.9%) had ventricular dysrhythmia during follow-up. The groups with and without ICD were compared regarding the percentages of events, but no significant statistical difference was found between them ($p = 0.59$) – Table 2.

In the group classified as level IIa in 2011 and IIb in 2014, despite the need for ICD implantation for primary prevention, the device was implanted in 4, but not in 7 patients. In both groups, all patients had dysrhythmic events ($p = 1.00$). The ICD implantation seems beneficial, but the sample is small – Table 2.

Regarding the patients classified as recommendation level IIa according to both guidelines, that is, indication for ICD implantation for primary prevention, of the total of 13 patients, 3 did not undergo the procedure, while 10 did. Of the 3 patients not undergoing ICD implantation,

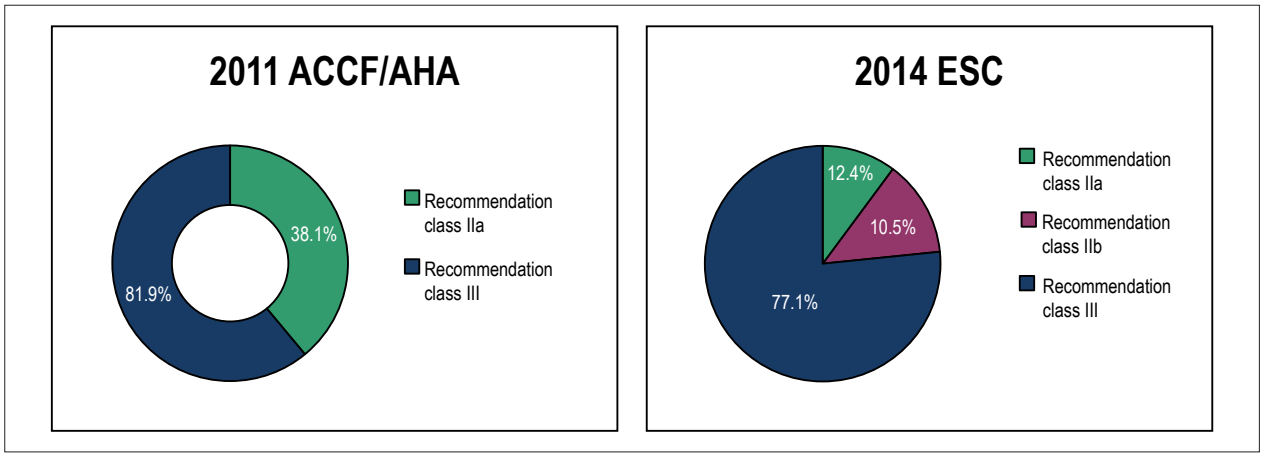


Figure 1 – *Comparison of risk stratification of SCD due to HCM according to the 2011 versus 2014 recommendations.*

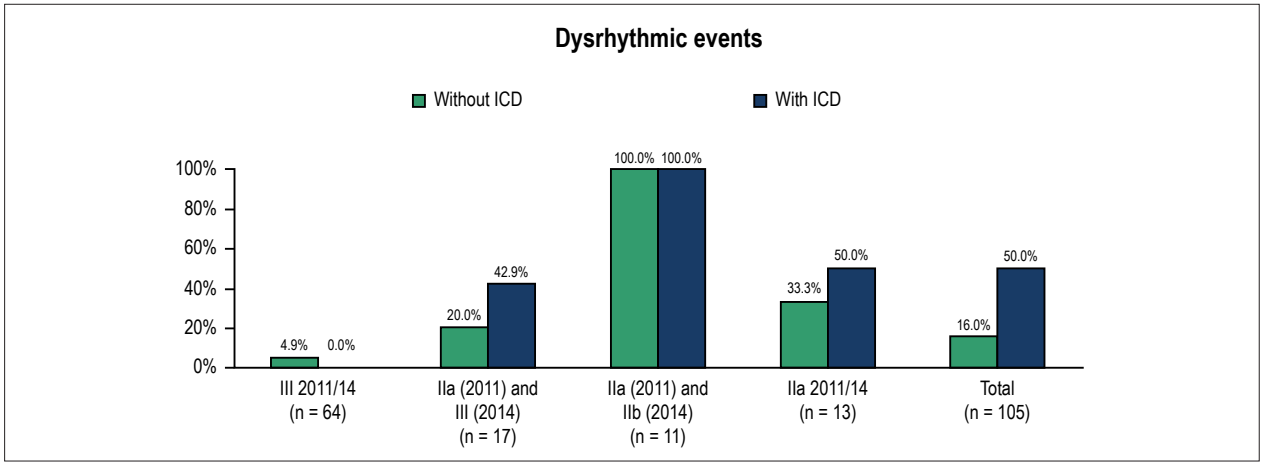


Figure 2 – *Comparison of the occurrence of dysrhythmic events during clinical follow-up.*

Table 2 – Comparison of dysrhythmic events in the different groups

Groups				Dysrhythmic events		Total	p
				No	Yes		
III in 2011 and 2014	ICD	No	N %	58 / 95.1	3 / 4.9	61 / 100	1.00
		Yes	N %	3 / 100	0 / 0	3 / 100	1.00
	Total		N %	61 / 95.3	3 / 4.7	64 / 100	1.00
IIa in 2011 and III in 2014	ICD	No	N %	8 / 80.0	2 / 20.0	10 / 100	0.59
		Yes	N %	4 / 57.1	3 / 42.9	7 / 100	
	Total		N %	12 / 70.5	5 / 29.4	17 / 100	0.59
IIa in 2011 and IIb in 2014	ICD	No	N %		7 / 100	7 / 100	1.00
		Yes	N %		4 / 100	4 / 100	
	Total		N %		11 / 100	11 / 100	1.00
IIa in 2011 and 2014	ICD	No	N %	2 / 66.7	1 / 33.3	3 / 100	
		Yes	N %	5 / 50.0	5 / 50.0	10 / 100	
	Total		N %	7 / 53.8	6 / 46.2	13 / 100	1.00
TOTAL	ICD	No	N %	68 / 84.0	13 / 46.2	81 / 100	0.001
		Yes	N %	12 / 50.0	12 / 50.0	24 / 100	
	Total		N %	80 / 76.2	25 / 23.8	105 / 100	0.001

1 (33.3%) had VT during follow-up. Of the 10 receiving an ICD, 5 (50,0%) had dysrhythmic events. The groups with and without ICD were compared regarding the percentages of events, but no statistical difference was found between them (p = 1.00) – Table 2.

Of the total population of 105 patients, those who underwent and those who did not undergo ICD implantation for primary prevention were compared regarding the percentages of events. Of the 81 patients who did not receive an ICD, 13 (16.0%) had dysrhythmic events. Of the 24 patients with an ICD, 12 (50.0%) had VT/ventricular fibrillation. Comparing the percentages of events in the two groups, there was a statistically significant difference (p = 0.001) – Table 2.

Discussion

Our sample of 'real world' patients with HCM had a 22.6% prevalence of ICD implantation. The proportion of patients with HCM and indication for ICD for primary prevention significantly decreased when comparing the 2011 and 2014 guidelines. During clinical follow-up, we detected the presence of complex ventricular dysrhythmia on Holter and/or ICD in some patients, of whom only a minority had a risk score of SCD due to HCM > 6%. In our population, 1 patient with a score < 4%/5 years died due to ventricular fibrillation. According to the literature, in Portugal, no other center has published a study with which we could compare our data and experience.

The gold-standard treatment for primary and secondary prevention of SCD in patients with HCM is ICD implantation, which proved effective in interrupting potentially lethal ventricular tachyarrhythmias, altering the disease's natural history.[1,7] The efficacy of that therapy has been consolidated since 2000, and has been recently reinforced in a meta-analysis examining the results of 16 studies published between 1998 and 2012, regarding ICD interventions and complications in primary and secondary preventions.[17-22]

The risk stratification of SCD in patients with HCM according to the 2011 ACCF/AHA recommendations was effective in identifying many patients who could benefit from ICD implantation. However, the method proved to be incomplete and some patients without the conventional risk factors were excluded and remained at risk for SCD.[23,24] Thus, the development of new SCD markers for risk stratification is required.[11] In 2013, a group of English researchers suggested a new risk score of SCD due to HCM at 5 years. It is a mathematical and statistically complex model.[13] That score has been rapidly incorporated into the 2014 ESC recommendations as the valid and independent method to select/exclude patients for ICD implantation in primary prevention.[1]

The major objective of any stratification method is its reliability to identify patients at major risk for events, being thus candidates for ICD implantation in primary prevention of SCD. It is worth noting that the new SCD risk model has incorporated arbitrarily two new risk markers (LV outflow tract gradient and left atrial diameter), which had not previously shown to be independent predictors of SCD due to HCM and are not included as risk markers for patients' assessment.[2,10,18]

This study was not aimed at validating (or invalidating) the risk score of SCD due to HCM, but at characterizing the clinical performance of that model individually in a population of Portuguese patients with HCM.

It is worth noting that this analysis showed that the risk model seems to have little sensitivity to identify patients at elevated risk for arrhythmic events and SCD, who, according to the conventional criteria, would be candidates for prophylactic ICD implantation. For example, in the sample of 28 patients with complex dysrhythmic events during the 6-year clinical follow-up, only 4.7% had a risk score > 6%/5 years, which would have justified ICD implantation in primary prevention. In addition, most patients had a score <4%/5 years, that is, no indication for treatment with ICD.

It is worth noting that HCM is a complex pathology, with a spectrum of histological findings and varied and unpredictable clinical manifestations, and a relatively low percentage of SCD.[1,2,10,22,24-29] Thus, intuitively it would not be expected that the clinical decision individualized for each patient could be based only on a complex mathematical formula, minimizing the fundamental clinical reasoning when facing a patient with HCM.

Being a genetic pathology, some specific mutations might pose a higher risk for SCD. However, it is difficult to determine the existence of a consistent genotype/phenotype correlation, explaining the inability to establish an accurate prognosis based on specific mutations.[4] Thus, given the inconsistency, they were not included as markers in the current risk model.

However, an important omission in this model is that of quantified late enhancement on CMRI, which several studies have shown to be an independent marker of adverse arrhythmic events (NSVT, VT, ventricular fibrillation) and SCD,[30-34] even in patients without the conventional risk factors.

Some individuals with HCM can develop LV apical aneurysms, associated with local healing and greater propensity to potentially lethal arrhythmias and SCD,[35] in addition to heart failure with systolic dysfunction[36] and coronary atherosclerotic disease,[37] which are not contemplated in the risk score of SCD. Some prediction inconsistency of the new risk model might be related to the inclusion of some variables, such as syncope, NSVT, left atrial diameter and LV outflow tract obstruction gradient (non-static variables).[11,24,38,39]

The strategy of conventional risk stratification prioritizes SCD prevention in patients with HCM *versus* excessive ICD implantation. On the contrary, the new risk score seems to identify many patients at low risk, who are not candidates for ICD implantation. There is, thus, a significant reduction in the number of devices implanted, but it seems at the cost of misclassifying some patients at high risk for arrhythmic events and SCD.

Study limitations

Our study has some limitations, because it is based on a single center, with a reduced number of patients and events. However, calculating the sample power ensured that the number of patients was sufficient to draw conclusions. As in any retrospective study, we were limited by the information available in the patients' medical records.

Conclusion

Hypertrophic cardiomyopathy is a complex pathology, with a wide and unpredictable clinical spectrum.

According to our data, the current risk stratification model seems to reduce the proportion of patients with indication for ICD implantation. It is worth noting that the decision based on a mathematical model that minimizes the individual clinical reasoning seems a little reliable strategy to identify patients at risk for events due to HCM.

Author contributions

Conception and design of the research and writing of the manuscript: Reis L; Acquisition of data: Reis L, Silva J; Analysis and interpretation of the data: Reis L, Teixeira R, Fernandes A, Almeida I, Madeira M, Silva J, Botelho A; Statistical analysis: Reis L, Teixeira R, Fernandes A, Almeida I, Madeira M, Silva J; Critical revision of the manuscript for intellectual content: Reis L, Teixeira R, Fernandes A, Almeida I, Madeira M, Silva J, Botelho A, Pais J, Nascimento J, Gonçalves L.

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 Comissão Nacional de Proteção de Dados (CNPD) under the protocol number 6416/ 2018. 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.

References

1. Elliot PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, et al. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy - The Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). Eur Heart J. 2014;35(39):2733-79.
2. Maron BJ, Maron MS. Hypertrophic cardiomyopathy. Lancet. 2013;381(9862):242-55.
3. Maron BJ. Hypertrophic cardiomyopathy: a systematic review. JAMA. 2002;287(10):1308-20.
4. Maron BJ, Maron MS, Semsarian C. Genetics of hypertrophic cardiomyopathy after 20 years: clinical perspectives. J Am Coll Cardiol. 2012;60(8):705-15.
5. Cardim N, Freitas A, Brito D. From hypertrophic cardiomyopathy centers to inherited cardiovascular disease centers in Europe. A small or a major step? A position paper from the Nucleus of the Working Group on Myocardial and Pericardial Diseases of the Portuguese Society of Cardiology. Rev Port Cardiol. 2011;30(11):829-35.
6. Monteiro S, Costa S, Monteiro P, Gonçalves L, Providência LA. Hypertrophic cardiomyopathy--state of the art in 2007. Rev Port Cardiol. 2008;27(5):625-37.
7. Efthimiadis GK, Zegkos T, Meditskou S, Hadjimiltiades S. Perspectives on sudden death prevention in hypertrophic cardiomyopathy. Cardiol Rev. 2014;22(5):210-6.
8. Magnusson P, Gadler F, Liv P, Mörner S. Risk markers and appropriate implantable defibrillator therapy in hypertrophic cardiomyopathy. Pacing Clin Electrophysiol. 2016;39(3):291-301.
9. Vriesendorp PA, Schinkel AF, Van Cleemput JV, Willems R, Jordaens LJ, Theuns DA, et al. Implantable cardioverter-defibrillators in hypertrophic cardiomyopathy: Patients outcomes, rate of appropriate and inappropriate interventions, and complications. Am Heart J. 2013;166(3):496-502.
10. Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS, et al; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. 2011 ACCF/AHA Guideline for the Diagnosis and Treatment of Hypertrophic Cardiomyopathy - A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the American Association for Thoracic Surgery, American Society of Echocardiography, American Society of Nuclear Cardiology, Heart Failure Society of America, Heart Rhythm Society, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. J Am Coll Cardiol. 2011;58(25):e212-60.
11. O'Mahony C, Tome-Esteban M, Lambiase PD, Pantazis A, Dickie S, McKenna WJ, et al. A validation study of the 2003 American College of Cardiology/European Society of Cardiology and 2011 American College of Cardiology Foundation/American Heart Association risk stratification and treatment algorithms for sudden cardiac death in patients with hypertrophic cardiomyopathy. Heart. 2013 Apr;99(8):534-41.
12. Beadle R, Williams L. Device therapy in hypertrophic cardiomyopathy. Expert Rev Cardiovasc Ther. 2010;8(12):1767-75.
13. Vriesendorp PA, Schinkel AF, Liebregts M, Theuns DA, van Cleemput J, Ten Cate FJ, et al. Validaton of the 2014 European Society of Cardiology Guidelines risk prediction model for the primary prevention of sudden cardiac death in hypertrophic cardiomyopathy. Circ Arrhythm Electrophysiol. 2015;8(4):829-35.
14. Maron BJ, Casey SA, Chan RH, Garberich RF, Rowin EJ, Maron MS. Independent assessment of the European Society of Cardiology sudden death risk model for hypertrophic cardiomyopathy. Am Heart J. 2015;116(5):757-64.
15. O'Mahony C, Jichi F, Pavlou M, Monserrat L, Anastasakis A, Rapezzi C, et al; Hypertrophic Cardiomyopathy Outcomes Investigators. A novel clinical risk prediction model for sudden cardiac death in hypertrophic cardiomyopathy (HCM Risk-SCD). Eur Heart J. 2014;35(30):2010-20.
16. Kelley-Hedgepeth A, Maron MS. Imaging techniques in the evaluation and management of hypertrophic cardiomyopathy. Curr Heart Fail Rep. 2009;6(3):135-41.
17. Maron BJ. Contemporary insights and strategies for risk stratification and prevention of sudden death in hypertrophic cardiomyopathy. Circulation. 2010;121(3):445-56.
18. Maron BJ, Shen WK, Link MS, Epstein AE, Almquist AK, Daubert JP, et al. Efficacy of implantable cardioverter–defibrillators for the prevention of sudden death in patients with hypertrophic cardiomyopathy. N Engl J Med. 2000;342(6):365-73.
19. Schinkel AF, Vriesendorp PA, Sijbrands EJ, Jordaens LJ, ten Cate FJ, Michels M. Outcome and complications after implantable cardioverter defibrillator therapy in hypertrophic cardiomyopathy: systematic review and meta-analysis. Circ Heart Fail. 2012;5(5):552-9.
20. Maron BJ, Spirito P, Ackerman MJ, Casey SA, Semsarian C, Estes NA 3rd, et al. Prevention of sudden cardiac death with implantable cardioverter-defibrillators in children and adolescents with hypertrophic cardiomyopathy. J Am Coll Cardiol. 2013;61(14):1527-35.
21. Maron BJ, Ommen SR, Semsarian C, Spirito P, Olivotto I, Maron MS. Hypertrophic cardiomyopathy: present and future, with translation into contemporary cardiovascular medicine. J Am Coll Cardiol. 2014;64(1):83-99. Erratum in: J Am Coll Cardiol. 2014;64(11):1188.
22. Maron BJ, Rowin EJ, Casey SA, Link MS, Lesser JR, Chan RH, et al. Hypertrophic cardiomyopathy associated with low cardiovascular mortality with contemporary management strategies. J Am Coll Cardiol. 2015;65(18):1915-28.
23. Spirito P, Autore C, Rapezzi C, Bernabò P, Badagliacca R, Maron MS, et al. Syncope and risk of sudden death in hypertrophic cardiomyopathy. Circulation. 2009;119(13):1085-92.
24. Bos JM, Maron BJ, Ackerman MJ, Haas TS, Sorajja P, Nishimura RA, et al. Role of family history of sudden death in risk stratification and prevention of sudden death with implantable defibrillators in hypertrophic cardiomyopathy. J Am Coll Cardiol. 2010;106(10):1481-6.
25. Elliot PM, Poloniecki J, Dickie S, Sharma S, Monserrat L, Varnava A, et al. Sudden death in hypertrophic cardiomyopathy: identification of high risk patients. J Am Coll Cardiol. 2000;36(7):2212-8.
26. Maron BJ, Spirito P, Ackerman MJ, Casey SA, Semsarian C, Estes NA 3rd, et al. Prevention of sudden cardiac death with implantable cardioverter-defibrillators in children and adolescents with hypertrophic cardiomyopathy. J Am Coll Cardiol. 2013;61(14):1527-35.

27. Spirito P, Bellone P, Harris KM, Bernabo P, Bruzzi P, Maron BJ. Magnitude of left ventricular hypertrophy and risk of sudden death in hypertrophic cardiomyopathy. N Engl J Med. 2000;342(24):1778-85.

28. Maron BJ, Rowin EJ, Casey AS, Haas TS, Chan RH, Udelson JE, et al. Risk stratification and outcome of patients with hypertrophic cardiomyopathy over 60 years of age. Circulation. 2013;127(5):585-93.

29. Chan RH, Maron BJ, Olivotto I, Pencina MJ, Assenza GE, Haas T, et al. Prognostic value of quantitative contrast-enhanced cardiovascular magnetic resonance for the evaluation of sudden death risk in patients with hypertrophic cardiomyopathy. Circulation. 2014;130(6):484-95.

30. Maron MS, Maron BJ. Clinical impact of contemporary cardiovascular magnetic resonance imaging in hypertrophic cardiomyopathy. Circulation. 2015;132(4):292-8.

31. O'Hanlon R, Grasso A, Roughton M, Moon JC, Clark S, Wage R, et al. Prognostic significance of myocardial fibrosis in hypertrophic cardiomyopathy. J Am Coll Cardiol. 2010;56(11):867-74.

32. Green JJ, Berger JS, Kramer CM, Salerno M. Prognostic value of the gadolinium enhancement in clinical outcomes for hypertrophic cardiomyopathy. JACC Cardiovasc Imaging. 2012;5(4):370-7.

33. Caetano F, Botelho A, Trigo J, Silva J, Almeida I, Venâncio M, et al. Phenotypic expression in hypertrophic cardiomyopathy and late gadolinium enhancement on cardiac magnetic resonance. Rev Port Cardiol. 2014;33(5):261-267.

34. Maron MS, Finley JJ, Bos JM, Hauser TH, Manning WJ, Haas TS, et al. Prevalence, clinical significance and natural history of left ventricular apical aneurysms in hypertrophic cardiomyopathy. Circulation. 2008;118(15):1541-9.

35. Harris KM, Spirito P, Maron MS, Zenovich AG, Formisano F, Lesser JR, et al. Prevalence, clinical profile, and significance of left ventricular remodeling in the end-stage phase of hypertrophic cardiomyopathy. Circulation. 2006;114(3):216-25.

36. Sorajja P, Ommen SR, Nishimura RA, Gersh BJ, Berger PB, Tajik AJ, et al. Adverse prognosis of patients with hypertrophic cardiomyopathy who have epicardial coronary artery disease. Circulation. 2003;108(19):2342-8.

37. Maron MS, Olivotto I, Betocchi s, Casey SA, Lesser JR, Losi MA, et al. Effect of left ventricular outflow tract obstruction on clinical outcome in hypertrophic cardiomyopathy. N Engl J Med 2003;348(4):295-303.

38. Efthimiadis GK, Parcharidou DG, Giannakoulas G, Pagourelias ED, Charalampidis P, Savvopoulos G, et al. Left ventricular outflow tract obstruction as a risk factor for sudden cardiac death in hypertrophic cardiomyopathy. Am J Cardiol. 2009;104(5):695-9.

39. Maron BJ, Haas TS, Maron MS, Lesser JR, Browning JA, Chan RH, et al. Left atrial remodeling in hypertrophic cardiomyopathy and susceptibility markers for atrial fibrillation identified by cardiovascular magnetic resonance. Am J Cardiol. 2014;113(8):1394-400.

Prevention of Sudden Death in Hypertrophic Cardiomyopathy

Edmundo Arteaga-Fernández and Murillo de Oliveira Antunes

Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (INCOR HC FMUSP), São Paulo, SP – Brazil

Short Editorial regarding the article: Prevention of Sudden Cardiac Death in Hypertrophic Cardiomyopathy: What has Changed in The Guidelines?

Hypertrophic cardiomyopathy (HCM) is the most common congenital disease, and sudden death (SD), its most feared complication, was already mentioned by Donald Teare[1] in the first description of the disease, being observed in 7 out of 8 patients. SD occurs during daily activities, after exercises and even during sleep; it may affect young athletes, which has a great impact on the media. This has required considerable effort by researchers in defining clinical factors and complementary tests that could be used in the screening of individuals at higher risk that could benefit from implantable cardioverter defibrillator (ICD) and also to prevent SD, since it is caused by tachycardia and ventricular fibrillation.[2] HCM favors the occurrence of ventricular arrythmias – hypertrophy causes repolarization dispersion; myocyte disarray and increased fibrosis create areas of conduction block and predispose to reentry arrhythmias; and abnormalities in ion fluxes, such as calcium, during repolarization may also trigger arrhythmias. In addition, this complex arrhythmogenic substrate may be modulated by impaired autonomic response, myocardial ischemia and left ventricular outflow tract obstruction.[2-4] If we consider deaths from cardiovascular causes, in patients with HCM, they account for 0.5%-1.5% deaths a year, which is near to that of the general population.[2] In HCM patients considered as high risk, SD may reach 2.5% of deaths a year.[5] However, the accurate identification of these patients for preventive therapy with ICD may be challenging.

Before the guidelines were published,[3] it was known that manifestations of HCM in children younger than 10 years old with diastolic or systolic dysfunction, SD in first-degree relatives younger than 50 years, nonsustained ventricular tachycardia, syncope and myocardial hypertrophy > 30 mm were factors associated with SD, and the last four fully considered as indications for ICD in the first guideline (2011).[2]

Today, we know that the positive predictive value of each of these factors is low, and there is little evidence suggesting a higher predictive value of any of these factors. However, some authors have considered only one risk factor for indication of ICD.[6]

The two largest multicentric studies grounded in the American guidelines[2] – one of adults (n = 506, mean age of 42; mean follow-up period of 3.7 years) showed that for primary prevention ICD indication, in 75% of cases, the devices were used in 4%/year, whereas for the secondary prevention, intervention rates were 12%/year in 25% of cases. Therapies were found in 20% of patients and inappropriate shocks in 27%, with 7% of complications.[7] The other study involved 224 children and adolescents (mean age of 14 years; mean follow-up of 4.3 years). Primary prevention was indicated in 84% of cases and secondary prevention indicated for 16% of cases. Intervention rates were similar to those in adults, with therapies and inappropriate shocks in 19% and 41% of cases, respectively.[8]

The 2014 European Guidelines (ESC) recommended a new sudden-death risk model based on a longitudinal, retrospective, multicenter study risk calculation model (n = 3,675) and seven variables – age, history of SD, syncope, wall thickness, left atrial diameter, left ventricular outflow gradient and nonsustained ventricular tachycardia. In primary prevention, the risk calculation encompasses three SD risk levels at five years – low, moderate and high – for patients older than 16 years.[3,9] This risk prediction model, validated in Europe[10] (n = 706) and in South America[11] (n = 502), was shown to better predict individual risks as compared with that used in North America and Canada societies. However, a study[12] using the ESC risk calculation model (n = 1,629, age > 16 years) showed that most patients with HCM or with previous ICD were classified as low risk and therefore would remain unprotected from SD. The authors concluded that the primary risk stratification using this model is unreliable for prediction of future SD events.[13]

In this issue of *Arquivos Brasileiros de Cardiologia*, in a cohort study (n = 105), Reis et al.[14] compared the American and the European guidelines in stratifying SD risk, and concluded that the European model reduces the proportion of patients with indication for ICD.

We can affirm that, despite continuous advances in knowledge,[10,15] the assessment of SD risk in HCM is limited to a small number of patients (5%) and is still a great challenge. The guidelines have so far included increasing number of risk factors[15] with low predictive value, and validated for a frequent, but still underdiagnosed disease, characterized by patients with a normal life cycle and free from SD.

Keywords

Cardiomyopathy, Hypertrophic; Death, Sudden, Cardiac / prevention & control; Heart Defects, Congenital.

Mailing address: Edmundo Arteaga-Fernández •
Rua Arruda Alvim, 49/111. Postal Code 05410-020, São Paulo, SP - Brazil
E-mail: Edmundo.arteaga@incor.usp.br

DOI: 10.5935/abc.20180101

Acknowledgements

We thank the colleagues Adriana Paula Tirone, Afonso Akio Shiozaki, Afonso Yoshikiro Matsumoto, Aloir Queiroz de Araujo, Julia Daher Carneiro Marsiglia, Paula de Cássia Buck and Paulo de Tarso Jorge Medeiros, for their contribution to the study of hypertrophic cardiomyopathy.

References

1. Teare D. Asymmetrical hypertrophy of the heart in young adults. Br Heart J. 1958;20(1):1-8.
2. Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS, et al; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines; American Association for Thoracic Surgery; American Society of Echocardiography; American Society of Nuclear Cardiology; Heart Failure Society of America; Heart Rhythm Society; Society for Cardiovascular Angiography and Interventions; Society of Thoracic Surgeons. 2011 ACCF/AHA guidelines for the diagnosis and treatment of hypertrophic cardiomyopathy: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation. 2011;124(24):2761-796.
3. Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, et al. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: the Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). Eur Heart J. 2014;35(39):2733-79.
4. Arteaga E, Ianni BM, Fernandes F, Mady C. Benign outcome in a long--term follow-up of patients with hypertrophic cardiomyopathy in Brazil. Am Heart J. 2005;149(6):1099-105.
5. Maron BJ, Casey SA, Poliac LC, Gohman TE, Almquist AK, Aeppli DM. Clinical course of hypertrophic cardiomyopathy in regional United States cohort. JAMA. 1999;281(7):650-5. Erratum in: JAMA. 1999;281(24):2288.
6. Maron BJ, Ommen SR, Semsarian C, Spirito P, Olivotto I, Maron MS. Hypertrophic cardiomyopathy: present and future, with translation into contemporary cardiovascular medicine. J Am Coll Cardiol. 2014;64(1):83-99. Erratum in: J Am Coll Cardiol. 2014;64(11):1188.
7. Maron BJ, Spirito P, Shen WK, Haas TS, Formisano F, Link MS, et al. Implantable cardioverter-defibrillators and prevention of sudden cardiac death in hypertrophic cardiomyopathy. JAMA. 2007;298(4):405-12. Erratum in: JAMA. 2007;298(13):1516.
8. Maron BJ, Spirito P, Ackerman MJ, Casey SA, Semsarian C, Estes NA 3rd, et al. Prevention of sudden cardiac death with the implantable cardioverter-defibrillators in children and adolescent with hypertrophic cardiomyopathy. J Am Coll Cardiol. 2013;61(14):1527-35.
9. O'Mahony C, Jichi F, Pavlou M, Monserrat L, Anastasakis A, Rapezzi C, et al; Hypertrophic Cardiomyopathy Outcomes Investigators. A novel clinical risk prediction model for sudden cardiac death in hypertrophic cardiomyopathy (HCM Risk-SCD). Eur Heart J. 2014;35(30):2010-20.
10. Vriesendorp PA, Schinkel AF, Liebregts M, Theuns DA, van Cleemput J, Ten Cate FJ, et al. Validation of the 2014 European Society of Cardiology Guidelines risk prediction mode for the primary prevention of sudden cardiac death in hypertrophic cardiomyopathy. Circ Arrhythm Eletrophysiol. 2015;8(4):829-35.
11. Fernandez A, Quiroga A, Ochoa JP, Mysuta M, Casabé JH, Biagetti M, et al. Validation of the 2014 European Society of Cardiology sudden cardiac death risk prediction model in hypertrophic cardiomyopathy in a reference center in south America. Am J Cardiol. 2016;118(1):121-6.
12. Maron BJ, Casey SA, Chan RH, Garberich RF, Rowin EJ, Maron MS. Independent assessment of the European Society of Cardiology sudden death risk model for hypertrophic cardiomyopathy. Am J Cardiol. 2015;116(5):757-64.
13. Maron BJ, Maron BS. Contemporary strategies for risk stratification and prevention of sudden death with the implantable defibrillator in hypertrophic cardiomyopathy. Heart Rhythm. 2016;13(5):1155-65.
14. Reis L, Teixeira R, Fernandes A, et al. Prevenção de morte súbita cardíaca em doentes com miocardiopatia hipertrófica: o que mudou nas guidelines? Arq Bras Cardiol. 2018; 110(6):524-531.
15. de Oliveira Antunes M, Samesima N, Pereira Filho HG, Matsumoto AY, Verrier RL, et al. Exercise-induced quantitative microvolt T-wave alternans in hypertrophic cardiomyopathy. J Electrocardiol. 2017;50(2):184-90.

Is There any Relationship Between Myocardial Repolarization Parameters and the Frequency of Ventricular Premature Contractions?

Kayihan Karaman,[1] Metin Karayakali,[1] Arif Arisoy,[1] Ilker Akar,[2] Mustafa Ozturk,[3] Ahmet Yanik,[4] Samet Yilmaz,[1] Atac Celik[1]

Gaziosmanpasa University Faculty of Medicine, Department of Cardiology,[1] Tokat - Turkey

Gaziosmanpasa University Faculty of Medicine, Department of Cardiovascular Surgery,[2] Tokat - Turkey

Erzurum Territorial Training and Research Hospital, Cardiology Clinic,[3] Erzurum - Turkey

Samsun Training and Research Hospital, Cardiology Clinic,[4] Samsun - Turkey

Abstract

Background: Ventricular premature contractions (VPCs) may trigger lethal ventricular arrhythmias in patients with structural heart disease. However, this role of VPCs in healthy people remains controversial once that not enough clinical trials are available. Recently, some myocardial repolarization markers, such as Tp-e interval, Tp-e/QT, and Tp-e/QTc ratios, have been reported to be useful for predicting lethal ventricular arrhythmias in various clinical disorders without structural heart disease.

Objective: In this study, we aimed to investigate the relation between VPC frequent and myocardial repolarization markers in individuals without structural heart disease.

Methods: This study included 100 patients who had complaints of dizziness and palpitations. Twelve-lead electrocardiography and 24-hour ambulatory Holter recordings were obtained from all patients. VPC burden was calculated as the total number of VPCs divided by the number of all QRS complexes in the total recording time. P-values < 0.05 were considered significant.

Results: Tp-e interval and Tp-e/QTc ratio were significantly higher in patients with higher VPC burden than in patients with lower VPC burden, and a positive correlation was found between these markers and VPC burden. Tp-e ($\beta = 1.318$, $p = 0.043$) and Tp-e/QTc ($\beta = -405.136$, $p = 0.024$) in the lead V5 were identified as independent predictors of increased VPC burden.

Conclusions: Tp-e interval and Tp-e/QTc ratio increased in patients with high VPC number. Our study showed that VPCs may have a negative effect on myocardial repolarization. This interaction may lead to an increased risk of malignant arrhythmias. (Arq Bras Cardiol. 2018; 110(6):534-541)

Keywords: Ventricular Premature Complexes; Arrhythmias, Cardiac; Electrocardiography / methods; Cardiovascular Diseases; Obesity; Ventricular Dysfunction, Left.

Introduction

Ventricular premature contractions (VPCs) are commonly seen in the electrocardiography (ECG) of patients with hypertension, obesity, and structural heart disease. Some studies reported VPCs to occur in about 4% of the general population.[1,2] As some patients may be asymptomatic, many patients suffer from VPC-related symptoms, such as palpitation, dizziness, dyspnea, and chest pain. In addition to these symptoms, frequent VPCs may cause more serious disorders. Recent studies on adults with frequent VPCs (> 20,000/24 h) have reported left ventricular dilation and/or dysfunction,[3,4] diastolic dysfunction,[5] and malignant ventricular arrhythmias in patients with structural heart disease.[6] However, whether frequent VPCs are associated with malignant arrhythmias in individuals without structural heart disease remains uncertain.

T wave is commonly used in assessing myocardial repolarization. Increased transmural dispersion of myocardial repolarization in a normal heart is associated with their tendency toward cardiac arrhythmias. Recently, some myocardial repolarization markers, such as QT interval (QT), corrected QT (QTc), QT dispersion (QTd), Tp-e interval (Tp-e), and Tp-e/QT ratio have been found to be useful in predicting life-threatening cardiac arrhythmias in several clinical disorders without structural heart disease. Some studies showed that increased Tp-e, Tp-e/QT, and Tp-e/QTc were related to the elevated risk of occurrence of malignant ventricular arrhythmias.[7,8] In this study, we investigated the relation between VPC burden and myocardial repolarization by using some ECG markers in individuals without structural heart disease.

Mailing Address: Kayihan Karaman •
Gaziosmanpasa University School of Medicine Training and Research Hospital, 60100, Tokat - Turkey
E-mail: drkkaraman55@gmail.com, kay55han@hotmail.com
Manuscript received June 19, 2017, revised manuscript October 18, 2017, accepted October 18, 2017

DOI: 10.5935/abc.20180079

Methods

Study population

One hundred patients with at least 1 VPC in the 12-lead ECG with diagnosis of dizziness, syncope, and palpitation without structural heart disease admitted to the Cardiology Department of our university hospital, between July 2016 and March 2017, were enrolled for this cross-sectional study. Twenty-four-hour ambulatory Holter recordings were obtained from all patients. VPC burden was calculated as the total number of VPCs divided by the number of all QRS complexes in the total recording time. A frequency of < 1% VPCs/24 h was denoted as "rare-group 1 (n = 32)", 1–5% VPCs/24 h was denoted as "occasional-group 2 (n = 36)", and > 5% VPCs/24 h was denoted as "frequent-group 3 (n = 32)". The exclusion criteria for all groups were non-reliable T waves on the ECG, atrial fibrillation, bundle branch block, moderate or severe valvular heart diseases, thyroid disorders, cardiomyopathies, congenital heart diseases, malignancy, pulmonary hypertension, electrolyte disturbances, acute coronary syndromes, heart failure, history of myocardial infarction, history of coronary artery bypass grafting, implanted permanent pacemaker, and LV segmental motion defect in the echocardiographic exam. The local ethics committee approval and informed consent from all patients were obtained.

Electrocardiography and Holter Recordings

Twelve-lead ECGs were obtained at rest at 10 mm/mV amplitude and 25 mm/sec (Cardiofax V; Nihon Kohden Corp., Tokyo, Japan) rate, with the patient in the supine position. All ECGs were transferred to a computer through a scanner and then used for × 300% magnification using the Paint software. Holter recordings were performed by using Lifecard CF recorders (Del-Mar Reynolds). Patients were warned not to smoke and not to consume coffee and/or alcohol during the Holter recording. Measurements were performed on the computer by two cardiologists who were blinded to the clinical data of each patient. Ventricular tachycardia (VT) was defined as the line-up of at least three or more consecutive VPCs. The ventricular couplet (Vc) was defined as a sequential ordering of two VPCs.

RR interval, QRS duration, QT, and QTd were measured in all derivations. QT was defined as the time from the start of the QRS to the point at which the T wave returns to the isoelectric line. The average value of at least two readings was calculated for each lead. QTc was calculated by using Bazett's formula:[9] $QTc = QT/\sqrt{R-R\ interval}$. QTd was defined as the difference between the longest and the shortest QT interval of the 12leads. Subjects with U waves in their ECGs were excluded from the study. In the measurement of Tp-e interval, the tail and tangent methods can be used, but the former is a better predictor of mortality than the latter.[10] Thus, the tail method was used in this study. The tail method was defined as the interval from the peak to the end of the T wave to the point where the wave reached the isoelectric line.[9] Measurement of the Tp-e interval was obtained from leads V2 and V5, which were corrected for heart rate (cTp-e).[11] The Tp-e/QT and Tp-e/QTc ratios were calculated from these measurements.

Echocardiographic examination

All echocardiography examinations (General Electric Vivid S5, Milwaukee, WI, USA) were performed by an experienced cardiologist in all subjects using a 2.5–3.5 MHz transducer in the left decubitus position. Two-dimensional and pulsed Doppler measurements were obtained using the criteria of the American Society of Echocardiography and the European Association of Cardiovascular Imaging.[12] Left ventricular ejection fraction (LVEF) was assessed using Simpson's method.

Statistical analysis

All tests were performed by using PASW Statistics (SPSS 18.0 for Windows, Inc., Chicago, IL, USA). Shapiro–Wilk test was used to test for normal distribution. Continuous variables were described as the mean (± standard deviation), and categorical variables were described as frequency (percentage). All continuous parameters were compared among groups by using one-way ANOVA. The post hoc Tukey's test was used for significant intergroup differences. Categorical factors were compared among groups using the χ^2 test for independence. Correlations between two variables were performed by Pearson's correlation. Multiple linear regression analysis was used to evaluate the association between an increased VPC burden and independent variables that differed significantly in Pearson's correlation analyses ($p < 0.1$). A multivariate logistic regression analysis was performed to demonstrate the effect of presence of CAD on ECG parameters. P-values < 0.05 were considered significant.

Results

The baseline demographics and laboratory characteristics of the three groups are summarized in Table 1. No significant difference was found among the three groups in terms of any baseline demographic or laboratory characteristic. Some baseline and ambulatory ECG parameters among the groups are shown in Table 2.

According to the comparison of the ECG parameters among the three groups in lead V2, QT interval was significantly longer in groups 2 and 3 than in group 1. Tp-e interval in group 3 was significantly longer than those in groups 1 and 2. The Tp-e/QTc ratio significantly increased in groups 2 and 3 in comparison with group 1. When the groups were compared, no significant difference was found in QTc interval and Tp-e/QT ratio (Table 2).

In the comparison of ECG parameters among the three groups in lead V5, QT interval was significantly longer in group 3 than in group 1. Tp-e interval was significantly longer in group 3 than in groups 1 and 2. Tp-e/QTc ratio was significantly increased in the group 3 when compared to the group1. When the groups were compared, no significant difference was found in QTc interval and Tp-e/QT ratio (Table 2).

A total of 28 patients had coronary artery disease (CAD) (7, 10, and 11 patients in groups 1, 2, and 3, respectively). Non-critical lesions that did not cause significant narrowing were evident in the angiographic reports. The presence of CAD was greater in group 3 than in groups 1 and 2, but statistical significance was not observed (p = 0.538). In the multivariate logistic regression

Table 1 – Baseline characteristics, laboratory and echocardiographic parameters of the study population

Variables	Group 1 (n = 32)	Group 2 (n = 36)	Group 3 (n = 32)	p*
Age, years	49.60 ± 16.50	51.40 ± 17.00	52.10 ± 12.90	0.805
Female sex, n (%)	16.00 (50.00)	19.00 (52.80)	14.00 (43.8)	0.752
Body mass index, kg/m^2	24.10 ± 2.50	23.60 ± 3.60	23.40 ± 4.40	0.657
Hypertension, n (%)	8.00 (25.00)	12.00 (33.30)	10.00 (31.3)	0.743
Diabetes Mellitus, n (%)	1.00 (3.10)	4.00 (11.10)	5.00 (15.6)	0.240
Coronary Artery Disease, n (%)	7.00 (21.90)	10.00 (27.80)	11.00 (34.4)	0.538
Smoking, n (%)	6.00 (18.80)	5.00 (13.90)	7.00 (21.9)	0.687
Systolic Blood Pressure (mmHg)	125.40 ± 15.40	125.10 ±14.30	122.80 ±14.00	0.737
Diastolic Blood Pressure (mmHg)	78.70 ± 7.50	77.50 ± 8.10	76.70 ± 8.90	0.638
Left Ventricle Ejection Fraction, (%)	62.80 ± 3.70	61.30 ± 4.20	60.90 ± 4.70	0.167
Interventricular Septum, (mm)	9.80 ± 0.70	10.20 ± 0.80	10.00 ± 0.80	0.460
Creatinine, mg/dl	0.82 ± 0.22	0.85 ± 0.22	0.83 ± 0.21	0.816
Neutrophil to Lymphocyte Ratio	1.90 ± 0.57	2.36 ± 1.05	2.26 ± 1.67	0.267
Hemoglobin, gr/dl	14.60 ± 1.60	14.00 ± 1.40	14.20 ± 1.80	0.345
β-blockers, n (%)	15.00 (46.90)	16.00 (44.40)	11.00 (34.4)	0.559
Angiotensin-converting Enzyme Inhibitors, n (%)	8.00 (25.00)	9.00 (25.00)	6.00 (18.8)	0.787
Angiotensin Receptor Blockers, n (%)	4.00 (12.50)	5.00 (13.90)	4.00 (12.5)	0.981
Number of patients with Vc, n (%)	9.00 (28.10)	21.00 (58.30)	21.00 (65.6)	0.006
Number of patients with VT, n (%)	3.00 (9.40)	11.00 (30.60)	12.00 (37.5)	0.028

*Vc: ventricular couplet; VT: ventricular tachycardia. Data are presented as mean ± SD, or n (%). Statistically significant p values shown in bold. *ANOVA and χ^2 tests were performed to study differences among the three groups.*

analysis, CAD had no effect on ECG parameters. Vc was observed in 51 patients (9, 21, and 21 patients in groups 1, 2, and 3, respectively) and VT in 26 patients (3, 11, and 12 patients in groups 1, 2, and 3, respectively). The QTd duration of group 3 was significantly longer than those in groups 1 and 2 ($p = 0.001$, $p = 0.015$, respectively).

