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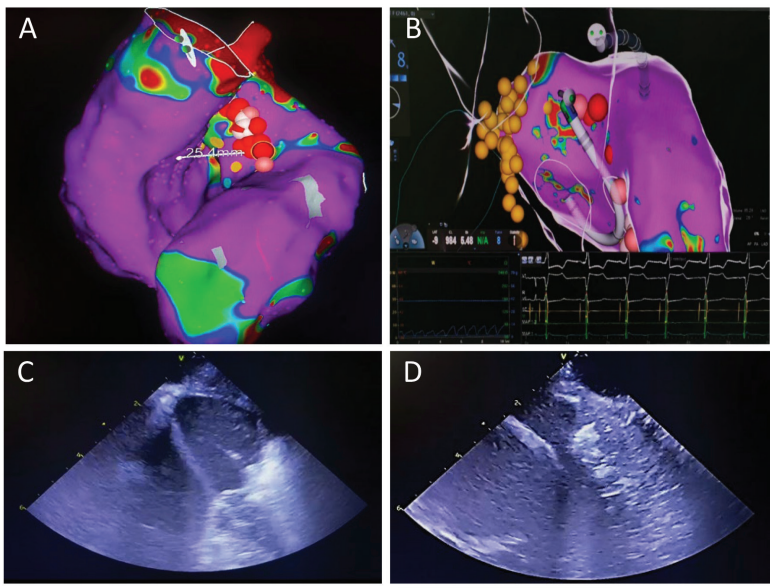

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Within-Visit Blood Pressure Variability and Cardiovascular Risk in ELSA-Brasil Study Participants

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Abstract

Background: **Blood pressure variability (BPV) is of prognostic value for fatal and non-fatal cardiovascular outcomes.**

Objective: **This study aimed to evaluate the association between within-visit BPV and cardiovascular risk among participants of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil).**

Methods: **The present cross-sectional study was carried out using baseline data (2008-2010) of 14,357 ELSA-Brasil participants with no prior history of cardiovascular disease. Within-visit BPV was quantified by the coefficient of variation of three standardized systolic blood pressure (SBP) measurements using an oscillometer. Anthropometric measurements and laboratory tests were also performed. Cardiovascular risk was assessed using the atherosclerotic cardiovascular disease risk estimator (ASCVD) and multivariate logistic regression analysis was employed with a significance level of 5%.**

Results: **Significantly higher cardiovascular risk was determined by increased BPV for both sexes. A significantly higher prevalence of high risk was found in men than women across all quartiles, with the highest difference observed in the fourth quartile of variability (48.3% vs. 17.1%). Comparisons among quartiles in each sex revealed a significantly higher cardiovascular risk for men in the third (OR=1.20; 95%CI: 1.02 - 1.40) and fourth quartiles (OR=1.46; 95%CI: 1.25 -1.71), and for women in the fourth quartile (OR=1.27; 95%CI: 1.03 - 1.57).**

Conclusion: **Analysis of baseline data of the ELSA-Brasil participants revealed that blood pressure variability was associated with increased cardiovascular risk, especially in men.**

Keywords: **Arterial Pressure; Heart Disease Risk Factors; Hypertension.**

Introduction

Hypertension is considered the main risk factor for cardiovascular disease, with increasing global mortality evidenced in recent years.[1-3] In Brazil, it is estimated that arterial hypertension affects approximately 36 million Brazilians, with epidemiological studies reporting a prevalence ranging from 21.4 - 35.8%.[4-7]

Although arterial hypertension is considered an important risk factor for stroke and acute myocardial infarction (AMI), evidence suggests that elevated blood pressure is not the only relevant pathophysiological factor involved in cardiovascular events.[8,9] Several studies have demonstrated the importance of blood pressure variability (BPV) in the association between arterial hypertension and cardiovascular risk.[10-24] BPV is a complex phenomenon, in which fluctuations in blood pressure readings can be influenced by the individual's environment, behavior, hormonal and central nervous system activity, among other factors.

BPV is assessed beat-by-beat through intra-arterial measurements, by physicians in a clinical setting, by using an ambulatory blood pressure monitoring (ABPM) device, or a home blood pressure monitor (HBPM) at very short, short, medium or long intervals.[25,26] Short-term (24 hours), medium-term (2+ days) and long term (at weekly, monthly or yearly intervals) BPV is associated with high cardiovascular risk,[12,13] left ventricular hypertrophy,[14,15] increased carotid intima-media thickness,[16,17] chronic renal failure,[18,19] and fatal and non-fatal cardiovascular events.[20-22]

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Some studies have demonstrated that the within-visit BPV can be positively associated with stroke and cardiovascular risk,[27,28] while others found no associations with cardiovascular or total mortality outcomes.[29,30] Previously published data from the Brazilian Longitudinal Study on Adult Health (ELSA-Brasil) reported an association between within-visit BPV and carotid intima-media thickness.[17] Despite the body of accumulated knowledge in the literature, it remains impossible to conclude that BPV represents an independent risk factor that should be modulated and controlled through antihypertensive treatment, or whether it is simply a marker that accompanies elevated blood pressure.[26] Thus, the present study aimed to establish associations between BPV in a single visit and cardiovascular risk in a cohort of participants at baseline of the ELSA-Brasil.[31]

Methods

Study design

The present cross-sectional study was carried out with baseline data (2008-2010) from the ELSA-Brasil. ELSA-Brasil is a cohort study initiated in 2008 involving public servants at higher education and research institutions located in six Brazilian capitals (Salvador, Vitória, Belo Horizonte, Porto Alegre, São Paulo, and Rio de Janeiro) and aims to investigate the incidence and progression of cardiovascular diseases and diabetes, as well as associated biological, environmental, psychological, and social factors. At baseline, data collection consisted of interviews, anthropometric measurements, clinical examinations and the collection of biological samples. Participants are contacted by phone annually to record health events, and every four years they are called back for new interviews and assessment of health status and outcomes.[32]

Population

Active and retired public servants from six institutions were included, aged between 35 and 74 years. Individuals with severe cognitive or communication impairment were considered ineligible, as well as those who intended to retire in the near future or had retired and then moved to a residence far from their respective local research center. Also, women who were pregnant or had given birth less than four months prior to their baseline visit were ineligible to participate. Of the 15,105 ELSA-Brasil baseline participants, we excluded 749 (5.0%) who self-reported previous stroke, myocardial infarction, revascularization, or heart failure. As a result, our study sample comprised 14,357 individuals. All participants signed an informed consent form, and the study protocol was approved by the institutional review board of each institution.

Study variables

BPV was considered the main independent variable, defined by the coefficient of variation of three systolic blood pressure (SBP) measurements obtained during the first visit of each participant at baseline.

The sociodemographic variables evaluated were sex, age, self-declared race/skin color (black, brown, white, asian, indigenous