According to Pearson's correlation test, positive correlations were observed between VPC burden and Tp-e (in leads V2 and V5) and Tp-e/QTc (in leads V2 and V5) ($r = 0.476$, $p < 0.001$; $r = 0.395$, $p = < 0.001$and $r = 0.296$, $p = 0.003$; $r = 0.256$, $p = 0.010$, respectively) (Table 3, Figure 1). Table 3 shows the results of the multiple linear regression analyses performed to identify the ECG parameters affecting VPC burden. Thus, Tp-e interval ($\beta = 1.318$, $p = 0.043$) and Tp-e/QTc ratio ($\beta = -405.136$, $p = 0.024$) in lead V5 were independent predictors of VPC burden.

Discussion

In this study, we demonstrated that Tp-e interval and Tp-e/QTc ratios were significantly higher in patients with higher VPC burden than in those with lesser VPC burden and that a positive correlation was observed between these markers and VPC frequency. However, we did not find a relationship between Tp-e/QT ratio and VPC burden. Tp-e interval and Tp-e/QTc ratio in lead V5 were identified as independent predictors of increased VPC burden. The prolongation of the duration of myocardial repolarization in patients with increased VPC burden is important because this condition may be related to the increased risk of life-threatening arrhythmia. According to our results, myocardial repolarization parameters deteriorated with increasing VPC frequency. Therefore, we concluded that both VPC frequency and stage of myocardial repolarization were affected by similar causes.

Idiopathic VPCs, generally regarded as a benign condition in healthy individuals without structural heart disease, are formed by the spread of an early stimulus originating from an ectopic focus. VPCs may cause serious complications, such as angina, syncope, or heart failure, when the ectopic beat number increases.[13-16] Although VPCs are known to be benign in individuals with a structurally normal heart, they have been shown to cause malignant arrhythmias in some cases. However, the clinical significance of VPC frequency in these individuals remains unclear once adequate human studies have not been performed.[17] Tilz et al.[18] found that ventricular fibrillation (VF) was triggered by VPCs after an implantable cardioverter-defibrillator was used on a 29-year-old patient, who was resuscitated following sudden cardiac death. All examinations, including echocardiography, angiography, ajmaline test, and myocardial biopsy, were normal. At the same time, some cases demonstrated that polymorphic VT and idiopathic VF were induced because specific VPCs with short coupling intervals could promote intracellular calcium overload.[19,20] In a study examining the records of 21 patients

Table 2 – Baseline and ambulatory Holter electrocardiography parameters of the study population

Variables	Group 1 (n = 32)	Group 2 (n = 36)	Group 3 (n = 32)	p values (groups)*		
				1 vs 2	1 vs 3	2 vs 3
Maximum Heart Rate (beats/min)	123.60 ± 17.10	120.40 ± 20.10	116.80 ± 13.20	0.720	0.259	0.671
Minimum Heart Rate (beats/min)	58.90 ± 7.40	54.90 ± 8.60	57.10 ± 7.30	0.097	0.638	0.481
Average Heart Rate (beats/min)	73.40 ± 13.40	72.40 ± 14.60	73.90 ± 12.00	0.940	0.980	0.855
Number of VPCs (median/24 h)	543.00 ± 288.00	2779 ± 1041	8358 ± 2911	< 0.001	< 0.001	< 0.001
Number of VPCs (median/h)	22.80 ± 12.40	117.50 ± 46.30	358.00 ± 125.20	< 0.001	< 0.001	< 0.001
Percent of VPC number (24 h)	0.50 ± 0.23	2.76 ± 1.03	7.90 ± 2.72	< 0.001	< 0.001	< 0.001
Lead V2						
QT (ms)	358.00 ± 22.80	378.10 ± 35.50	387.00 ± 25.30	0.013	< 0.001	0.419
QTc (ms)	414.30 ± 32.20	410.50 ± 27.00	427.30 ± 33.80	0.867	0.222	0.071
Tp-e (ms)	94.30 ± 9.40	100.50 ± 9.70	106.50 ± 7.90	0.016	< 0.001	0.023
cTp-e (ms)	108.60 ± 14.80	110.00 ± 16.30	117.70 ± 11.50	0.923	0.038	0.079
Tp-e/QT	0.26 ± 0.02	0.27 ± 0.03	0.28 ± 0.02	0.854	0.239	0.493
Tp-e/QTc	0.23 ± 0.02	0.24 ± 0.03	0.25 ± 0.03	0.007	0.001	0.689
Lead V5						
QT (ms)	363.70 ± 26.20	380.50 ± 41.50	389.30 ± 20.50	0.075	0.004	0.485
QTc (ms)	421.00 ± 37.00	413.00 ± 29.30	429.70 ± 29.10	0.554	0.524	0.084
Tp-e (ms)	91.30 ± 9.20	94.00 ± 12.20	101.10 ± 8.80	0.519	0.001	0.015
cTp-e (ms)	106.50 ± 15.10	102.3 ± 13.9	112.0 ± 14.0	0.453	0.280	0.018
Tp-e/QT	0.25 ± 0.02	0.25 ± 0.03	0.26 ± 0.03	0.895	0.372	0.163
Tp-e/QTc	0.22 ± 0.02	0.23 ± 0.03	0.24 ± 0.03	0.244	0.021	0.465
QTd (ms)	23.30 ± 6.40	26.3 ± 13.1	34.3 ± 13.4	0.537	0.001	0.015

*QTc: corrected QT; QTd: QT dispersion; Tp-e: T wave peak-to-end interval; cTp-e: corrected Tp-e; ms: millisecond; VPC: ventricular premature contraction; Data are presented as mean ± SD. Statistically significant p values shown in bold; *ANOVA test was performed to study differences among the three groups. The post hoc Tukey's test was performed after ANOVA to study between groups differences for group 1 vs. group 2, group 1 vs. group 3 and group 2 vc. group 3.*

who experienced cardiac arrest during ambulatory ECG recording, heart rate and VPC frequency increased before the onset of VF.[21] Savelieva et al.[22] found significant QT turbulence after VPC in individuals with a structurally healthy heart. Although these data provide information on the cause of malignant arrhythmias for VPC, they do not provide enough information about the importance of VPC frequency.

Several mechanisms have been proposed to explain the relationship between VPC and life-threatening arrhythmias. VPC may play a key role in the initiation of malignant cardiac arrhythmias. Various factors such as increased sympathetic tonus, altered hemodynamic status, or electrolyte imbalances (e.g., the hypokalemia and hypercalcemia), which all disrupt the stability of the myocardium, may cause a transition from VPC to malignant arrhythmia.[17] Increased sympathetic tonus due to anxiety or physiological stress may cause the release of catecholamines such as adrenaline. This condition causes the flow of calcium from an extracellular space into the myocyte cells by increasing the production of cyclic AMP (cAMP). The contraction force of the myocytes increases, and the myocyte is depolarized rapidly. For this reason, myocytes become more sensitive than normal and may depolarize spontaneously without sino-atrial node depolarization. Therefore, VPC formation and frequency may increase.[23,24] Armaganijan et al.[25] reported the relationship of sympathetic activation with patients with ventricular arrhythmias and suggested the effectiveness of renal sympathetic denervation by catheter to reduce arrhythmic burden.

Another factor that increases the frequency of VPC is excessive caffeine consumption. Caffeine, a phosphodiesterase inhibitor, is also a central stimulant that can enhance sympathetic activity. It can increase intracellular calcium concentration by inhibiting the enzyme that catalyzes the breakdown of cAMP. Animal studies showed that caffeine administration at high doses could induce and increase the frequency of VPCs.[26,27]

Prolongation in the dispersion of myocardial repolarization predisposes the malignant ventricular arrhythmia and has prognostic importance in terms of sudden cardiac death (SCD). Prolongation of QT and QTd durations may be associated with polymorphic ventricular tachycardia, Torsades de pointes, and SCD.[28,29] Recently, some myocardial repolarization markers, such as Tp-e interval, Tp-e/QT, and Tp-e/QTc ratios, have been reported to be useful in predicting lethal ventricular arrhythmias in various clinical disorders without structural

Table 3 – Relationship between ventricular premature contractions (VPC) burden and clinical and electrocardiographic parameters

Variables	VPC burden			
	Pearson correlation coefficient	p	Beta regression coefficient	p
Age	-0.026	0.797	-	-
Female sex	0.089	0.380	-	-
CAD	0.065	0.520	-	-
QTd	0.256	0.010	0.035	0.190
Lead V2				
QT	0.362	< 0.001	0.067	0.749
QTc	0.243	0.015	0.148	0.382
Tp-e	0.476	< 0.001	-0.665	0.260
Tp-e/QT	0.171	0.088	-48.643	0.734
Tp-e/QTc	0.296	0.003	-366.464	0.059
Lead V5				
QT	0.292	0.003	-0.151	0.449
QTc	0.173	0.085	-0.154	0,309
Tp-e	0.395	< 0.001	1.318	0.043
Tp-e/QT	0.185	0.066	-100.943	0.585
Tp-e/QTc	0.256	0.010	-405.136	0.024

QTc: corrected QT; QTd: QT dispersion; Tp-e: T wave peak-to-end interval; VPC: ventricular premature contraction. Pearson's correlation and linear regression analyses.

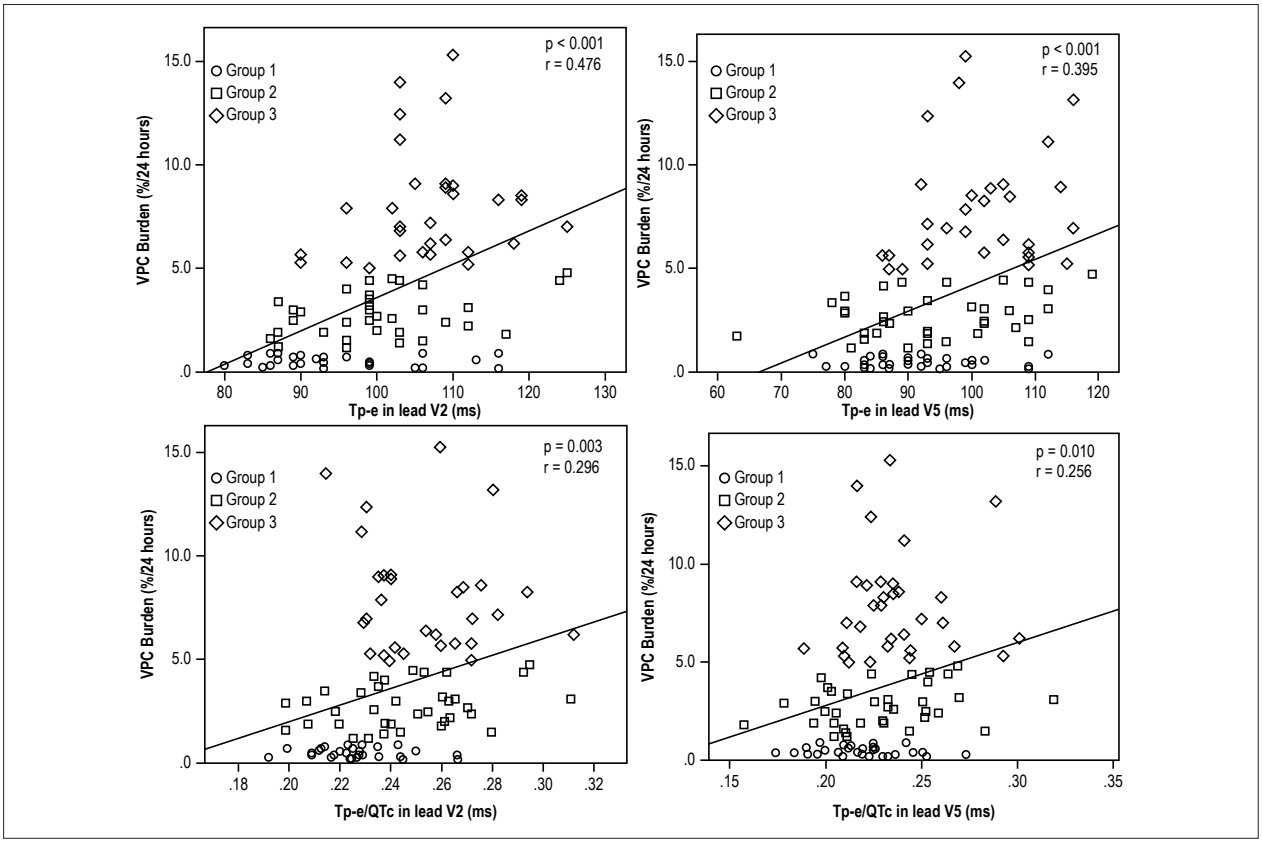


Figure 1 – *Scatter analysis of the correlation between the Tp-e interval and Tp-e/QTc ratio (in the leads V2 and V5) and the VPC burden. ms: millisecond; QTc: corrected QT; Tp-e: T wave peak-to-end interval; VPC: ventricular premature contraction.*

heart disease.[7,30,31] Tp-e interval is considered a new marker of increased risk of SCD. Yamaguchi et al.[32] showed that Tp-e interval is more significant than QTd or QTc in predicting Torsade de Pointes in patients with acquired long QT syndrome. At the same time, an increase in Tp-e interval and Tp-e/QT ratios was shown to be associated with Brugada syndrome.[8] Tp-e/QT and Tp-e/QTc ratios were found to be relatively more constant than other markers because they were not affected by changes in heart rate and body weight.[9]

Although we found an increase in Tp-e interval and Tp-e/QTc ratios as VPC frequency increased, the slight increase observed in Tp-e/QT ratio was not statistically significant. Yayla et al.[33] assessed the myocardial repolarization parameters before and after RFA in patients with a VPC burden of more than 5% on a 24 h Holter recording. After the successful procedure, Tp-e interval, Tp-e/QT ratio, and Tp-e/QTc ratio significantly decreased more than before RFA (all $p < 0.001$). In accordance with this data, the higher detection of Tp-e interval in patients with increased VPC frequency suggests that the risk of malignant arrhythmias might be higher in these patients. In our study, malignant arrhythmias, such as Vc and VT, were seen more in group 3 patients, thus supporting our predictions. This important link can be used to closely follow up on and manage their treatment of patients with increased VPC frequency.

Study limitations

Our study has several major limitations. First, our study was single centered and included a small number of patients. Therefore, statistical power was limited. The results should be verified in a larger prospective cohort study. Second, because we did not have other ambulatory Holter measures, such as heart rate variability and heart rate turbulence, we could not exclude the effect of these measurements on the VPC frequency. Third, we did not have data on cardiac event rates for this study because we could not follow up on the patients prospectively for future arrhythmic events. Fourth, we aimed to record a relatively young patient profile to exclude occult CAD in our study. However, we abandoned this goal because of the limited number of patients. Further comprehensive studies should be conducted with a larger number of patients and a longer follow-up time to increase the consistency of our results.

Conclusions

In conclusion, Tp-e interval and Tp-e/QTc ratios increased in patients with high VPC number. Our study showed that VPCs could have a negative effect on myocardial repolarization. This interaction could lead to an increased risk of malignant arrhythmias.

Author contributions

Conception and design of the research: Karaman K, Karayakali M, Arisoy A; Acquisition of data: Karaman K, Akar O, Ozturk M, Yanik A, Yilmaz S; Analysis and interpretation of the data: Karaman K, Karayakali M, Arisoy A, Yilmaz S, Celik A; Statistical analysis: Karaman K, Karayakali M, Arisoy A, Akar O, Celik A; Obtaining financing: Karaman K, Arisoy A, Akar O; Writing of the manuscript: Karaman K, Arisoy A, Yanik A; Critical revision of the manuscript for intellectual content: Karaman K, Karayakali M, Ozturk M, Yanik A, Celik A.

Potential Conflict of Interest

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

Sources of Funding

There were no external funding sources for this study.

Study Association

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

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Gaziosmanpasa University Faculty of Medicine under the protocol number 83116987-252. 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.

References

1. Kennedy HL, Whitlock JA, Sprague MK, Kennedy LJ, Buckingham TA, Goldberg RJ. Long-term follow-up of asymptomatic healthy subjects with frequent and complex ventricular ectopy. N Engl J Med. 1985;312(4):193-7.
2. Cheriyath P, He F, Peters I, Li X, Alagona P Jr, Wu C, et al. Relation of atrial and/or ventricular premature complexes on a two-minute rhythm strip to the risk of sudden cardiac death: the Atherosclerosis Risk in Communities [ARIC] study. Am J Cardiol. 2011;107(2):151-5.
3. Bogun F, Crawford T, Reich S, Koelling TM, Armstrong W, Good E, et al. Radiofrequency ablation of frequent, idiopathic premature ventricular complexes: comparison with a control group without intervention. Heart Rhythm. 2007;4(7):863-7.
4. Duffee DF, Shen WK, Smith HC. Suppression of frequent premature ventricular contractions and improvement of left ventricular function in patients with presumed idiopathic dilated cardiomyopathy. Mayo Clin Proc. 1998;73(5):430-3.
5. Topaloglu S, Aras D, Cagli K, Yildiz A, Cagirci G, Cay S, et al. Evaluation of left ventricular diastolic functions in patients with frequent premature ventricular contractions from right ventricular outflow tract. Heart Vessels. 2007;22(5):328-34.
6. Moss AJ, Akiyama T. Prognostic significance of ventricular premature beats. Cardiovasc Clin. 1974;6(1):273-98.
7. Karaman K, Altunkas F, Çetin M, Karayakali M, Arisoy A, Akar I, et al. New markers for ventricular repolarization in coronary slow flow: Tp-e interval, Tpe/QT ratio, and Tp-e/QTc ratio. Ann Noninvasive Electrocardiol. 2015;20(4):338-44.
8. Gupta P, Patel C, Patel H, Narayanaswamy S, Malhotra B, Green JT, et al. T(p-e)/QT ratio as an index of arrhythmogenesis. J Electrocardiol. 2008;41(6):567-74.
9. Antzelevitch C, Viskin S, Shimizu W, Yan G-X, Kowey P, Zhang L, et al. Does Tpeak-Tend provide an index of transmural dispersion of repolarization? Heart Rhythm. 2007;4(8):1114-9.
10. Tatlisu MA, Özcan KS, Güngör B, Ekmekçi A, Çekirdekçi EI, Aruğarslan E, et al. Can the T-peak to T-end interval be a predictor of mortality in patients with ST elevation myocardial infarction? Coron Artery Dis. 2014;25(5):399-404.
11. Castro Hevia J, Antzelevitch C, Tornés Bárzaga F, Dorantes Sánchez M, Dorticós Balea F, Zayas Molina R, et al. Tpeak-Tend and TpeakTend dispersion as risk factors for ventricular tachycardia/ventricular fibrillation in patients with the Brugada syndrome. J Am Coll Cardiol. 2006;47(9):1828-34.
12. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2015;28(1):1-39.
13. Simpson RJ Jr, Cascio WE, Schreiner PJ, Crow RS, Rautaharju PM, Heiss G. Prevalence of premature ventricular contractions in a population of African American and white men and women: The Atherosclerosis Risk In Communities (ARIC) study. Am Heart J. 2002;143(3):535-40.
14. Wang K, Hodges M. The premature ventricular complex as a diagnostic aid. Ann Intern Med. 1992;117(9):766-70.
15. Shiraishi H, Ishibashi K, Urao N, Tsukamoto M, Hyogo M, Keira N, et al. A case of cardiomyopathy induced by premature ventricular complexes. Circ J. 2002;66(11):1065-7.
16. Chugh SS, Shen WK, Luria DM, Smith HC. First evidence of premature ventricular complex-induced cardiomyopathy: a potentially reversible cause of heart failure. J Cardiovasc Electrophysiol. 2000;11(3):328-9.
17. Myerburg RJ, Kessler KM, Castellanos A. Sudden cardiac death: epidemiology, transient risk, and intervention assessment. Ann Intern Med. 1993;119(12):1187-97.
18. Tilz RR, Lin T, Makimoto H, Ouyang F. Successful epicardial ablation of electrical storms due to recurrent ventricular fibrillation triggered by premature ventricular contractions. Heart Rhythm. 2014;11(1):146-9.
19. Haïssaguerre M, Shah DC, Jaïs P, Shoda M, Kautzner J, Arentz T, et al. Role of Purkinje conducting system in triggering of idiopathic ventricular fibrillation. Lancet. 2002;359(9307):677-8.
20. Tsuchiya T, Nakagawa S, Yanagita Y, Fukunaga T. Transition from purkinje fiber-related rapid polymorphic ventricular tachycardia to sustained monomorphic ventricular tachycardia in a patient with a structurally normal heart: a case report. J Cardiovasc Electrophysiol. 2007;18(1):102-5.
21. Nikolic G, Bishop RL, Singh JB. Sudden death recorded during Holter monitoring. Circulation. 1982;66(1):218-25.
22. Savelieva I, Wichterle D, Camm JA. QT-interval turbulence induced by atrial and ventricular extrastimuli in patients with ventricular tachycardia. Pacing Clin Electrophysiol. 2005 Jan;28 Suppl 1:S187-92.
23. Adams JC, Srivathsan K, Shen WK. Advances in management of premature ventricular contractions. J Interv Card Electrophysiol. 2012;35(2):137-49.
24. Lee GK, Klarich KW, Grogan M, Cha YM. Premature ventricular contraction-induced cardiomyopathy: a treatable condition. Circ Arrhythm Electrophysiol. 2012;5(1):229-36.
25. Armaganijan LV, Staico R, Moreira DA, Lopes RD, Medeiros PT, Habib R, et al. 6-month outcomes in patients with implantable cardioverter-defibrillators undergoing renal sympathetic denervation for the treatment of refractory ventricular arrhythmias. JACC Cardiovasc Interv. 2015;8(7):984-90.
26. DeBacker G, Jacobs D, Prineas R, Crow R, Vilandre J, Kennedy H, et al. Ventricular premature contractions: a randomized non-drug intervention trial in normal men. Circulation. 1979;59(4):762-9.
27. Dobmeyer DJ, Stine RA, Leier CV, Greenberg R, Schaal SF. The arrhythmogenic effects of caffeine in human beings. N Engl J Med. 1983;308(14):814-6.
28. Sarı İ, Zengin S, Özer O, Davutoğlu V, Yıldırım C, Aksoy M. Chronic carbon monoxide exposure increases electrocardiographic P-wave and QT dispersion. Inhal Toxico. 2008;20(9):879-84.
29. Shimizu H, Ohnishi Y, Inoue T, Yokoyama M. QT and JT dispersion in patients with monomorphic or polymorphic ventricular tachycardia/ ventricular fibrillation. J Electrocardiol. 2001;34(2):119-25.
30. Soylu K, İnci S, Aksan G, Nar G, Yüksel EP, Ocal HS, et al. Evaluation of inhomogeneities of repolarization in patients with psoriasis vulgaris. Arch Med Sci. 2016;12(6):1225-31.
31. Kaplan O, Kurtoglu E, Nar G, Yasar E, Gozubuyuk G, Dogan C, et al. Evaluation of Electrocardiographic T-peak to T-end Interval in Subjects with Increased Epicardial Fat Tissue Thickness. Arq Bras Cardiol. 2015;105(6):566-72.
32. Yamaguchi M, Shimizu M, Ino H, Terai H, Uchiyama K, Oe K, et al. T wave peak-to-end interval and QT dispersion in acquired long QT syndrome: a new index for arrhythmogenicity. Clin Sci. 2003;105(6):671-6.
33. Yayla Ç, Özcan F, Aras D, Turak O, Özeke Ö, Çay S, et al. Tp-e interval and Tp-e/QT ratio before and after catheter ablation in patients with premature ventricular complexes. Biomark Med. 2017;11(4):339-46.

Original Article

Cardiovascular Risk in Xavante Indigenous Population

Luana Padua Soares,[1,2] Amaury Lelis Dal Fabbro,[1] Anderson Soares Silva,[1] Daniela Saes Sartorelli,[1] Luciana Ferreira Franco,[3] Patrícia Chamadoira Kuhn,[3] Regina Santiago Moises,[3] João Paulo Botelho Vieira-Filho,[3] Laércio Joel Franco[1]

Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo,[1] São Paulo, SP - Brazil

Universidade Federal de Uberlândia,[2] Uberlândia, MG - Brazil

Escola Paulista de Medicina – Universidade Federal de São Paulo,[3] São Paulo, SP - Brazil

Abstract

Background: The prevalence of cardiovascular risk factors is little known in Brazilian indigenous populations. In the last two decades, important changes have occurred in the lifestyle and epidemiological profile of the Xavante people.

Objective: to assess the prevalence of cardiovascular risk factors in Xavante adults in São Marcos and Sangradouro/Volta Grande reserves, in the state of Mato Grosso, Brazil.

Methods: Cross-sectional study carried out with 925 Xavante people aged ≥ 20 years between 2008 and 2012. The following indicators were assessed: triglycerides (TG), total, LDL and HDL-cholesterol, Castelli index I and II, TG/HDL-cholesterol ratio, apo B / Apo A1 ratio, Framingham risk score, C-reactive protein, body mass index (BMI), waist circumference (WC), hypertriglyceridemic waist (HW), glycemia and blood pressure. Kolmogorov-Smirnov, Student's t test and Chi-square test (χ^2) were used for statistical analysis, and significance level was set at 5%.

Results: High prevalence of elevated cardiovascular risk was observed in men and women according to HDL-cholesterol (66.2% and 86.2%, respectively), TG (53.2% and 51.5%), TG/HDL-cholesterol ratio (60.0% and 49.1%), C-reactive protein (44.1% and 48.1%), BMI (81.3% and 81.7%), WC (59.1% and 96.2%), HW (38.0% and 50,6%) and glycemia (46.8% and 70.2%). Individuals aged 40 to 59 years had the highest cardiovascular risk.

Conclusions: The Xavante have a high cardiovascular risk according to several indicators evaluated. The present analysis of cardiovascular risk factors provides support for the development of preventive measures and early treatment, in attempt to minimize the impact of cardiovascular diseases on this population. (Arq Bras Cardiol. 2018; 110(6):542-550)

Keywords: Cardiovascular Diseases / epidemiology; Risk Factors; Indigenous Population; Adult; Obesity; Dyslipidemias.

Introduction

Cardiovascular diseases (CVDs) are the main cause of mortality and morbidity in Brazil and the world. Approximately one third of deaths are caused by CVDs. Besides, they constitute one of the main causes of long hospital stay and health costs in Brazil.[1,2]

Most CVDs result from unhealthy lifestyle and modifiable risk factors. Altered lipid profile, diabetes mellitus, smoking, advanced age, family history, sedentary lifestyle and weight excess are the main predisposing factors for CVDs.[1-3] CVDs start in early stages of life and progress silently until first manifestations in advanced stages. The earlier the risk factors are identified, the higher the possibility of prevention to prevent and reduce complications.[2]

Mailing Address: Luana Padua Soares •
Av. Pará, 1720 - Bloco 2U - Curso de Nutrição. Postal Code 38405-320, Umuarama, Uberlândia. MG – Brazil
E-mail: luanapaduasoares@hotmail.com, luanaps@ufu.br
Manuscript received April 26, 2017, revised mansucript September 18, 2017, accepted December 19, 2017

DOI: 10.5935/abc.20180090

The prevalence of cardiovascular risk factors is still poorly investigated in indigenous populations in Brazil. In the last decades, considerable changes in eating habits and physical activity level have occurred in Xavante people, contributing to increased prevalence of non-communicable chronic diseases in this population.[4,5] However, despite significant literature on health conditions, there are no studies on cardiovascular risk in this indigenous group.

Considering that CVDs increase the risk of premature deaths, disabilities and decreased quality of life, and exert an economic impact for families, communities and society, determining the prevalence of cardiovascular risk factors would be valuable for the establishment of prevention strategies.[2]

The aim of this study was to evaluate the prevalence of cardiovascular risk factors in Xavante adults from São Marcos and Sangradouro/Volta Grande indigenous reserves in Mato Grosso state, Brazil.

Methods

This was a cross-sectional study of Xavante adults living in São Marcos and Sangradouro/Volta Grande indigenous reserves in Mato Grosso State, Brazil.

The study was approved by the Research Ethics Committee of Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto - USP, Escola Paulista de Medicina - UNIFESP, CONEP and FUNAI.

Xavante communities live in eight indigenous reserves located in Mato Grosso State, Brazil. The study was conducted by periodic visits made to these communities from October 2008 to January 2012. Total population of indigenous in these reserves is estimated to be 4,020 people, 1,582 of them aged 20 years or older.[6] All subjects aged 20 years or older were invited to participate in the study.

Physical examination, including anthropometry, and collection of blood samples were performed in the villages. After being informed about the study objectives, the tribe chiefs and participants gave their consent, mostly written. To illiterate participants (14%), the consent forms were read by community health agents, and fingerprints were used to confirm their agreement to participate in the study.

The following variables were assessed: sex, age, weight (Kg), height (m), waist circumference (WC) (cm), triglyceride levels (TG) (mg/dL), total cholesterol (TC) (mg/dL), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), apolipoproteins A1 and B (apo 1 and apo B) (mg/dL), capillary blood glucose levels at baseline and at 2 hours (mg/dL), systolic and diastolic arterial pressure (mm/Hg), high-sensitivity C-reactive protein (hs-CRP) (mg/dL).

Body weight was measured using an electronic scale (Plenna®), with maximum capacity of 150 Kg, and height was measured using a portable stadiometer (Alturexata®). Weight and height values were used for body mass index (BMI) calculation (weight (kg)/height(m)^2].[7] WC was measured using an inelastic measuring tape at the midpoint between the lowest rib and iliac crest, at standing position.

Venous blood was collected after an 8-10 hour fast, using sterile disposable tubes (Vacutainer ®). Samples were stored at -20°C and transported to a laboratory in Sao Paulo, Brazil. Measurements of serum TG, TC, LDL-c, HDL-c, apo A1 and apo B were determined by enzymatic methods, and hs-CRP levels were determined by immunoturbidimetry.

Blood pressure (BP) was measured on the left arm in the sitting position after 5 minutes at rest, using an automatic digital monitor (OMRON HEM-742INTC®). Measurements were taken three times, and the mean of the last two measurements was considered for analysis.

Capillary blood glucose at baseline and two hours after a 75 g anhydrous glucose overload (Glutol®) were measured using a portable glucose meter (HemoCue® Glucose 201, HemoCue AB).

Castelli index I (TC/HD/l-c ratio) and II (LDL-c/HDL-c ratio)[8], TG/HDL-c,[9] ApoB/ApoA1[10] and Framingham risk score[11] were calculated. Hypertriglyceridemic waist (HW) was defined as the simultaneous presence of increased WC and increased TG levels.[12]

Indicators of cardiovascular risk used in the study are described in Chart 1.[7-17]

Statistical analysis

The *Kolmogorov-Smirnov* test was used to test normality of variable distributions. Continuous variables were described as mean and standard deviation, and Student's t-test was used to compare the variable means between men and women. Categorical variables were expressed as absolute and relative frequencies, and the chi-square test (χ^2) was used for comparison of proportions. Analyses were formed using the *Statistical Package for Social Sciences* (SPSS) software version 17, and significance level was set at 5%.

Results

Study population was composed of 925 Xavante people, 455 (49.2%) men and 470 (50.8%) women. Most (57.0%) of them were aged between 20 and 39 years.

Cardiovascular risk indicators are presented as mean and standard deviations in Table 1. Mean apo A1, WC, BMI and glucose levels were higher in women than men, whereas mean Castelli index I and II, Framingham score, Apo B/Apo A-I ratio and systolic and diastolic BP were higher in men than in women.

We found a high prevalence of elevated cardiovascular risk according to HDL-c, TG, TG/HDL-c ratio, CRP-hs, BMI, WC, HW and glucose levels, although a small number of participants had increased levels of TC or LDL-c. In general, participants aged between 40 and 59 years were the most exposed to cardiovascular risk factors (Tables 2 and 3).

Discussion

Our findings show that Xavante people have an increased risk for CVDs according to HDL-c, TG, TG/HDL-c ratio, CRP-hs, BMI, WC, HW and glucose levels. Based on this, the prevalence of these diseases and consequently the risk of death, disabilities, and reduced quality of life may increase in this population in the next years.

Although several methods and indicators may be used to estimate cardiovascular risk, none of them can predict cardiovascular risk alone, and hence, should be evaluated together.

One of the cardiovascular risk factors evaluated in our study was lipid profile. The risk for atherosclerotic disease is associated with increased TC and LDL-c levels and low HDL-c levels.[13] With respect to TG, however, there is no consensus on whether they are a direct cause of atherosclerosis or a marker of other high-risk conditions.[18] Only a small percentage of Xavante people had increased TC and LDL-c levels. Nevertheless, similarly to other indigenous populations,[19,20] the Xavantes showed a high prevalence of increased TG and decreased HDL-c levels.

Castelli index I (CT/HDL-c) and II (LDL-c/HDL-c) and the TG/HDL-c ratio have been used to assess the combined influence of cardiovascular risk factors.[8,9] We did not find an increased cardiovascular risk according to these indexes in the study population; however, values of TG/HDL-c ratio in

Chart 1 – Cardiovascular risk indicators

Indicators	RISK
Total cholesterol (mg/dl)[13]	≥ 200 mg/dl
HDL-cholesterol (mg/dl)[13]	< 50 mg/dl in women and < 40 mg/dl in men
LDL-cholesterol (mg/dl)[13]	≥ 130 mg/dl
Triglycerides (mg/dl)[13]	≥ 150 mg/dl
Castelli index I[8]	> 4.4 for women and > 5.1 for men
Castelli index II[8]	> 2.9 for women and > 3,3 for men
TG/HDL-C ratio[9]	≥ 3.8
ApoB/ApoA1 ratio[10]	> 0.8 for women and > 0.9 for men
Framingham risk score[11]	Low risk – probability < 10%
	Intermediate risk – probability between 10% and 20%
	High risk – probability > 20%
Hs-CRP (mg/L)[14]	Low risk - < 1.0 mg/L
	Intermediate risk – 1.0 – 3.0 mg/L
	High risk - >3.0 mg/L
BMI (kg/m^2)[7,15]	≥ 25.0 kg/m^2 for adults
	≥ 27.0 kg/m^2 for elderly subjects
Waist circumference (cm)[7]	≥ 94 cm in men and ≥ 80 cm in women
Hypertriglyceridemic waist[7,13]	Increased WC (≥ 94 cm in men and ≥ 80 cm in women) and TG ≥ 150 mg/dl
Glycemia (mg/dL)[16]	Casual glucose level ≥ 200 mg/dL and/or
	Glucose after 2 hours ≥ 140 mg/dL and/or
	using oral antidiabetic drugs or insulin
Blood pressure (mm/Hg)[17]	Systolic arterial pressure ≥ 140mmHg and/or
	Diastolic arterial pressure ≥ 90mmHg and/or
	Use of anti-hypertensive agents

WC: waist circumference; TG: triglycerides; hs-CRP: high sensitivity C-reactive protein; BMI: body mass index

49.1% of women and 60.0% of men were indicative of high cardiovascular risk, corroborating the increased levels of TG and decreased levels of HDL-c observed in the population.

Plasma apolipoproteins A1 and B and the apo B/apo A1 ratio have been described as the best predictors of cardiovascular risk as compared with lipid and lipoprotein levels or the Castelli index I and II.[21,22] Apolipoproteins are structural and functional components of lipoproteins. Apo A1 constitutes non-atherogenic lipid fractions (HDL-c), whereas apo B constitutes atherogenic ones (chylomicrons, LDL, IDL and VLDL). Thus, apo B/apo A1 ratio represents the balance between atherogenic and antiatherogenic lipoproteins.[21,22] Increased apo B and apoB/A1 and reduced apo A1 levels have been consistently associated with risk for CVDs.[22] In our study group, 12.2% of women and 9.3% of men had an apo B/apo A1 ratio indicative of cardiovascular risk. We have not found any studies evaluating these indicators in other indigenous populations.

CRP, an acute-phase protein released into blood in response to inflammatory cytokines and a biomarker of systemic inflammation, was also evaluated in the current study. Increased CRP levels have been associated with coronary disease and stroke, even in patients with normal lipid profile.[14] Approximately half of Xavante people had CRP-hs levels indicative of high cardiovascular risk. However, caution is needed in interpreting these data, as other inflammatory diseases can also increase CRP levels. Infectious and parasite diseases are common in indigenous populations, including the Xavante people, which may have influenced the results.

Framingham score is one of the algorithms used in detecting the risk for CVDs.[11] In our study, 15.2% of men and 5.7% of women have increased risk of developing CVDs in the next 10 years according to this score. Although this score has been developed for subjects aged 30 years or older, in the current study, patients aged between 20 and 29 years were also included, corresponding to 28.0% of the study population. In the "age" component of Framingham score calculation, these subjects received the rating assigned for individuals aged between 30 and 34 years (zero). No participant aged between 20 and 39 years showed increased cardiovascular risk. Despite its high predictive value, Framingham score does not consider weight excess or sedentary lifestyle, both considered important cardiovascular risks.[23]

Table 1 – Cardiovascular risk indicators (mean and standard deviation) by sex in Xavante adults in Sao Marcos and Sangradouro reserves, Brazil, 2008-2012

Variables	Mean ± SD			p-value*
	Total	Women	Men	
Age (years)	42.8 ± 19.2	42.5 ± 19.4	43.2 ± 19.0	0.586
Total cholesterol (mg/dl)	146.4 ± 43.1	146.8 ± 43.2	146.0 ± 43.0	0.757
HDL-cholesterol (mg/dl)	38.9 ± 8.0	40.6 ± 8.2	37.1 ± 7.5	< 0.001
LDL-cholesterol (mg/dl)	70.4 ± 24.6	70.0 ± 23.3	70.8 ± 26.0	0.621
Triglycerides (mg/dl)	199.1 ± 171.2	196.4 ± 180.0	202.1 ± 161.7	0.615
Castelli index I (CT/HDL-c)	3.9 ± 1.3	3.7 ± 1.3	4.0 ± 1.3	< 0.001
Castelli index II (LDL-c/HDL-c)	1.8 ± 0.7	1.8 ± 0.6	2.0 ± 0.8	< 0.001
TG/HDL-C ratio	5.4 ± 5.1	5.2 ± 5.3	5.7 ± 4.8	0.107
Framingham risk score	5.7 ± 6.5	5.1 ± 6.8	6.3 ± 6.1	0.006
Apo B (mg/dl)	72.9 ± 18.9	73.2 ± 17.8	72.5 ± 17.9	0.577
Apo A1 (mg/dl)	106.8 ± 4.7	110.1 ± 14.4	103.4 ± 14.1	< 0.001
ApoB/ApoA1 ratio	0.69 ± 0.18	0.67 ± 0.16	0.71 ± 0.18	0.001
High-sensitivity C-reactive protein	6.1 ± 11.6	6.3 ± 12.7	5.8 ± 10.3	0.543
Waist circumference (cm)	97.3 ± 10.9	98.6 ± 11.1	95.9 ± 10.4	< 0.001
Body mass index (kg/m^2)	30.3 ± 5.1	30.7 ± 5.6	29.9 ± 4.6	0.011
Baseline glucose level (mg/dL)	152.5 ± 104.9	163.7 ± 112.4	140.8 ± 95.3	0.001
Glucose level at 2 hours (mg/dL)	148.9 ± 51.8	158.6 ± 49.0	140.2 ± 52.8	< 0.001
Diastolic blood pressure (mm/Hg)	72.7 ± 10.8	71.5 ± 10.6	74.0 ± 10.9	< 0.001
Systolic blood pressure (mm/Hg)	122.3 ± 17.4	119.7 ± 18.4	125.1 ± 15.8	< 0.001

** Student's t-test; LDL: low-density lipoprotein; HDL: high-density lipoprotein.*

Studies have reported a considerable increase in the prevalence of overweight and obesity in indigenous populations.[4,5,24] Studies conducted in specific populations have shown a high proportion of overweight and obese adults, greater than 50% in some age groups.[25-27]

Obesity is an important risk factor for CVDs. It is independently associated with risk for coronary disease, atrial fibrillation and heart failure. On the other hand, obesity, particularly abdominal or visceral obesity, is associated with other factors known to increase cardiovascular risk, such as systemic arterial hypertension (SAH), diabetes mellitus, hypertriglyceridemia and low HDL-c.[23]

More recently, HW has also been used as an indicator of cardiometabolic risk. HW is defined as the simultaneous presence of increased WC and increased TG levels, and may be used in the screening of patients likely to have the atherogenic metabolic triad – fasting hyperinsulinemia; hyperapolipoprotein B; and high proportion of small, dense LDL-c. For this reason, HW has been used as a practical, viable, low-cost tool in the identification of patients with high cardiovascular risk.[12,28] The prevalence of HW found in our study group (50.6% in women and 38.0% in men) was higher than that reported in other Brazilian studies.[29,30]

Diabetic subjects have from twice to three times the risk to suffer a cardiovascular event.[31] Besides, cardiovascular and cerebrovascular diseases are important causes of death in diabetes mellitus patients, accounting for up to 80% of deaths.[32,33]

Altered glucose levels is a health problem of large magnitude in Xavante people. In the present study, 70.2% of women and 46.8% of men had diabetes and decreased glucose tolerance, indicating that they constitute a vulnerable group. This was a much higher prevalence as compared with that in the Brazilian population.[34]

SAH is also an important risk factor for CVDs.[17] The prevalence of SAH in Xavante people – 14.7% in women and 18.0% in men – was lower than mean values reported in Brazilian adult populations, ranging from 20.0%[35] to 24.1%.[36]

As compared with the Xavantes of Pimentel Barbosa reserve, there was a tendency of increase in the prevalence of SAH. In 1962, no cases of HAS was observed in this population.[37] In 2009, however, the prevalence reached 8.1% among men and 5.8% among women.[38] This may result from social, cultural, economic and environmental changes in Xavante people, that culminated in reduction of physical activity and changes in eating habits with increased consumption of packaged foods high in sugar, fat and sodium.[4,27]

This study has some limitations. Despite the large sample size, it corresponded to only 60% of the total estimated subjects aged 20 years or older in these communities, suggesting

Table 2 – Frequency of cardiovascular risk factors by age range in Xavante women in São Marcos and Sangradouro reserves, Brazil, 2008-2012

Cardiovascular risk indicators	20 – 39 years	40 – 59 years	≥ 60 years	Total	p-value*
Total cholesterol (mg/dl)					**0.039**
Normal	254 (95.5)	94 (94.9)	93 (88.6)	441 (93.8)	
Risk	12 (4.5)	5 (5.1)	12 (11.4)	29 (6.2)	
HDL-cholesterol (mg/dl)					**0.015**
Normal	38 (14.3)	6 (6.1)	21 (20.0)	65 (13.8)	
Risk	228 (85.7)	93 (93.9)	84 (80.0)	405 (86.2)	
LDL-cholesterol (mg/dl)					**0.620**
Normal	254 (99.2)	86 (98.9)	98 (98.0)	438 (98.9)	
Risk	2 (0.8)	1 (1.1)	2 (2.0)	5 (1.1)	
Triglycerides (mg/dl)					**< 0.001**
Normal	161 (60.5)	31 (31.3)	36 (34.3)	228 (48.5)	
Risk	105 (38.5)	68 (68.7)	69 (65.7)	242 (51.5)	
Castelli index I					**0.054**
Normal	230 (86.5)	81 (81.8)	80 (76.2)	391 (83.2)	
Risk	36 (13.5)	18 (18.2)	25 (23.8)	79 (16.8)	
Castelli index II					**0.571**
Normal	247 (96.5)	82 (94.3)	97 (97.0)	426 (96.2)	
Risk	9 (3.5)	5 (5.7)	3 (3.0)	17 (3.8)	
TG/HDL-C ratio					**< 0.001**
Normal	160 (60.2)	35 (35.4)	44 (41.9)	239 (50.9)	
Risk	106 (39.8)	64 (64.6)	61 (58.1)	231 (49.1)	
ApoB/ApoA1 ratio					**0.018**
Normal	242 (91.3)	85 (85.7)	85 (81.0)	411 (87.8)	
Risk	23 (8.7)	14 (14.3)	20 (19.0)	57 (12.2)	
Framingham					**< 0.001**
Low risk	266 (100.0)	85 (85.9)	29 (27.6)	380 (80.9)	
Intermediate risk	0 (0.0)	12 (12.1)	51 (48.6)	63 (13.4)	
High risk	0 (0.0)	2 (2.0)	25 (23.8)	27 (5.7)	
CRP (mg/L)					**0.650**
Low risk	40 (15.0)	11 (11.1)	17 (16.2)	68 (14.5)	
Intermediate risk	102 (38.3)	34 (34.3)	40 (38.1)	176 (37.4)	
High risk	124 (46.6)	54 (54.5)	48 (45.7)	226 (48.1)	
BMI (kg/m^2)					**< 0.001**
Normal	25 (9.4)	7 (7.1)	54 (51.4)	86 (18.3)	
Risk	241 (90.6)	92 (92.9)	51 (48.6)	384 (81.7)	
Waist circumference (cm)					**0.071**
Normal	12 (4.5)	0 (0.0)	6 (5.7)	18 (3.8)	
Risk	254 (95.5)	99 (100.0)	99 (94.3)	452 (96.2)	
Hypertriglyceridemic waist					**<0.001**
Normal	162 (60.9)	31 (31.3)	39 (37.1)	232 (49.4)	
Risk	104 (39.1)	68 (68.7)	66 (62.9)	238 (50.6)	
Glycemia (mg/dL)					**< 0.001**
Low risk	104 (39.1)	15 (15.2)	21 (20.0)	140 (29.8)	
High risk	162 (61.9)	84 (84.8)	84 (80.0)	330 (70.2)	
Blood pressure (mm/Hg)					**< 0.001**
Low risk	254 (95.5)	76 (76.8)	71 (67.6)	401 (85.3)	
High risk	12 (4.5)	23 (23.2)	34 (32.4)	69 (14.7)	

** Chi square test (χ^2); TG: triglycerides; BMI: body mass index LDL: low-density lipoprotein; HDL: high-density lipoprotein.*

Table 3 – Frequency of cardiovascular risk factors by age range in Xavante men in São Marcos and Sangradouro reserves, Brazil, 2008-2012

Cardiovascular risk indicators	20 – 39 years	40 – 59 years	≥ 60 years	Total	p-value*
Total cholesterol (mg/dl)					**0.871**
Normal	238 (91.2)	100 (90.9)	78 (92.9)	416 (91.4)	
Risk	23 (8.8)	10 (9.1)	6 (7.1)	39 (8.6)	
HDL-cholesterol (mg/dl)					**0.035**
Normal	78 (29.9)	38 (34.5)	38 (45.2)	154 (33.8)	
Risk	183 (70.1)	72 (65.5)	46 (54.8)	301 (66.2)	
LDL-cholesterol (mg/dl)					**0.448**
Normal	242 (98.8)	93 (96.9)	77 (98.7)	412 (98.3)	
Risk	3 (1.2)	3 (3.1)	1 (1.3)	7 (1.7)	
Triglycerides (mg/dl)					**0.003**
Normal	120 (46.0)	41 (37.3)	52 (61.9)	213 (46.8)	
Risk	141 (54.0)	69 (62.7)	32 (38.1)	242 (53.2)	
Castelli index I					**0.128**
Normal	225 (86.2)	94 (85.5)	79 (94.)	398 (87.5)	
Risk	36 (13.8)	16 (14.5)	5 (6.0)	57 (12.5)	
Castelli index II					**0.033**
Normal	227 (92.7)	94 (97.9)	77 (98.7)	398 (95.0)	
Risk	18 (7.3)	2 (2.1)	1 (1.3)	21 (5.0)	
TG/HDL-C ratio					**< 0.001**
Normal	98 (37.5)	35 (31.8)	49 (58.3)	182 (40.0)	
Risk	163 (62.5)	75 (68.2)	35 (41.7)	274 (60.0)	
ApoB/ApoA1 ratio					**0.128**
Normal	229 (88.4)	102 (92.7)	79 (95.2)	410 (90.7)	
Risk	30 (11.6)	8 (7.3)	4 (4.8)	42 (9.3)	
Framingham					**< 0.001**
Low risk	261 (100.0)	79 (71.8)	1 (1.2)	34 (74.9)	
Intermediate risk	0 (0.0)	24 (21.8)	21 (25.0)	45 (9.9)	
High risk	0 (0.0)	7 (6.4)	62 (73.8)	69 (15.2)	
Hs-CRP (mg/L)					**0.867**
Low risk	47 (18.0)	19 (17.3)	17 (20.5)	83 (18.3)	
Intermediate risk	102 (39.1)	42 (38.2)	27 (32.5)	171 (37.7)	
High risk	112 (42.9)	49 (44.5)	39 (47.0)	200 (44.1)	
BMI (kg/m^2)					**< 0.001**
Normal	33 (12.6)	10 (9.1)	42 (50.0)	85 (18.7)	
Risk	228 (87.4)	100 (90.9)	42 (50.0)	370 (81.3)	
Waist circumference (cm)					**< 0.001**
Normal	118 (45.2)	27 (24.5)	41 (48.8)	186 (40.9)	
Risk	143 (54.8)	83 (75.5)	43 (51.2)	269 (59.1)	
Hypertriglyceridemic waist					**0.001**
Normal	164 (62.8)	54 (49.1)	64 (76.2)	282 (62.0)	
Risk	97 (37.2)	56 (50.9)	20 (23.8)	173 (38.0)	
Glycemia (mg/dL)					**< 0.001**
Low risk	160 (61.3)	46 (41.8)	36 (42.9)	242 (53.2)	
High risk	101 (38.7)	64 (58.2)	48 (57.1)	213 (46.8)	
Blood pressure (mm/Hg)					**< 0.001**
Low risk	236 (90.4)	86 (78.2)	51 (60.7)	373 (82.0)	
High risk	25 (9.6)	24 (21.8)	33 (39.3)	82 (18.0)	

** Chi square test (χ^2); TG: triglycerides; hs-CRP: high sensitivity C-reactive protein; BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein.*

a selection bias, since healthier individuals tend to be less interested in participating in the study. In addition, some smaller, less accessible indigenous communities were not included in the study, affecting the participation rate. Limitations regarding communication between indigenous people and investigators, which may have been a source of bias, were partly prevented by participation of health professionals, members of the indigenous community in data collection. Also, due to cultural differences, we cannot assure that all volunteers were in fasting conditions on blood collection day despite instructions to do so; in addition to a more irregular eating pattern, they may have not understood the importance of such condition for laboratory tests. Thus, caution is need in interpreting TG levels and TG/HDL ratio and HW values. Another limitation was the fact that we did not evaluate smoking habit, which is a key cardiovascular risk factor, not only isolated but also as a Framingham score component. All subjects were rated as non-smokers in the score calculation, and hence the possibility that cardiovascular risk by this indicator was underestimated cannot be ruled out.

These results are significant for this population and, to our knowledge, this is the first study to evaluate cardiovascular risk using all these indicators.

Conclusions

Xavante people have high cardiovascular risk according to indicators such as HDL-c, TG/HDL-c ratio, BMI, WC, HW and glucose levels.

Considering that CVD patients are initially asymptomatic, and that CVDs are important causes of morbidity and mortality, the present analysis of cardiovascular risk factors may be used as a basis for the planning of preventive measures and early treatment to minimize the impact of these diseases on this population.

Author contributions

Conception and design of the research: Soares LP, Moises RS, Vieira-Filho JPB, Franco LJ; Acquisition of data, Analysis and interpretation of the data, Statistical analysis and Critical revision of the manuscript for intellectual content: Soares LP, Dal Fabbro AL, Silva AS, Sartorelli DS, Franco LF, Kuhn PC, Moises RS, Vieira-Filho JPB, Franco LJ; Obtaining financing: Franco LJ; Writing of the manuscript: Soares LP, Franco LJ.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Proc. 476347/2007-6) and Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Proc. 2010/05634-0).

Study Association

This article is part of the thesis of Doctoral submitted by Luana Padua Soares, from Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo.

Ethics approval and consent to participate

This study was approved by the Comissão Nacional de Ética em Pesquisa (CONEP) under the protocol number 598/2008 (CONEP 14914 / Process no 25000.103891/2008-41). 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.

References

1. Simão AF, Precoma DB, Andrade JP, Correa Fº H, Saraiva JF, Oliveira GM, et al; Sociedade Brasileira de Cardiologia. [I Brazilian Guidelines for cardiovascular prevention]. Arq Bras Cardiol. 2013;101(6 Suppl 2):1-63.
2. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Análise de Situação de Saúde. Plano de ações estratégicas para o enfrentamento das doenças crônicas não transmissíveis (DCNT) no Brasil 2011-2022. Brasília; 2011.
3. Grundy SM, Balady GJ, Criqui MH, Fletcher G, Greenland P, Hiratzka LF, et al. Primary prevention of coronary heart disease: guidance from Framingham: a statement for healthcare professionals from the AHA task force on risk reduction. Circulation. 1998;97(18):1876-87.
4. Coimbra Jr CE, Flowers NM, Salzano FM, Santos RV. The Xavante in Transition: Health, Ecology and Bioanthropology in Central Brazil. Ann Arbor: University of Michigan Press; 2002.
5. Santos RV, Cardoso AM, Garnelo L, Coimbra Jr CE, Chaves MB. Saúde dos povos indígenas e políticas públicas no Brasil. In: Escorel S, Lobato LV, Noronha JC, Carvalho AI. (Org.). Políticas e Sistema de Saúde no Brasil. Rio de Janeiro: Editora Fiocruz; 2008. p. 1035-56.
6. Brasil, Instituto Brasileiro de Geografia e Estatística. (IBGE). Censo Demográfico 2010 – Características gerais dos indígenas. Rio de Janeiro; 2010. p.1- 245.
7. World Health Organization. (WHO). Obesity: preventing and managing the global epidemic. Report of a World Health Organization Consultation. Geneva; 2000. p. 252. (WHO Obesity Technical Report Series, n. 284).
8. Castelli WP, Abbott RD, McNamara PM. Summary estimates of cholesterol used to predict coronary heart disease. Circulation. 1983;67(4):730-4.
9. Hanak V, Munoz J, Teague J, Stanley A Jr, Bittner V. Accuracy of the triglyceride to high-density lipoprotein cholesterol ratio for prediction of the low-density lipoprotein phenotype B. Am J Cardiol. 2004;94(2):219-22.
10. Walldius G, Jungner I. Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. J Intern Med. 2004;255(2):188-205.
11. D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008;117(6):743-53.
12. Lemieux I, Pascot A, Couillard C, Lamarche B, Tchernof A, Almeras N, et al. Hypertriglyceridemic waist: a marker of the atherogenic metabolic triad (Hyperinsulinemia; hyperapolipoprotein B; small, dense LDL) in men? Circulation. 2000;102(2):179-84.

13. Xavier HT, Izar MC, Faria Neto JR, Assad MH, Rocha VZ, Sposito AC, et al; Sociedade Brasileira de Cardiologia. [V Brazilian Guidelines on Dyslipidemias and Prevention of Atherosclerosis]. Arq Bras Cardiol. 2013;101(4 Suppl 1):1-20.

14. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, et al. Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003;107(3):499-511.

15. Lipschitz DA. Screening for nutritional status in the elderly. Prim Care. 1994;21(1):55-67.

16. World Health Organization. (WHO). Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. part 1: diagnosis and classification of diabetes mellitus. Geneva; 1999.

17. Sociedade Brasileira de Cardiologia, Sociedade Brasileira de Hipertensão, Sociedade Brasileira de Nefrologia. VI Diretrizes Brasileiras de hipertensão. Arq Bras Cardiol. 2010;95(1 supl. 1):1-51.

18. Oberman A, Kreisberg RA. Hypertrigliceridemia and coronary heart disease. J Clin Endocrinol Metab. 2000;85(6):2089-112.

19. Rocha AK, Bós AJ, Huttner E, Machado DC. Prevalence of metabolic syndrome in indigenous people over 40 years of age in Rio Grande do Sul, Brazil. Rev Panam Salud Publica. 2011;29(1):41-5.

20. Santos KM, Tsutsui ML, Galvão PP, Mazzucchetti L, Rodrigues D, Gimeno SG. Degree of physical activity and metabolic syndrome: a cross-sectional study among the Khisêdjê group in the Xingu Indigenous Park, Brazil. Cad Saude Publica. 2012;*28*(12):2327-38.

21. Walldius G, Jungner I. The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy - a review of the evidence. J Intern Med. 2006;259(5):493-519.

22. Lima LM, Carvalho MG, Sousa MO. Apo B/apo A-I ratio and cardiovascular risk prediction. Arq Bras Cardiol. 2007;88(6):e187-90.

23. Zalesin KC, Franklin BA, Miller WM, Peterson ED, McCullough PA. Impact of obesity on cardiovascular disease. Endocrinol Metab Clin North Am. 2008;37(3):663-84.

24. Brasil. Ministério da Saúde – Fundação Nacional de Saúde (FUNASA). Inquérito nacional de saúde e nutrição dos povos indígenas. Relatório Final. Brasília; 2009.

25. Salvo VL, Rodrigues D, Baruzzi RG, Pagliaro H, Gimeno SG. Metabolic and anthropometric profile of Suyá. Xingu Indigenous Park, Central Brazil. Rev Bras Epidemiol. 2009;12(3):458-68.

26. Lourenço AE, Santos RV, Orellana JD, Coimbra CE Jr. Nutrition transition in Amazonia: obesity and socioeconomic change in the Suruí Indians from Brazil. Am J Hum Biol. 2008;20(5):564-71.

27. Welch JR, Ferreira AA, Santos RV, Gugelmin SA, Werneck G, Coimbra CE Jr. Nutrition transition, socioeconomic differentiation, and gender among adult Xavante Indians, Brazilian Amazon. Hum Ecol. 2009;37(1):13-26.

28. Arsenault BJ, Lemieux I, Despres JP, Wareham NJ, Kastelein JJ, Khaw KT, et al. The hypertriglyceridemic-waist phenotype and the risk of coronary artery disease: results from the EPIC-Norfolk prospective population study. CMAJ. 2010;182(13):1427-32.

29. Mendes MS, Melendez JG. Cintura hipertrigliceridêmica e sua associação com fatores de risco metabólicos [Dissertação]. Belo Horizonte: Universidade Federal de Minas Gerais; 2009.

30. Oliveira JL, Lopes LL, Pelúzio, MC, Hermsdorff HH. Hypertriglyceridemic waist phenotype and cardiometabolic risk in dyslipidemic subjects. Rev Bras Cardiol. 2014;27(6):395-402.

31. Kannel WB, McGee DL. Diabetes and cardiovascular risk factors: the Framingham Study. Circulation. 1979;59(1):8-13.

32. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care. 1993;16(2):434-44.

33. Malerbi D, Franco LJ. Multicenter study of the prevalence of diabetes mellitus and impaired glucose tolerance in the urban Brazilian population aged 30 to 69 yr. The Brazilian Cooperative Group on the Study of Diabetes Prevalence. Diabetes Care. 1992;15(11):1509-16.

34. Oliveira GF, Oliveira TR, Rodrigues FF, Corrêa LF, Ikejiri AT, Casulari LA. Prevalence of diabetes mellitus and impaired glucose tolerance in indigenous people from Aldeia Jaguapiru, Brazil. Rev Panam Salud Publica. 2011;29(5):315-21.

35. Passos VM, Assis TD, Barreto SM. Hypertension in Brazil: estimates from population-based prevalence studies. Epidemiol Serv Saúde. 2006;15(1):35-45.

36. Brasil. Ministério da Saúde. VIGITEL Brasil 2013: Vigilância de fatores de risco e proteção para doenças crônicas por inquérito telefônico. Brasília; 2014.

37. Neel JV, Salzano FM, Junqueira PC, Ketter F, Maybury-Lewis D. Studies on the Xavante Indians of the Brazilian Mato Grosso. Am J Hum Genet 1964 Mar;16:52-140.

38. Oliveira MVG. Níveis tensionais e prevalência de hipertensão entre os Xavante, Terra Indígena Pimentel Barbosa, Mato Grosso [Dissertação]. Rio de Janeiro: Escola Nacional de Saúde Pública Sergio Arouca. Fundação Oswaldo Cruz; 2011.

Behavior of Blood Pressure Variables in Children and Adolescents with Duchenne Muscular Dystrophy

Fabiane R. R. H. Marui, Henrique Tria Bianco, Maria Teresa N. Bombig, Natascha G. F. Palmeira, José M. Thalenberg, Fernando Focaccia Povoa, Maria Cristina de O. Izar, Francisco Antonio H. Fonseca, Acary S. B. de Oliveira, Rui M. S. Povoa

Universidade Federal de São Paulo, São Paulo, SP - Brazil

Abstract

Background: Duchenne muscular dystrophy is an X-chromosome-linked genetic disorder (locus Xp21). Involvement of the cardiovascular system is characterized by fibrous degeneration/replacement of myocytes with consequent ventricular hypertrophy and arterial hypertension.

Objective: To assess, by using 24-hour ambulatory blood pressure monitoring, the behavior of blood pressure variables in children and adolescents with a confirmed diagnosis of Duchenne muscular dystrophy.

Methods: Prospective observational cohort study, which selected 46 patients followed up on an outpatient basis, divided according to age groups. Blood pressure was classified according to the age percentile. The monitoring interpretation includes systolic and diastolic blood pressure means, systolic and diastolic blood pressure loads, and nocturnal dipping. The blood pressure means were calculated for the 24-hour, wakefulness and sleep periods. Nocturnal dipping was defined as a drop in blood pressure means during sleep greater than 10%. The significance level adopted was $p < 0.05$.

Results: Nocturnal dipping for systolic blood pressure was present in 29.9% of the participants. Approximately 53% of them had attenuated nocturnal dipping, and 15%, reverse nocturnal dipping. The age groups of 9-11 years and 6-8 years had the greatest percentage of attenuation, 19.1% and 14.9%, respectively. Regarding diastolic blood pressure, nocturnal dipping was identified in 53.2% of the children, being extreme in 27.7% of those in the age group of 6-11 years.

Conclusions: The early diagnosis of blood pressure changes can allow the appropriate and specific therapy, aimed at increasing the life expectancy of patients with Duchenne muscular dystrophy. (Arq Bras Cardiol. 2018; 110(6):551-557)

Keywords: Cardiovascular Diseases / genetics; Muscular Dystrophy, Duchenne / genetics; Hypertension; Child; Male.

Introduction

Duchenne muscular dystrophy (DMD) is an X-chromosome-linked genetic disorder that affects approximately 1 in every 3500 live-born boys.[1]

It is clinically characterized by progressive and irreversible muscle weakness consequent to dystrophin deficiency or absence. It is the most frequent neuromuscular disease in human beings, and, although predominating in the male sex, it is occasionally reported in females due to inactivation or abnormalities of the X chromosome. That anomaly is present in the short arm of the X chromosome (*locus Xp21*). Its global prevalence can reach 63 cases per one million individuals. Duchenne muscular dystrophy has a high spontaneous mutation velocity, and approximately one third of the cases are estimated to be due to new mutations.[2-4] The first clinical signs manifest at an early age as frequent falls, difficulty climbing stairs, running and getting up from a lying or sitting position, and mainly calf hypertrophy. The muscle impairment is symmetric, initiates in the pelvic girdle muscles (hip and legs) and reaches the upper limbs.

In addition, cardiomyopathy is frequent in DMD. While some studies have estimated its incidence in 25% at the age of 6 years, and 59% at the age of 10 years, others have reported its beginning at the age of 14 and 15 years.[5,6] Cardiac involvement occurs in 90% of the patients, being the cause of death in 50% of them. However, its clinical identification can be hindered by severe muscle weakness and thoracic deformities. Cardiac histological changes include hypertrophy of myocytes and myocardial fibrosis, with replacement with connective and fatty tissues.[7] Dystrophin deficiency or absence in cardiomyocytes hinders the function of membrane ion channels, notably in sarcolemma, which is activated by stretching, responding to mechanical stress. When cardiomyocytes, with or without dystrophin deficiency, are stretched during ventricular filling, the ion channels do not open properly, increasing calcium influx. Excessive intracellular calcium activates a group of calcium-induced proteases, the calpains, which degrade troponin I and hinder contraction.[8-10]

The importance of the cardiomyopathy in children with DMD has grown in past decades, mainly due to the increase in survival, consequent to advances in ventilatory

Mailing Address: Henrique Tria Bianco •
Setor de Lípides, Aterosclerose, Biologia Vascular e Hipertensão Arterial. Disciplina de Cardiologia da Universidade Federal de São Paulo (UNIFESP). Rua Loefgren, 1350. Postal Code 04040-001, Vila Clementino, São Paulo, SP – Brazil
E-mail: henriquetria@uol.com.br
Manuscript received July 14, 2017, revised mansucript October 24, 2017, accepted Novembe 16, 2017

DOI: 10.5935/abc.20180085

and orthopedic supports.[11] In the absence of ventilatory intervention, death usually occurs by the end of the second or beginning of the third decade. Diastolic dysfunction can be present even before systolic dysfunction is detected. The use of drugs that act on the renin-angiotensin-aldosterone axis, such as angiotensin-converting-enzyme inhibitors or angiotensin-receptor blockers, should be considered, aiming at reducing afterload before symptom onset.[12]

Ambulatory Blood Pressure Monitoring (ABPM) allows indirect and intermittent blood pressure (BP) recording for 24 hours, during wakefulness and sleep. In adults, ABPM is a well-established diagnostic and follow-up method, considered "gold standard" in BP assessment.[13] In 2008, the American Heart Association (AHA) published recommendations for the use of ABPM in the pediatric population, which were reviewed in 2014.[14,15] Many recommendations of ABPM use for adults can be applied to children. Because of the difficulty of conducting randomized clinical trials in the pediatric population, the recommendations used are based on expert opinions. However, it is worth considering some aspects, such as equipment selection, which should be light (weight between 168 and 457 grams), with appropriate cuff size.[16]

The use of corticosteroid, known to increase BP, has been widely studied. Left and/or right ventricular hypertrophy can cause arterial hypertension (AH) and/or pulmonary hypertension or mitral and/or tricuspid regurgitation, sometimes culminating in ventricular failure.[17,18] A study by Braat et al. has assessed the renal function of 20 individuals with DMD and undergoing ABPM, 9 of whom had elevated BP (over the 95th percentile), 8 of whom were on corticosteroids, and 13 had no nocturnal dipping (ND), 10 of whom were on corticosteroids.[19]

Knowing BP behavior in such patients is fundamental, mainly because it enables early treatment, contributing to improve the quality of life, aiming at reducing the high morbidity rates of those patients. Thus, our study aimed at assessing the behavior of BP variables, by using 24-hour ABPM, in children and adolescents diagnosed with DMD, followed up at a university-affiliated outpatient clinic specialized in muscular dystrophies.

Methods

This is a descriptive study comprising all 46 boys with a confirmed diagnosis of DMD followed up on an outpatient basis. Because DMD is a rare disease, we chose to assess all children and adolescents followed up on a university-affiliated outpatient clinic. The boys were divided into five age groups, considering the distribution of normal BP levels for age, according to the previously reported AHA suggestion.[14,15] The research project was approved by the local Ethics Committee, the information was provided by the parents or guardians, and written informed consent was provided by all participants. Prior to ABPM, the patients' clinical history was collected, and the height and weight measured in the children who could walk, while, for wheelchair-bound patients, historical height was used. Blood pressure was measured at the medical office with the OMROM digital device (HEM 742INT® model) and appropriate cuff size, respecting the proportion width/length 1:2, corresponding to 40% of arm circumference and at least 80% of its length. The Spacelabs 90207® ABPM monitor was installed on the "nondominant" arm, with appropriate cuff size, by a trained nurse, and programmed to take BP every 15 minutes during wakefulness and every 30 minutes during sleep. The parents/guardians received a diary to record the most important events in 24 hours, mainly the bedtime and wake-up time.

The following variables were assessed in ABPM interpretation: systolic and diastolic BP (SBP and DBP, respectively) means; systolic and diastolic blood pressure loads (SBPL and DBPL, respectively); and ND. The SBP and DBP means were calculated for the 24-hour, wakefulness and sleep periods. The SBPL and DBPL were calculated considering the proportion of readings over the 95th percentile. Nocturnal dipping was defined as a drop greater than 10% in BP means during sleep. All parameters were compared to the normal range values to determine whether BP was normal or elevated, as was the presence or absence of ND. In addition, ND was stratified as follows: "present", BP drop during sleep between 10% and 20% as compared to wakefulness; "absent", no BP drop during sleep; "attenuated", BP drop > 0% and < 10% during sleep; "reverse", BP during sleep higher than that during wakefulness; and "extreme", BP drop > 20%.

Statistical analysis

The continuous variables with normal distribution were presented as means ± standard deviation, while those without normal distribution were presented as medians and interquartile range. The categorical variables were presented as absolute numbers and percentages. The significance level adopted in the statistical analyses was 0.05. Kolmogorov-Smirnov test was used to assess the normality of the variables, and Pearson chi-square test was used to assess the association between corticosteroid use and BP classification.

We used as comparator the BP values of the 95th percentile from the AHA recommendations, as shown in Table 1, which classified children and adolescents according to age groups. Values of $p < 0.05$ were considered significant. All statistical analyses were performed with the SPSS software, version 17.0 (SPSS Inc. Chicago, IL, USA)®.

Results

Table 2 shows the major characteristics of the participants with DMD. Of the 46 children, 57.4% were wheelchair-bound, 69.6% were on corticosteroids, and 6.4% had been previously diagnosed with AH, as reported by their parents/guardians. The diagnosis of DMD was established at around 7 years of age, while the first symptoms appeared approximately at the age of 2.7 years. Other family members were reported to have DMD in 6.4% of the cases. A significant part (63.8%) of the participants was undergoing specific physical therapy, and 40.5% used some type of respiratory support, such as artificial manual breathing unit (AMBU) and/or bilevel positive airway pressure (bipap).

Table 3 shows BP behavior, distribution of the SBP and DBP means with their respective standard deviations in the five age groups, as well as SBPL and DBPL. Regarding SBP,

Table 1 – Classification of blood pressure (BP) levels in children

Classification	Office BP	Mean SBP and DBP on ABPM	Blood pressure loads (SBP and DBP)
Normal BP	< 90th percentile	< 95th percentile	< 25%
White coat hypertension	≥ 95th percentile	< 95th percentile	< 25%
Prehypertension	≥ 90th percentile or > 120/80 mm Hg	< 95th percentile	≥ 25%
Masked hypertension	< 95th percentile	> 95th percentile	≥ 25%
Ambulatory hypertension	> 95th percentile	> 95th percentile	25-50%
Severe ambulatory hypertension	> 95th percentile	> 95th percentile	> 50%

Adapted from: A Scientific Statement From the American Heart Association. Hypertension.[15] SPB: systolic blood pressure; DBP: diastolic blood pressure; ABPM: ambulatory blood pressure monitoring.

Table 2 – Baseline characteristics of the patients

Age groups	
3 - 5 years, n (%)	3 (6.5)
6 - 8 years, n (%)	15 (32.6)
9 - 11 years, n (%)	18 (39.1)
12 - 14 years, n (%)	7 (15.2)
15 - 17 years, n (%)	3 (6.5)
Clinical characteristics	
Previous diagnosis of SAH,* n (%)	3 (6.5)
Other family members with DMD†	3 (6.5)
Use of bipap‡	6 (13)
Use of AMBU	12 (26)
Wheelchair-bound	26 (56.5)
Motor physical therapy	29 (63)
Use of corticosteroid	32 (69.6)
Age of the first symptoms of DMD, md (IQR)	7 (5-8)
Age of diagnosis of DMD, md (IQR)	2.5 (1.2-4.5)

SAH: systemic arterial hypertension; DMD†: Duchenne muscular dystrophy; bipap‡: Bilevel Positive Airway Pressure. Data expressed as numbers (n) and percentages (%); age in median (md) and interquartile range (IQR).*

ND was present in 29.9% of the children with DMD. More than half of the participants (53.1%) had attenuated ND, while 15% had reverse ND. The age groups of 9-11 years and 6-8 years concentrated the highest percentage of ND attenuation, 19.1% and 14.9% respectively. Regarding DBP, ND was present in 53.2% of the boys; 27.7% had extreme ND (highest percentage in the age group of 6-11 years: 19.1%), while 14.9% had attenuated ND.

For BP stratification, the BP measurements taken at the office and on ABPM were considered. Although the recommendations of the specialized guidelines suggest classifying BP in one of the ABPM periods (wakefulness or sleep) or in 24 hours, we classified BP in all periods (Table 4). Regarding the use of corticosteroids, there was no association between corticosteroid use and BP classification in 24 hours ($p = 0.904$), during wakefulness ($p = 0.720$) and sleep ($p = 0.996$).

Discussion

Duchenne muscular dystrophy is a disease of poor prognosis, whose survival extends to the second decade of life.[20] However, the advances in treatment, such as non-invasive ventilation and physical therapy, have enabled boys with DMD to reach 30 years of age. Considering this increase in life expectancy, other aspects, in addition to neuromuscular impairment, need to be assessed.[20]

Although office BP classification in pediatrics was standardized by the National High Blood Pressure Education Program in 2004, the classification of BP obtained from ABPM in children and adolescents has not been standardized. Thus, we used the recommendations based on expert opinions, such as those published by the AHA in 2008. Blood pressure classification in children, according to those recommendations, should consider, in addition to measurements taken at the office, those from ABPM during 24 hours, wakefulness or sleep, and SBPL or DBPL.[21,22]

A study on the BP of boys with DMD has reported the prevalence and correlations of low BP levels measured at the office with a possible autonomic dysfunction due to DMD.[23] Regarding office BP measurement, more than 50% of the boys had stage 1 or 2 AH, and 12.8% had borderline BP levels. The VII Brazilian Guidelines on Hypertension replaced the term 'borderline' with 'prehypertension', and estimates its prevalence between 10% and 15% of the pediatric population. However, established AH affects 3% to 5% of the children.[24] In our study, the highest SBP mean during wakefulness was 126.7 ± 10.0 in the age group of 12-14 years, followed by 122 ± 18.6 for office BP in children aged 6-8 years. Considering the percentile for age, the BP mean on ABPM during wakefulness was within the expected range. However, the BP mean at the office was above the 95th percentile. Regarding DBP, the highest mean was observed in the 95th percentile (78.5 ± 12.4) in the age group of 6-8 years on ABPM during wakefulness, followed by 77.3 ± 9.1 in the age group of 15-17 years on ABPM, which is above the estimated percentile. Regarding SBPL and DBPL, all age groups had median > 25% in 24 hours, wakefulness or sleep. Considering only wakefulness, 38.3% of the children had normal BP, 21.3% had severe AH, and 21.3% had white coat hypertension.

Table 3 – Distribution of the blood pressure variables (office and ABPM) according to age groups

Age groups (years)	3-5 years n = 3	6-8 years n = 15	9-11 years n = 18	12-14 years n = 7	15-17 years n = 3
Office SBP* (mm Hg)	117.3 ± 17.0	118.3 ± 14.6	118.2 ± 22.2	119.8 ± 18.2	123.3 ± 12.6
Office DBP† (mm Hg)	69.3 ± 9.5	73.2 ± 8.4	76.4 ± 16.6	71.4 ± 9.1	74.3 ± 4.5
ABPM: 24h SBP (mm Hg)	122.6 ± 20.0	117.7 ± 15.6	119.3 ± 19.6	117.4 ± 8.3	114.3 ± 8.5
ABPM: 24h DBP (mm Hg)	73.3 ± 10.1	71.2 ± 15.6	71.2 ± 13.3	71.7 ± 15.1	69.3 ± 5.9
ABPM: wakefulness SBP (mm Hg)	125.0 ± 19.5	121.0 ± 13.7	121.3 ± 19.8	120.1 ± 8.3	117.3 ± 6.5
ABPM: wakefulness DBP (mm Hg)	76.7 ± 13.0	75.5 ± 8.7	71.4 ± 13.6	74.8 ± 8.3	73 ± 4.4
ABPM: sleep SBP (mm Hg)	119 ± 20.1	111.7 ± 18.1	114.2 ± 19.8	110.4 ± 10.8	109 ± 8.9
ABPM: sleep DBP (mm Hg)	66.7 ± 10.7	61.2 ± 18.7	66.2 ± 13.8	62.9 ± 6.7	59.7 ± 8.1
24h SBPL‡ (> 50%), n (%)	2 (66.6)	5 (33.3)	7 (38.9)	0 (0)	0 (0)
24h DBPL§ (> 50%), n (%)	1 (33.3)	7 (46.7)	7 (38.9)	3 (21.4)	0 (0)
Wakefulness SBPL (> 50%), n (%)	2 (66.6)	5 (33.3)	6 (33.3)	0 (0)	0 (0)
Wakefulness DBPL (> 50%), n (%)	1 (33.3)	5 (33.3)	5 (27.8)	1 (14.3)	0 (0)
Sleep SBPL (> 50%), n (%)	2 (66.6)	7 (46.7)	4 (22.2)	0 (0)	0 (0)
Sleep DBPL (> 50%), n (%)	1 (33.3)	2 (13.3)	3 (16.7)	1 (14.3)	0 (0)

SBP: systolic blood pressure; DBP†: diastolic blood pressure; SBPL‡: systolic blood pressure load; DBPL§: diastolic blood pressure load. Data presented as numbers (n) and percentages; and mean ± standard deviation.*

Table 4 – Distribution of the participants with Duchenne muscular dystrophy according to blood pressure classification on ABPM during 24hours, wakefulness and sleep

Classification	24h ABPM n (%)		Wakefulness ABPM n (%)		Sleep ABPM n (%)	
Normal	13	28.3	17	36.9	16	34.8
Normal with BPL* > 25%	2	4.3	1	2.2	1	2.2
Prehypertension	6	13.0	4	8.7	7	15.2
Prehypertension without increased BPL	4	8.7	0	0	0	0
White coat SAH†	9	19.6	10	21.7	6	13
Masked hypertension	1	2.2	2	4.3	3	6.5
Masked hypertension with SBPL‡ > 50%	1	2.2	1	2.2	2	4.3
Severe SAH	11	23.9	10	21.8	8	17.4
High wakefulness or sleep SBP§ without increased BPL	0	0	1	2.2	0	0
SAH only on ABPM	0	0	0	0	2	4.3
SAH with SBPL < 25%	0	0	0	0	1	2.2
Total	46	100	46	100	46	100

BPL: blood pressure load; SAH†: systemic arterial hypertension; SBPL‡: systolic blood pressure load; SBP§: systolic blood pressure. Data presented as numbers (n) and percentages.*

The median ND was lower than 10% for SBP and higher than 10% for DBP in all age groups. It is worth noting that 68% of the boys had no 10% ND for SBP. In adults, the absence of ND is considered a risk factor for target-organ damage, in addition to increasing the cardiovascular risk of hypertensive and normotensive individuals. Although the use of corticosteroid can lead to weight gain and BP elevation, it is the only drug that can delay the progression of muscle weakness, reduce the development of scoliosis, and delay respiratory failure. Its mechanism is based on the hypothesis that its anti-inflammatory property and immunosuppressive action promote the proliferation of myoblasts and a reduction in necrosis.[25] Our study showed no association of corticosteroid use and AH, blood pressure load elevation and ND. Although a significant part of the boys was on prednisone, its administration was intermittent and on the first days of the month.

However, if corticosteroid did not influence BP behavior, what was the factor responsible for the elevated number of hypertensives and BP measurements out of the normal range

among those children? The mdx mouse develops X-linked recessive muscular dystrophy (*locus Xp21*) and does not express dystrophin. Although it does not show intense fibrosis and fatty tissue accumulation in muscles, it is considered the most appropriate animal model of DMD. A mechanistic study with those mice has shown that the absence of dystrophin interferes with nitric oxide (NO)-dependent vascular dilatation. When submitted to pressure variations, the vessel showed no adaptation. Because the endothelium is essential for the arteries to adapt to chronic changes in blood flow, in the long run that deficiency might affect the flow-induced vascular remodeling, with consequence to vascular resistence.[26] Other studies have shown an abnormal sympathetic neurovascular control in dystrophin-deficient muscle, which is evidenced during physical exercise, when sympathetic vasoconstriction is normally absent in active muscles, due to the action of local vasodilating substances, such as NO.[27,28] The neuronal deficiency of NO sintase, which is reduced in the absence of dystrophin, seems to be the major cause of vasoregulation deficiencies. However, the vascular tone modulation can also be hindered by dystrophin deficiency in arterial smooth muscle cells. Dystrophin is usually expressed in the tunica media of blood vessels, being absent in the vessels of mdx mice.[29,30]

Considering those findings, we might explain the high number of individuals with DMD and BP changes in our study. Another aspect that can be related to endothelial change is the absence or attenuation of ND in those children. Duchenne muscular dystrophy is usually accompanied by changes in the respiratory pattern during sleep, such as obstructive sleep apnea (OSA), causing deleterious effects to the cardiovascular system. In addition, excessive sympathetic activity occurs in OSA, which can contribute to the lack of decrease in BP levels during the night.

Study limitations

Because DMD is a rare disease, our study can be considered a potential generator of hypotheses. It is worth noting the lack of standardization for ABPM use for children. Thus, we followed the AHA recommendation, which was based on expert opinions. However, such data need to be validated in further studies, with a greater sample power. In addition, it is worth noting the difficulties in measuring the height of the children, because many of them were wheelchair-bound. Thus, we used the historical height, reported by the parents or legal guardians.

Another limitation of our study was the patients' stratification based on age groups, by use of convenience sampling, because of the low incidence of DMD. Because of the difficulty of conducting randomized clinical trials in the pediatric population, the recommendations used were based on expert opinions.

Conclusion

The analysis of BP variables, obtained mainly from ABPM, is a useful tool to identify patients with DMD at higher risk. Considering the cardiovascular changes of those patients, the early identification of BP changes would allow the appropriate and specific therapeutic intervention. In addition, we suggest the regular and multidisciplinary follow-up of those patients to identify their BP changes, ensuring improvement in their life expectancy and comfort.

Author contributions

Conception and design of the research: Marui FRRH, Povoa RMS; Acquisition of data: Marui FRRH, Thalenberg JM; Analysis and interpretation of the data: Marui FRRH, Bianco HT, Oliveira ASB, Povoa RMS; Statistical analysis: Marui FRRH, Bianco HT, Palmeira NGF, Povoa RMS; Obtaining financing: Marui FRRH, Povoa RMS; Writing of the manuscript: Marui FRRH, Bombig MTN, Povoa FF, Izar MCO, Fonseca FAH, Povoa RMS; Critical revision of the manuscript for intellectual content: Bianco HT, Bombig MTN, Palmeira NGF, Thalenberg JM, Povoa FF, Izar MCO, Fonseca FAH, Povoa RMS.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by CNPq.

Study Association

This article is part of the thesis of Doctoral submitted by Fabiane R. R. H. Marui, from Universidade Federal de São Paulo.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Universidade Federal de São Paulo (UNIFESP) under the protocol number CEP 0199/10, 05/21/10. 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.

References

1. Emery AE. Population frequencies of inherited neuromuscular diseases—a world survey. Neuromuscul Disord. 1991;1(1):19-29.
2. McDonald CM. Physical activity, health impairments, and disability in neuromuscular disease. Am J Phys Med Rehabil. 2002;81(11 Suppl):108-20.
3. Childers MK, Okamura CS, Bogan DJ, Bogan JR, Sullivan MJ, Kornegay JN. Myofiber. Injury and regeneration in a canine homologue of Duchenne muscular dystrophy. Am J Phys Med Rehabil. 2001;80(3):175-81.
4. Kueh SL, Head SI, Morley JW. GABA(A) receptor expression and inhibitory post-synaptic currents in cerebellar Purkinje cells in dystrophin-deficient mdx mice. Clin Exp Pharmacol Physiol. 2008;35(2):207-10.
5. Nigro G, Comi LI, Politano L, Bain RJ. The incidence and evolution of cardiomyopathy in Duchenne muscular dystrophy. Int J Cardiol. 1990;26(3):271-7.
6. Connuck DM, Sleeper LA, Colan SD, Cox GF, Towbin JA, Lowe AM, et al; Pediatric Cardiomyopathy Registry Study Group. Characteristics and outcomes of cardiomyopathy in children with Duchenne or Becker muscular dystrophy: a comparative study from the Pediatric Cardiomyopathy Registry. Am Heart J. 2008;155(6):998-1005.
7. Cox GF, Kunkel LM. Dystrophies and heart disease. Curr Opin Cardiol. 1997;12(3):329-43.
8. Feng J, Schaus BJ, Fallavollita JA, Lee TC, Canty JM Jr. Preload induces troponin I degradation independently of myocardial ischemia. Circulation 2001;103(16):2035-7.
9. Woolf PJ, Lu S, Cornford-Nairn R, Watson M, Xiao XH, Holroyd SM, et al. Alterations in dihydropyridine receptors in dystrophin-deficient cardiac muscle. Am J Physiol Heart Circ Physiol. 2006;290(6):H2439-45.
10. Williams IA, Allen DG. Intracellular calcium handling in ventricular myocytes from mdx mice. Am J Physiol Heart Circ Physiol. 2007; 292(2):H846-55.
11. American Academy of Pediatrics, Section on Cardiology and Cardiac Surgery. Cardiovascular health supervision for individuals affected by Duchenne or Becker muscular dystrophy. Pediatrics. 2005;116(6):1569-73.
12. Viollet L, Thrush PT, Flanigan KM, Mendell JR, Allen HD. Effects of angiotensin-converting enzyme inhibitors and/or beta blockers on the cardiomyopathy in Duchenne muscular dystrophy. Am J Cardiol. 2012;110(1):98-102.
13. Sociedade Brasileira de Cardiologia. V Diretrizes Brasileiras de Monitorização Ambulatorial da Pressão Arterial (MAPA) e III Diretrizes Brasileiras de Monitorização Residencial da Pressão Arterial (MRPA). Rev Bras Hipertens. 2011;18(1):18-25.
14. Urbina E, Alpert B, Flynn J, Hayman L, Harshfield GA, Jacobson M, et al. American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee. Ambulatory blood pressure monitoring in children and adolescents: recommendations for standard assessment: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee of the council on cardiovascular disease in the young and the council for high blood pressure research. Hypertension. 2008;52(3):433-51.
15. Flynn JT, Daniels SR, Hayman LL, Maahs DM, McCrindle BW, Mitsnefes M, et al; American Heart Association Atherosclerosis, Hypertension and Obesity in Youth Committee of the Council on Cardiovascular Disease in the Young. Update: ambulatory blood pressure monitoring in children and adolescents: a scientific statement from the American Heart Association Atherosclerosis, Hypertension and Obesity in Youth Committee of the Council on Cardiovascular Disease in the Young. Hypertension. 2014;63(5):1116-35.
16. Varda NM, Gregoric A. Twenty-four-hour ambulatory blood pressure monitoring in infants and toddlers. Pediatr Nephrol. 2005;20(6):798-802.
17. Manzur AY, Kinali M, Muntoni F. Update on the management of Duchenne muscular dystrophy. Arch Dis Child. 2008;93(11):986-90.
18. Manzur AY, Kuntzer T, Pike M, Swan AV. Glucocorticoid corticosteroids for Duchenne muscular dystrophy. Cochrane Database Syst Rev. 2008;23(1):CD003725.
19. Braat E, Hoste L, De Waele L, Gheysens O, Vermeersch P, Goffin K, et al. Renal function in children and adolescents with Duchenne muscular dystrophy. Neuromuscul Disord. 2015;25(5):381-7.
20. American Academy of Pediatrics, Section on Cardiology and Cardiac Surgery. Cardiovascular health supervision for individuals affected by Duchenne or Becker muscular dystrophy. Pediatrics. 2005;116(6):1569-73.
21. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004;114(2 Suppl 4th Report):555-76.
22. O'Brien E, Asmar R, Beilin L, Imai Y, Mancia G, Mengden T, et al. European Society of Hypertension Working Group on Blood Pressure Monitoring. Practice guidelines of the European Society of Hypertension for clinic, ambulatory and self blood pressure measurement. J Hypertens. 2005;23(4):697-701.
23. Masood SA, Kazmouz S, Heydemann P, Li H, Kenny D. Under-recognition of Low Blood Pressure Readings in Patients with Duchenne Muscular Dystrophy. Pediatr Cardiol. 2015;36(7)1489-94.
24. Malachias MV, Souza WK, Plavnik FL, Rodrigues CI, Brandão AA, Neves MF, et al; Sociedade Brasileira de Cardiologia. 7a Diretriz Brasileira de hipertensão arterial. Arq Bras Cardiol. 2016;107(3 supl 3):1-83.
25. Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al; DMD Care Considerations Working Group. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. Lancet Neurol. 2010;9(2):177-89.
26. Loufrani L, Levy BI, Henrion D. Defect in microvascular adaptation to chronic changes in blood flow in mice lacking the gene encoding for dystrophin. Circ Res. 2002;91(12):1183-9.
27. Thomas GD, Sander M, Lau KS, Huang PL, Stull JT, Victor RG. Impaired metabolic modulation of alpha-adrenergic vasoconstriction in dystrophin-deficient skeletal muscle. Proc Natl Acad Sci USA. 1998;95(25):15090-5.
28. Thomas GD, Shaul PW, Yuhanna IS, Froehner SC, Adams ME. Vasomodulation by skeletal muscle-derived nitric oxide requires alpha-syntrophin-mediated sarcolemmal localization of neuronal Nitric oxide synthase. Circ Res. 2003;92(5):554-60.
29. Brenman JE, Chao DS, Xia H, Aldape K, Bredt DS. Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. Cell 1995;82(5):743-52.
30. Chang WJ, Iannaccone ST, Lau KS, Masters BS, McCabe TJ, McMillan K, et al. Neuronal nitric oxide synthase and dystrophin-deficient muscular dystrophy. Proc Natl Acad Sci USA. 1996;93(17):9142-7.

Endothelial Dysfunction and Inflammation Precedes Elevations in Blood Pressure Induced by a High-Fat Diet

Jorge Camargo Oishi,[1] Cynthia Aparecida Castro,[1] Karina Ana Silva,[1] Victor Fabricio,[1] Evelin Capelari Cárnio,[2] Shane A. Phillips,[3] Ana Claudia Garcia de Oliveira Duarte,[1] Gerson Jhonatan Rodrigues[1]

Universidade Federal de São Carlos (UFSCar),[1] São Paulo, SP - Brazil

Universidade de São Paulo (USP), Ribeirão Preto,[2] SP - Brazil

University of Chicago Medical Center,[3] Chicago, Illinois - USA

Abstract

Background: **Obesity leads to a chronic inflammatory state, endothelial dysfunction and hypertension.**

Objective: **To establish the time-course of events regarding inflammatory markers, endothelial dysfunction, systolic blood pressure (SBP) in obesity in only one experimental model.**

Methods: **We fed male Wistar rats (eight-week age) with a standard diet (Control - CT, n = 35), or palatable high-fat diet (HFD, n = 35) for 24 weeks. Every six weeks, 7 animals from each group were randomly selected for euthanasia. SBP and serum levels of interleukin -6, tumor necrosis factor-α, C-reactive protein, adiponectin and nitric oxide were determined. Endothelial and vascular smooth muscle functions were determined in dissected aorta and lipid peroxidation was measured. Statistical significance was set at $p < 0.05$.**

Results: **Levels of pro-inflammatory cytokines began to increase after six weeks of a high-fat diet, while those of the anti-inflammatory cytokine adiponectin decreased. Interestingly, the endothelial function and serum nitric oxide began to decrease after six weeks in HFD group. The SBP and lipid peroxidation began to increase at 12 weeks in HFD group. In addition, we showed that total visceral fat mass was negatively correlated with endothelial function and positively correlated with SBP.**

Conclusion: **Our results show the time-course of deleterious effects and their correlation with obesity. (Arq Bras Cardiol. 2018; 110(6):558-567)**

Keywords: **Hypertension; Endothelium / abnormalities; Diet, High-Faties; Nitric Oxide; Dyslipidemias.**

Introduction

Currently, obesity and associated comorbidities are one of the major health problems in developed and developing countries, reducing both the quality and quantity of life and increasing the risk of mortality.[1] Obesity is characterized by excessive fat tissue storage and is strongly associated with the development of cardiovascular diseases, dyslipidemia and hypertension. There is an associated pro-inflammatory environment that appears to worsen cardiovascular outcomes[2,3] and according to the World Health Organization,[4] cardiovascular diseases are currently one of the major causes of mortality in the world.

A great number of metabolic disorders are caused by obesity; among them endothelial dysfunction plays an important role in the development of insulin resistance and hypertension.[5] Almost thirty-five years ago, it was discovered that endothelial cells could modulate relaxations and contractions of the underlying vascular smooth muscle, which allowed for the concept that vascular tonus control is endothelium-dependent of the underlying vascular smooth muscle.[6-8]

The endothelium produces several "relaxing factors" (EDRFs, endothelium-derived relaxing factors), hyperpolarizing factors (EDHFs), as well as contractile factors (EDCFs). Through a fine balance between the release of EDRFs and EDCFs, the endothelium plays a vital role in maintaining circulatory homeostasis. Any change in this balance may result in endothelial dysfunction.[5,8]

Previous studies have demonstrated the onset of hypertension and endothelial dysfunction in obesity induced by a high-fat diet.[9,10] However, whether and in which order they appear has not been well defined and the temporal relationships between weight gain, endothelial dysfunction and blood pressure following a high-fat diet have not been determined. Therefore, the aim of the present study was to determine the time course of inflammation, endothelial dysfunction and the increase in blood pressure following a high-fat diet designed to induce obesity.

Mailing Address: Jorge Camargo Oishi •
Rua Rosalino Belline, 455. Postal Code 13564-050, Santa Paula, São Carlos, SP – Brazil
E-mail: jorgeoishi@hotmail.com
Manuscript received July 18, 2017, revised manuscript September 14, 2017, accepted October 06, 2017

DOI: 10.5935/abc.20180086

Methods

Animals and dietary treatments

The experimental protocol was in accordance with the guidelines of the Brazilian College for Animal Experimentation (COBEA) and was approved by the Ethical Committee of the Federal University of São Carlos (026/2013).

Seventy male (8-week-old) Wistar rats (250–300 g) were assigned to two experimental groups with food and water *ad libitum* for 24 weeks: Control (CT, n = 35) was fed a standard diet or HFD (n = 35) fed with high-fat diet, that consisted of a standard rat diet plus peanuts, milk chocolate, and biscuits at a proportion of 3:2:2:1 as previously described.[11] Standard diet and high-fat diet contained, respectively, 20/20% of protein, 4.5/20% of fat and 55/40% of carbohydrate.[11] The caloric values of the diets were approximately 4.07 kcal/g for the standard diet and 5.12 Kcal/g for HFD. At time 0 and after every 6 weeks, 7 rats from CT and 7 from HFD group were randomly euthanized, and blood was collected for experimental analysis.

Blood pressure measurements in Conscious Rats

Indirect systolic blood pressure (SBP) was measured two days before euthanasia every 6 weeks using tail-cuff plethysmography (Power Lab 8/35, AD Instruments, Pty Ltda, CO), as described by Rodrigues et al.[12] Mean SBP was calculated from an average of four successive measurements in each animal.

Vascular reactivity studies

The animals were anaesthetized with isoflurane and euthanized by decapitation. Thoracic aortas were isolated and cleaned of adherent connective tissues, and placed in a Krebs solution, as described previously.[13] Aortas were carefully mounted as ring preparations (≅ 4 mm in length) and placed in bath chambers containing Krebs solution at 37°C continuously bubbled with 95% O_2 and 5% CO_2, pH 7.4 in an isometric myograph (model 610 DMT-USA) and recorded by a PowerLab8/SP data acquisition system (AD Instruments Pty Ltd., Colorado). The aortic rings were submitted to a tension of 1.5 g, which was readjusted every 15 min during a 60 min equilibration period before adding the given drug. Experiments were done in aortic rings with intact endothelium and also in denude endothelium aortic rings. Endothelial integrity was assessed by the degree of relaxation induced by 1 μmol/l acetylcholine (ACH) in the presence of contractile tone induced by phenylephrine (0.1 μm/l). The ring was considered with intact endothelium if the relaxation with acetylcholine was higher than 80%. In endothelium-denuded aortas, the relaxation to ACH was less than 5%. After the endothelial integrity test, aortic rings were pre-contracted with phenylephrine (100 nM). When the plateau was reached, concentration–effect curves to acetylcholine (0.1nM to 0.1mM) in intact endothelium aortic rings or concentration–effect curves for NO donor sodium nitroprusside (SNP) in denude endothelium aortic rings were constructed. Concentration curves were fitted with a sigmoidal dose-response equation which disclosed the maximal effect (MaxE) and the negative logarithm of the agonist that produces half-maximal response (pD2) using GraphPad Prism (GraphPad Software In, USA).

Body fat composition

Visceral adipose tissue (VAT) was dissected (mesenteric, epididymal and retroperitoneal white adipose tissues) and weighed to evaluate central adiposity.

Aorta lipid peroxidation (Ferrous oxidation-Xylenol Orange – FOX)

Thoracic aortas were isolated and cleaned of adherent connective tissues. The methodology was described by Jiang et al.[14] The ferrous oxidation−xylenol orange (FOX), measures lipid peroxides (cumene hydroperoxide – CHP), one of the main products of lipid peroxidation. For the standard assay, the following reagents were added sequentially: 0.25 mM $FeSO_4$, 25 mM $H2SO_4$, 0.1 mM xylenol orange, and water to a total of 0.9 ml. A sample of tissue extract (20-100 μL) was added, and the final volume was adjusted to 1 ml with water. Blanks were prepared by replacing tissue extract with water. Samples were incubated at room temperature until the reaction was complete (40 min), and absorbance at 560 nm was measured.

Serum nitrite and nitrate (NOx)

Serum nitric oxide levels were obtained by measuring the serum concentrations of its stable end-products nitrite (NO_2^-) and nitrate (NO_3^-), collectively known as NOx, as described previously.[15] The NO/ozone chemiluminescence method was performed using the NO Analyzer 280i (Sievers, Boulder, CO, USA).

Determination of adiponectin and inflammatory cytokines

Quantification of adiponectin and inflammatory cytokines tumor necrosis factor-α (TNF- α), interleukin 6 (IL-6) and C-reactive protein (CRP) in serum was performed using the enzyme-linked immunosorbent assay (ELISA) kit. IL-6 and TNF-α were evaluated using commercial OptEIA kits (BD Biosciences, Pharmingen, USA). Adiponectin and CRP were analyzed using Duo Set kits (R&D Systems, USA). All kits were used according to the manufacturers' instructions, and the results were expressed in pg/mL for all cytokines evaluated.

Morphological and histological evaluation

Aorta segments were quickly cleaned from the surrounding tissues and blood, cut into rings fixed in formalin 37% and embedded in paraffin blocks. Later, 4-μm thick sections were cut with a microtome (Leitz 1512, IMEB, USA), placed onto glass microscope slides and stained with hematoxylin and eosin using the standard methods. Images of transverse sections of the arterial segments were captured using a camera connected to an optical microscope (Leica DM 2000). External diameter (ED) was obtained by measuring the surfaces of the adventitia and internal diameter (ID) from the endothelium surface. The media thickness was obtained by dividing the difference ED - ID by 2 (δ = ED - ID/2). The media/lumen ratio was calculated from the area data. The images were analyzed using the ImageJ analysis software, as described previously.[16]

Statistical analysis

The normality of distribution (of all quantitative and continuous variables) was checked using the Kolmogorov-Smirnov test. A sample of 7 animals in each group was required to provide 85% statistical power with a two-tailed alpha of 0.05 for pD2 and 90% for all other variables analyzed in this study. Differences between the CT and HFD groups were compared using two-way repeated measures analysis of variance ANOVA. When differences were indicated, a Newman-Keuls post hoc analysis was used with a statistical significance set at $p < 0.05$. These data were expressed as mean ± SD (Statistica software 7.0, StatSoft. Inc, USA). Vascular reactivity data of pD2 and Emax were expressed as mean ± SD with a statistical significance set at $p < 0.05$ (Graphpad Prism 3.0). Pearson correlation was made between pD2 and the SBP, pD2 and VAT, blood pressure and VAT, IL-6 and pD2, TNF-α and pD2, CRP and pD2 and between adiponectin and pD2, with a statistical significance of 5%.

Results

Total visceral adipose tissue

The sum of the weight of the retroperitoneal, visceral and epididymal adipose tissues – (VAT) was higher in HFD than in the CT group at 6 weeks. At 24 weeks, fat weight was 300% higher in the HFD than the CT group. VAT in the CT group increased at 12 weeks compared to 6 weeks, but remained unchanged for the rest of the experimental period (Figure 1).

Inflammatory status

The inflammatory cytokines IL-6, TNF-α and CRP were increased in serum of HFD animals in 6, 12, 18 and 24 weeks when compared to the CT group (Figure 2 A, B, C). On the other hand, the levels of serum adiponectin decreased in the HFD group after 6, 12, 18 and 24 weeks of the experimental protocol (Figure 2 D). In the CT group, no changes were found in these cytokine levels.

Vascular reactivity

No differences were found (Figure 3A) in the endothelium-dependent relaxation induced by acetylcholine (pD2) in the CT group over the entire experimental period. On the other hand, the pD2 was impaired in aortas of obese animals at 6, 12, 18 and 24 weeks compared to CT rats. Moreover, we observed a decrease in pD2 throughout the experimental period in HFD group (Figures 3B, C).

No differences were observed in the maximum relaxant effect (MaxE) in both CT and HFD groups. In endothelium-denuded aortic rings, there were no differences in the pD2 and MaxE to endothelium-independent relaxation induced by SNP in the CT and HFD groups in all the weeks evaluated (Table 1).

There was a strong negative correlation between pD2 and SBP ($r = -0.722$, $p < 0.01$). Moreover, we found a negative correlation between pD2 and VAT ($r = -0.729$, $p < 0.01$), between pD2 and inflammatory cytokines (pD2 and IL-6, $r = -0.74$; pD2 and TNF-α, $r = -0.86$; pD2 and CRP, $r = -069$, $p < 0.05$) and a positive correlation between pD2 and adiponectin ($r = 0.77$, $p < 0.01$).

Serum nitric oxide (NO) and aorta lipid peroxidation

By quantification of serum NO metabolites, we observed that NO level decreased at 6 weeks in HFD rats and remained lower throughout the experimental period when compared to the CT group. The time of experiment had no effect on NO concentrations in CT and the HFD groups (Figure 4).

Levels of lipid peroxidation in aorta increased at 12 weeks of a high-fat diet and remained high throughout the experimental period when compared to the CT group. In the HFD group, there was an increase in lipid peroxidation at 12 weeks when compared to 6 weeks (Figure 5).

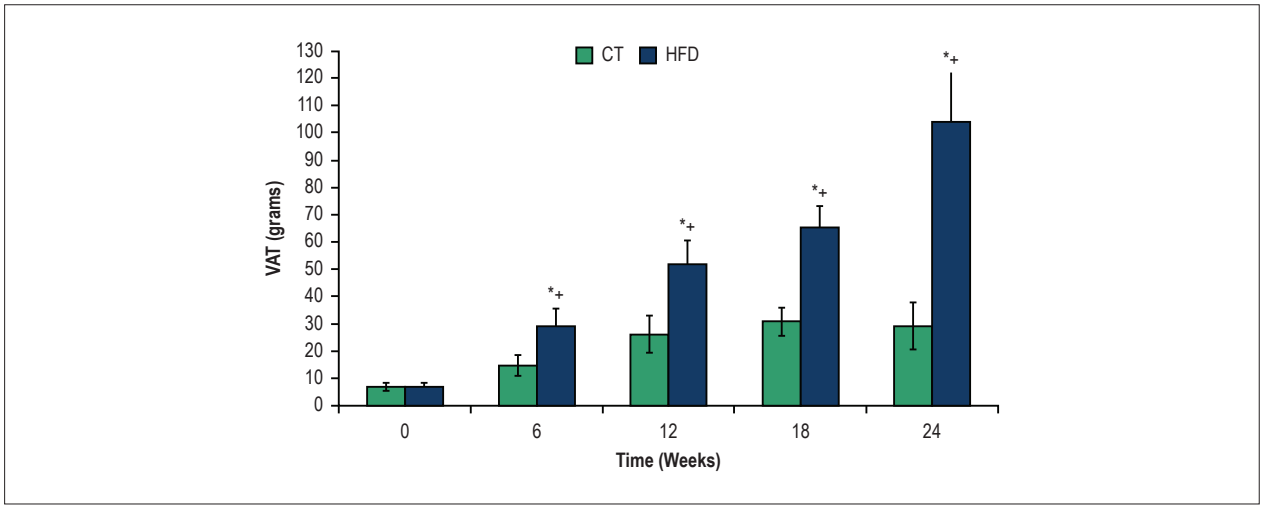


Figure 1 – *Visceral adipose fat (VAT) in control (CT) and high-fat diet (HFD) groups over the weeks. *P < 0.05, compared with CT; + p < 0.05, within-group comparison (0 vs. 6, 6 vs. 12, 12 vs. 18, 18 vs. 24 weeks). Seven rats from each group were compared at each time point.*

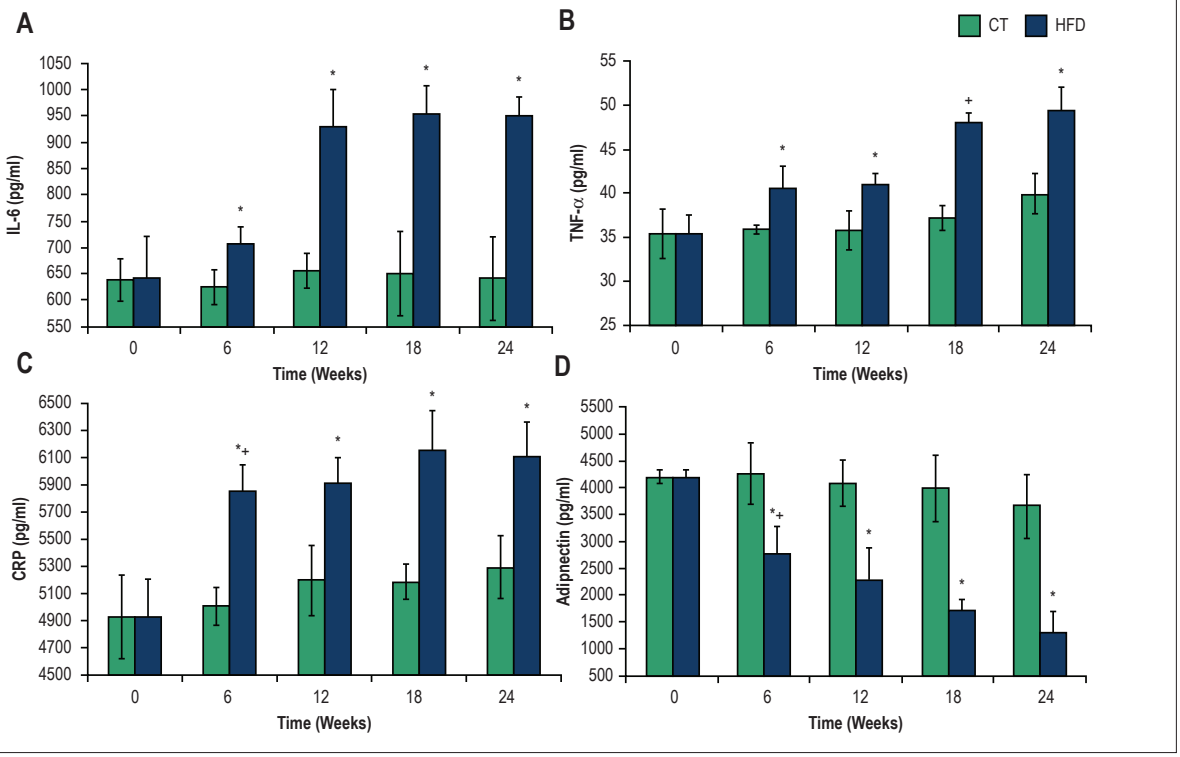


Figure 2 – *Serum interleukin-6 (IL-6) (A), tumor necrosis factor-α (TNF-α) (B), C-reactive protein (CRP) (C) and adiponectin (D) in the control (CT) and high-fat diet (HFD) groups over time. *P < 0.05, CT compared with HFD group; + p < 0.05, within-group comparison (0 vs. 6, 6 vs. 12, 12 vs. 18, 18 vs. 24 weeks). Seven rats from each group were compared at each time point*

Systolic blood pressure

As shown in Figure 6, high-fat diet induced an increase in SBP at 12, 18 and 24 weeks in the HFD group when compared to the CT group. Moreover, a positive correlation was found between SBP and VAT ($r = 0.756$, $p < 0.01$) in the HFD, and no significant differences in blood pressure were found in the CT group.

Alterations in the vascular structure

Table 2 shows that high-fat diet induced an increase in aortic medial thickness after 18 weeks and 24 weeks, and decreased the ID after 24 weeks in HFD compared to CT group ($p < 0.05$), resulting in an increase in the media thickness/lumen ratio after 18 and 24 weeks. In HFD group, there was an increase in the intima-media thickness after 18 weeks of high-fat diet, a decrease in the ID after 12 weeks, and an increase in the media thickness/lumen ratio in the aorta after 18 weeks of high-fat diet.

Discussion

To the best of our knowledge, this is the first study that has detected the time course of vascular function, vascular structure, oxidative stress and inflammatory status during the obesity progression in just one experimental model. Our findings showed that inflammatory state and endothelial dysfunction precedes the development of high blood pressure induced by high-fat diet. Obesity progression was associated with increased predisposition to pathological conditions and to common features of cardiovascular risk factors, including hypertension and endothelial dysfunction.[17]

The high-fat diet used in this study induced differences in adiposity between HFD and CT groups, validating our experimental model. The risk of developing obesity-related derangements is proportional to the degree of adiposity[18] and, in particular, to the accumulation of fat in the visceral region.[19] In this study, the HFD group had greater VAT mass at 6, 12, 18 and 24 weeks than the CT group.

In obesity, the inflammatory status is distinctive,[19] and is characterized by low-grade inflammation, which results in tissue remodeling and systemic metabolic deterioration over time.[20] Thus, detecting the time of increased inflammation is important for the development of therapeutic intervention.

Adipose tissue is fundamental to the development of inflammation by inducing the increase of pro-inflammatory cytokines, including TNF-α and IL-6,[21] and a decrease in anti-inflammatory chemokines such as adiponectin.[22] In addition, it has been described that TNF-α contributes to CRP elevation, which is a marker of low-grade inflammatory state, but also has a close relationship with dyslipidemia and endothelial dysfunction.[23] In mice, the HFD induced an elevation of IL-6 after 2, 4 and 6 months,[24] and an increase in plasma levels of pro-inflammatory mediators TNF-α, IL-6 after 15 weeks.[25]

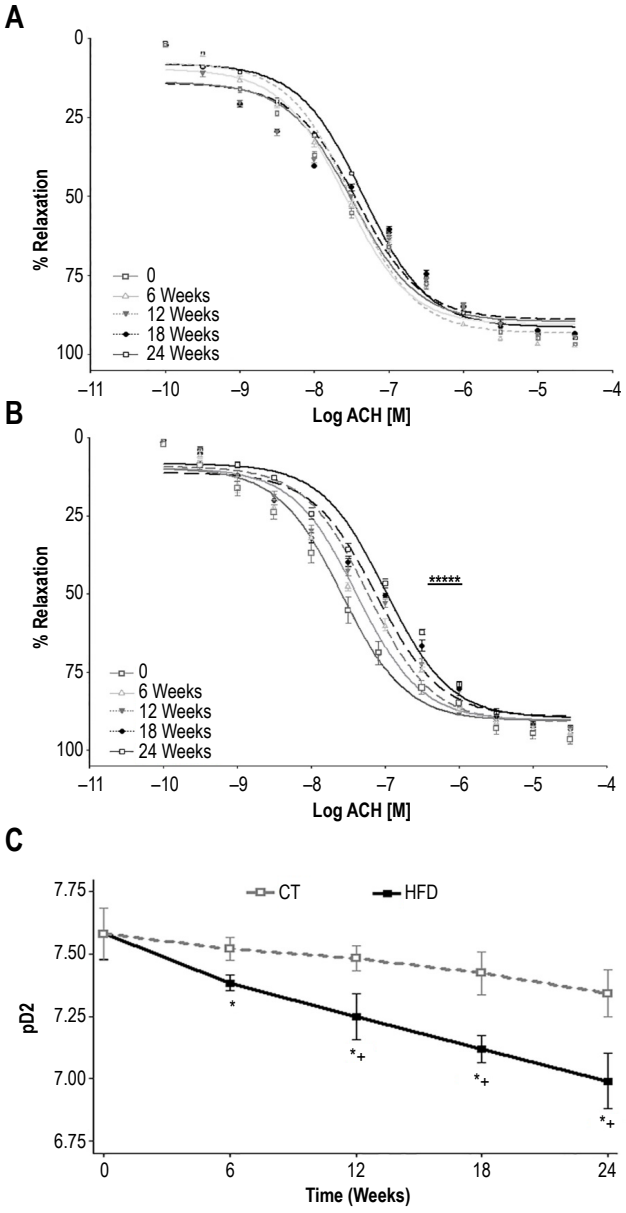


Figure 3 – *Concentration-response curve to acetylcholine (endothelium-dependent relaxation) in aortic rings of rats of the control (CT) (A) and high-fat diet (HFD) group (B) groups and half-maximal response pD2 (C) in both groups. *P < 0.05, CT compared with HFD group in each 6 weeks; + p < 0.05, within-group comparison (0 vs. 6, 6 vs. 12, 12 vs. 18, 18 vs. 24 weeks); seven rats from each group were compared at each time point*

In the present study, we detected that the levels of inflammatory cytokines TNF-α, IL-6 and CRP increased after 6 weeks in the HFD group and remained higher up to 24 weeks, while the adiponectin concentration was reduced and remained lower in the same period. These results indicate an early development of a low-grade inflammation state in this animal model. TNF-α is involved in the systemic inflammatory response and its levels are increased in the adipose tissue of obese mice compared with lean controls.[20] On the other hand, adiponectin, which improves cardiovascular functions and has anti-inflammatory effects[22] decreased after 6 weeks in HFD group and remained lower up to 24 weeks.

Obesity is also associated with an impairment of endothelial cell function and promotes endothelial dysfunction through an array of metabolic disorders including the accumulation of adipose tissue, high blood pressure, dyslipidemia and diabetes, which are linked to vascular oxidative stress.[25] The endothelium comprises the inner lining of blood vessels, and forms the interface between the circulating blood and the

Table 1 – Half-maximal response (pD2) and maximal effect (MaxE) in aortic rings of the rats of the control (CT) and high-fat (HFD) groups. *P < 0.05, compared with CT group; + p < 0.05, within-group comparison (0 *vs.* 6, 6 *vs.* 12, 12 *vs.* 18, 18 *vs.* 24 weeks)

Weeks	Intact Endothelium				Denude Endothelium			
	pD2		MaxE(%)		pD2		MaxE(%)	
	CT	HFD	CT	HFD	CT	HFD	CT	HFD
0	7.58 ± 0.25	7.58 ± 0.22	90.67 ±7.40	90.87 ± 7.14	8.69 ± 0.13	8.68 ± 0.23	103.8 ± 2.77	104.6 ± 3.38
6	7.52 ± 0.07	7.37 ± 0.18*+	93.42 ±6.80	90.31 ± 7.64	8.67 ± 0.21	8.66 ± 0.22	98.3 ± 4.10	100.2 ± 7.67
12	7.48 ± 0.18	7.23 ± 0.11*+	89.17 ±8.80	90.90 ± 7.67	8.69 ± 0.10	8.71 ± 0.13	102.5 ± 2.48	103.9 ± 3.43
18	7.42 ± 0.22	7.12 ± 0.15*+	88.98 ±9.90	89.34 ± 10.05	8.71 ± 0.10	8.69 ± 0.07	105.8 ± 3.70	104.3 ± 1.85
24	7.34 ± 0.19	6.99 ± 0.23*+	91.46 ±6.61	89.80 ± 8.59	8.69 ± 0.07	8.68 ± 0.14	105.9 ± 2.98	105.9 ± 2.11

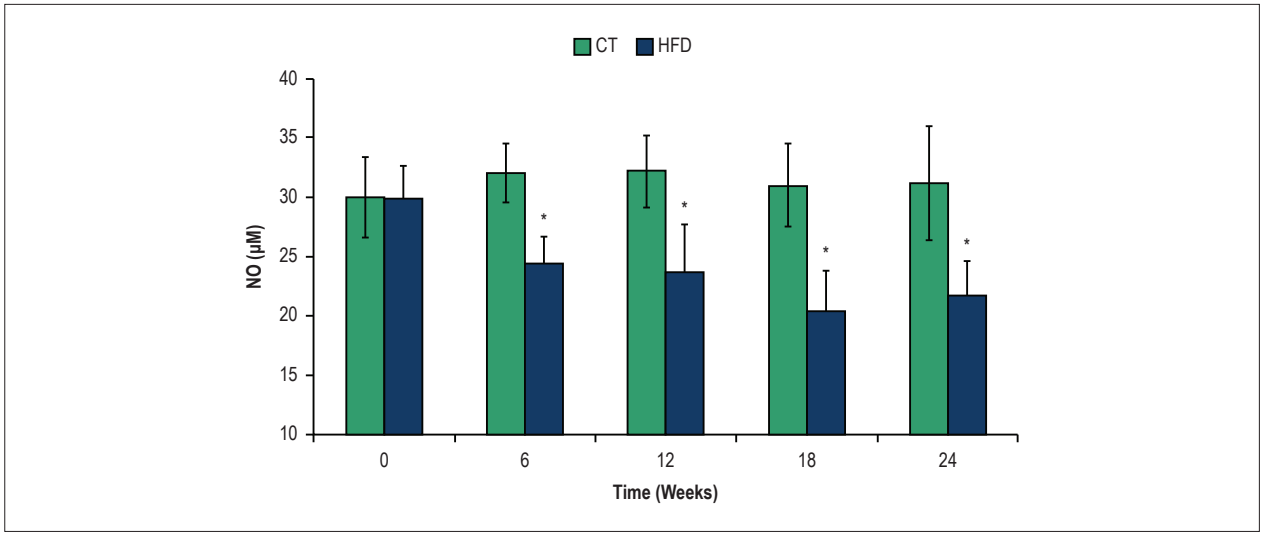


Figure 4 – *Serum nitric oxide (NO) concentration in rats of the control (CT) and high-fat diet (HFD) groups. *P < 0.05, compared with CT group; + p < 0.05, within-group comparison (0 vs. 6, 6 vs. 12, 12 vs. 18, 18 vs. 24 weeks); seven rats from each group were compared at each time point*

vascular wall. It also acts as an endocrine and paracrine organ, which regulates vascular function by secreting a variety of trophic and vasoactive factors that regulate vascular tone, cell adhesion, smooth muscle cell proliferation and inflammation of the vascular wall.[8]

Endothelial dysfunction has a key role in the development of various cardiovascular diseases. In obesity, many factors could negatively affect the endothelium function, which include changes in blood pressure, glucose levels, lipid metabolism and inflammatory system, elevated levels of free fatty acids and oxidative stress, which in turn causes a reduction in the availability of NO. [26-28]

We observed that 6 weeks of high-fat diet was sufficient to induce endothelial dysfunction. Moreover, our results suggest that the impaired relaxation to acetylcholine observed in aortas from obese rats is related to a reduction of NO production. The HFD group showed the lowest serum concentration of NO at 6 weeks, which remained low up to 24 weeks. Consistent with our observations, various studies have shown obesity-induced impairment of endothelial function at different points of obesity development. Boustany-Kari et al.[29] observed impaired endothelial function in rats fed for 11 weeks on a high-fat diet. In addition, 16 weeks of a high-fat diet in mice led to endothelial dysfunction and increases in systolic pressure in animals.[30]

Moreover, levels of TNF-α are strongly correlated with adiposity and diminished vasodilation in resistance arteries of rats, and IL-6 levels are proportional to adiposity whose elevations result in direct impairments of endothelial function.[31] On the other hand, the decreased adiponectin levels are associated with dyslipidemia and cardiovascular diseases. Furthermore, adiponectin can upregulate NO production by modulation of Ser1177 phosphorylation through AMPK and, conversely, IL-6 and TNF-α decrease eNOS Ser1177 phosphorylation, resulting in diminished eNOS activity and less NO generation.[32]

In addition, we found a strong correlation between inflammatory cytokines (TNF-α, IL-6, CRP) and endothelial function (pD_2).

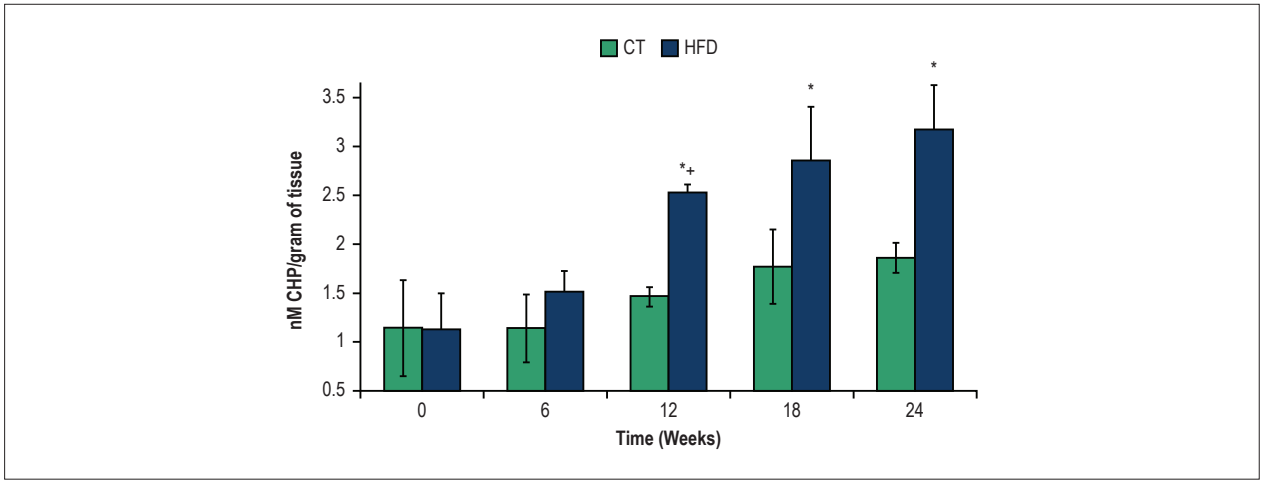


Figure 5 – *Lipid peroxidation in aortic rings from rats of control (CT) and high-fat diet (HFD) groups. *P < 0.05, compared with CT group; + p < 0.05, within-group comparison (0 vs. 6, 6 vs. 12, 12 vs. 18, 18 vs. 24 weeks); seven rats from each group were compared at each time point. CHP: cumene hydroperoxide.*

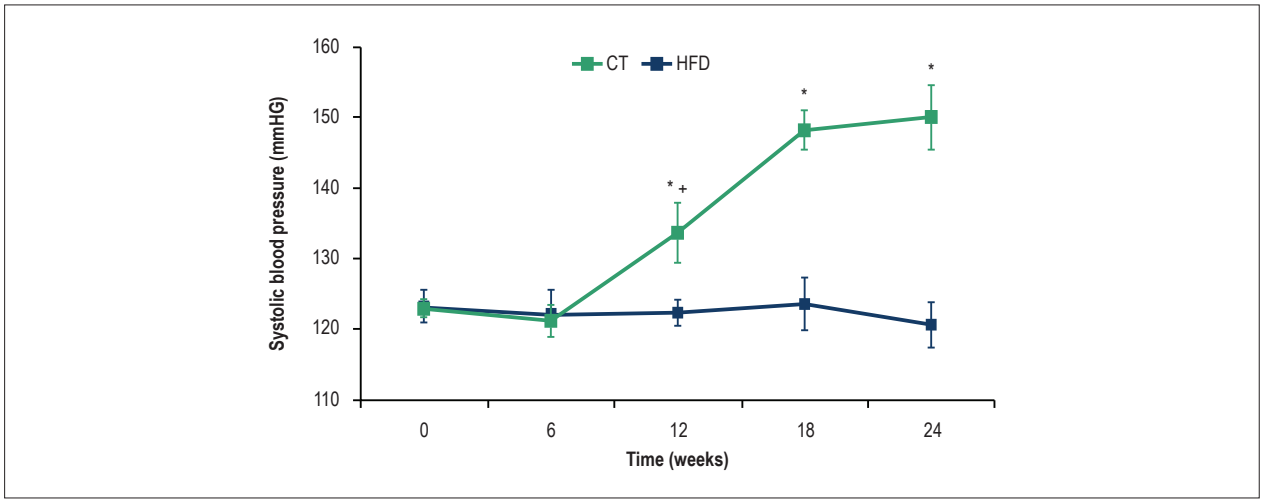


Figure 6 – *Systolic blood pressure in rats of control (CT) and high-fat diet (HFD) groups over 24 weeks. *P < 0.05, compared with CT group; + p < 0.05, within-group comparison (0 vs. 6, 6 vs. 12, 12 vs. 18, 18 vs. 24 weeks); seven rats from each group were compared at each time point*

Our findings are consistent with literature, showing that a high-fat diet treatment for 6 weeks was able to increase VAT. Interestingly, an inverse correlation was observed between VAT and endothelium function (pD_2). In addition, the levels of these adipokines were altered at 6 weeks in the HFD group, confirming the concept of obesity-related endothelial dysfunction. We showed here that these events occur at an early stage of obesity development.

Obesity is also strongly associated with hypertension, which is a major risk factor for the development of coronary heart diseases. In fact, 79% of hypertension in men was a direct result of excess weight.[33] Hypertension, characterized by chronic high blood pressure, has a multifactorial origin and an endothelial dysfunction can contribute to its genesis and maintenance.[5] In the present study, the high-fat diet induced an increase in the SBP at 12 weeks and it continued to increase reaching the maximum values at 18 weeks. These results are in accordance with Boustany et al. [34] that observed a rise in blood pressure, and increased activity of adipose and systemic renin angiotensin system after 11 weeks of high-fat diet in rats. The Framingham Heart Study reported a close connection between body fat levels and blood pressure in both men and women, and that adiposity emerged as a major factor which can be controlled and that contributes to hypertension.[34] The same occurred in our study, which showed a strong correlation between SBP and VAT.

Interestingly, in the present study, structural alterations in the aorta occurred after a rise in blood pressure. It is well known that hypertension is associated with structural alterations in arteries that could contribute to maintaining hypertension.[35] In addition, although not significantly, the media/lumen ratio starts to increase at 12 weeks, coinciding with the rise of blood pressure, and at 18 and 24 weeks, this increase becomes

Table 2 – Quantitative values obtained from morphometrical analysis of thoracic aorta thickness from control group (CT, n = 7) and high-fat group (HFD, n = 7) rats. Results are expressed as means ± SD. * P < 0.05, compared with CT group; + p < 0.05, within-group comparison (0 *vs.* 6, 6 *vs.* 12, 12 *vs.* 18, 18 *vs.* 24 weeks)

Weeks	Media Thickness (μm)		Internal diameter (ID) (μm)		Media:lumen ratio	
	CT	HFD	CT	HFD	CT	HFD
0	157.99 ± 7.18	157.88 ± 4.75	2830.64 ± 75.20	2832.64 ± 75.98	0.056 ± 0.00	0.056 ± 0.01
6	163.51 ± 7.51	163.64 ± 11.98	2967.21 ± 177.85+	2919.31 ± 145.46	0.054 ± 0.00	0.056 ± 0.00
12	162.82 ± 6.67	164.64 ± 9.64	2976.80 ± 167.73	2876.36 ± 99.89+	0.055 ± 0.01	0.057 ± 0.00
18	161.65 ± 9.95	178.20 ± 5.26 *+	2987.53 ± 156.18	2854.40 ± 133.40	0.054 ± 0.00	0.062 ± 0.00*+
24	164.21 ± 9.51	181.96 ± 9.73 *	3045.25 ± 168.01	2835.53 ± 167.74*	0.054 ± 0.00	0.064 ± 0.01*

significant. Chen et al.[36] found that the high-fat diet induced the increase in media thickness after 9 weeks. Our findings are in good agreement with these reports.

High-fat diet can also induce vascular pathogenesis, including effects on the aorta, leading to changes in vascular structure. Clinical and experimental studies have shown that increased body mass index is often associated with stiffening and increased arterial wall thickness.[37] These alterations found in this study are important predictors of increased cardiovascular mortality.

Previous studies in animals suggested that hypertension is associated with an increased formation of reactive oxygen species (ROS) from all layers of the vascular wall.[38] In agreement with these results, our findings showed an increase in lipid peroxidation (used as a marker of oxidative stress) in aortic rings at the same time that SBP increased, starting at 12 weeks. Moreover, Kobayasi et al.[30] found a reduced antioxidant activity, increased local vascular inflammation and impaired endothelium-dependent relaxation in mice fed on a high fat diet at 16 weeks. The release of IL-6, mainly from abdominal adipocyte sources might have a pivotal role in the relationship between oxidative stress and endothelial dysfunction. IL-6 and TNF-α contribute to CRP elevation, a marker of low-grade inflammatory state, and also have a close relationship with endothelial dysfunction.[23]

As mentioned earlier, obesity is commonly associated with oxidative stress,[39] which is able to modify vascular tonus by impeding NO bioavailability and/or signaling.[38] We have observed that 6 weeks of high-fat diet decreased NO circulating levels without significant effects on aortic lipid peroxidation at this point of obesity progression. Thus, these results suggest that the decrease in circulating NO levels precedes the increase in oxidative stress. During the oxidative stress state, excessive production of ROS reduces the bioactivity of NO due to its rapid oxidative inactivation by the ROS superoxide (O_2^-).[38]

According to Victor et al.,[40] while visceral fat stores expand, adipocytes generate increasing levels of ROS. In the present study, the high-fat diet induced the accumulation of abdominal fat that could trigger lipid peroxidation in the aorta at 12 weeks, which persists up to 24 weeks.

One limitation of this study was the fact that visceral fat mass was evaluated by dissection of adipose tissue. Dual-energy X-ray absorptiometry (DXA), the gold standard method for assessment of body fat mass, would provide more comprehensive data of body composition; but, unfortunately, the method could not be performed in this study.

Our data suggest that even at early stages of development, obesity (6 weeks) can trigger chronic inflammation and impairment of endothelial function. This impairment appears most closely related to inflammatory cytokines and expansion of VAT.

Conclusion

In conclusion, development of obesity first led to a reduction of endothelial function, which continued to decline over the weeks, and to systemic inflammation, followed by an increase in blood pressure, lipid peroxidation and changes in aortic structure. Our work is relevant in showing the relationship of obesity with chronic inflammation, endothelial dysfunction and hypertension. Despite many studies in this area, the results we found are a further step towards to the development of therapeutic strategies to prevent these abnormalities.

Author contributions

Conception and design of the research and Obtaining financing: Oishi JC, Duarte ACGO, Rodrigues GJ; Acquisition of data: Oishi JC, Castro CA, Silva KA, Fabricio V, Cárnio EC, Duarte ACGO, Rodrigues GJ; Analysis and interpretation of the data: Oishi JC, Castro CA, Silva KA, Fabricio V, Cárnio EC, Phillips SA, Duarte ACGO, Rodrigues GJ; Statistical analysis: Oishi JC; Writing of the manuscript: Oishi JC, Castro CA, Silva KA, Fabricio V, Duarte ACGO, Rodrigues GJ; Critical revision of the manuscript for intellectual content: Oishi JC, Castro CA, Silva KA, Cárnio EC, Phillips SA, Duarte ACGO, Rodrigues GJ.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by CNPq and partially funded by FAPESP.

Study Association

This article is part of the thesis of Post Doctoral submitted by Jorge Camargo Oishi, from Universidade Federal de São Carlos.

Ethics approval and consent to participate

This study was approved by the Ethics Committee on Animal Experiments of Universidade Federal de São Carlos under the protocol number 026/2013.

References

1. Beleigoli AM, Boersma E, Diniz MD, Lima-Costa MF, Ribeiro AL. Overweight and class I obesity are associated with lower 10-year risk of mortality in Brazilian older adults: the Bambuí Cohort Study of Ageing. PLoS One. 2012;7(12):e52111. doi: 10.1371/journal.pone.0052111.
2. Field AE, Coakley EH, Must A, Spadano JL, Laird MA, Dietz WH, et al. Impact of overweight on the risk of developing common chronic diseases during a 10 year period. Arch Intern Med. 2001;161(13):1581-6. doi:10.1001/archinte.161.13.1581.
3. Iantorno M, Campia U, Di Daniele N, Nistico S, Forleo GB, Cardillo C, et al. Obesity, inflammation and endothelial dysfunction. J Biol Regul Homeost Agents. 2014;28(2):169-76. PMID: 25001649.
4. World Health Organization. (WHO) Cardiovascular diseases. [Cited in 2016 Feb 10]. Available from: http://www.who.int/cardiovasculardiseases/en
5. Davel AP, Wenceslau CF, Akamine EH, Xavier FE, Couto, GK, Oliveira HT, et al. Endothelial dysfunction in cardiovascular and endocrine-metabolic diseases: an update. Braz J Med Biol Res. 2011;44(9):920-32. doi: http://dx.doi.org/10.1590/S0100-879X2011007500104.
6. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980;288(5789):373-6. PMID: 6253831.
7. Vanhoutte PM. The endothelium – modulator of vascular smooth-muscle tone. N Engl J Med. 1988;319(8):512-3. doi: 10.1056/NEJM198808253190809.
8. Furchgott RF, Vanhoutte PM. Endothelium-derived relaxing and contracting factors. FASEB J. 1989;3(9):2007-18. PMID: 2545495.
9. Costa RR, Villela NR, Souza MG, Boa BC, Cyrino FZ, Silva SV, et al. High fat diet induces central obesity, insulin resistance and microvascular dysfunction in hamsters. Microvasc Res. 2011;82(3):416-22. doi: 10.1016/j.mvr.2011.08.007.
10. Kurtel H, Rodrigues SF, Yilmas CE, Yildirim A, Granger DN. Impaired vasomotor function induced by combination of hypertension and hypercholesterolemia. J Am Soc Hypertens. 2013;7(1):14-23. doi:10.10.16/j.jash.2012.11.005.
11. Estadella D, Oyama LM, Bueno AA, Habitante CA, Souza GI, Ribeiro EB, et al. A palatable hyperlipidic diet causes obesity and affects brain glucose metabolism in rats. Lipids Health Dis. 2011;Sep 23;10:168. doi: 10.1186/1476-511X-10-168.
12. Rodrigues GJ, Pereira AC, Vercesi JA, Lima RG, Silva RS, Bendhack LM. Long-lasting hypotensive effect in renal hypertensive rats induced by nitric oxide released from a ruthenium complex. J Cardiovasc Pharmacol. 2012;60(2):193-8. doi: 10.1097/FJC.0b013e31825bacc4.
13. Oishi JC, Buzinnari TC, Pestana CR, De Moraes TF, Vatanabe IP, Wink DA, et al. In vitro treatment with cis-[(Ru(H-dcbpy-)2(Cl)(NO)]) improves the endothelial function in aortic rings with endothelial dysfunction. J Pharm Pharm Sci. 2015;18(5):696-704. PMID: 26670366.
14. Jiang ZY, Woollard AC, Wolff S. Lipid hydroperoxide measurement by oxidation of $Fe2^{+}$ in the presence of xylenol orange. Comparison with the TBA Assay and a Iodometric Method. Lipids. 1991;26(10)853-6. PMID: 1795606.
15. Pereira FH, Batalhão ME, Cárnio EV. Correlation between body temperature, blood pressure and plasmatic nitric oxide in septic patients. Rev Lat Am Enfermagem. 2014;22(1):123-8. doi: 10.1590/0104-1169.2896.2392.
16. Coura MA, Pacheco ME, Simões HF, Moraes JF, Campbell CS. Estudo morfoquantitativo da parede da aorta de ratos wistar idosos treinados com exercício aeróbio. Motri. 2012;8(4):71-9. doi: http://dx.doi.org/10.6063/motricidade.8(4).1554.
17. Huang PL. eNOS, metabolic syndrome and cardiovascular disease. Trends Endocrinol Metab. 2009;20(6):295-302. doi: 10.1016/j.tem.2009.03.005.
18. Gomez-Ambrosi J, Silva C, Galofre JC, Escalada J, Santos S, Millán D, et al. Body mass index classification misses subjects with increased cardiometabolic risk factors related to elevated adiposity. Int J Obes. (Lond). 2012;36(2):286-94. doi: 10.1038/ijo.2011.100.
19. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013;93(1):359-404. doi: 10.1152/physrev.00033.2011.
20. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol. 2011;29:415-45. doi: 10.1146/annurev-immunol-031210-101322.
21. Jung UJ, Choi MS. Obesity and Its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. J Mol Sci. 2014;15(4):6184-223. doi:10.3390/ijms15046184.
22. Li FY, Lam KS, Xu A. Therapeutic perspectives for adiponectin: an update. Curr Med Chem. 2012;19(32):5513-23. doi: 10.2174/092986712803833173.
23. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature. 2006;444(7121):875-80. doi: 10.1038/nature05487.
24. Barbosa-da-Silva S, Fraulob-Aquino JC, Lopes JR, Mandarim-de-Lacerda CA, Aguila MB. Weight cycling enhances adipose tissue inflammatory responses in male mice. PLoS One. 2012;7(7):e39837. doi: 10.1371/journal.pone.0039837.
25. Carvalho KM, Marinho Filho JD, de Melo TS, Araújo AJ, Quetz Jda S, da Cunha Mdo P, et al. The resin from protium heptaphyllum prevents high-fat diet-induced obesity in mice: scientific evidence and potential mechanisms. Evid Based Complement Alternat Med. 2015;2015:106157. doi: 10.1155/2015/106157.
26. Chantemele EJ, Stepp DW. Influence of obesity and metabolic dysfunction on the endothelial control in the coronary circulation. Mol Cell Cardiol. 2012;52(4):840-7. doi:10.1016/j.yjmcc.2011.08.018.
27. Bray GA. Medical consequence of obesity. J Clin Endocrinol Metab. 2004;89(6):2583-9. doi: 10.1210/jc.2004-0535.
28. De Kreutzenberg SV, Crepaldi C, Marchetto S, Calò L, Tiengo A, Del Prato S, et al. Plasma free fatty acids and endothelium-dependent vasodilation: effect of chain-length and cyclooxygenase inhibition. J Clin Endocrinol Metab. 2000;85(2):793-8. doi: 10.1210/jcem.85.2.6352.
29. Boustany-Kary CM, Gong M, Akers WS, Guo Z, Cassis LA. Enhanced vascular contractility and diminished coronary artery flow in rats made hypertensive from diet-induced obesity. Int J Obes (Lond). 2007;31(11):1652-9. doi: 10.1038/sj.ijo.0803426.
30. Kobayasi R, Akamine EH, Davel AP, Rodrigues MA, Carvalho CR, Rossoni LV. Oxidative stress and inflammatory mediators contribute to endothelial dysfunction in high-fat diet-induced obesity in mice. J Hypertens. 2010;28(10):2111-9 doi: 10.1097/HJH.0b013e32833ca68c.

31. Stapleton PA, James ME, Goodwill AG, Frisbee JC. Obesity and vascular dysfunction. Pathophysioloy. 2008;15(2):79-89. doi: 10.1016/j.pathophys.2008.04.007.

32. Prieto D, Contreras C, Sánchez A. Endothelial dysfunction, obesity and insulin resistance. Curr Vasc Pharmacol. 2014;12(3):412-26. doi: 10.2174/1570161112666140423221008.

33. Garrison RJ, Kannel WB, Stokes J 3rd, Castelli WP. Incidence and precursors of hypertension in young adults: the Framingham Offspring Study. Prev Med. 1987;16(2):235-51. doi: https://doi.org/10.1016/0091-7435(87)90087-9.

34. Boustany CN, Bharadwaj K, Daugherty A, Brown DR, Randall DC, Cassis LA. Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. Am J Physiol Regul Integr Comp Physiol. 2004;287(4):R943-9. doi: 10.1152/ajpregu.00265.2004.

35. Arribas SM, Hinek A, Gonzalez MC. Elastic fibres and vascular structure in hypertension. Pharmacol Ther. 2006;111(3):771-91. doi: 10.1016/j.pharmthera.2005.12.003.

36. Chen J, Wang S, Luo M, Zhang Z, Dai X, Kong M, et al. Zinc deficiency worsens and supplementation prevents high-fat diet induced vascular inflammation, oxidative stress, and pathological remodeling. Toxicol Sci. 2016;153(1):124-36. doi: 10.1093/toxsci/kfw110.

37. Martínez-Martínez E, Miana M, Jurado-López R, Bartolomé MV, Souza Neto FV, Salaices M, et al. The potential role of leptin in the vascular remodeling associated with obesity. Int J Obes (Lond). 2014;38(12):1565-72. doi: 10.1038/ijo.2014.37.

38. Schulz E, Gori T, Münzel T. Oxidative stress and endothelial dysfunction in hypertension. Hypertens Res. 2011;34(6):665-73. doi:10.1038/hr.2011.39

39. Vincent HK, Innes KE, Vincent KR. Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. Diabetes Obes. Metab. 2007;9(6):813-39. doi: 10.1111/j.1463-1326.2007.00692.x.

40. Victor VM, Apostolova N, Herance R, Hernandez-Mijares A, Rocha M. Oxidative stress and mitochondrial dysfunction in atherosclerosis: mitochondria-targeted antioxidants as potential therapy. Curr Med Chem. 2009;16(35):4654-67. doi: 10.2174/092986709789878265.

Pressure Overload-induced Cardiac Hypertrophy Varies According to Different Ligation Needle Sizes and Body Weights in Mice

Zhen Jia,[1] Zhijun Chen,[2] Hongfei Xu,[1] Malik Akuffu Armah,[1] Peng Teng,[1] Weidong Li,[1] Dongdong Jian,[1] Liang Ma,[1] Yiming Ni[1]

Department of Cardio-Thoracic Surgery, The First Affiliated Hospital, School of Medicine, Zhejiang University,[1] Zhejiang - China

Department of Cardio-Thoracic Surgery, Zhoushan Hospital,[2] Zhejiang - China

Abstract

Background: The cardiac hypertrophy (CH) model for mice has been widely used, thereby providing an effective research foundation for CH exploration.

Objective: To research the effects of CH modeling under abdominal aortic constriction (AAC) using different needles and weights in mice.

Methods: Four needles with different external diameters (0.35, 0.40, 0.45, and 0.50 mm) were used for AAC. 150 male C57BL/6 mice were selected according to body weight (BW) and divided into 3 weight levels: 18 g, 22 g, and 26 g (n = 50 in each group). All weight levels were divided into 5 groups: a sham group (n = 10) and 4 AAC groups using 4 ligation intensities (n = 10 per group). After surgery, survival rates were recorded, echocardiography was performed, hearts were dissected and used for histological detection, and data were statistically analyzed, $P < 0.05$ was considered statistically significant.

Results: All mice died in the following AAC groups: 18g/0.35 mm, 22 g/0.35 mm, 26 g/0.35 mm, 22 g/0.40 mm, and 26 g/0.40 mm. All mice with AAC, those ligated with a 0.50-mm needle, and those that underwent sham operation survived. Different death rates occurred in the following AAC groups: 18 g/0.40 mm, 18 g/0.45 mm, 18 g/0.50 mm, 22 g/45 mm, 22 g/0.50 mm, 26 g/0.45 mm, and 26 g/0.50 mm. The heart weight/body weight ratios (5.39 ± 0.85, 6.41 ± 0.68, 4.67 ± 0.37, 5.22 ± 0.42, 4.23 ± 0.28, 5.41 ± 0.14, and 4.02 ± 0.13) were significantly increased compared with those of the sham groups for mice with the same weight levels.

Conclusion: A 0.45-mm needle led to more obvious CH than did 0.40-mm and 0.50-mm needles and caused extraordinary CH in 18-g mice. (Arq Bras Cardiol. 2018; 110(6):568-576)

Keywords: Cardiomegaly; Body Weight; Heart Failure; Needles/utilization; Rats.

Introduction

Cardiac hypertrophy (CH) is a compensatory pathological change that is usually induced by pressure overload (PO), neurohumoral abnormality, and the effects of cytokines. It is characterized by cardiomyocyte hypertrophy and interstitial hyperplasia, and it results in an enlarged heart and thickening of the heart walls. Clinically, CH is involved in the development of many diseases, such as valvular disease, hypertension, arterial stenosis, and primary myocardial hypertrophy. If these diseases develop at their own pace, then cardiac function (CF) will gradually decompensate, leading to heart failure (HF), which severely lowers the quality of life and increases the mortality rate. Therefore, CH is a widespread concern and has been explored at the molecular level by researchers. Due to the high genomic homology between mice and humans, an established CH model for mice has been widely used in animal experiments, thereby providing an effective research foundation for CH exploration.

Currently, PO-induced CH is a common way to establish the model. Abdominal aortic constriction (AAC) is highly recommended by researchers because of the high success rate and the ability to perform surgery without the need for thoracotomy or a ventilator. However, the modeling effects with different ligating intensities for certain body weights (BWs) have not yet been reported. Therefore, we used 3 frequently used mice BWs (18 g, 22 g, and 26 g) and 4 different needle sizes (0.35, 0.40, 0.45, and 0.50 mm) to establish the CH model for each weight level for AAC, summarized the survival rates, and evaluated the CH effects.

Mailing Address: Yiming Ni •
Department of Cardio-Thoracic Surgery, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310003 – China
E-mail: 1183020@zju.edu.cn
Manuscript received August 21, 2017, revised manuscript October 25, 2017, accepted November 24, 2017

DOI: 10.5935/abc.20180088

Methods

Animal groups and handling

One-hundred fifty male C57BL/6 wild-type mice were obtained from the Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China). All animals were treated and cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, Washington, DC, 1996). Experimental protocols were approved by our Institutional Animal Care and Use Committee of Zhejiang University (Hangzhou, China). Mice were selected according to weights of approximately 18 g (range, 17.3-18.7 g), 22 g (range, 20.8-23.0 g), and 26 g (range, 25.1-27.0 g), and they were divided into the following 3 weight levels: 18 g (18.0 ± 0.3 g; n = 50), 22 g (22.0 ± 0.6 g; n = 50), and 26 g (26.1 ± 0.5 g; n = 50). All weight levels were divided using sortition randomization method to create a sham group (n = 10) and 4 AAC groups according to ligating intensities (0.35, 0.40, 0.45, and 0.50mm; n = 10 per group). Regarding BW, no significant differences were found among the 5 groups for each weight level (Table S1), and the preoperative BWs of mice that died and those that survived were not significant (Table S2).

Mice were anesthetized with 4% chloralhydrate (0.1ml/1g BW, intraperitoneal injection). When the mice did not respond when their toe was pinched, the limbs were fixed on the operating board in the supine position and the skin was prepared by shaving and disinfection with alcohol. Sterile gauze was placed on the right side of the abdomen and a ventrimesal incision approximately 1.5 cm was created starting from the xiphoid. The skin was fixed with a spreader and the viscera was pulled out gently with a swab and placed on the gauze. Then, the abdominal aorta was isolated using a blunt dissection technique with curved microforceps under a microscope. A 6–0 silk suture was snared and pulled back around the aorta 1mm above the superior mesenteric artery. A 2-mm blunt acupuncture needle (external diameters: 0.35 mm, 0.40 mm, 0.45 mm, and 0.50 mm; Huatuo; Suzhou Medical Appliance Factory, Suzhou, China; criterion number GB2024-1994) was then placed next to the aorta. The suture was tied snugly around the needle and the aorta. The needle was removed immediately after ligation, the viscera were replaced, the peritoneum and skin were sutured, and the mice were allowed to recover. Aortic ligation was omitted only for the sham group. After surgery, the ears were cut to differentiate the mice. Then, mice were placed in an incubator at 30°C until they woke, and they were returned to their cages. Survival status was recorded daily. To observe the physical development of mice under different conditions, BW differences before surgery and at week 8 post-surgery were calculated as the change in BW.

Echocardiography imaging

After post-surgery weeks 4 and 8, mice were weighed and anesthetized with 4% chloralhydrate and placed on a warming pad after skin preparation. Transthoracic 2-dimensional (2D) echocardiography was performed using the GE Vivid E9 Ultrasound echocardiographic system (General Electric Company, Fairfield, CT, USA) with the GE 9L probe (8-MHz linear array transducer; General Electric Company). M-mode parasternal long-axis scans of the left ventricle at the mitral chordae level were used to quantify the interventricular septum thickness at end-diastole (IVSd), interventricular septum thickness at end-systole (IVSs), left ventricular internal dimension at end-diastole (LVIDd), left ventricular internal dimension at end-systole (LVIDs), left ventricular posterior wall thickness at end-diastole (LVPWd), left ventricular posterior wall thickness at end-systole (LVPWs), ejection fraction (EF), and fractional shortening (FS). All mice were tested using the same parameters.

Heart weight, heart weight/body weight, and heart weight/tibial length

After echocardiographic analysis at 8 weeks post-surgery, mice were sacrificed by cervical dislocation and the hearts were dissected. Then, atrial and vascular tissues were snipped carefully, leaving the ventricles. The hearts were rinsed with phosphate-buffered saline (PBS), drained by gently squeezing on absorbent paper, weighed, photographed under natural light, and fixed in 4% paraformaldehyde. The tibial lengths (TLs; mean value of the bilateral tibia) were recorded. Heart weight (HW), BW, and TL were measured, and the HW/BW ratio and HW/TL ratio were calculated to evaluate the hypertrophic response to PO.

Histological examination of the heart

Extracted hearts were fixed in 4% paraformaldehyde for 24h and dehydrated. After routine histologic procedures, the hearts were embedded in paraffin and cut into 4-μm sections. Sections were stained with hematoxylin and eosin (HE) and picrosirius red (PSR). Cardiac cross-sections were captured at 20 × microscopic views from HE sections, and 5 thicknesses of the left ventricle in each view were selected in systematic sampling, and measured using Image-Pro Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA). Then, the mean values were calculated. Cardiomyocyte morphological changes were captured at 400×microscopic views from HE sections. Interstitial and/or perivascular collagen depositions were captured at 200×microscopic views under standard lights. Collagen was stained red using PSR, thereby indicating fibrosis. At least 6 views were selected in a blinded manner, and each photograph was analyzed to reveal the ratio of red collagen to the entire tissue area using Image-Pro Plus 6.0. Then, the mean values were calculated.

Statistical Analysis

SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. The Kolmogorov-Smirnov (K-S) test was used to verify the normality of the quantitative variables as appropriate. Data are presented as mean ± standard deviation (SD). One-way ANOVA and post-hoc Tukey tests were used to evaluate differences between groups. $p < 0.05$ was considered statistically significant.

Results

Excessive AAC may lead to death

We monitored mice deaths after surgery according to acute heart failure (AHF) criteria. Data (Table 1) showed that all deaths occurred within 5 days, and a high incidence of death occurred during the initial 24h post-surgery.

AAC increases cardiac dimensions and reduces cardiac function

Echocardiography was performed at the end of post-operative weeks 4 and 8. At week 4 post-surgery, data (Table 2) showed a trend of heart enlargement for mice with AAC, including thickening of the ventricular wall and an increase in chamber dilation; however, differences in EF and FS were not significant, indicating that changes in the heart structure did not have a pronounced effect on cardiac function at that time point. At week 8 post-surgery, the trend of heart enlargement continued; however, the EF and FS values for the AAC groups decreased significantly. This change in cardiac function from week 4 to week 8 was consistent with systolic function beginning to be markedly affected at week 4 after PO surgery.

AAC increases HW, HW/BW, and HW/TL ratio

Generally, the increased HW, HW/BW, and HW/TL ratio are the three main indicators of CH. In our study, as shown in Table 3, we found that AAC significantly increased HW and caused a significantly higher HW/BW ratio and HW/TL ratio compared to the sham groups for all weight levels. The HW, HW/BW, and HW/TL values for the AAC0.45 mm groups were significantly higher than those for the AAC0.50 mm groups. These HW-related indices for the 18 g/0.45 mm groups were even significantly higher than those for the 18 g/0.40 mm groups.

AAC leads to cardiomyocyte hypertrophy and increases collagen depositions

For mice undergoing AAC surgery, the hearts demonstrated different degrees of enlargement (Figure 1A), enlargement of the papillary muscles, and thickening of the ventricular walls (Figure 1B). Wall thickening increased significantly compared with that of the sham group (Table 4). The sham groups showed normal architecture of the cardiomyocytes compared with the AAC groups. Pathological changes including enlarged, disarrayed, and eosinophilic cardiomyocytes and cardiomyocytes rich in cytoplasm and trachychromatic and pantomorphic nuclei were observed in each of the AAC groups (Figure 1C). Scattered collagen depositions in the interstitial and perivascular spaces were observed in the sham groups. In comparison, in some AAC groups, a larger quantity and wider range of red deposits were observed in the interstitial space (Figure 1D), and thickened collagen was observed in the perivascular space, especially in the external vascular wall (Figure 1E). Statistical analysis indicated that the AAC group had a significantly greater collagen area than the sham group (Table 5). These results imply that AAC is capable of inducing PO-induced CH and fibrosis.

AAC may restrict physical development

Analysis showed that with AAC 0.45 mm, BW significantly increased in 18-g mice compared to 22-g and 26-g mice (Table 6), indicating that the 18-g groups had higher development potential. In the 18-g mice groups, data showed that the value of 18 g/0.40 mm was significantly lower than that of the 18 g/0.45 mm and 18 g/sham groups, and that there were no significant differences between the 18 g/0.45 mm and 18 g/sham groups (Table 7), indicating that the 18 g/0.45 mm group had nearly normal physical development. Development of the 18 g/0.40 mm group was limited.

Discussion

In this study, we performed AAC according to 4 different ligating intensities for mice of 3 different weight levels to evaluate the survival rates of mice and CH induced by PO under different conditions. This is the first study showing that CH diversities exist among groups under different ligations and BW.

AAC is widely used in the modeling of CH induced by PO in mice. Needle ligation is usually used, and the efficiency of modeling is highly dependent on ligation intensity. Nevertheless, excessive constriction will lead to death,[1] and our research findings (Table 1) demonstrated this point. In this study, a 0.35-mm needle caused the death of all mice in the 3 weight levels, and the 0.40-mm needle caused the death of all mice in 22-g and 26-g groups. Contrarily, all mice with AAC that underwent surgery with a 0.50-mm needle or sham operation survived. Mice in the other groups had different mortality rates. Regarding the selection of needles for the BW ranges of this study, a needle smaller than 0.35mm in diameter caused stronger constriction and death. However, a needle larger than 0.50 mm in diameter did not alternatively affect the survival rate, but it did reduce the efficiency of CH because of the reduced PO from weaker constriction. This is why we chose needles between 0.35 mm and 0.50 mm.

Table 1 – Mice deaths after surgery

	Needles (mm) for 18 g			Needles (mm) for 22 g			Needles (mm) for 26 g		
	0.35	0.40	0.45	0.35	0.40	0.45	0.35	0.40	0.45
0-24 h	10	4	1	10	7	1	10	8	3
24 h-3 d	0	0	0	0	2	0	0	1	2
3 d-5 d	0	0	1	0	1	2	0	1	1

There were no mice deaths in the AAC0.50-mm group or the sham group. Deaths were recorded during 3 time periods (0-24h, 24h-3d, and 3-5 d); 54 deaths occurred within 0-24h post-surgery. The total number of deaths was 65.

Table 2 – Echocardiographic outcomes of 18-g, 22-g, and 26-g mice

	18 g/0.40 mm (n = 6)	18 g/0.45 mm (n = 8)	18 g/0.50 mm (n = 10)	18 g/Sham (n = 10)	22 g/0.45 mm (n = 7)	22 g/0.50 mm (n = 10)	22 g/Sham (n = 10)	26 g/0.45 mm (n = 4)	26 g/0.50 mm (n = 10)	26 g/Sham (n = 10)
Week 4										
IVSd	0.92 ± 0.05*	0.96 ± 0.05*	0.86 ± 0.05	0.81 ± 0.04	0.82 ± 0.06	0.83 ± 0.04	0.78 ± 0.04	0.84 ± 0.03	0.80 ± 0.04	0.80 ± 0.04
IVSs	1.12 ± 0.05	1.24 ± 0.15*	1.08 ± 0.10	1.05 ± 0.04	1.06 ± 0.07*	1.00 ± 0.04	0.98 ± 0.03	1.15 ± 0.08	1.11 ± 0.07	1.08 ± 0.08
LVIDd	3.21 ± 0.31	3.33 ± 0.26	3.00 ± 0.16	3.08 ± 0.28	3.04 ± 0.20	3.01 ± 0.17	3.00 ± 0.19	3.40 ± 0.11*	3.11 ± 0.15	2.96 ± 0.22
LVIDs	2.13 ± 0.26	2.37 ± 0.26*	2.03 ± 0.11	2.09 ± 0.21	1.92 ± 0.13	1.98 ± 0.14	2.07 ± 0.18	2.24 ± 0.18*	2.02 ± 0.13	1.93 ± 0.14
LVPWd	0.94 ± 0.04*	1.01 ± 0.08*	0.87 ± 0.04	0.81 ± 0.09	0.85 ± 0.04	0.87 ± 0.05*	0.81 ± 0.07	0.90 ± 0.06	0.89 ± 0.05	0.87 ± 0.06
LVPWs	1.13 ± 0.06	1.24 ± 0.06*	1.08 ± 0.09	1.04 ± 0.03	1.04 ± 0.04	1.00 ± 0.07	1.04 ± 0.10	1.27 ± 0.04*	1.12 ± 0.07	1.10 ± 0.05
EF %	70.7 ± 3.8	66.0 ± 3.9	67.9 ± 3.5	68.5 ± 2.6	72.1 ± 4.5	68.3 ± 3.7	70.6 ± 3.6	70.5 ± 5.4	71.2 ± 3.1	73.1 ± 4,.7
FS %	34.5 ± 2.4	31.2 ± 2.1*	34.5 ± 2.3	34.3 ± 2.4	36.9 ± 2.3	35.7 ± 3.0	36.7 ± 2.8	36.3 ± 2.6	37.5 ± 2.3	36.6 ± 3.2
Week 8										
IVSd	0.93 ± 0.08*	0.97 ± 0.05*	0.88 ± 0.04	0.83 ± 0.07	0.99 ± 0.06*	0.91 ± 0.02*	0.84 ± 0.07	0.93 ± 0.08	0.91 ± 0.06	0.85 ± 0.06
IVSs	1.18 ± 0.20	1.33 ± 0.14*	1.15 ± 0.07	1.10 ± 0.10	1.26 ± 0.07*	1.16 ± 0.10*	1.04 ± 0.08	1.19 ± 0.10	1.17 ± 0.11	1.14 ± 0.07
LVIDd	3.34 ± 0.24	4.12 ± 0.34*	3.30 ± 0.41	3.14 ± 0.15	3.26 ± 0.13	3.15 ± 0.13	3.23 ± 0.15	3.50 ± 0.12*	3.29 ± 0.16	3.20 ± 0.15
LVIDs	2.13 ± 0.11	3.02 ± 0.27*	2.21 ± 0.40	1.94 ± 0.18	2.25 ± 0.11	2.11 ± 0.14	2.19 ± 0.12	2.50 ± 0.15*	2.33 ± 0.26	2.14 ± 0.15
LVPWd	0.96 ± 0.08*	1.03 ± 0.08*	0.93 ± 0.04*	0.84 ± 0.08	0.99 ± 0.05*	0.96 ± 0.04*	0.90 ± 0.06	1.02 ± 0.07*	0.96 ± 0.04*	0.90 ± 0.04
LVPWs	1.23 ± 0.08*	1.35 ± 0.13*	1.20 ± 0.07*	1.07 ± 0.07	1.22 ± 0.06*	1.14 ± 0.07	1.07 ± 0.08	1.30 ± 0.08*	1.13 ± 0.05*	1.04 ± 0.06
EF %	64.7 ± 4.6*	60.9 ± 2.4*	67.6 ± 4.7*	75.5 ± 5.5	63.3 ± 3.0*	67.7 ± 3.3*	74.2 ± 3.2	62.8 ± 2.6*	67.5 ± 5.3*	73.1 ± 2.9
FS %	33.2 ± 3.0*	29.4 ± 1.9*	35.8 ± 4.3*	41.0 ± 5.5	32.9 ± 1.6*	35.5 ± 2.4*	40.8 ± 3.1	31.0 ± 3.2*	32.7 ± 3.8*	36.9 ± 2.5

*IVSd: interventricular septum thickness at end-diastole; IVSs: interventricular septum thickness at end-systole; LVIDd: left ventricular internal dimension at end-diastole; LVIDs: left ventricular internal dimension at end-systole; LVPWd: left ventricular posterior wall thickness at end-diastole; LVPWs: left ventricular posterior wall thickness at end-systole; EF: ejection fraction; FS: fractional shortening. The cardiac dimensions (incldng IVSd, IVSs, LVIDd, LVIDs, LVPWd, and LVPWs) (mm) and functional indices (incldng EF and FS) changes measured by echocardiography. Data were statistically analyzed and presented as the mean ± SD. At week 4, cardiac dimensions for the AAC groups significantly increased compared with the sham groups (*p < 0.05); at week 8 , more cardiac dimensions significantly increased, and that EF and FS values for the AAC groups all decreased significantly compared to those of the sham groups for 3 weight levels (*p < 0.05).*

Table 3 – Heart weight–related indices of 18-g, 22-g, and 26-g mice

	18 g/0.40 mm (n = 6)	18 g/0.45 mm (n = 8)	18 g/0.50mm (n = 10)	18 g/Sham (n = 10)	22 g/0.45 mm (n = 7)	22 g/0.50 mm (n = 10)	22 g/Sham (n = 10)	26 g/0.45 mm (n = 4)	26 g/0.50 mm (n = 10)	26 g/Sham (n = 10)
HW	136.5 ± 22.3*	170.0 ± 21.4**	124.0 ± 9.9*	103.5 ± 7.0	137.1 ± 7.4**	115.5 ± 7.6*	104.3 ± 7.4	153.5 ± 4.8**	114.3 ± 5.1*	103.2 ± 5.6
HW/BW	5.39 ± 0.85*	6.41 ± 0.68**	4.67 ± 0.37*	3.86 ± 0.18	5.22 ± 0.42**	4.23 ± 0.28*	3.62 ± 0.26	5.41 ± 0.14**	4.02 ± 0.13*	3.59 ± 0.16
HW/TL	63.8 ± 10.3*	74.4 ± 9.3**	57.6 ± 4.6*	47.8 ± 3.6	59.6 ± 3.3**	54.2 ± 3.8*	48.3 ± 3.9	65.6 ± 1.3**	49.2 ± 2.6*	44.1 ± 2.8

*HW: heart weight; BW: body weight; TL: tibial length. HW(mg), HW/BW(mg/g), and HW/TL(mg/cm) were measured and calculated from AAC and sham groups of 3 BW levels. Data are presented as the mean ± SD. *Compared to the sham group in the same BW level, the heart weight–related indices of AAC groups increased significantly (p < 0.05). **Compared to the rest groups in the same BW level, the heart weight–related indices of the AAC 0.45-mm group increased significantly (p < 0.05).*

Table 4 – Thickness of the left ventricle (mm) based on weight and needle size

Weight	0.40 mm	0.45 mm	0.50 mm	Sham
18 g	1.81 ± 0.30*	1.86 ± 0.17*	1.59 ± 0.09*	1.27 ± 0.07
22 g		1.69 ± 0.24*	1.55 ± 0.19*	1.22 ± 0.14
26 g		1.82 ± 0.30*	1.59 ± 0.22*	1.34 ± 0.07

*Data are presented as the mean ± SD (n = 5). *p < 0.05 represents a significant difference between the abdominal aortic constriction (AAC) and sham groups.*

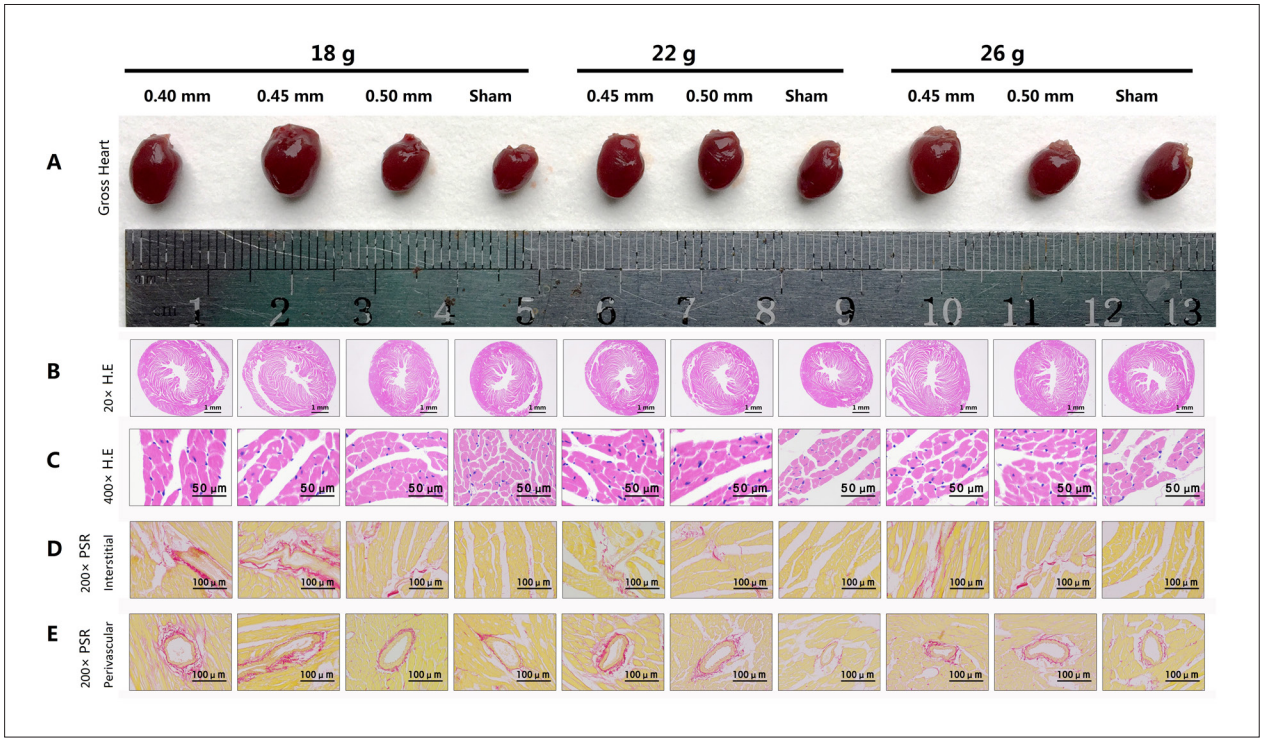


Figure 1 – *Cardiomyocyte hypertrophy and collagen deposition histological examination. (A) Gross hearts under natural light. (B) The 20 × microscopic views of HE sections. (C) The 400 × microscopic views of HE sections. (D) Representative 200×microscopic views under standard lights of PSR sections in the interstitial space. (E) Representative 200 × microscopic views under standard lights of PSR sections in the perivascular space. Fibrosis is presented as red in the PSR sections.*

Death can occur after AAC. Undoubtedly, AAC increases cardiac afterloading. To cope with the additional biodynamics, the heart exerts a series of adaptive changes, including activation and hypertrophy of cardiomyocytes and hyperplasia of the extracellular matrix.[2] This compensational mechanism maintains cardiac output (CO) effectively for a period of time while maintaining the survival of the organism; it is also the basis for the establishment of the CH model. However, when the sudden afterloading is out of the range of cardiomyocyte adjustment, the bloodstream will be limited and cause constriction, resulting in AHF. AHF is typically characterized by rapid changes in heart failure (HF) symptoms.[3] Sato et al.[4] considered the incidence of death within 5 days as an assessment criterion of AHF. AHF could moderately or markedly improve by the second day if effectively controlled. AHF leads to high ventricular pressure, and high ventricular pressure leads to high pulmonary blood pressure, thus leading to pulmonary congestion, which is one of the causes of death after AAC.[5] Liao et al.[6] suggested that cardiogenic pneumo-edema is the main cause of postoperative death for PO mice. Additionally, arrhythmia may occur as part of the electrophysiological changes,[7] and cardiomyocyte sarcomeres may be disordered during the pathological changes.[8] These are all severe threats to the survival rate after AAC. Our record of mice death times (Table 1) showed the phenomenon of all deaths occurring within 5 days. A high incidence of death occurred during the initial 24h, which is in accordance with the aforementioned AHF criteria. In addition, there is a positive correlation between CO and BW;[9,10] therefore, compared with the low-weight mice, high-weight mice require more CO and will have cardiac afterloading that is more increased than that of low-weight mice with the same aortic constriction. Results of the current study (Table 1) indicate that higher-weight mice had poorer tolerance for AAC, which is reflected in their mortality rates. Regarding mice with AAC that underwent ligation with a 0.40-mm needle, all mice in the 22-g and 26-g groups died. However, 6 out of 10 mice survived in the 18-g group.

Table 5 – Percentage of collagen deposition in the left ventricle based on weight and needle size

Weight	0.40 mm	0.45 mm	0.50 mm	Sham
18 g	5.8 ± 2.2*	8.9 ± 1.3*	5.1 ± 1.3*	2.6 ± 1.0
22 g		5.2 ± 1.6*	4.9 ± 1.5*	2.5 ± 0.9
26 g		6.1 ± 1.0*	5.3 ± 1.8*	3.1 ± 0.8

*Data are presented as the mean ± SD (n = 6). *p < 0.05 represents a significant difference between the abdominal aortic constriction (AAC) and sham groups.*

Table 6 – Body weight changes with AAC under 0.45 mm needle

	18 g/0.45 mm (n = 8)	22 g/0.45 mm (n = 7)	26 g/0.45 mm(n = 4)
Change in BW(g)	8.4 ± 0.8*	4.4 ± 0.8	2.4 ± 0.3

*BW: body weight; AAC: abdominal aortic constriction. Data are presented as the mean ± SD. *p < 0.05 represents a significant difference between the 18 g/0.45 mm group and the 22 g/0.45 mm and 26 g/0.45 mm groups.*

Table 7 – Body weight and BW changes in 18-g mice

	18 g/0.40 mm (n = 6)	18 g/0.45 mm (n = 8)	18 g/0.50 mm (n = 10)	18 g/Sham (n = 10)
BW before surgery (g)	18.1 ± 0.4	18.1 ± 0.3	18.0 ± 0.4	17.9 ± 0.4
BW at week 8 (g)	25.3 ± 0.4*	26.5 ± 0.9	26.6 ± 0.8	26.8 ± 0.9
BW change (g)	7.2 ± 0.6*	8.4 ± 0.8	8.6 ± 0.6	8.8 ± 0.9

*BW: body weight. BW changes of 18-g mice before and after surgery for 8 weeks. Data are presented as the mean ± SD. *p < 0.05 represents a significant difference between the 18 g/0.40 mm group and the rest groups after surgery.*

The diagnosis of CH usually depends on changes in cardiac function and morphology.[11] Echocardiography can be performed in vitro noninvasively during the first assessment of CH, and it is especially used to monitor changes in cardiac function.[12] We performed echocardiographic examinations of mice at the end of week 4 and week 8 post-surgery. Data (week 4 data in Table 2) showed that at the end of week 4, the phenomena of thickened ventricular walls, enlarged ventricular chambers, and decreased cardiac functions were emerging in each AAC group compared with the sham groups, and this diversity was consistent with the characteristic cardiac changes that occur with chronic pressure overload.[13,14] These trends became more pronounced at the end of week 8 (week 8 data in Table 2), when EF and FS, which represent cardiac function, were significantly lower compared with the sham groups. CH also increased HW. In our study, the HW, HW/BW ratio, and HW/TL ratio for the AAC groups were significantly increased (Table 3). Cardiac remodeling is the most typical pathological change of CH, including cardiomyocyte hypertrophy and the extracellular matrix increases.[15] Our histological results showed increased external diameters and ventricular thickness in gross hearts and cross-sections under AAC (Figures 1A and B). HE staining of the AAC groups displayed the hypertrophic pathology of cardiomyocytes and nuclei (Figure 1C). PSR staining of the AAC groups displayed extensive collagen depositions (Figure 1D), particularly in the perivascular space (Figure 1E). Statistical analysis showed that the thickness of the left ventricle (Table 4) and the percentage of collagen deposition (Table 5) were significantly increased in the AAC groups compared to the sham group. Regarding the formation of collagen, Kuwahara et al.[16] indicated that cardiac fibroblasts are activated on day 3 after PO, and that the neoformative fibrous tissues mainly affect the diastolic function rather than the systolic function during the initial 4 weeks. Then, excessive myocardial fibrosis is implicated in systolic dysfunction because of its more intensive traction, and cardiac function begins to deteriorate significantly. Regarding EF and FS values for the AAC groups (Table 2), the downward trends from week 4 to week 8 conform to this theory.

Choosing the proper needle is critical for establishing the CH model. Based on these results, we found that all mice with AAC died when a 0.35-mm needle was used for ligation for all 3 weight levels and when a 0.40-mm needle was used for ligation for the 22-g and 26-g groups; therefore, these 5 groups of weight–needle pairings were clearly unsuitable for use. The 18g/0.40mm group had obvious CH compared with the sham group, and its survival rate was acceptable (6 out of 10). However, it should still be excluded because the 18 g/0.45 mm group showed more obvious CH and higher survival rates (8 out of 10) (Table 1, Table 3). The 0.45-mm and 0.50-mm needles are available for all 3 weight levels, but both can result in definite myocardial hypertrophy. However, the values of the HW, HW/BW ratio, and HW/TL ratio for the AAC mice when using the 0.45-mm needle were significantly higher than those when using a 0.50-mm needle for each weight level (Table 3). Therefore, for all 3 weight levels of our study, a CH model can be established using a 0.50-mm needle and the survival rate of the mice will not be threatened. However, a 0.45-mm needle leads to more effective CH model, and higher mortality than the 0.50-mm needle.

Normally, with the PO-induced CH model, thinner needles creates more severe aortic stenosis and lead to more pronounced CH, and vice versa. However, we observed an interesting phenomenon: the CH level of the 18 g/0.45 mm group was abnormally significantly higher than that of the 18 g/0.40 mm group (18-g mice in Table 3). Regarding the analysis of BW data with AAC (Table 6), the changes in BW in 18-g mice during weeks 0 to 8 were significantly higher than those for the 22-g and 26-g mice, indicating that 18-g mice have greater potential for physical development after surgery and that physical development is often accompanied by organ development.[17] Therefore, the heart of 18-g mice also has greater development potential. For the same weight level, the BW change of the 18 g/0.45 mm group during weeks 0 to 8 was significantly higher than that of the 18 g/0.40 mm group (BW change in Table 7). As mentioned, BW is positively related to CO; therefore, perhaps the greater ligation limited CO in the 18 g/0.40 mm group, which also limited physical development and organ development, including development of the heart. At the end of week 8, there was no significant difference in BW for the 18g/0.45mm group and 18g/sham groups; both had significantly higher BW than the 18 g/0.40 mm group (BW at week 8 in Table 7). The 0.45-mm needle had no obvious limits in18-g mice, but the BW advantage for the 18 g/0.45 mm group compared to the 18 g/0.40 mm group depends on greater CO and requires more hypertrophic myocardium for support. So, to establish CH models for AAC in mice that have developmental potential, such as 18-g mice, there may be a special ligating intensity region that can cause more obvious CH than the two adjacent regions. However, this phenomenon must comprise multiple factors and is worth further study.

Conclusion

We established CH models using 4 ligation needle sizes and 3 weights for mice. Data showed that both of 0.45-mm and 0.50-mm needles lead to CH. However, 0.45mm needle brings more effective model and causes obvious CH in 18-g mice.

Author contributions

Conception and design of the research: Zhen J, Yiming N; Acquisition of data: Zhen J, Chen Z, Hongfei X, Dongdong J; Analysis and interpretation of the data: Zhen J, Chen Z; Statistical analysis: Zhen J, Chen Z, Peng T; Obtaining financing: Hongfei X; Writing of the manuscript: Zhen J, Dongdong J; Critical revision of the manuscript for intellectual content: Armah MA, Weidong L, Liang M.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by The National Natural Science Foundation of China (number 81570343).

Study Association

This article is part of the thesis of Doctoral submitted by Zhen Jia, from Zhejiang University.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University under the protocol number 2014-17. 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.

References

1. Zhao CZ, Zhao XM, Yang J, Mou Y, Chen B, Wu HD, et al. Inhibition of farnesyl pyrophosphate synthase improves pressure overload induced chronic cardiac remodeling. Sci Rep. 2016 Dec 23;6:39186. doi: 10.1038/srep39186.
2. Frey N, Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. Annu Rev Physiol. 2003;65:45-79. doi: 10.1146/annurev.physiol.65.092101.142243.
3. Alla F, Zannad F, Filippatos G. Epidemiology of acute heart failure syndromes. Heart Fail Rev. 2007;12(2):91-5. doi: 10.1007/s10741-007-9009-2.
4. Sato N, Lam CS, Teerlink JR, Greenberg BH, Tsutsui H, Oh BH, et al. Evaluating the efficacy, safety, and tolerability of serelaxin when added to standard therapy in Asian patients with acute heart failure: design and rationale of RELAX-AHF-ASIA trial. J Card Fail. 2017;23(1):63-71. doi: 10.1016/j.cardfail.2016.10.016.
5. Yamamoto K, Ohishi M, Katsuya T, Ito N, Ikushima M, Kaibe M, et al. Deletion of angiotensin-converting enzyme 2 accelerates pressure overload-induced cardiac dysfunction by increasing local angiotensin II. Hypertension. 2006;47(4):718-26. doi: 10.1161/01.HYP.0000205833.89478.5b.
6. Liao Y, Bin J, Asakura M, Xuan W, Chen B, Huang Q, et al. Deficiency of type 1 cannabinoid receptors worsens acute heart failure induced by pressure overload in mice. Eur Heart J. 2012;33(24):3124-33. doi: 10.1093/eurheartj/ehr246.
7. Martinez-Rubio A, Schwammenthal Y, Schwammenthal E, Block M, Reinhardt L, Garcia-Alberola A, et al. Patients with valvular heart disease presenting with sustained ventricular tachyarrhythmias or syncope: results of programmed ventricular stimulation and long-term follow-up. Circulation. 1997;96(2):500-8. doi: https://doi.org/10.1161/01.CIR.96.2.500.
8. Massengill MT, Ashraf HM, Chowdhury RR, Chrzanowski SM, Kar J, Warren SA, et al. Acute heart failure with cardiomyocyte atrophy induced in adult mice by ablation of cardiac myosin light chain kinase. Cardiovasc Res. 2016;111(1):34-43. doi: 10.1093/cvr/cvw069.
9. Foppa M, Arora G, Gona P, Ashrafi A, Salton CJ, Yeon SB, et al. Right ventricular volumes and systolic function by cardiac magnetic resonance and the impact of sex, age, and obesity in a longitudinally followed cohort free of pulmonary and cardiovascular disease: the Framingham Heart Study. Circ Cardiovasc Imaging. 2016;9(3):e003810. doi: 10.1161/CIRCIMAGING.115.003810.

10. Karimian S, Stein J, Bauer B, Teupe C. Impact of severe obesity and weight loss on systolic left ventricular function and morphology: assessment by 2-dimensional speckle-tracking echocardiography. J Obes. 2016;2016:2732613. doi: 10.1155/2016/2732613.

11. Elliott P, McKenna WJ. Hypertrophic cardiomyopathy. Lancet. 2004;363(9424):1881-91. doi: 10.1016/S0140-6736(04)16358-7.

12. Zhao B, Wang S, Chen J, Ji Y, Wang J, Tian X, et al. Echocardiographic characterization of hypertrophic cardiomyopathy in Chinese patients with myosin-binding protein C3 mutations. Exp Ther Med. 2017;13(3):995-1002. doi: 10.3892/etm.2017.4089.

13. Hunter JJ, Chien KR. Signaling pathways for cardiac hypertrophy and failure. N Engl J Med. 1999;341(17):1276-83. doi: 10.1056/NEJM199910213411706.

14. Huang CK, Chen BY, Guo A, Chen R, Zhu YQ, Kutschke W, et al. Sildenafil ameliorates left ventricular T-tubule remodeling in a pressure overload-induced murine heart failure model. Acta Pharmacol Sin. 2016;37(4):473-82. doi: 10.1038/aps.2016.13.

15. Tsotetsi OJ, Woodiwiss AJ, Netjhardt M, Qubu D, Brooksbank R, Norton GR. Attenuation of cardiac failure, dilatation, damage, and detrimental interstitial remodeling without regression of hypertrophy in hypertensive rats. Hypertension. 2001;38(4):846-51. doi: https://doi.org/10.1161/hy1001.092649.

16. Kuwahara F, Kai H, Tokuda K, Kai M, Takeshita A, Egashira K, et al. Transforming growth factor-beta function blocking prevents myocardial fibrosis and diastolic dysfunction in pressure-overloaded rats. Circulation. 2002;106(1):130-5. doi: https://doi.org/10.1161/01.CIR.0000020689.12472.E0.

17. Minior VK, Levine B, Ferber A, Guller S, Divon MY. Nucleated red blood cells as a marker of acute and chronic fetal hypoxia in a rat model. Rambam Maimonides Med J. 2017;8(2). doi: 10.5041/RMMJ.10302.

Supplementary Materials

Table S1 – Mice body weights before surgery

Weight	0.35 mm	0.40 mm	0.45 mm	0.50 mm	Sham
18 g	18.0 ± 0.4	18.0 ± 0.3	18.1 ± 0.3	18.0 ± 0.4	17.9 ± 0.4
22 g	22.1 ± 0.4	22.0 ± 0.6	21.9 ± 0.6	22.2 ± 0.7	22.1 ± 0.6
26 g	26.0 ± 0.7	26.1 ± 0.5	26.0 ± 0.5	25.9 ± 0.3	26.3 ± 0.5

No significant differences were found among the 5 groups according to pair-wise comparisons of each weight level ($p > 0.05$); therefore, body weight could be considered one index for the same weight level. Data are presented as the mean ± SD (g) (n = 10). Body weights did not differ significantly from each other ($p > 0.05$).

Table S2 – Mice body weights before abdominal aortic constriction

Weight	Survival	Death
18 g	18.1 ± 0.3 (n = 24)	18.0 ± 0.3 (n = 16)
22 g	22.1 ± 0.7 (n = 17)	22.0 ± 0.5 (n = 23)
26 g	25.9 ± 0.4 (n = 14)	26.1 ± 0.6 (n = 26)

Retrospective data showed that the difference between weight at death and survival were not significant for each weight level ($p > 0.05$), indicating that individual weight differences for the same weight level had no influence on postoperative death. Data are presented as mean ± SD (g).

Pulmonary Ultrasound in Patients with Heart Failure - Systematic Review

Rafael Tostes Muniz,[1,2] Evandro Tinoco Mesquita,[1] Celso Vale Souza Junior,[2] Wolney de Andrade Martins[1,2]

Programa de Pós-graduação em Ciências Cardiovasculares da Universidade Federal Fluminense (UFF),[1] Niterói, RJ - Brazil

Complexo Hospitalar de Niterói,[2] Niterói, RJ – Brazil

Abstract

Pulmonary congestion is an important clinical finding in patients with heart failure (HF). Physical examination and chest X-ray have limited accuracy in detecting congestion. Pulmonary ultrasound (PU) has been incorporated into clinical practice in the evaluation of pulmonary congestion. This paper aimed to perform a systematic review of the use of PU in patients with HF, in different scenarios. A search was performed in the MEDLINE and LILACS databases in February 2017 involving articles published between 2006 and 2016. We found 26 articles in the present review, 11 of which in the emergency setting and 7 in the outpatient setting, with diagnostic and prognosis defined value and poorly studied therapeutic value. PU increased accuracy by 90% as compared to physical examination and chest X-ray for the diagnosis of congestion, being more sensitive and precocious. The skill of the PU performer did not interfere with diagnostic accuracy. The presence of B-lines ≥ 15 correlated with high BNP values (≥ 500) and E/e' ratio ≥ 15, with prognostic impact in IC patients at hospital discharge and those followed up on an outpatient basis. In conclusion, when assessing pulmonary congestion in HF, PU has an incremental value in the diagnostic and prognostic approach in all scenarios studied.

Introduction

Heart failure (HF) is one of the major causes of hospitalization of adults in Brazil. The BREATHE Registry is the first to include a large sample of hospitalized patients with decompensated HF of different regions from Brazil,[1] that being the first cause of hospitalization of patients older than 65 years,[2] one fourth of whom are readmitted to the hospital within 30 days.[3] In Europe, 44% of the patients with HF are readmitted at least once every 12 months.[4] Acute or progressive dyspnea due to pulmonary congestion is the major reason why patients seek care in emergency units.[5] Subclinical congestion is associated with a worse clinical outcome.[3,4]

Physical examination and chest X-ray are widely used by emergency doctors; however, they have low accuracy to diagnose pulmonary congestion. In addition, chest X-ray often depends on the radiologist's assessment, which delays decision-making.[6]

Pulmonary ultrasound (PU) was previously considered of little clinical usefulness in classic cardiology textbooks.[5] However, since the study by Daniel Lichtenstein in 1997,[6] PU has become widely used to assess alveolar-interstitial syndrome, which encompasses pulmonary congestion of cardiac origin,[6] in intensive care and emergency settings, for hospitalized patients before hospital discharge, and for patients with HF undergoing outpatient follow-up.

The major use of PU for the cardiologist is to assess B-lines.[7-9] The analysis of B-lines (ultrasound lung comets) allows the detection of alveolar-interstitial syndrome and the access to extravascular lung water.[6,7] The B-lines are laser-like vertical hyperechoic reverberation artifacts that arise from the pleural line, extend to the bottom of the screen without fading and move synchronously with lung sliding.[10] Several B-lines are present in pulmonary congestion and can aid the detection, semiquantification and monitoring of extravascular lung water, the differential diagnosis of dyspnea and the prognostic stratification of chronic and acute HF.[6,11] When three or more B-lines are identified, the zone or field is considered positive.[7,10,12]

Different methodologies have been applied to PU to analyze B-lines, from the prehospital setting, where only 2 lung fields are assessed,[13,14] to more detailed assessments with 28 fields, as described by Jambrik[12,15] (Figure 1). Most studies, however, have used the 8-field methodology as shown in Figure 2.

Pulmonary ultrasound has shown better accuracy than physical examination and lung X-ray for the diagnosis of pulmonary congestion, even when performed by physicians lacking training in the method or physicians other than radiologists.[16,17] This method adds value to neuropeptides [brain natriuretic peptide (BNP) and NTpro-BNP] for the diagnosis,[18] prognosis and treatment of patients with decompensated HF.

This study was aimed at conducting a systematic review about the use of PU for patients with HF in different clinical scenarios, to identify its role in the diagnosis, prognosis and treatment of the condition. We hypothesized that PU applied to the analysis of pulmonary congestion in different clinical scenarios for patients with HF can contribute to clinical practice.

Methods

Bibliographic search

The search was conducted in the MEDLINE (accessed via PubMed) and LILACS databases. The descriptors used were "heart failure", "pulmonary ultrasound", "thoracic ultrasound". The search in the databases used the following

Keywords

Heart Failure; Pulmonary Congestion, Extravascular Lung Water / diagnostic imaging; Lung / diagnostic imaging; Ultrassonography; Lung / radiography.

Mailing Address: Rafael Tostes Muniz •
Hospital Universitário Antônio Pedro - Rua Marques de Paraná, 330, 4º andar - prédio anexo. Postal Code 24240-670, Niterói, RJ - Brazil
E-mail: dr.rtmuniz@gmail.com
Manuscript received November 14, 2017, revised manuscript March 13, 2018, accepted March 21, 2018

DOI: 10.5935/abc.20180097

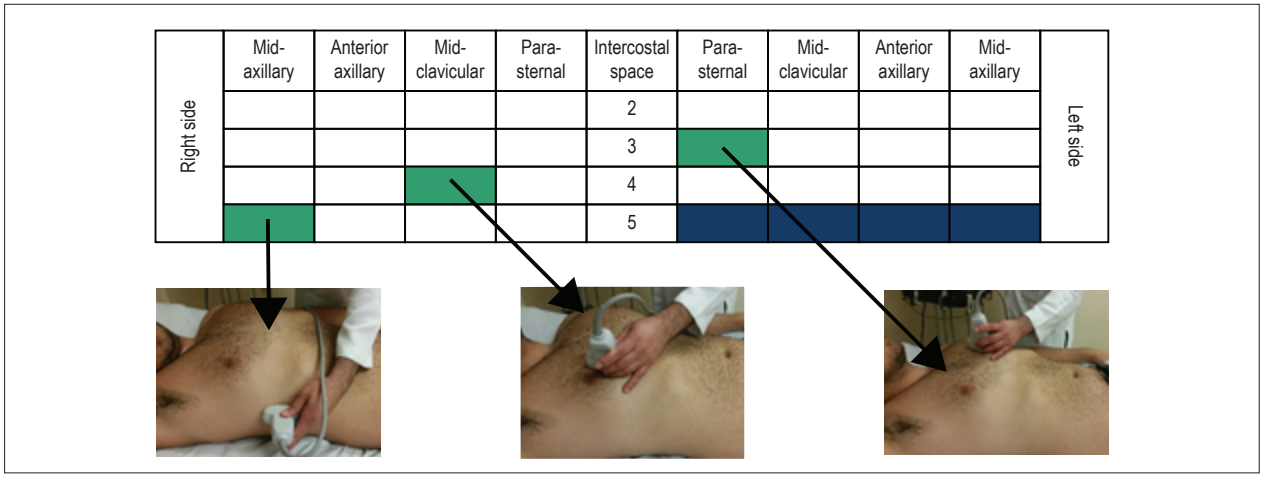


Figure 1 – *Methodology for pulmonary ultrasound assessment: 28 fields (zones). Modified from Jambrik et al.*[15]

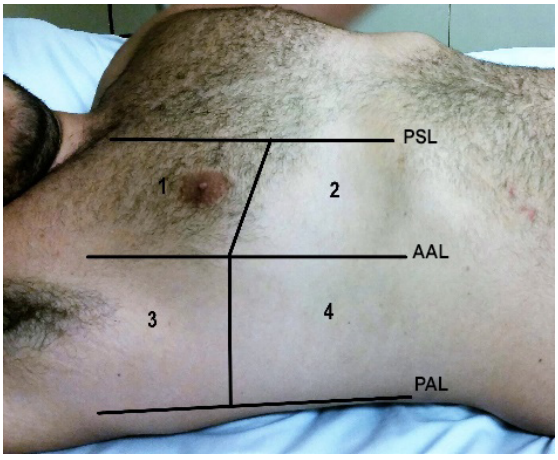


Figure 2 – *Methodology for pulmonary ultrasound assessment: 8 fields (zones). Modified from Volpicelli et al.*[12] *PSL: para-sternal line; AAL: anterior axillary line; PAL: posterior axillary line.*

connectors: (heart failure) AND (pulmonary ultrasound) AND (thoracic ultrasound). The inclusion criteria adopted in the studies were: articles written in English, Portuguese or Spanish, approaching PU for the assessment of dyspnea or congestion in patients with HF. The data were extracted in a standardized way, by two independent researchers responsible for assessing the methodological quality of the manuscripts. Duplicate articles, reviews, editorials, letter to the editor, and studies conducted on animals and populations younger than 18 years were excluded. The search in the literature was performed in February 2017 and included articles from 2006 to 2016.

The articles were selected in two steps. In the first, the abstracts were read and those not meeting the inclusion criteria were excluded. In the second step, the studies selected based on their abstracts were fully read, and those not meeting the inclusion criteria were excluded, according to the PRISMA model (Figure 3).

Results

Interobserver assessment in pulmonary ultrasound and comparison with other diagnostic methods

Gustafsson et al.[19] have observed that nurses specialized in HF and trained in PU for 4 hours achieved a substantial level of interobserver analysis when compared to cardiologists (k = 0.71 and 0.66) to assess B-lines and pleural effusion, respectively.[19] Those results and other data are shown in Table 1.

Platz et al.,[20] assessing the B-lines with Doppler echocardiographic data, have found a correlation with left ventricular (LV) end-diastolic diameter (EDD - p = 0.036) and LV end-systolic diameter (p = 0.026), with septal wall thickening (p = 0.009), LV mass index (p = 0.001), left atrial volume index (p = 0.005), tricuspid valve regurgitation velocity (p = 0.005) and systolic pulmonary artery pressure (SPAP, p = 0.003).

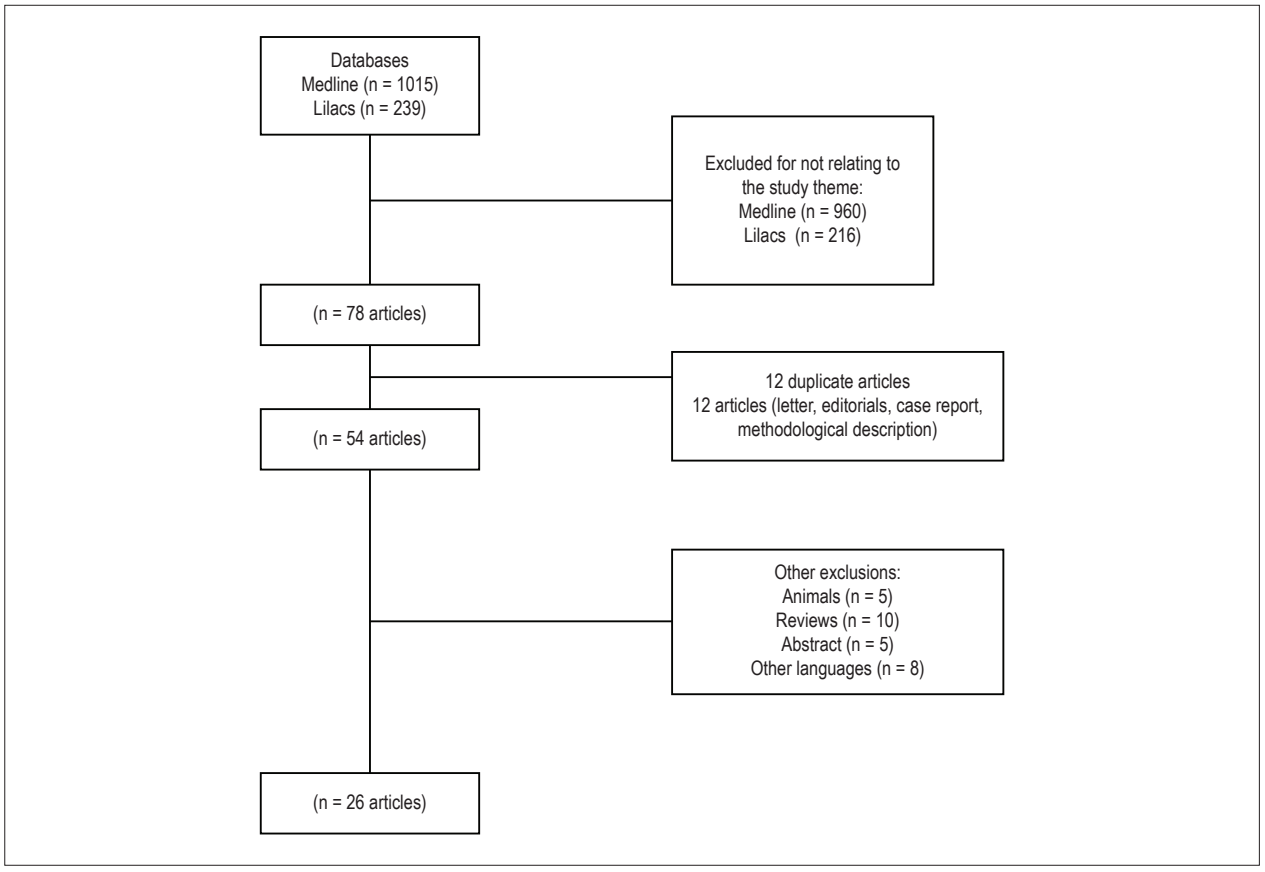


Figure 3 – *Structured search according to the PRISMA model of systematic reviews.*

In two distinct studies, Platz et al.[21,22] have concluded that the clip duration is more important than the type of device used to analyze B-lines, and that the number of B-lines correlate with right atrial pressures, diastolic and systolic pulmonary artery pressures and central venous pressure, but correlated with neither pulmonary artery occlusion pressure nor cardiac index.

In our initial experience, pulmonary congestion detected on PU correlated better with SPAP than with EDD, 86% and 58%, respectively.

Pulmonary ultrasound and diagnostic assessment

A study has identified pleural effusion in 100% of the patients with decompensated HF in the prehospital setting,[13] and another by Prosen et al.[18] has concluded that PU can differentiate cardiac from pulmonary dyspnea, mainly when associating with the use of BNP, observing an increase in diagnostic sensitivity and specificity for the association of PU and BNP.

In the emergency setting, Pivetta et al.[23] have observed an increase in diagnostic accuracy, with reclassification of the diagnosis in 19% of the patients after PU. Russel et al.[24] have found a change in treatment in the acute phase of around 47% of the cases. Gallard et al.[25] have reported an accuracy of 90% when PU was compared to the clinical examination (67%, $p = 0.001$), as well as compared to the combination of clinical examination with NT-proBNP and chest X-ray (81%, $p = 0.04$). Oskan et al.,[26] when comparing the diagnostic performance of PU and auscultation for the diagnosis of decompensated HF and pneumonia, have found sensitivity of 100% and 89% vs. 75% and 73%, respectively. Gullet et al.[16] and Chiem et al.[17] have found agreement between the little or newly trained observer and the highly trained observer in the interobserver analysis for the diagnosis of patients with dyspnea in the emergency setting. Regarding the diagnosis of decompensated HF in patients with dyspnea in the emergency setting, Anderson et al.[27] have found similar values for PU (S = 70%) and BNP > 500 pg/mL (S = 75%). Martindale et al.[28] have reported the superiority of PU (74%) *versus* chest X-ray (58%) in the global agreement with the gold-standard method for the diagnosis of pulmonary edema. Kajimoto et al.[29] have reported that inferior vena cava (IVC) ultrasound associated with PU increases diagnostic sensitivity in acute HF *versus* primary pulmonary disease. Jang et al.[30] have reported that the longitudinal and cross-sectional measures of the internal jugular vein at the end of exhalation is a sensitive test to identify pulmonary edema on chest X-ray in patients with suspected HF. Liteplo et al.[31] have reported the superiority of PU as compared to NT-proBNP to differentiate chronic HF from chronic obstructive pulmonary disease with a positive likelihood ratio (LR)(+) of 3.88 (99% CI = 1.55 – 9.73), while NT-proBNP had a LR(+) of 2.3 (95% CI = 1.41 – 3.76).

Table 1 – Summary of the articles selected and their results.

Diagnostic assessment of dyspnea in prehospital settings (AHF or DCHF)
PU was useful for the diagnosis in 68% of dyspneic patients in the prehospital setting with no delay in treatment and/or transportation, PE being present in 100% of those with decompensated HF, in 17% of patients with ACS, and in 20% of patients with COPD (p < 0.01), PE thus being a diagnostic marker in patients with decompensated HF.[13] In the diagnosis of HF on PU, the S = 100% and E = 95% were comparable to those of NT-proBNP (> 1.000 pg/mL), S = 92% and E = 89%, and superior to those of the modified Boston criteria, S = 85% and E = 86%. The combination of PU and NT-proBNP showed S and E of 100%.[18]
Diagnostic assessment of dyspnea in emergency settings (AHF or DCHF)
Studies reported S ranging from 70% to 96.2% and E from 54% to 75%,[23-25,27,29,31] diagnostic reclassification ranging from 19% to 47%,[23,24] with change in treatment in 43% of the cases,[24] figures comparable to those of BNP > 500 (S = 75% and E = 83%).[27] PU accuracy of 90% versus 67% (p = 0.0001) for clinical examination, and 81% (p = 0.04) for the combination of clinical examination + NT-proBNP + X-ray.[25] PU was better for the diagnosis of DCHF (S = 100%) and of PNM (S = 75%) as compared to stethoscope auscultation (S = 89% and S = 73%, respectively).[26] Interobserver agreement was better in the anterior/superior thoracic zones for both pairs expert/expert and expert/beginner,[16] and the PU performed by beginners versus experts had S and E of 79-85% and 84-88%, respectively,[17,37] and PPV of 64-75% and NPV of 90.9-94%.[17,29] Global agreement with the gold-standard method for pulmonary edema interpretation on PU was 74%, higher than that with X-ray (58%, p< 0.0001).[28] A combination of PU and US of IVC had S = 94.3%, E = 91.9%, NPV = 91.9% and PPV = 94.3% to differentiate AHF from pulmonary disease,[29] and JVD-US is a sensitive test (S = 98.2%) to identify pulmonary edema in dyspneic patients with suspicion of congestive AHF.[30] Studies have shown an LR(+) of PU of 3.88-4.8% and an LR(-) of PU of 0.20-0.50%[24,31] for the diagnosis of AHF or DCHF, being higher than the LR(+) of NT-proBNP [= 2.3] and similar to the LR(-) of NT-proBNP [= 0.24].[31]
Diagnostic assessment in intensive care settings (AHF or DCHF)
Agreement of PU with the final diagnosis was 84%, with S = 86% and E = 87% for cardiac pulmonary edema,[32] and IVC values > 9 mm on B mode had S = 84.4% and E = 92.9% [LR(+) = 11.8, LR(-) = 0.16] for the diagnosis of cardiac dyspnea.[33]
Diagnostic assessment in outpatient settings
Primary outcome (hospitalization due to DCHF and all-cause death) was 4x more frequent in patients of the third tertile than in patients of the first tertile with B-lines ≥ 3 (p < 0.001), whose time alive or outside the hospital was shorter (p< 0.001).[36] The finding of B-lines or PE or both increased the risk of death or hospitalization (p< 0.05)[19] and correlated in a paired way with the estimates of PCWP (p < 0.001) and with the fluid impedance index (p < 0.001); the impedance monitoring alert detected clinical deterioration of HF with S = 92%, while B-lines ≥ 5 showed S = 83%.[35] HF decompensation was present in 68% of the patients when the number of B-lines ≥ 15, and correlated with NT-proBNP > 1000 (p < 0.0001) and with an E/e' ratio > 15 (p < 0.0001).[34]
Prognostic assessment
Event-free survival (all-cause death and re-hospitalization) of patients with HF and B-lines ≥ 30 was shorter than that of patients with B-lines < 30 (p < 0.0001) in 3 months[10] and of patients with B-lines ≥ 15 in 6 months,[11] and the presence of B-lines ≥ 30 was a predictor of death with BNP > 700 (p = 0.002).[10]
Therapeutic assessment
The number of B-lines reduced with treatment (p < 0.05), and the PU score showed a linear correlation with the radiologic (p < 0.05) and clinical scores (p < 0.05) and with BNP levels (p < 0.05).[8]
Assessment of PU as compared to other diagnostic methods
An increase in the number of B-lines correlated with LVEDV (p = 0.036);[20] LV end-systolic diameter (p = 0.026);[20] PW (p = 0.009);[20] LV mass index (p = 0.001);[20] RA volume index (p = 0.005);[20] TR velocity (p = 0.005);[20] measures of RA, DPAP, MPAP, PVR, all p < 0,005,[21] and SPAP (p = 0.003-0,005),[20-21] and, for each B-line, there was an increase of 1 mm Hg in SPAP and of 0.1 Woods units in RVP.[21] In the analysis of the number of B-lines, the US device types used did not statistically differ (4 or 8 zones assessed; p= 0.67),[22] but the clip duration did differ: 4 versus 2 seconds (p < 0.001 for 4 and 8 zones) and 6 versus 4 seconds (p = 0.057 for 4 zones; and p = 0.018 for 8 zones).[22]

AHF: acute heart failure; DCHF: decompensated chronic heart failure; HF: heart failure; PU: pulmonary ultrasound; COPD: chronic obstructive pulmonary disease; PE: pleural effusion; ACS: acute coronary syndrome; S: sensitivity; E: specificity; NPV: negative predictive value; PPV: positive predictive value; NT-proBNP: N-terminal pro-brain natriuretic peptide; LR(+): positive likelihood ratio; LR(-): negative likelihood ratio; US: ultrasound; X-ray: chest X-ray; PNM: pneumonia; IVC: inferior vena cava; JVD-US: jugular vein distension on ultrasound; PCWP: pulmonary capillary wedge pressure; BNP: brain natriuretic peptide; LVEDV: left ventricular end-diastolic volume; PW: posterior wall; LV: left ventricular; LA: left atrium; TR: tricuspid regurgitation; RA: right atrium; DPAP: diastolic pulmonary artery pressure; MPAP: mean pulmonary artery pressure; PVR: pulmonary vascular resistance; SPAP: systolic pulmonary artery pressure.

In the intensive care setting, Dexheimer Neto et al.,[32] using the BLUE protocol in dyspneic patients, have found an 84% agreement between PU and the final diagnosis of pneumonia or acute pulmonary edema (total kappa = 0.81). Yamanoglu et al.[33] have detected the cardiac origin of dyspnea by using the caval index (sensitivity= 84.4% and specificity= 92.9%).

In our clinical practice, we observed that PU increases the diagnostic accuracy of pulmonary congestion, being better than the stethoscope auscultation in both the emergency and the cardiac intensive care unit settings.

In the outpatient care setting, Miglioranza et al.[34] have reported that a number of B-lines ≥15 correlates with NT-proBNP > 1000 (p < 0.001), E/e' ratio >15 (p = 0.001) and clinical assessment (p < 0.001), with sensitivity of 85% and specificity of 83%, for the risk of decompensated HF. Maines et al.[35] have reported a correlation between the presence of B-lines and the impedance fluid index (p < 0.001) of patients with HF at regular outpatient follow-up.

Pulmonary ultrasound and prognostic assessment

In the outpatient clinic context, Platz et al.[36] have identified that patients with more than three B-lines had a four-fold increase in the chance of hospitalization due to HF or of all-cause death, being worth noting that 81% of those

patients had no compatible alteration in lung auscultation. Gustafsson et al.,[37] studying 104 patients, have identified that the presence of B-lines or pleural effusion or both correlated with the increased risk of death or hospitalization (HR: 3-4; $p < 0.05$). In 2015, Gargani et al.[9] and Corio et al.[10] found prognostic value on hospital discharge for the number of B-lines ≥ 30 and ≥ 15, respectively, for all-cause death or event-free hospitalization in 3 and 6 months ($p < 0.001$ for both).

We found a mean number of B-lines of 12.2 ± 7.3 on hospital discharge. Five patients were hospitalized again in 90 days, with an event-free mean of 63.6 ± 25.7 days and a mean BNP value of 450.10 ± 409.96 pg/mL.

Pulmonary ultrasound and therapeutic assessment

Volpicelli et al.[8] have concluded that B-line pattern mostly clears after medical treatment and correlates with other parameters, such as radiologic ($p < 0.05$) and clinical ($p < 0.05$) scores of congestion and BNP levels ($p < 0.05$).

Discussion

This systematic review was aimed at identifying scientific evidence about PU in HF. The results showed it increases the HF diagnosis accuracy in the prehospital and hospital settings with incremental prognostic value on the discharge of patients with decompensated HF and might play a role in guiding the treatment of patients with HF.

Figure 4 shows the progressive increase in the number of publications on PU in HF over the past 10 years; however, several studies were clinical reviews,[7,38,39] others were editorials, and there was a methodological description.[40]

There are several scenarios for the applicability of PU in assessing dyspneic patients with decompensated or presumed HF. As shown in Figure 5, the emergency application of PU was the most studied. It is believed that one of the reasons for that would be the low accuracy of physical examination and of chest X-ray[6] for a rapid and more accurate diagnosis.[23,24] A review study with 100 patients in the emergency department and using a pocket-sized cardiac ultrasound device has shown that PU can rapidly aid the diagnosis of HF, providing a more adequate and early treatment.[38]

In that context of emergency assessment, Miglioranza et al.[34] and Facchini et al.[41] have reported positive correlations between PU data and neuropeptide levels. That information can be useful, mainly when the measurement of natriuretic peptides is not available for the initial assessment. Another author,[42] using PU in the emergency setting, has reported that the identification of multiple B-lines bilaterally was a sensitive, but not specific, predictor of BNP elevation > 500 pg/mL. That was the first study correlating B-lines with BNP.[42] In addition, it was confirmed that the presence of alveolar-interstitial syndrome, identified by the presence of B-lines, can represent a precise and reproducible test to discriminate between cardiac and noncardiac dyspnea in the emergency setting, with sensitivity of 93.6%, specificity of 84%, positive predictive value of 87.9% and negative predictive value of 91.3%.[43] Those findings also correlate with the NYHA functional class, left ventricular ejection fraction and grade of diastolic dysfunction.[44]

Several studies[5,18,23,24] have correlated the presence of B-lines on PU with a sensitive marker for the diagnosis of decompensated HF; however, B-lines are not an exclusivity of decompensated HF. They can appear in adult respiratory distress syndrome and pulmonary interstitial fibrosis.[12]

Another review study of patients with HF followed up on an outpatient basis has concluded that PU has great diagnostic potential for identifying pulmonary congestion signs at the bedside, can become a state-of-the-art marker of interstitial fluid, and that the B-line pattern usually disappears after proper treatment of acute HF, revealing itself as an alternative diagnostic tool of easy use and therapeutic

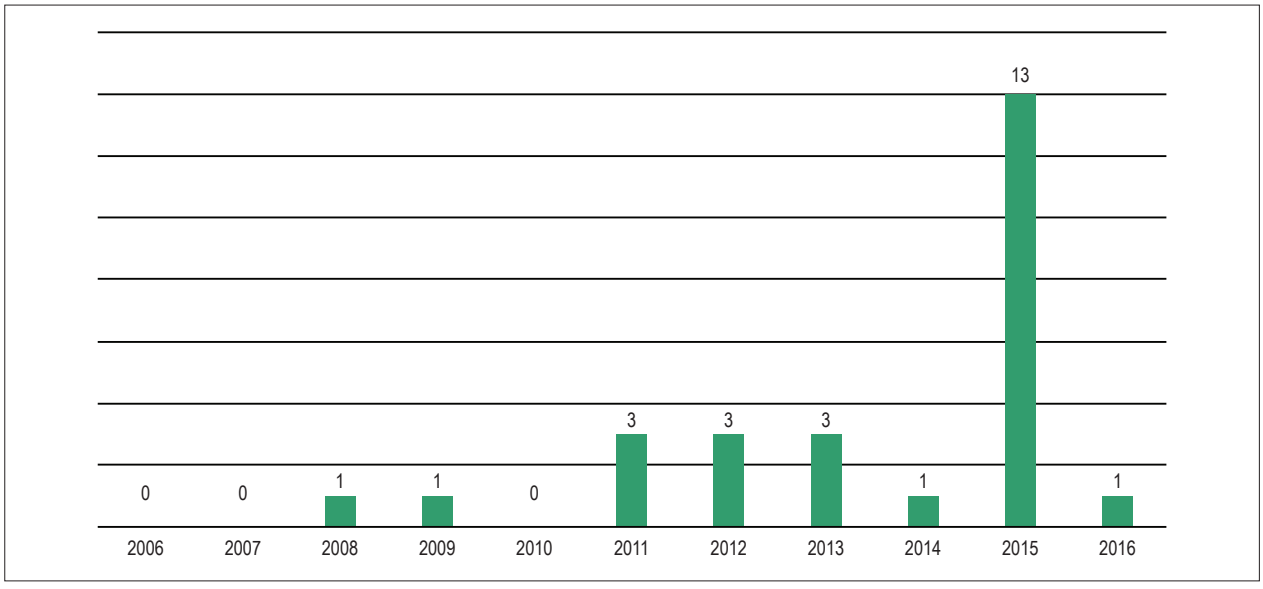


Figure 4 – *Distribution of specific publications about pulmonary ultrasound in heart failure in the 2006-2016 period.*

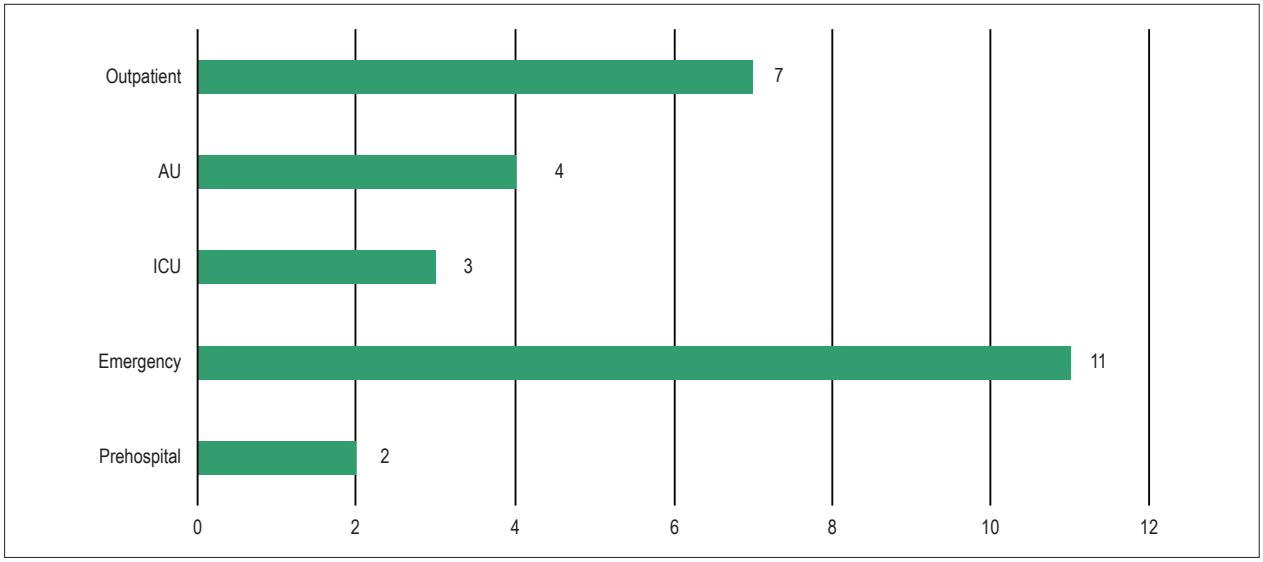


Figure 5 – *Distribution of the number of publications about pulmonary ultrasound in heart failure according to the assessment setting. AU: admission unit; ICU: intensive care unit.*

applicability.[8] A recent systematic review has shown that the PU findings can rapidly change with therapy for HF, and that the identification of residual congestion in patients with acute HF at hospital discharge or in patients with chronic HF followed up on an outpatient basis can indicate those at higher risk for adverse events.[45]

Gullet et al.[16] and Bedetti et al.[46] have reported the excellent correlation between two observers with different specific expertise regarding PU for the analysis of B-lines at the bedside of patients with known or presumed HF.

In a study on stable patients undergoing dialysis, the identification of B-lines on PU correlated with pre-dialysis diastolic blood pressure ($p = 0.015$) and with the combination of reduced ejection fraction and reduced blood volume percentage at the end of hemodialysis ($p = 0.028$).[47]

We trained two non-specialized physicians on PU to assess congestion. We concluded that 4 hours of theoretical training and performing 15 tests were sufficient for them to develop similar accuracy in quantifying pulmonary congestion. Our tests are validated by a specialist radiologist (AMB), emphasizing our commitment with performance areas and need for proficiency-training.

In addition, in our medical practice, we identified the superiority of PU over stethoscope auscultation to assess pulmonary congestion. Furthermore, the presence of B-lines (mean value of 12.2 ± 7.3) was a marker of re-admission for one fourth of the patients in 90 days, and the presence of moderate congestion was a predictor of re-admission in 100% of the cases.

Pulmonary ultrasound and evidence-based recommendations

Volpicelli et al.[12] have proposed the first document to provide evidence-based recommendations for clinical use of point-of-care PU. In that document, those authors have determined the levels of evidence for each applicability, establishing that, when assessing interstitial syndrome, the ultrasonographic technique consists ideally of the assessment of 8 regions (range: from 2 to 28). A positive region is defined by the presence of at least three B-lines on a longitudinal plane between two ribs.

The ultrasonographic definition of B-line and the positive zone criterion (presence of ≥ 3 B-lines per field analyzed) were criteria used by all the authors of the present review. In addition, the criterion to define alveolar-interstitial syndrome (≥ 3 B-lines per field analyzed bilaterally) was common among the authors.

Limitations

The present systematic review had as limitation the small sample size. The lack of standardization of the scores used for semiquantitative analysis was also a limiting factor.

Conclusion

The use of PU to assess dyspneic patients and those with HF in different clinical settings increases the sensitivity, specificity and accuracy of the diagnosis and prognosis of pulmonary congestion in patients with HF.

Pulmonary ultrasound adds value to the diagnosis, facilitating decision-making in the assessment of acutely dyspneic patients, to whom HF is one of the differential diagnoses, minimizing treatment errors and improving the clinical outcome of this patient model.

Author contributions

Conception and design of the research: Muniz RT, Mesquita ET; Acquisition of data and analysis and interpretation

of the data: Muniz RT, Mesquita ET, Souza Junior CV; Writing of the manuscript and critical revision of the manuscript for intellectual content: Muniz RT, Mesquita ET, Martins WA.

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 Rafael Tostes Muniz, from Universidade Federal Fluminense.

Ethics approval and consent to participate

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

References

1. Albuquerque DC, Neto JD, Bacal F, Rohde LE, Bernardez-Pereira S, Berwanger O, et al. I Brazilian registry of heart failure - clinical aspects, care quality and hospitalization outcomes. Arq Bras Cardiol. 2015;104(6):433-42.
2. Tavares LR, Victer H, Linhares JM, de Barros CM, Oliveira MV, Pacheco LC, et al. Epidemiology of decompensated heart failure in the city of Niterói - EPICA - Niterói Project. Arq Bras Cardiol. 2004;82(2):125-8, 121-4.
3. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, et al; American College of Cardiology Foundation; American Heart Association Task Force on Practice Guidelines. ACCF/AHA Guideline for Management of Heart Failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2013;62(16):e147-239.
4. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail. 2016;18(8):891-975.
5. Leidi F, Casella F, Cogliati C. Bedside lung ultrasound in the evaluation of acute decompensated heart failure. Intern Emerg Med. 2016;11(4):597-601.
6. Lichtenstein D, Mézière G, Biderman P, Gepner A, Barré O. The comet-tail artifact, an ultrasound sign of alveolar-interstitial syndrome. Am J Respir Crit Care Med. 1997;156(5):1640-6.
7. Picano E, Frassi F, Agricola E, Gligorova S, Gargani L, Mottola G. Ultrasound lung comets: a clinically useful sign of extravascular lung water. J Am Soc Echocardiogr. 2006;19(3):356-63.
8. Volpicelli G, Caramello V, Cardinale L, Mussa A, Bar F, Frascisco MF. Bedside ultrasound of the lung for the monitoring of acute decompensated heart failure. Am J Emerg Med. 2008;26(5):585-91.
9. Gargani L, Pang PS, Frassi F, Miglioranza MH, Dini FL, Landi P, et al. Persistent pulmonary congestion before discharge predicts rehospitalization in heart failure: a lung ultrasound study. Cardiovasc Ultrasound. 2015 Sep 4;13:40.
10. Coiro S, Rossignol P, Ambrosio G, Carluccio E, Alunni G, Murrone A, et al. Prognostic value of residual pulmonary congestion at discharge assessed by lung ultrasound imaging in heart failure. Eur J Heart Fail. 2015;17(11):1172-81.
11. Gargani L. Lung ultrasound: a new tool for the cardiologist. Cardiovascular Ultrasound. 2011 Feb 27;9:6.
12. Volpicelli G, Elbarbary M, Blaivas M, Lichtenstein DA, Mathis G, Kirkpatrick AW, et al; International Liaison Committee on Lung Ultrasound (ILC-LUS) for International Consensus Conference on Lung Ultrasound (ICC-LUS). International evidence-based recommendations for point-of-care lung ultrasound. Intensive Care Med. 2012;38(4):577-91.
13. Neesse A, Jerrentrup A, Hoffmann S, Sattler A, Görg C, Kill C, et al. Prehospital chest emergency sonography trial in Germany: a prospective study. Eur J Emerg Med. 2012;19(3):161-6.
14. Laursen CB, Hänselmann A, Posth S, Mikkelsen S, Videbæk L, Berg H. Prehospital lung ultrasound for the diagnosis of cardiogenic pulmonar oedema: a pilot study. Scand J Trauma Resusc Emerg Med. 2016 Aug 2;24:96.
15. Jambrik Z, Monti S, Coppola V, Agricola E, Mottola G, Miniati M, et al. Usefulness of ultrasound lung comets as a nonradiologic signo f extravascular lung water. Am J Cardiol. 2004;93(10):1265-70.
16. Gullett J, Donnelly JP, Sinert R, Hosek B, Fuller D, Hill H, et al. Interobserver agreement in the evaluation of B lines using bedside ultrasound. J Crit Care. 2015;30(6):1395-9.
17. Chiem AT, Chan CH, Ander DS, Kobylivker AN, Manson WC. Comparison of expert and novice sonographers' performance in focused lung ultrasonography in dyspnea (FLUID) to diagnose patients with acute heart failure syndrome. Acad Emerg Med. 2015;22(5):564-73.
18. Prosen G, Klemen P, Štrnad M, Grmec S. Combination of lung ultrasound (a comettail sign) and Nterminal probrain natriuretic peptide in differentiating acute heart failure from chronic obstructive pulmonary disease and asthma as cause of acute dyspnea in prehospital emergency setting. Crit Care. 2011;15(2):R114. Erratum in: Crit Care. 2011;15(6):450.
19. Gustafsson M, Alehagen U, Johansson P. Pocketsized ultrasound examination of fluid imbalance in patients with heart failure: a pilot and feasibility study of heart failure nurses without prior experience of ultrasonography. Eur J Cardiovasc Nurs. 2015;14(4):294-302.
20. Platz E, Hempel D, Pivetta E, Rivero J, Solomon SD. Echocardiographic and lung ultrasound characteristics in ambulatory patients with dyspnea or prior heart failure. Echocardiography. 2014;31(2):133-9.
21. Platz E, Lattanzi A, Agbo C, Takeuchi M, Resnic FS, Solomon SD, et al. Utility of lung ultrasound in predicting pulmonary and cardiac pressures. Eur J Heart Fail. 2012;14(11):1276-84.
22. Platz E, Pivetta E, Merz AA, Peck J, Rivero J, Cheng S. Impact of device selection and clip duration on lung ultrasound assessment in patients with heart failure. Am J Emerg Med. 2015;33(11):1552-6.
23. Pivetta E, Goffi A, Lupia E, Tizzani M, Porrino G, Ferreri E. Lung ultrasound implemented diagnosis of acute decompensated heart failure in the ED: A SIMEU Multicenter Study. SIMEU Group for Lung Ultrasound in the Emergency Department in Piedmont. Chest. 2015;148(1):202-10.
24. Russell FM, Ehrman RR, Cosby K, Ansari A, Tseeng S, Christain E, et al. Diagnosing acute heart failure in patients with undifferentiated dyspnea: a lung and cardiac ultrasound (LuCUS) Protocol. Acad Emerg Med. 2015;22(2):182-91.

25. Gallard E, Redonnet JP, Bourcier JE, Deshaies D, Largeteau N, Amalric JM, et al. Diagnostic performance of cardiopulmonary ultrasound performed by the emergency physician in the management of acute dyspnea. Am J Emerg Med. 2015;33(3):352-8.

26. Özkan B, Ünlüer EE, Akyol PY, Karagöz A, Bayata MS, Akoğlu H, et al. Stethoscope versus point-of-care ultrasound in the differential diagnosis of dyspnea: a randomized trial. Eur J Emerg Med. 2015;22(6):440-3.

27. Anderson KL, Jenq KY, Fields JM, Panebianco NL, Dean AJ. Diagnosing heart failure among acutely dyspneic patients with cardiac, inferior vena cava, and lung ultrasonography. Am J Emerg Med. 2013;31(8):1208-14.

28. Martindale JL, Noble VE, Liteplo A. Diagnosing pulmonary edema: lung ultrasound versus chest radiography. Eur J Emerg Med. 2013;20(5):356-60.

29. Kajimoto K, Madeen K, Nakayama T, Tsudo H, Kuroda T, Abe T. Rapid evaluation by lung, cardiac and inferior vena cava (LCI) integrated ultrasound for differentiating heart failure from pulmonary disease as the cause of acute dyspnea in the emergency setting.Cardiovasc Ultrasound. 2012;10(1):49.

30. Jang T, Aubin C, Naunheim R, Lewis LM, Kaji AH. Jugular vein ultrasound and pulmonary oedema in patients with suspected congestive heart failure. Eur J Emerg Med. 2011;18(1):41-5.

31. Liteplo AS, Marill KA, Villen T, Miller RM, Murray AF, Croft PE, et al. Emergency thoracic ultrasound in the differentiation of the etiology of shortness of breath (ETUDES): sonographic B-lines and N-terminal pro-brain-type natriuretic peptide in diagnosing congestive heart failure. Acad Emerg Med. 2009;16(3):201-10.

32. Dexheimer Neto FL, Andrade JM, Raupp AC, Townsend RS, Beltrami FG, Brisson H, et al. Diagnostic accuracy of the Bedside Lung Ultrasound in Emergency protocol for the diagnosis of acute respiratory failure in spontaneously breathing patients. J Bras Pneumol. 2015;41(1):58-64.

33. Yamanoğlu A, Çelebi Yamanoğlu NG, Parlak İ, Pınar P, Tosun A, Erkuran B, et al. The role of inferior vena cava diameter in the differential diagnosis of dyspneic patients, best sonographic measurement method? Am J Emerg Med. 2015;33(3):396-401.

34. Miglioranza MH, Gargani L, Sant'Anna RT, Rover MM, Martins VM, Mantovani A. Lung ultrasound for the evaluation of pulmonary congestion in outpatients: a comparison with clinical assessment, natriuretic peptides, and echocardiography. JACC Cardiovasc Imaging. 2013;6(11):1141-51.

35. Maines M, Catanzariti D, Angheben C, Valsecchi S, Comisso J, Vergara G. Intrathoracic impedance and ultrasound lung comets in heart failure deterioration monitoring. Pacing Clin Electrophysiol. 2011;34(8):968-74.

36. Platz E, Lewis EF, Uno H, Peck J, Pivetta E, Merz AA, et al. Detection and prognostic value of pulmonary congestion by lung ultrasound in ambulatory heart failure patients. Eur Heart J. 2016:37(15):1244-51.

37. Gustafsson M, Alehagen U, Johansson P. Imaging congestion with a pocket ultrasound device: prognostic implications in patients with chronic heart failure. J Card Fail. 2014;21(7):548-54.

38. Mancuso FJ, Siqueira VN, Moisés VA, Gois AF, Paola AA, Carvalho AC, et al. Focused cardiac ultrasound using a pocket-size device in the emergency room. Arq Bras Cardiol. 2014;103(6):530-7

39. Volpicelli G, Melniker LA, Cardinale L, Lamorte A, Frascisco MF. Lung ultrasound in diagnosing and monitoring pulmonary interstitial fluid. Radiol Med. 2013;118(2):196-205.

40. Frasure SE, MatilSky DK, Siadecki SD, Platz E, Saul T, Lewiss RE. Impact of patient positioning on lung ultrasound findings in acute heart failure. Eur Heart J Acute Cardiovasc Care. 2015;4(4):326-32

41. Facchini C, Malfatto G, Giglio A, Facchini M, Parati G, Branzi G. Lung ultrasound and transthoracic impedance for noninvasive evaluation of pulmonary congestion in heart failure. J Cardiovasc Med (Hagerstown). 2016;17(7):510-7.

42. Manson WC, Bonz JW, Carmody K, Osborne M, Moore Cl. Identification of sonographic B-lines with linear transducer predicts elevated B-type natriuretic peptide level. West J Emerg Med. 2011;12(1):102-6.

43. Cibinel GA, Casoli G, Elia F, Padoan M, Pivetta E, Lupia E, et al. Diagnostic accuracy and reproducibility of pleural and lung ultrasound in discriminating cardiogenic causes of acute dyspnea in the Emergency Department. Intern Emerg Med. 2012;7(1):65-70.

44. Frassi F, Gargani L, Gligorova S, Ciampi Q, Mottola G, Picano E. Clinical and echocardiographic determinants of ultrasound lung comets. Eur J Echocardiogr. 2007;8(6):474-9.

45. Platz E, Merz AA, Jhund PS, Vazir A, Campbell R, McMurray JJ. Dynamic changes and prognosis value of pulmonary congestion by ultrasound in acute and chronic heart failure: systematic review. Eur J Heart Fail. 2017;19(9):1154-63.

46. Bedetti G, Gargani L, Corbisiero A, Frassi F, Poggianti E, Mottola G. Evaluation of ultrasound lung comets by hand-held echocardiography. Cardiovasc Ultrasound. 2006 Aug 31;4:34-9.

47. Weitzel WF, Hamilton J, Wang X, Bull JL, Vollmer A, Bowman A, et al. Quantitative lung ultrasound comet measurement: method and initial clinical results. Blood Purif. 2015;39(1-3):37-44.

Update of the Impact of Consumption of Whole Chicken Eggs on the Lipid Profile: to What Extent are They Impacting?

Heitor Oliveira Santos
Universidade Federal de Uberlândia (UFU), Uberlândia, MG - Brazil

Introduction

The older literature (mid 1980-90) shows that increased dietary cholesterol intake can raise total serum cholesterol and LDL levels.[1,2] Some more current positions question the recommendation of daily cholesterol intake, as well as the impact of the whole chicken egg, inquiring whether it is a harmful or beneficial classification food in this context.[3,4] In the midst of controversial issues, it is essential to analyze variables such as food consumption in general and how much dietary cholesterol intake is impacting on the parameters of the lipid profile, thus obtaining a more reliable information, mainly when the focus is the clinical conduct.

One of the foods most known to contain cholesterol is the egg, and it is in the yolk where the cholesterol is concentrated. The chicken egg is the most consumed worldwide, being a food of affordable price, and, above all, of practical cooking and good nutritional profile.[3]

Among the types of cooking, the fried chicken egg, stir-fried, cooked, pochê, roast, in the form of omelet and soufflés, besides being an ingredient of various preparations, stands out. The nutritional aspects of the chicken egg are extensive. It is a source rich in proteins of high biological value, unsaturated fats, liposoluble vitamins (mainly vitamin A and E), vitamin B12 and antioxidant components.[3]

I carried out studies of great sample character, exhibiting interesting adjustments through the ingestion of chicken eggs. Data from observational methodology and human intervention support new research.[5,6]

Hence, we sought to analyze the impact of the intake of whole chicken eggs on the lipid profile from older and recent studies.

Pre-established studies: discussion of research in the mid-2000s

In 2000, McNamara[7] through a review encompassing a survey of 167 studies, whereof cholesterol intake in more than 4000 individuals was analyzed, shows that every 100 mg of dietary cholesterol intake the total plasma cholesterol increased only 2.2 mg/dL. A whole chicken egg unit (~ 50 g) contains a cholesterol content equivalent to the amount analyzed in the McNamara study,[7] presenting approximately 100-150 mg of cholesterol,[8] therefore, being one of the main foods that provided the increase of the cholesterol intake.[7,8]

Regarding the LDL and HDL lipoproteins, the addition of 100 mg of cholesterol per day from the diet in the general McNamara[7] population increased the LDL levels by 1.9mg/dL and HDL levels by 0.4 mg/dL. Nevertheless, the mean change in the proportion of LDL: HDL per 100mg of dietary daily cholesterol in the patients was 2.60 to 2.61, that is, both values were literally negligible for the outcome of cardiovascular problems, such as plaque atheroma and stroke.[7]

McNamara[7] also shows the relationship of dietary cholesterol response to heterogeneity, whose people are divided into hypersensitive and hypersensitive. According to the author, 15% to 25% of the population is sensitive to dietary cholesterol, in which there is a genetic increase in the production of apolipoprotein E4 and apolipoprotein B. However, the impact of daily dietary cholesterol intake per 100mg increased by only 1.4 mg/dL of total cholesterol levels in hypo-responsive individuals and 3.9 mg/dL in hyperresponsive individuals.[7]

However, McNamara[7] does not emphasize whether the main food responsible for dietary cholesterol was chicken egg. A subject to analyze is the response of the lipid profile of patients with dyslipidemia to the eggs ingestion. In 1997, Knopp et al.[9] already had data for this issue.

Knopp et al.[9] conducted a very well controlled study involving 130 patients with hypercholesterolemia and combined hyperlipidemia. For six weeks was used a diet based on traditional recommendations, and from that period randomized patients to receive two whole chicken eggs per week or at most one whole chicken egg per week, lasting 12 weeks. They analyzed the lipid profile before and after the intervention and observed that patients with hypercholesterolemia who consumed two eggs daily increased their HDL levels by an average of 48 to 52 ng/dL ($p = 0.003$), while they did not change other markers such as LDL, total cholesterol, VLDL and apolipoprotein B. On the other hand, patients with combined hyperlipidaemia who consumed two eggs per day increased mean total cholesterol from 238 to 250 ng/dL ($p = 0.001$), LDL from 150 to 162 ng/dL ($p = 0.001$), HDL from 42 to 45 ng/dL ($p = 0.02$), and apolipoprotein B ($p = 0.05$). A decrease in VLDL from 103 to 95 mg/dL ($p = 0.007$) was observed in this study, which was probably not expected.[9]

Current studies: from large population samples to intervention with good control of intakes of whole chicken eggs

A recently published study showed that consumption between two to four ($n = 4493$) and greater than four

Keywords

Cholesterol / chemistry; Eggs / utilization; Egg Proteins; Egg Proteins, Dietary; Antioxidants.

Mailing Address: Heitor Oliveira Santos •
Av. Pará, 1720. Postal Code 38400-902, Uberlândia, MG – Brazil
E-mail: heitoroliveirasantos@gmail.com
Manuscript received July 10, 2017, revised manuscript September 02, 2017, accepted September 29, 2017

DOI: 10.5935/abc.20180092

whole chicken eggs (n = 214) per week did not increase the incidence of cardiovascular disease compared to individuals with a consumption habit of less than two eggs per week (n = 2509). It is noteworthy that this study is of the renowned group PREDIMED (PREvención con DIeta MEDiterránea), in which the individuals followed a Mediterranean food style, therefore, with habitual consumption of olive oil and oilseeds. In this way, the increase in egg consumption was accompanied by a good dietary style, which was confirmed by the control of macronutrients and type of ingested lipids, as a relevant percentage of monounsaturated fats (~20% of total caloric value) and moderate in saturated fats (~10% of the total caloric value).[5]

Despite the study by the PREDIMED group,[5] in a study with a considerable sample (n = 1231) it is stated that the higher the consumption of whole chicken eggs, the greater the area of the atheroma plaque in the carotid (weekly consumption between one and five eggs exhibited larger area of the plaque compared to half egg weekly). However, in the study lack physical exercise control, waist circumference and mainly, dietary habits. Nevertheless, the increase in plaque area was related to an older age and history of smoking.[10]

Another recent study showed that the daily intake of two to three whole chicken eggs increased the functionality of HDL and plasma carotenoids, which are anti-inflammatory and antioxidant factors. Thirty-eight healthy participants participated in a study in which initially they stayed a period of 2 weeks without eating egg, and later consumed an entire egg of chicken for 4 weeks, and progressively two and three whole eggs daily every 4 weeks; the intervention lasted 14 weeks. Compared with the time of deprivation of egg ingestion, consumption of one to three eggs/day resulted in increased concentrations of large LDL (21-37%), large HDL (6-13%) and apolipoprotein AI (9-15%) whereas ingestion of two to three eggs/day promoted an increase in apolipoprotein AII by 11% and lutein and plasma zeaxanthin by 20-31%, whereas the ingestion of three eggs/day resulted in an increase of 9-16% in serum paraoxonase-1 activity compared to the intake of one to two eggs/day. Intake of one egg/day was sufficient to increase HDL function and particle concentration of large LDL. In general, the daily intake of less than three eggs/day favored a better LDL particle profile, improved the function of HDL and increased plasma antioxidants in healthy young adults.[6]

This recent intervention adds to the literature a more biologically detailed impact as a function of the consumption of whole chicken eggs, since it goes beyond classic lipid profile markers in clinical practice, analyzing lipoprotein precursors and antioxidant character.[6] The increase of paraoxonase-1 and apolipoprotein AI levels as a function of egg ingestion are beneficial, since they are precursors of HDL formation, providing greater functionality.[11,12] In reference to the increase of large LDL, it does not mean a bad factor, but a beneficial modulation of the molecule, because the higher the volume, the lower is the propensity for endothelial penetration in the arteries, unlike LDL of lower volume (ie, sdLDL, small dense low-density lipoprotein particles).[13]

Positioning of guidelines regarding cholesterol consumption

The V Brazilian Guideline on Dyslipidemias and Prevention of Atherosclerosis encourages the ingestion of cholesterol < 300 mg/d for patients in general, and for dyslipidemics the incentive is < 200 mg/d.[14] In agreement with the most recent recommendations in the medical literature, a new consensus from the American Heart Association,[15] based mainly on the dietary guidelines of the Dietary Guidelines for Americans,[16] in the period from 2015 to 2020, cholesterol consumption is still limited to that advocated by the V Brazilian Guideline on Dyslipidemias and Prevention of Atherosclerosis.[14] However, such recommendations do not specify where dietary cholesterol comes from, for example, whether it is primarily from a Western-style diet rich in fritters as a whole, or from a type of nutrient-rich diet rich in functional substances. The American Heart Association's recent positioning encourages Mediterranean feeding, citing the PREDIMED group, which, in parallel, can be based on a considerable weekly frequency of intakes of whole eggs.[15,16]

Discussion

Analyzing a food in isolation requires detailed adjustments, and the egg is a food that undoubtedly remains controversial. There are two strands, one more cautious and another one that overestimates the potential of the egg as food. Following the traditional recommendations of cholesterol intake is of some importance; however, one should consider the whole lifestyle.

Probably, in individuals who exercise and have good dietary control, the routine intake of whole eggs will not cause harm to the lipid profile, since, presumably, the body is in a good redox balance, being a protective factor for cardiovascular outcomes.[17]

Even in elderly individuals, the daily consumption of eggs, at least non-abusively, may be insignificant in altering the lipid profile.[18] In a cross-over study of 33 elderly (mean age 79 years), the daily consumption of a whole chicken egg for five weeks did not change any traditional lipid profile marker compared to the same period without the ingestion of eggs, and serum antioxidant markers (+26% lutein and + 38% zeaxanthin).[19]

Given the importance of redox balance as a protector to the cardiovascular side, perhaps the consumption of whole eggs is also not of concern for patients with dyslipidemias, because as was quoted, its consumption exhibits beneficial antioxidant modulation to lipoproteins. The PREDIMED study is a good baseline, encompassing middle-aged and large sample patients. Regarding whole eggs intake in the style of Mediterranean food, two to four eggs per week are consumed on average, whereas less than two servings of sweetmeats and red meat and less than a portion of processed meat are consumed. The white meat intake is two servings and the portions of vegetables, fish or shellfish are two or more per week.[19] Thus, like the PREDIMED study, if the individual has good eating habits as a whole, the intake of whole eggs with considerable weekly frequency seems to be safe. Above all, the prescription of eggs in clinical practice is a very individual factor, mainly depending on lipid and protein adjustments.

Taken together, dietary cholesterol mainly by using the egg as a source can alter the lipid profile by increasing the markers in general. However, in assessing the actual biological impact this appears to be practically insignificant. Genetic factors can increase the cholesterol, LDL and triglycerides levels of individuals because of higher cholesterol intake, but nonetheless not quite alarming.

Author contributions

Conception and design of the research, Acquisition data, Analysis and interpretation of the data, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Santos HO.

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.

References

1. Ginsberg HN, Karmally W, Siddiqui M, Holleran S, Tall AR, Blaner WS, et al. Increases in dietary cholesterol are associated with modest increases in both LDL and HDL cholesterol in healthy young women. Arterioscler Thromb Vasc Biol. 1995;15(2):169-78. PMID: 7749822.
2. Edington J, Geekie M, Carter R, Benfield L, Fisher K, Ball M, et al. Effect of dietary cholesterol on plasma cholesterol concentration in subjects following reduced fat, high fibre diet. Br Med J (Clin Res Ed). 1987;294(6568):333-6. PMID: 3028545.
3. Griffin BA. Eggs: good or bad? Proc Nutr Soc. 2016;75(3):259-64. doi: 10.1017/S0029665116000215.
4. Fernandez ML. Rethinking dietary cholesterol. Curr Opin Clin Nutr Metab Care. 2012;15(2):117-21. doi: 10.1097/MCO.0b013e32834d2259.
5. Díez-Espino J, Basterra-Gortari FJ, Salas-Salvadó J, Buil-Cosiales P, Corella D, Schröder H, et al; PREDIMED Investigators. Egg consumption and cardiovascular disease according to diabetic status: the PREDIMED study. Clin Nutr. 2017;36(4):1015-21. doi: 10.1016/j.clnu.2016.06.009.
6. DiMarco DM, Norris GH, Millar CL, Blesso CN, Fernandez ML. Intake of up to 3 eggs per day is associated with changes in HDL function and increased plasma antioxidants in healthy, young adults. J Nutr. 2017;147(3):323-9. doi: 10.3945/jn.116.241877.
7. McNamara DJ. The impact of egg limitations on coronary heart disease risk: do the numbers add up? J Am Coll Nutr. 2000;19(5 Suppl):540S-548S. PMID: 11023005.
8. Miranda JM, Anton X, Redondo-Valbuena C, Roca-Saavedra P, Rodriguez JA, Lamas A, et al. Egg and egg-derived foods: effects on human health and use as functional foods. Nutrients. 2015;7(1):706-29. doi: 10.3390/nu7010706.
9. Knopp RH, Retzlaff BM, Walden CE, Dowdy AA, Tsunehara CH, Austin MA, et al. A double-blind, randomized, controlled trial of the effects of two eggs per day in moderately hypercholesterolemic and combined hyperlipidemic subjects taught the NCEP step I diet. J Am Coll Nutr. 1997;16(6):551-61. PMID: 9430083.
10. Spence JD, Jenkins DJ, Davignon J. Egg yolk consumption and carotid plaque. Atherosclerosis. 2012;224(2):469-73. doi: 10.1016/j.atherosclerosis.2012.07.032.
11. Reddy ST, Wadleigh DJ, Grijalva V, Ng C, Hama S, Gangopadhyay A, et al. Human paraoxonase-3 is an HDL-associated enzyme with biological activity similar to paraoxonase-1 protein but is not regulated by oxidized lipids. Arterioscler Thromb Vasc Biol. 2001;21(4):542-7. PMID: 11304470.
12. DiDonato JA, Huang Y, Aulak K, Even-Or O, Gerstenecker G, Gogonea V, et al. The Function and Distribution of Apolipoprotein A1 in the Artery Wall are Markedly Distinct from those in Plasma. Circulation. 2013;128(15):1644-55. doi: 10.1161/CIRCULATIONAHA.113.002624
13. Krauss RM. Dietary and genetic probes of atherogenic dyslipidemia. Arterioscler Thromb Vasc Biol. 2005;25(11):2265-72. doi: 10.1161/01.ATV.0000186365.73973.f0.
14. Xavier HT, Izar MC, Faria Neto JR, Assad MH, Rocha VZ, Sposito AC, et al; Sociedade Brasileira de Cardiologia. [V Brazilian Guidelines on Dyslipidemias and Prevention of Atherosclerosis]. Arq Bras Cardiol. 2013;101(4 Suppl 1):1-20. doi: http://dx.doi.org/105935/abc20135010.
15. Sacks FM, Lichtenstein AH, Wu JH, Appel LJ, Creager MA, Kris-Etherton PM, et al; American Heart Association. Dietary fats and cardiovascular disease: a presidential advisory from the American Heart Association. Circulation. 2017;136(3):e1-e23. doi: 10.1161/CIR.0000000000000510.
16. Office of Disease Prevention and Health Promotion (ODPHP). Advisory Report. Report Index. [Internet]. [Accessed in 2017 Jun 24]. Available from: https://health.gov/dietaryguidelines/2015-scientific-report/
17. Hunter PM, Hegele RA. Functional foods and dietary supplements for the management of dyslipidaemia. Nat Rev Endocrinol. 2017;13(5):278-88. doi: 10.1038/nrendo.2016.210.
18. Goodrow EF, Wilson TA, Houde SC, Vishwanathan R, Scollin PA, Handelman G, et al. Consumption of one egg per day increases serum lutein and zeaxanthin concentrations in older adults without altering serum lipid and lipoprotein cholesterol concentrations. J Nutr. 2006;136(10):2519-24. PMID: 16988120.
19. Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, et al; Mediterranean Diet Foundation Expert Group. Mediterranean diet pyramid today. Science and cultural updates. Public Health Nutr. 2011;14(12A):2274-84. doi: 10.1017/S1368980011002515.

Case 3/2018 – A 60-year-old Female with Chagasic Heart Disease, Admitted Due to Heart Failure Decompensation, Cachexia and Pulmonary Infection

Gustavo Alonso Arduine and Vera Demarchi Aiello

Instituto do Coração (InCor) do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP), São Paulo, SP - Brazil

The patient is a 60-year-old female with Chagas heart disease and cachexia, admitted due to heart failure decompensation attributed to bronchopneumonia. She eventually had a cardiac arrest with pulseless electrical activity after lung biopsy.

The patient was being followed up at InCor since the age of 48 years, diagnosed with Chagas disease. She initially complained of palpitations, which subsided after amiodarone was prescribed. In addition, she had systemic arterial hypertension.

Her laboratory tests revealed: potassium, 5.2 mEq/L; sodium, 144 mEq/L; creatinine, 0.8 mg/L; hemoglobin, 16.2 g/dL; hematocrit, 48%; glycemia, 87 mg/dL; cholesterol, 200 mg/dL; triglycerides, 53 mg/dL; TSH, 1.16 microIU/mL; free T4, 1.1 ng/dL; ALT, 8 IU/L; AST, 10 IU/L.

At the time, the ECG revealed diffuse ventricular repolarization changes.

The echocardiogram (August 2004) showed the following: aorta, 28 mm; left atrium, 52 mm; septal thickness, 11 mm; posterior wall, 7 mm; left ventricle (diast/syst), 71/62 mm; left ventricular ejection fraction (LVEF), 26%, with posterior (basal), inferior (basal) and lateral (basal) akinesia, and small apical aneurysm; right ventricle, 28 mm (dilated and hypokinetic); severe mitral regurgitation; and right ventricular systolic pressure, 65 mm Hg.

The chest X-ray (2012) showed cardiomegaly (Figure 1).

The Holter ECG at that time showed frequent ventricular extrasystoles and nonsustained ventricular tachycardia.

The patient remained asymptomatic until 2013 (57 years of age) using hydrochlorothiazide (25 mg), spironolactone (25 mg), carvedilol (12.5 mg), enalapril (20 mg) and amiodarone (100 mg) daily. In April 2013, she had a resuscitated cardiac arrest, preceded by malaise and sustained ventricular tachycardia, receiving an implantable cardioverter defibrillator (ICD) with cardiac pacemaker programing for bradycardia episodes (ICD-T – ICD with antibradycardia and antitachycardia pacing and shock), being prescribed 600 mg of amiodarone daily.

Five days before that episode, she had upper digestive bleeding with hematemesis, when endoscopy revealed gastric ulcer with no active bleeding, and clean base (Forrest III), with a lower risk for rebleeding.

The patient experienced appropriate shock in May 2014. She was receiving an amiodarone dose lower than prescribed, thus the dose was increased, but the patient did not tolerate it because of dyspepsia. A cardiac electrophysiology study was indicated, aimed at the possible ablation of the sustained ventricular tachycardia pathways.

The chest X-ray revealed pulmonary congestion and more severe cardiomegaly (Figure 2).

The echocardiogram (August 4, 2014) revealed: aorta, 27 mm; left atrium, 43 mm; ventricular septal thickness, 9 mm, posterior wall, 8 mm; left ventricular diameters, 68/57 mm; LVEF, 30%. The left ventricle showed eccentric hypertrophy and reduced systolic function due to an inferolateral wall aneurysm (mid and basal segments) and an apical aneurysm. The right ventricle was normal. There was moderate mitral valve regurgitation. The pulmonary artery pressure was 25 mm Hg.

Her coronary tomography angiography (July 28, 2014) evidenced no coronary lesion. The cardiac electrophysiology study (July 31, 2014) identified poorly-tolerated sustained ventricular tachycardia triggered by extra-stimuli, which required electric cardioversion. The electroanatomic mapping revealed a scar associated with low and slow late potentials in the lateral (mid and basal segments), antero-lateral (mid and basal segments) and inferolateral (mid and basal segments) walls. Because of the proximity to the circumflex sub-branches, the radiofrequency pulses were not delivered through the epicardium, but through the endocardium. After the procedure, the new stimuli no longer triggered ventricular tachycardia similar to that initially observed. However, several poorly tolerated tachycardias of different morphologies were triggered, requiring cardioversion.

On outpatient follow-up (November 2014), the patient complained of dyspnea on exertion and dizziness when changing from supine to orthostatic position.

Her physical examination (November 11, 2014) revealed: weight, 70 kg; height, 1.7 m; arterial blood pressure, 102/80 mmHg; heart rate, 68 bpm. The pulmonary auscultation was normal. On cardiac auscultation, her heart rhythm was regular with no abnormal heart sound, and a systolic heart murmur was heard over the mitral area (++/4+). The abdominal examination was normal. There was no edema, and her pulses were palpable and symmetrical. Because of her complaints

Keywords

Chagas Disease; Chagas Cardiomyopathy; Heart Failure; Cachexia; Pneumonia.

Section editor: Alfredo José Mansur (ajmansur@incor.usp.br)

Associate editors: Desidério Favarato (dclfavarato@incor.usp.br)

Vera Demarchi Aiello (vera.aiello@incor.usp.br)

Mailing Address: Vera Demarchi Aiello •
Avenida Dr. Enéas de Carvalho Aguiar, 44, subsolo, bloco I, Cerqueira César. Postal Code 05403-000, São Paulo, SP – Brazil
E-mail: demarchi@cardiol.br, vera.aiello@incor.usp.br

DOI: 10.5935/abc.20180100

Anatomopathological Correlation

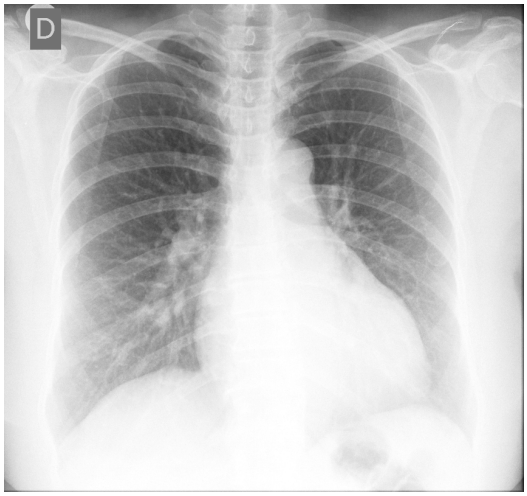


Figure 1 – *Chest X-ray (posterior-anterior view): increased pulmonary vasculature and cardiomegaly (+++).*

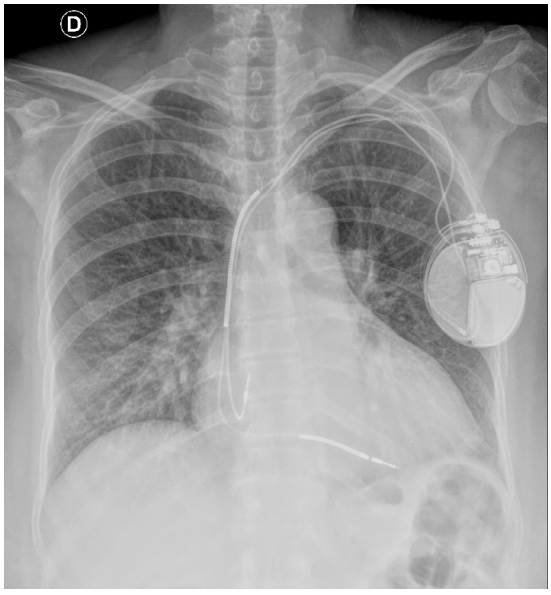


Figure 2 – *Chest X-ray (posterior-anterior view): presence of cardiac pacemaker, worse pulmonary congestion, cardiomegaly (+++).*

and arterial blood pressure levels, hydrochlorothiazide was suspended, and the other medications, maintained.

Her laboratory tests (October 2014) showed: hemoglobin, 13.2 g/dL; hematocrit, 43%; leukocytes, 7220/mm^3 (normal differential count); platelets, 200000/mm^3; total cholesterol, 180 mg/dL; HDL-cholesterol, 73 mg/dL; LDL-cholesterol, 88 mg/dL; triglycerides, 95 mg/dL; glycemia, 94 mg/dL; creatinine, 0.96 mg/dL; sodium, 141 mEq/L; potassium, 4.8 mEq/L; AST, 79 IU/L; ALT, 111 IU/L; uric acid, 4.2 mg/dL; C-reactive protein, 6.78 mg/L; TSH, 2.85 μg/mL; free T4, 1.62 mg/dL.

On outpatient follow-up visits (October 2015 and March 18, 2016), she denied dyspnea, chest pain, palpitations and syncope, but complained of dizziness. The ICD/pacemaker assessment was normal.

Her chest X-ray (2015) revealed pulmonary congestion and cardiomegaly (Figure 3).

On September 22, 2016, the patient was admitted due to decompensated heart failure and bronchopneumonia. She reported progressive worsening of dyspnea, which was then triggered on mild exertion. She denied chest pain and fever, but reported dry cough, lack of appetite and weight loss.

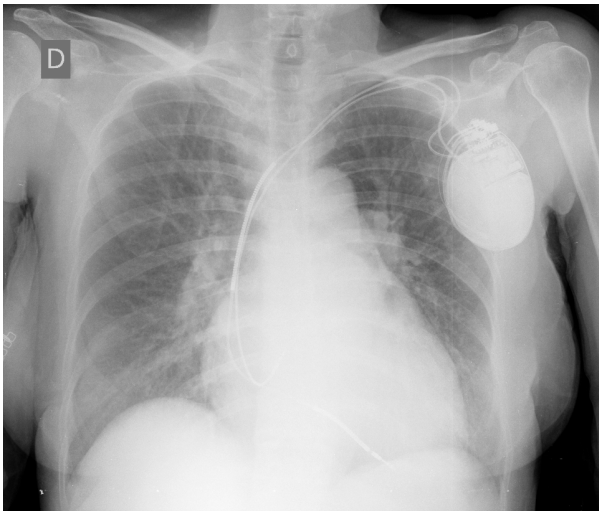


Figure 3 – *Chest X-ray (posterior-anterior view): increase and cephalization of the pulmonary vasculature.*

Her physical examination revealed an emaciated patient (53 kg), on regular general condition, tachypneic, with heart rate of 72 bpm, arterial blood pressure of 95/60 mm Hg, and O_2 saturation of 92%. Her auscultation revealed rales over the pulmonary bases, rhythmic heart sounds, and systolic heart murmur over the mitral area (++++/6+). Her abdomen was flaccid, with a tender liver, palpated 2 cm from the costal margin. There was lower limb edema (+++/4+).

The patient was using amiodarone (200 mg), enalapril (5 mg), hydrochlorothiazide (25 mg), levothyroxine (25 μg), metoprolol (100 mg), simvastatin (20 mg), warfarin (5 mg).

Her ECG (September 22) showed: sinus rhythm; heart rate, 63 bpm; indirect signs of right atrial overload (Peñaloza-Tranchesi); low-voltage QRS complexes on the frontal plane; probable electrically inactive lateral area; left anterior-superior hemiblock (Figure 4). After a few days, the new ECG revealed an operational pacemaker with atrial stimulus propagating to the ventricle (AAI) (Figure 5).

Her chest X-ray (September 22, 2016) evidenced the presence of an ICD/pacemaker with electrodes in the left atrium and ventricle, pulmonary congestion, opacity area suggestive of pneumonia in the right pulmonary lower field (air bronchogram), elevation of the left main bronchus (suggestive of enlarged left atrium), and global enlargement of the heart area, attributed mainly to the right ventricle (Figure 6).

Her laboratory tests (September 22) revealed: hemoglobin, 8.2 g/dL; hematocrit, 26%; leukocytes, 17500/mm^3 (neutrophils 79%, eosinophils 0%, lymphocytes 15% and monocytes 6%); platelets, 344000/mm^3; urea, 33 mg/dL; creatinine, 0.71 mg/dL; AST, 148 IU/L; ALT, 136 IU/L; gamma GT, 36 IU/L; alkaline phosphatase, 75 mg/dL; total proteins, 6.9 g/dL; albumin, 3.1 g/dL; C-reactive protein, 124.39 mg/L; sodium, 140 mEq/L; potassium, 3.6 mEq/L. Arterial blood gas analysis: pH, 7.54; pCO_2, 31.1 mm Hg; pO_2, 62.3 mmHg; O_2 saturation, 93%; bicarbonate, 26.2 mmol/L; base excess, 3.9 mmol/L.

Because of the diagnostic suspicion of pneumonia, ceftriaxone and clarithromycin were initiated.

The dyspnea and edema improved, arterial hypotension episodes occurred, and the dry cough and mild hyperthermia (37.6°C) persisted.

Assessment of the ICD/pacemaker revealed 14 episodes of ventricular tachycardia in July 2016: 12 abolished by burst (high-frequency stimuli) and 2 abolished with 31-J shocks.

Her echocardiogram (September 26, 2016) showed: aorta, 26 mm; left atrium, 60 mm; basal and mid right ventricle, 43 mm and 33 mm, respectively; ventricular septal thickness, 10 mm; posterior wall, 7 mm; left ventricle (diast/syst), 73/60 mm; LVEF, 40%. The atria were severely enlarged, the left ventricle was dilated with dyskinesia of the lateral wall (basal segment), akinesia of the inferior wall (basal segment), and apical aneurysm. The right ventricular function was normal. The mitral and tricuspid valves showed severe regurgitation due to inadequate leaflet coaptation. Systolic pulmonary artery pressure was estimated at 47 mmHg. No pericardial change was observed.

On the days following admission, her laboratory tests continued to show leukocytosis (around 19000), anemia, hypoalbuminemia (1.9 g/dL) and elevated C-reactive protein (above 150 mg/L).

A new chest X-ray detected an opaque nodule in the right base (Figure 7).

Chest tomography (September 28, 2016) evidenced: pacemaker with endocavitary electrodes in the right chambers; dilation of the pulmonary trunk (41 mm); permeable trachea and main bronchi of normal caliber; diffuse thickening of the bronchial walls; enlarged pulmonary hila; and hilar calcifications that could be lymph node sequelae. There was an irregular nodule measuring 2.3 x 2.5 x 1.9 cm in the transition between the medial and lateral segments of the middle lobe, in

Anatomopathological Correlation

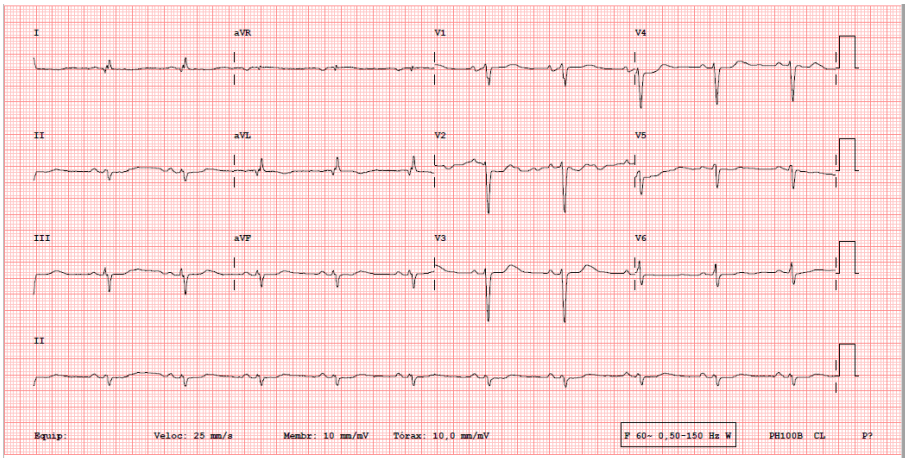


Figure 4 – *ECG: sinus rhythm, low-voltage QRS complexes on the frontal plane, probable electrically inactive lateral area, right bundle-branch block, left anterior-superior hemiblock.*

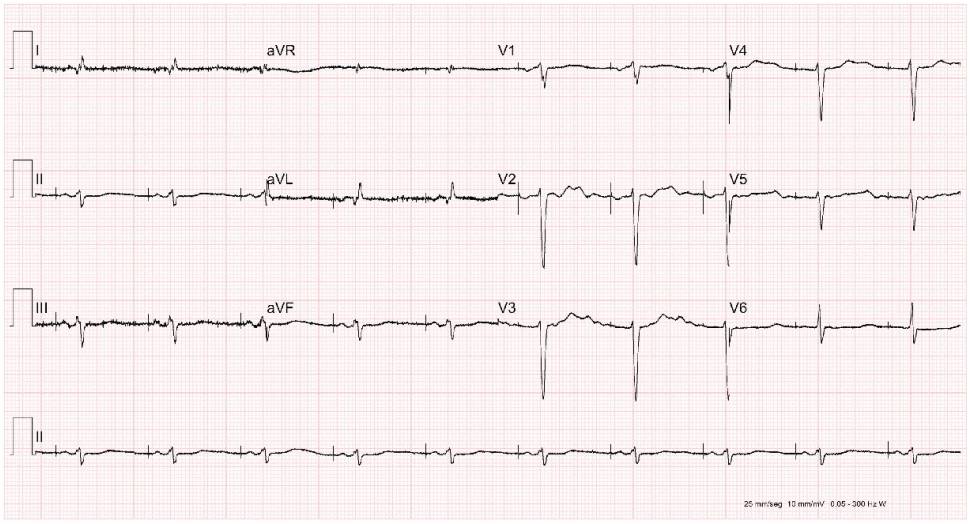


Figure 5 – *ECG: operational pacemaker with atrial stimulus propagating normally to the ventricle.*

addition to diffuse ground-glass opacity with septal thickening, mainly in the bases, compatible with congestion. Furthermore, there was a peripheral hyperdense area, close to the pleural surface of the right superior lobe, suggestive of subacute parenchymal bleeding or accumulation of amiodarone. The pulmonary vascularization was increased, and there was small bilateral pleural effusion. In the liver, low-density nodules were identified, but their assessment was limited due to lack of contrast media imaging. In addition, there were sparse nodular calcifications in the liver parenchyma, whose density was diffusely increased (suggestive of amiodarone deposition). Dilation of the inferior vena cava and of the hepatic veins, as well as ectasia of the gallbladder, was observed. No lymph node enlargement was identified. The heart was diffusely enlarged, with predominance of the left atrium.

Because the patient was emaciated, had moderate reduction of the LVEF and altered pulmonary imaging, the search for consumptive syndrome causes other than heart failure began. Sputum tests (three samples on different days) were negative for acid-fast bacillus. The bacteriologic examination of the sputum revealed the usual local flora: Gram-positive bacilli, Gram-positive cocci, Gram-negative bacilli, Gram-negative diplococci and Gram-negative coccobacilli.

The patient was submitted to biopsy of the pulmonary nodule (October 12, 2016), which revealed a granulomatous chronic inflammatory process with extensive area of necrosis. The search for fungi was negative and that for acid-fast bacillus was ongoing.

On the night of October 12, 2016, 12 hours after the biopsy, the patient woke up complaining of ill-defined malaise. Her initial examination revealed arterial blood pressure of 50/40 mmHg, O_2 saturation of 99% with O_2 catheter, tachycardia and tachypnea. The patient had a cardiac arrest with pulseless electrical activity. She was resuscitated but developed asystole and died.

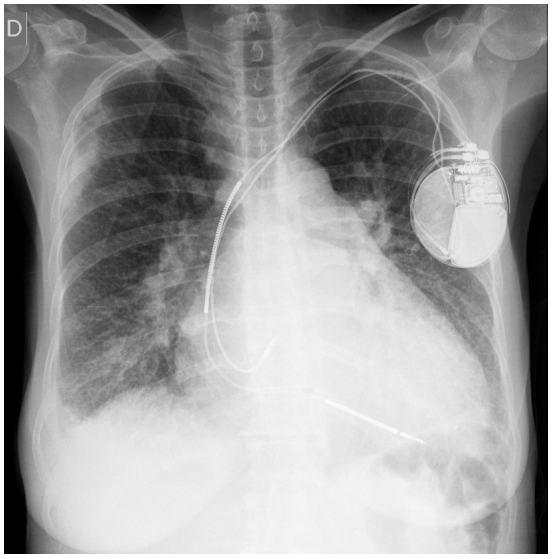


Figure 6 – *Chest X-ray (posterior-anterior view): presence of cardiac pacemaker, pulmonary congestion, and cardiomegaly (++++/4).*

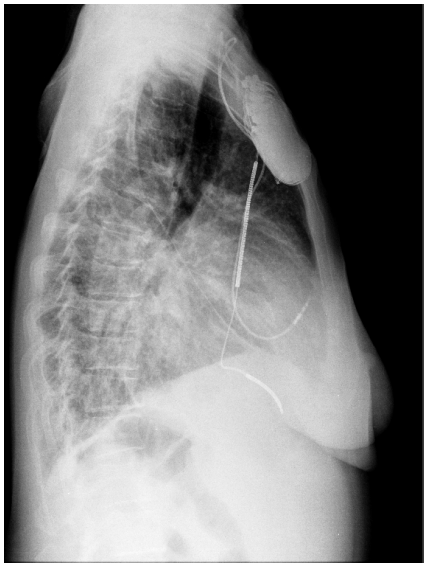


Figure 7 – *Chest X-ray (lateral view): similar to the previous ones, the only difference being the presence of an opaque nodule in the right base.*

Clinical aspects

This 60-year-old patient had the following problems: dyspnea, dry cough, weight loss, slightly elevated body temperature, anemia, enlarged pulmonary hila, hilar calcifications (lymph nodes) and pulmonary nodule (granuloma with necrosis). In addition, she had the following antecedents: Chagas disease (not confirmed, because no serology for Chagas disease was performed), systemic arterial hypertension, heart failure with reduced ejection fraction, and previous akinetic areas, apical aneurysm and sustained ventricular tachycardia.

Of the granulomatous diseases, sarcoidosis has unspecific symptoms, such as fever, weight loss, nocturnal sweating and fatigue. Other symptoms depend on the organs or parts of the body affected, such as the lungs (dry cough, dyspnea, chest pain), eyes (eye pain, blurred vision), skin, musculoskeletal system (joint pain, myalgias) and lymph nodes (swelling).[1] Although heart involvement is diagnosed in 5% to 10% of

the cases, on postmortem examination, it can range from 10% to 76%, causing bundle-branch block, repolarization disorders, arrhythmias and cardiomyopathy.[2,3] Isolated cardiac sarcoidosis can occur in up to 25% of the cases; thus, absence of extracardiac sarcoidosis does not exclude heart involvement.[4,5]

The most common clinical characteristic of cardiac sarcoidosis is biventricular heart failure, with or without evidence of noncardiac involvement. In addition, mitral regurgitation can be severe and caused by the involvement of the papillary muscle, which could explain this patient's mitral regurgitation.[6] Ventricular arrhythmias (sustained or non-sustained ventricular tachycardia and premature ventricular beats) are the second most common clinical presentation of cardiac sarcoidosis, occurring in approximately 30% of the cases.[7]

The echocardiographic findings in patients with cardiac sarcoidosis vary and can include focal areas of edema, resulting in wall thickening and hypertrophic cardiomyopathy mimicking (for example, asymmetric septal hypertrophy), or, in more advanced patterns of involvement, focal areas of akinesia, dyskinesia or aneurysm.[8]

Although cardiac sarcoidosis has been described as a restrictive cardiomyopathy, its most common phenotype is dilated cardiomyopathy, with occasional aneurysm formation.[6]

Because the above described disease has many findings in common with those of our patient, our clinical hypothesis is systemic sarcoidosis with cardiac and pulmonary involvement. However, infectious causes for the respiratory impairment cannot be ruled out, the most common and prevalent in Brazil being tuberculosis, which also forms granulomas.

It is worth noting, however, that sarcoid granulomas are usually "non-caseating", that is, have no necrosis. Thus, the lymph node biopsy of our patient does not support that diagnosis. (Gustavo Alonso Arduine, MD)

Diagnostic hypothesis: syndromic: heart failure with reduced ejection fraction; etiological: systemic sarcoidosis (cardiac and pulmonary). (Gustavo Alonso Arduine, MD)

Final finding: Mixed septic and cardiogenic shock (Gustavo Alonso Arduine, MD)

Postmortem examination

The postmortem examination revealed an emaciated female patient, with a cutaneous sign of thoracic needle puncture on the right side. Chest opening evidenced blood clots on the parietal pleura and blood collection in the right pleural cavity (total of 1400 mL).

The heart was moderately enlarged (450 g), with mild biventricular dilation and an aneurysm formation with thinning of the left lateral myocardial wall (approximately 4x3 cm) (Figure 8). That area evidenced fibrous replacement of the myocardium. The mitral valve showed signs of regurgitation secondary to annulus dilation. In the right chambers, the

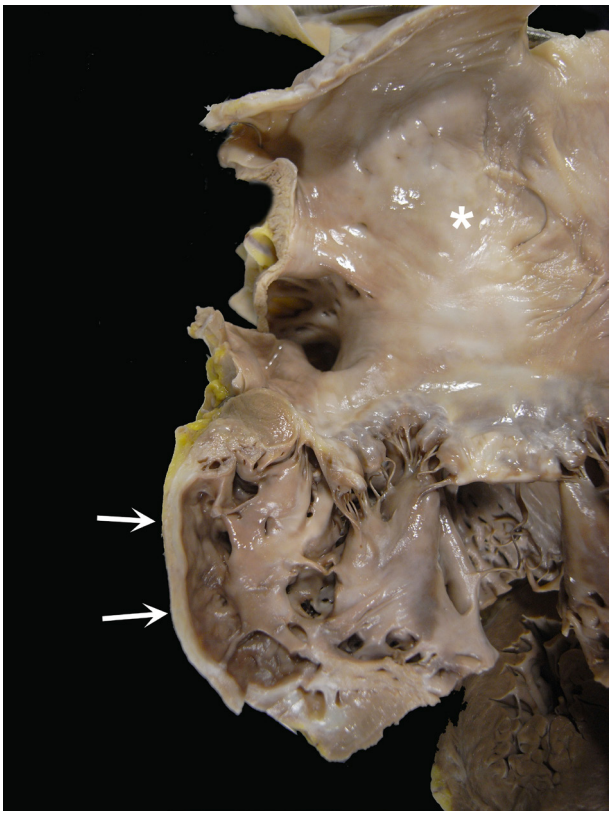


Figure 8 – *Gross examination: aneurysmal thinning of the left ventricular lateral wall, with fibrous replacement of the myocardium (arrows). The left atrium (asterisk) is extremely dilated.*

pacemaker metallic leads could be seen, one attached to the atrium and the other to the trabecular portion of the ventricle (Figure 9). On its way through the tricuspid valve, the lead was adhered and covered by a whitish sheath. No cavitary thrombus was seen.

The examination of the lungs evidenced an ill-defined brownish nodule, with necrotic center, in the right middle lobe, measuring 2.5 cm in its long axis (Figure 10).

The hilar and subcarinal lymph nodes were enlarged, confluent, and had extensive nodular, whitish areas (Figure 11).

The liver weighed 2223 g and had a finely granular surface.

The microscopic study of the myocardium showed hypertrophied cardiomyocytes, varied focal fibrosis, and focal and mild inflammatory infiltrate.

The microscopic study of the lungs and lymph nodes showed extensive chronic granulomatous inflammation with caseating necrosis, including in the nodular area of the pulmonary parenchyma (Figure 12). The search for acid-fast bacilli was positive, with a small number of bacilli in the caseating lesions (not shown).

The microscopic study of the liver evidenced diffuse nodular transformation, expansion of the portal spaces and diffusely damaged hepatocytes, characterized by the presence of multiple eosinophilic inclusions in their cytoplasm (Mallory bodies) (Figure 13). (Vera Demarchi Aiello, Prof., M.D.)

Anatomopathological diagnoses:

- Chronic heart disease, probably of Chagasic etiology, with an aneurysm of the lateral wall;
- Productive-caseating tuberculosis in the lungs and mediastinal lymph nodes;
- Chronic liver disease progressing to cirrhosis, with characteristics of cellular damage secondary to the chronic use of amiodarone;
- Hemothorax. (Vera Demarchi Aiello, Prof., M.D.)

Comments

The patient had chronic heart disease with arrhythmia, having received specific treatment with pacemaker implantation and prescription of antiarrhythmic drugs. Her clinical condition worsened due to the development of productive-caseating tuberculosis in the lungs and mediastinal lymph nodes, which motivated the final diagnostic investigation. The clinical hypothesis of sarcoidosis was ruled out by the finding of the infectious agent (acid-fast bacilli) in the granulomatous lesions.

Despite the lack of diffuse myocarditis on the microscopic study, Chagasic heart disease is the most probable diagnosis, considering the gross morphological aspect of the heart, with fibrous replacement of the left ventricular lateral/inferior wall and the presence of diffuse interstitial fibrosis, although such findings are not characteristic.

The microscopic findings in the liver parenchyma point to a type of cell damage related to drug toxicity. Because of the report of this patient having received amiodarone during her disease, we concluded that her liver damage is related to that drug. That type of lesion is characterized by nodular transformation (cirrhosis) and hepatitis with several Mallory

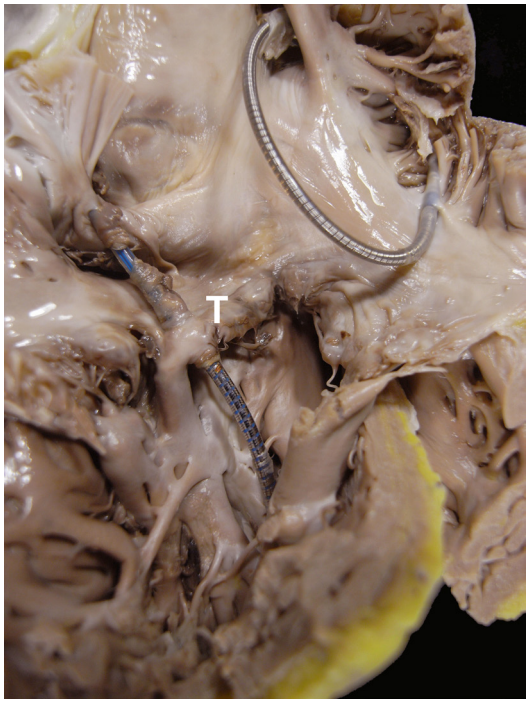


Figure 9 – *Right chambers showing two endocardial pacemaker metallic leads, one attached to the atrium and the other to the ventricular apex. T- Tricuspid valve.*

Anatomopathological Correlation

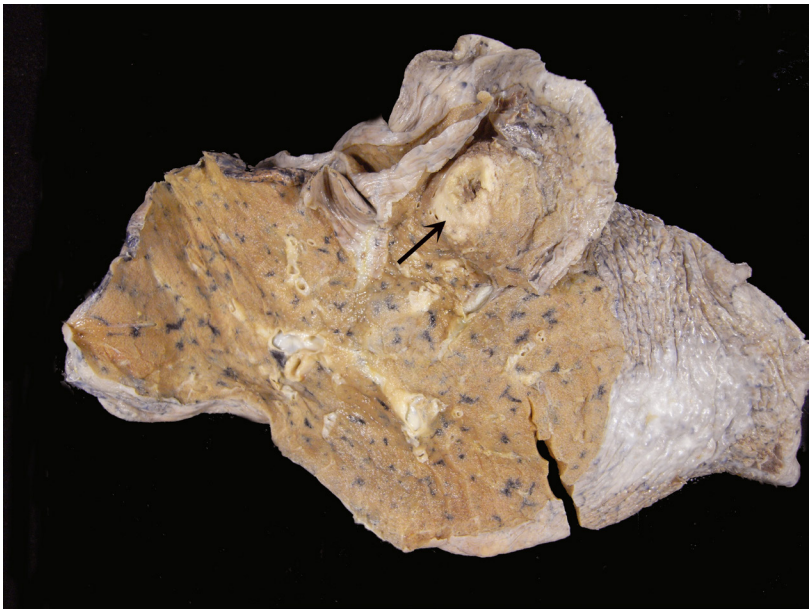


Figure 10 – *Cut surface of the lung showing an ill-defined nodule with necrotic center (arrow).*

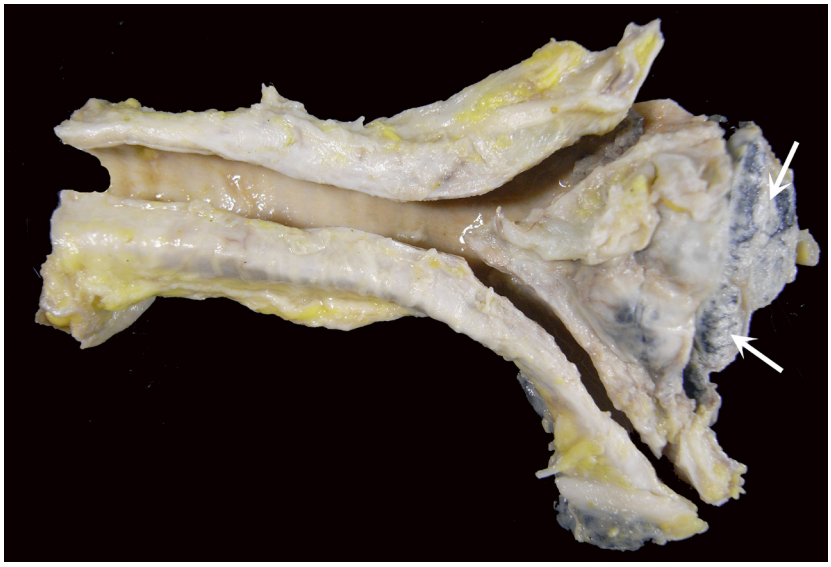


Figure 11 – *Gross examination of the longitudinally opened trachea on its posterior face. In the subcarinal region, confluent lymph nodes with whitish nodular areas are seen (arrows).*

bodies. In the past, such bodies were known to be associated with alcoholic liver disease. However, several studies have shown the pathogenic role of amiodarone and its metabolites on the development of liver disease.[9,10] Those substances accumulate in the hepatocytes, Kupffer cells and ductal cells, resulting in inhibition of the removal of lysosomal lipids. Hepatotoxicity occurs in 1% to 3% of the patients treated with amiodarone and seems to be dose-dependent (cumulative). However, the prevalence of pulmonary toxicity is higher, estimated at 5% to 7%. We believe that the liver damage contributed to the changes in coagulation that culminated in hemothorax.

There was no time to initiate the specific treatment against tuberculosis, which might have had a positive impact on this patient's outcome. (Vera Demarchi Aiello, Prof., M.D.)

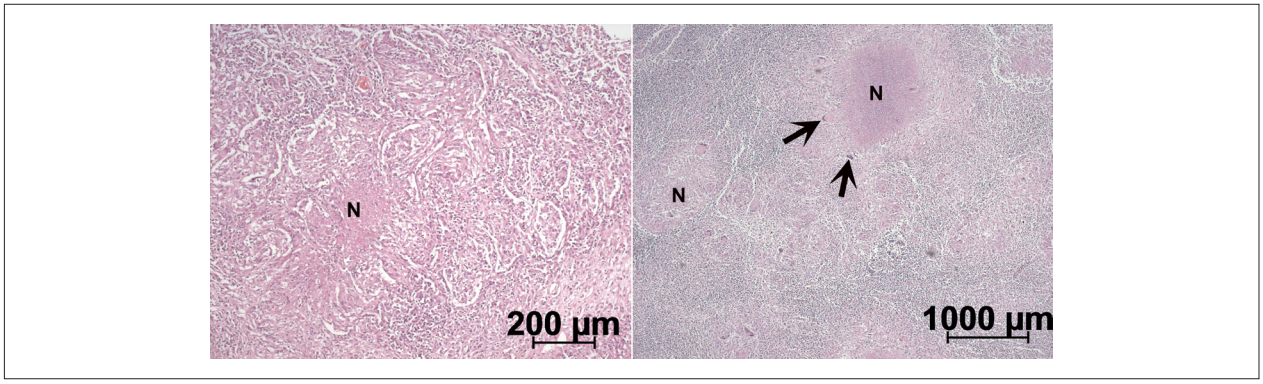


Figure 12 – *Microscopic examination: granulomatous chronic inflammation of the lung (left panel) and lymph node (right panel). Note the numerous giant cells (arrows) and foci of caseating necrosis (N). Hematoxylin-eosin, 10X and 5X.*

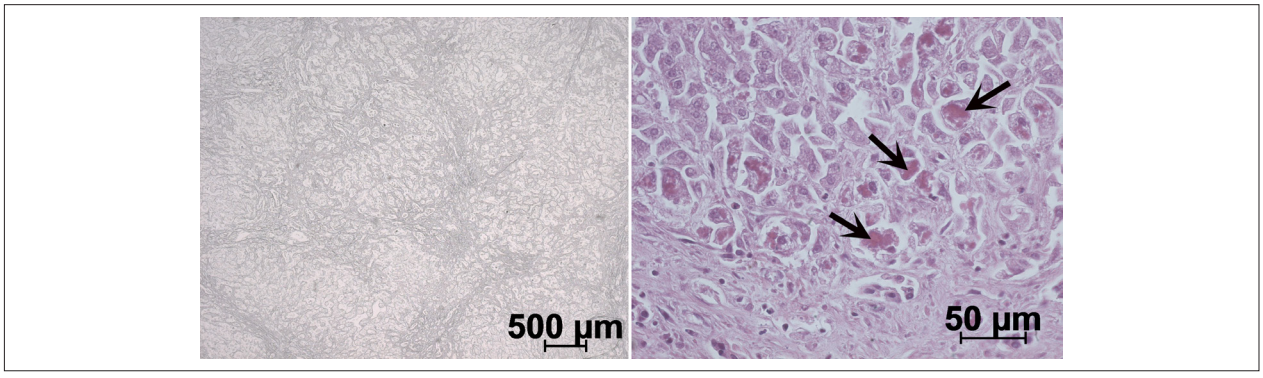


Figure 13 – *Photomicrograph of the liver. Left panel: nodular transformation of the parenchyma, reticulin stain, 5X. Right panel: hepatocytes with multiple eosinophilic bodies (Mallory bodies - arrows), hematoxylin-eosin, 40X.*

References

1. Thomas KW, Hunninghake GW. Sarcoidosis. JAMA. 2003;289(24):3300-03.
2. Newman LS, Rose CS, Maier LA. Sarcoidosis. N Engl J Med. 1997;336(17):1224-34. Erratum in: N Engl J Med. 1997;337(2):139.
3. Dengue JC, Baughman RP, Lynch JP 3rd. Cardiac involvement in sarcoidosis. Semin Respir Crit Care Med. 2002;23(6):513-27.
4. Okada DR, Bravo PE, Vita T, Agarwal V, Osborne MT, Taqueti VR, et al. Isolated cardiac sarcoidosis: A focused review of an under-recognized entity. J Nucl Cardiol. 2016 Sep 9. [Epub ahead of print].
5. Kandolin R, Lehtonen J, Graner M, Schildt J, Salmenkivi K, Kivistö SM, et al. Diagnosing isolated cardiac sarcoidosis. J Intern Med. 2011;270(5):461-8.
6. Juneau D, Nery P, Russo J, de Kemp RA, Leung E, Beanlands RS, et al. How common is isolated cardiac sarcoidosis? Extra-cardiac and cardiac findings on clinical examination and whole-body ^{18}F–fluorodeoxyglucose positron emission tomography. Int J Cardiol. 2018 Feb 15;253:189-193.
7. Kandolin R, Lehtonen J, Airaksinen J, Vihinen T, Miettinen H, Ylitalo K, et al. Cardiac sarcoidosis: epidemiology, characteristics, and outcome over 25 years in a nationwide study. Circulation. 2015;131(7):624-32.
8. Agarwal A, Sulemanjee NZ, Cheema O, Downey FX, Tajik AJ. Cardiac sarcoid: a chameleon masquerading as hypertrophic cardiomyopathy and dilated cardiomyopathy in the same patient. Echocardiography. 2014;31(5):E138-41.
9. Vorperian VR, Havighurst TC, Miller S, January CT. Adverse effects of low dose amiodarone: a meta-analysis. J Am Coll Cardiol. 1997;30(3):791-8.
10. Hussain N, Bhattacharyya A, Prueksaritanond S. Amiodarone-induced cirrhosis of liver: what predicts mortality? ISRN Cardiol. 2013;2013:617943.

Eosinophilic Myocarditis: Clinical Case and Literature Review

Paulo Dinis, Rogério Teixeira, Luís Puga, Carolina Lourenço, Maria Carmo Cachulo, Lino Gonçalves
Centro Hospitalar e Universitário de Coimbra, Hospital Geral, Coimbra - Portugal

Introduction

Eosinophilic myocarditis is a rare and potentially lethal disease characterized by eosinophil infiltration of the myocardium.[1] The association between eosinophilia and myocardial injury is well established and may present several etiologies, from hypersensitivity and autoimmune diseases to neoplasias and infections.[1,2] In some cases the etiology remains unknown, and it is denominated idiopathic hypereosinophilic syndrome. Clinical manifestations present a wide spectrum, ranging from mild symptomatology to severe symptoms such as retrosternal pain, rhythm disturbances, and sudden death.[2,3] The definitive diagnosis is made through endomyocardial biopsy.[1] Cardiac magnetic resonance imaging is a valid alternative, identifying the main structural changes caused by myocarditis.[4] Treatment includes neurohumoral therapy, management of cardiac complications, and in cases selected, systemic corticosteroid therapy.[5] Next, we present the case of a patient with symptomatology suggestive of myocardial infarction, but who in the course of the investigation had the diagnosis of eosinophilic myocarditis.

Case report

Patient 79 years old, female, who came to the Emergency Department with complaints of epigastralgia with two weeks of evolution and aggravation last night. She denied another accompanying symptomatology. As personal background, she presented unmedicated dyslipidemia and intrinsic asthma with onset in adulthood. She was medicated with bronchodilators and an association of a B2-agonist with inhaled corticosteroids at low doses.

The objective examination showed tachycardia, confirmed on electrocardiogram with sinus rhythm of 125 beats per minute. Analytically had leukocytosis (13.2 x 10^3/uL) and eosinophilia (2.8 x 10^3/uL or 23%), C-reactive protein (0.8 mg/dL) and elevation of markers of myocardial necrosis (troponin I of 7.6 ng/mL). Transthoracic echocardiography revealed severe left ventricular systolic dysfunction with an ejection fraction estimated at 30-35%, ventricular septal hypocontractility and an increase in the concentric thickness of the ventricular walls.

Keywords

Eosinophilia; Myocarditis; Hypereosinophilic Syndrome / mortality; Hypereosinophilic Syndrome / drug therapy; Magnetic Resonance Imaging.

Mailing Address: Paulo Dinis
Rua Vila de Manteigas, nº 28, 2º Dto. Posta Code 6300-617, Guarda – Portugal
E-mail: paulogdinis@gmail.com
Manuscript received May 09, 2017, revised manuscript June 08, 2017, accepted June 13, 2017

DOI: 10.5935/abc.20180089

Valvular disease was not evident. It was placed as a first hypothesis that it was an acute coronary syndrome, so anti-ischemic therapy with double platelet antiaggregation, enoxaparin, was started and the patient was assigned to an invasive strategy. Coronary angiography did not reveal epicardial coronary disease. After this, the diagnosis of eosinophilic myocarditis in a patient with a known atopic component was likely. She was admitted to hospital for treatment and study. Neuro-humoral, beta-blocker and diuretic therapy were initiated, maintaining aspirin.

On the third day of hospitalization, cardiac magnetic resonance was performed which identified subepicardial foci of edema and late enhancement in the left ventricular myocardium (Figure 1); she also showed a small pericardial effusion in the free wall of the right ventricle. The ejection fraction was quantified by 33%. On the same day, she underwent an endomyocardial biopsy and collection of right ventricular infarct fragments, which confirmed the diagnosis of eosinophilic myocarditis (Figure 2). Systemic corticosteroid therapy was started with intravenous prednisolone (1 mg/kg/day) with progressive improvement of general condition. On the 12th day of hospitalization, the echocardiogram showed a slight improvement in left ventricular global systolic function (ejection fraction estimated at 35-40%). She was discharged to home with prednisolone in weaning, and with follow-up consultation of cardiology and autoimmune diseases.

The autoimmune serological study was negative. After seven months of corticotherapy, the echocardiogram showed a significant improvement (ejection fraction estimated at 45-50%), and a decrease in concentric hypertrophy.

Discussion

In the case described, the patient had a history of asthma, which may have been the starting point for hypereosinophilia. She also presented an epigastric discomfort, which may be an atypical presentation of an acute coronary syndrome.[6] Electrocardiographic findings, sinus tachycardia, are neither specific nor sensitive.[1] Analytically, leukocytosis and eosinophilia with troponin I elevation were evident and explained by infiltration of eosinophils into the myocardium. This infiltration allows the release of toxic granules, cationic proteins, pro-inflammatory cytokines, and oxygen free radicals that will cause mitochondrial dysfunction, myocyte injury and necrosis.[7]

Complementary diagnostic tests are important in the evaluation of this pathology. The echocardiogram allows excluding other causes, to monitor dimension of cavities, thickness of the ventricular walls, presence of pericardial effusion and to evaluate left ventricular systolic and diastolic function. Cardiac magnetic resonance provides a combination of safety, anatomical definition, and tissue characterization of the myocardium.[4] It allowed the identification of edema

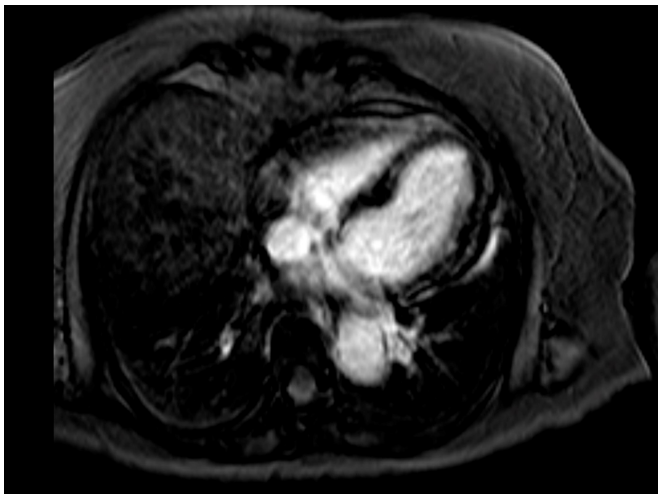


Figure 1 – *Cardiac magnetic resonance with subepicardial foci of edema and late enhancement of the myocardium in the left ventricle.*

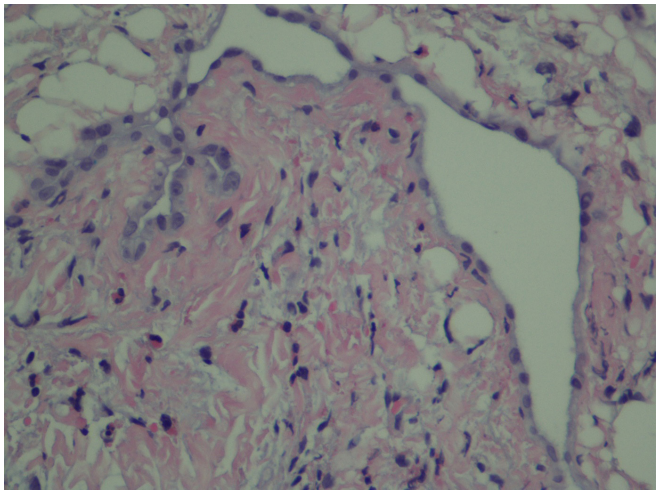


Figure 2 – *Endomyocardial biopsy with presence of eosinophils.*

and diffuse foci of late enhancement reflecting myocardial necrosis and fibrosis.[4] The presence of pericardial effusion and left ventricular systolic dysfunction reinforce the evidence of myocarditis. In stable patients, it is reasonable to perform cardiac magnetic resonance imaging prior to biopsy, since the former may help to identify focal pathology through late enhancement. However, in unstable patients the biopsy should be a priority.[1] Endomyocardial biopsy is the only method that allows definitive diagnosis and identification of the underlying etiology. It has a sensitivity estimated at 50% due to sample errors.[1,2] Although it is the gold standard, in clinical practice it is not always performed, existing recommendations[1,8] for its execution, which are dependent on the clinic and the results of the complementary tests. The pseudo-ischemic presentation of the patient, with elevation of markers of myocardial necrosis and exclusion of epicardial coronary disease, and alterations in the imaging tests done, fulfilled the criteria for performing the biopsy.[1,8,9] In these cases, magnetic resonance imaging cardiac and endomyocardial biopsy together present synergies that go beyond the limitations that each exam presents separately.[9]

The treatment and prognosis of eosinophilic myocarditis depends on its etiology. In the acute phase, restriction of physical activity is important.[1] In selected patients, particularly those with negative virology and suspected autoimmune etiology, early treatment with corticosteroids has shown favorable results.[5,10] Due to the clinical and hemodynamic stability of the patient, and after infective exclusion, we decided to postpone the onset of corticosteroids until confirmation of eosinophilic myocarditis. In the literature, it is described that a period of immunosuppressive therapy of six months can bring significant improvements in the left ventricular function (increase of 15-20% on the ejection

fraction),[10] which was verified in this case. The question remains whether this improvement is due only to the corticosteroid or if it is associated with initiation of therapy for heart failure, in particular beta-blockers. The mechanism of action of corticosteroids in myocarditis is not fully understood, however it is thought that they interfere with eosinophilia; antagonize the development and maturation pathways; and promote the redistribution of peripheral blood eosinophils.[10]

During follow-up, all patients should be submitted to clinical evaluations with electrocardiogram and echocardiogram. If clinical or imaging worsening occurs, hospital readmission and repeat cardiac magnetic resonance and / or endomyocardial biopsy may be required.[1,9]

Conclusion

Eosinophilic myocarditis is a rare, undiagnosed condition that can be fatal if not detected and treated in time.

Author contributions

Acquisition of data and Writing of the manuscript: Dinis P, Puga L; Critical revision of the manuscript for intellectual content: Dinis P, Teixeira R, Lourenço C, Cachulo MC, Gonçalves L.

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.

References

1. Caforio A, Pankuweit S, Arbustini E, Basso C, Gimeno-Blanes J, Felix S, et al; European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2013;34(33):2636-48, 2648a-2648d. doi: 10.1093/eurheartj/eht210.
2. Rezaizadeh H, Sanches-Ross M, Kaluski E, Klapholz M, Haider B, Gerula C. Acute eosinophilic myocarditis: diagnosis and treatment. Acute Card Care. 2010;12(1):31-6. doi: 10.3109/17482940903578998.
3. Thambidorai SK, Korlakunta HL, Arouni AJ, Hunter WJ, Holmber MJ. Acute eosinophilic myocarditis mimicking myocardial infarction. Tex Heart Inst J 2009;36(4):355-7. PMID: 19693316.
4. Friedrich MG, Sechtem U, Schultz-Menger J, Holmvang G, Alakija P, Cooper LT, et al; International Consensus Group on Cardiovascular Magnetic Resonance in Myocarditis. Cardiovascular magnetic resonance in myocarditis: A JACC White paper. J Am Coll Cardiol. 2009;53(17):1475-87. doi: 10.1016/j.jacc.2009.02.007.
5. Aggarwal H, Jain D, Kaverappa V, Jain P, Kumar A, Yadav S, et al. Idiopathic hypereosinophilic syndrome presenting as severe Loeffler's endocarditis. Arq Bras Cardiol. 2013;100(4):e43-6. PMID: 23681213.
6. El-Menyar A, Zubaid M, Sulaiman K, AlMahmeed W, Singh R, Alsheikh-Ali AA, et al; Gulf Registry of Acute Coronary Events (Gulf RACE) Investigators. Atypical presentation of acute coronary syndrome: a significant independent predictor of in-hospital mortality. J Cardiol. 2011;57(2):165-71. doi: 10.1016/j.jjcc.2010.11.008.
7. Arima M, Kanoh T. Eosinophilic myocarditis associated with dense deposits of eosinophilic cationic protein (ECP) in endomyocardium with high serum ECP. Heart. 1999;81(6):669-71. PMID: 10336931.
8. Butterfield JH, Kane GC, Weiler CR. Hypereosinophilic syndrome: endomyocardial biopsy versus echocardiography to diagnose cardiac involvement. Postgrad Med. 2017;129(5):517-23. doi: 10.1080/00325481.2017.1317215.
9. Baccouche H, Mahrholdt H, Meinhardt G, Merher R, Voehringer M, Hill S, et al. Diagnostic synergy of non-invasive cardiovascular magnetic resonance and invasive endomyocardial biopsy in troponin-positive patients without coronary artery disease. Eur Heart J. 2009;30(23):2869-79. doi: 10.1093/eurheartj/ehp328.
10. Frustaci A, Russo M, Chimenti C. Randomized study on the efficacy of immunosuppressive therapy in patients with virus-negative inflammatory cardiomyopathy: the TIMIC study. Eur Heart J. 2009;30(16):1995-2002. doi: 10.1093/eurheartj/ehp249.

Challenging Diagnosis of Myocardial Infarction Due to Anomalous Left Circumflex Artery

Maksymilian P. Opolski,[1] Kajetan Grodecki,[2] Mateusz Spiewak,[1] Mariusz Furmanek,[3] Ilona Michalowska[1]

Institute of Cardiology,[1] Warsaw – Poland

Medical University of Warsaw,[2] Warsaw – Poland

Medical Center for Postgraduate Education,[3] Warsaw – Poland

A 45-year-old male without past medical history presented with retrosternal chest pain and ST-segment elevation in inferolateral leads at ECG. Invasive coronary angiography, along with optical coherence tomography performed as part of the clinical study, showed normal coronaries, and myocardial infarction with non-obstructive coronary arteries (MINOCA) was diagnosed (Figure 1 A-B). Due to ongoing chest pain, triple-rule-out computed tomography angiography (CTA) was undertaken to exclude aortic dissection and pulmonary embolism. Incidentally, anomalous left circumflex artery (LCx) originating from the right sinus of Valsalva with a suspicion on severe stenosis was detected (Figure 1 C-E). Selective angiography of the LCx confirmed severe lesion in the distal vessel segment (Figure 1 F), however given the resolution of patient's symptoms, a decision on medical therapy with dual antiplatelet agents was undertaken. At discharge, cardiac magnetic resonance disclosed mildly reduced left ventricular ejection fraction (53%) with myocardial edema and transmural infarction of the basal-to-mid lateral wall (Figure 1 G-H).

LCx arising from the right aortic sinus is the most frequent coronary artery anomaly (CAA) found in up to 0.7% of the population. Although anomalous LCx is considered benign, the severe angle and tortuous vessel course may predispose it to accelerated atherosclerosis. Herein, the anomalous LCx was overlooked due to super-selective cannulation of the right coronary artery, and a large intermediate branch was incorrectly classified as LCx leading to deferred revascularization and irreversible myocardial injury. This case highlights that CAA could be included in the differential diagnosis of MINOCA, and unveils the potential for triple-rule-out CTA in detecting CAA.

Keywords

Myocardial Infarction / Diagnosis; Coronary Artery Anomalies; Coronary Angiography; Cardiac Magnetic Resonance.

Mailing Address: Maksymilian P. Opolski •
Alpejska 42, 04-628, Warsaw – Poland
E-mail: opolski.mp@gmail.com, maxopol@gmail.com
Manuscript received August 19, 2017, revised manuscript December 12, 2017, accepted December 12, 2017

DOI: 10.5935/abc.20180093

Author contributions

Conception and design of the research: Opolski MP, Spiewak M; Acquisition of data: Opolski MP, Spiewak M, Furmanek M, Michalowska I; Analysis and interpretation of the data and critical revision of the manuscript for intellectual content: Opolski MP, Grodecki K, Spiewak M, Furmanek M, Michalowska I; Obtaining financing: Opolski MP; Writing of the manuscript: Opolski MP, Grodecki K.

Potential Conflict of Interest

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

Sources of Funding

This study was partially funded by Research Grant "Iuventus Plus" from the Polish Ministry of Science and Higher Education [IP2014 034073].

Study Association

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

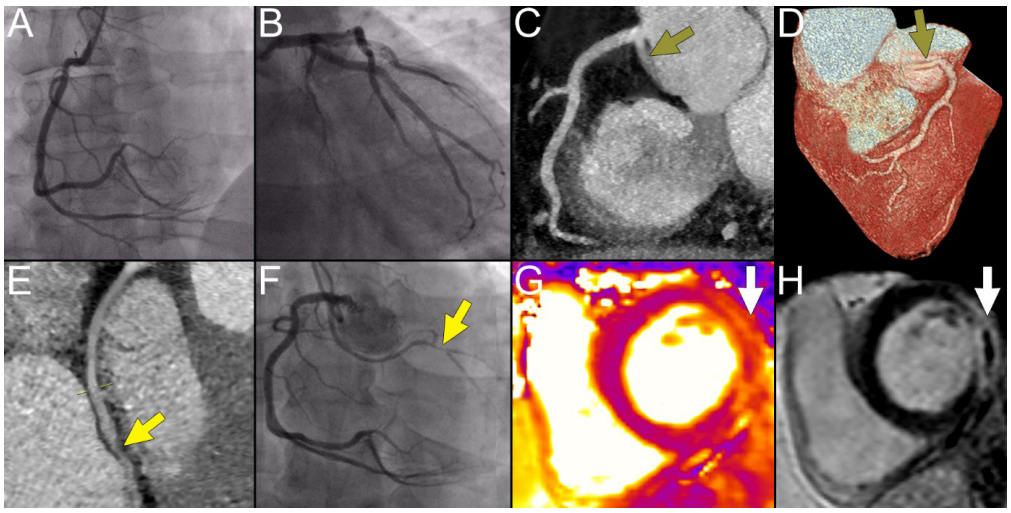


Figure 1 – *Coronary angiography, coronary computed tomography angiography and cardiac magnetic resonance findings of the patient with challenging diagnosis of myocardial infarction and anomalous left circumflex artery.*

QTc and QTcd Measurements and Their Relationships with Left Ventricular Hypertrophy in Hemodialysis Patients

Sora Yasri[1] and Viroj Wiwanitkit[2]
KMT Primary Care Center,[1] Bangkok, Tailândia
Hainan Medical University,[2] Haikou, China

Editor,

We read the publication on "QTc and QTcd Measurements and Their Relationships with Left Ventricular Hypertrophy in Hemodialysis Patients", which is very interesting.[1] Alonso et al.[1] concluded that "We found that QTc interval, in contrast to QTcd, is a reproducible and reliable measure and had a weak but positive correlation with LVMi in HD patients." This report used an unmatched control group; hence, the selection bias can be expected. In fact, the hypertrophy might be expected in a hemodialysis patient who might have underlying metabolic syndrome and vascular disease.[2] To test the reproducibility, the repeated analysis is needed and there is a need to assess the within-run and between-run precision. In the present report, one cannot conclude that the test has a good reproducibility.

Keywords

Hypertrophy, Left Ventricular; Renal Dialysis; Electrocardiography.

Mailing Address: Sora Yasri •
KMT Primary Care Center. 10330, Bangkok - Thailand
Email: sorayasri@outlook.co.th
Manuscript received October 02, 2017, revised manuscript March 14, 2018, accepted March 14, 2018

DOI: 10.5935/abc.20180096

References

1. Alonso MAG, Lima VAC, Carreira MAM, Lugon JR. Reproducibility and reliability OF QTe and QTed measurements and their relationships with left ventricular hypertrophy in hemodialysis patients. Arq Bras Cardiol.2017;109(3):222-30.
2. Chirakarnjanakorn S, Navaneethan SD, Francis GS, Tang WH. Cardiovascular impact in patients undergoing maintenance hemodialysis:clinical management considerations. Int J Cardiol. 2017;232:12-23.

Reply

Thank you for your comments regarding our paper.

Our report was a case-control study with hemodialysis patients recruited from a single dialysis center and a control group matched by gender and age without overt kidney disease. For evaluation of the reproducibility and reliability of QTc and QTcd measures, intra- and inter-observer correlation and concordance tests were performed employing Pearson´s correlation, Cohen's Kappa coefficient and Bland Altman diagram. Two observers (unaware of the results from each other) manually measured the QT interval and its dispersion in the same electrocardiographic tracing at two different times with a one-week interval between measurements. Most of the previous studies that considered the reproducibility of the QTc and QTcd measurements, used only one method of evaluation, especially the correlation coefficient test without contemplating concordance tests. We applied three different types of tests, with two different observers coming to a likely conclusion of good QTc reproducibility. In contrast, QTcd does not seem to be a reliable and reproducible measurement.

The present study carries some limitations such as the relatively small number of patients and the exclusion criteria. Further studies performed on larger patient populations are needed to determine the optimal time to measure these parameters (pre-dialysis, during dialysis, or after dialysis), as well as the standardization of cutoff points for these parameters, techniques of measurements and correction for heart rate.

Sincerely,

Maria Angélica Gonçalves Alonso
Valentine de Almeida Costa de Castro Lima
Maria Angela Magalhães de Queiroz Carreira
Jocemir Ronaldo Lugon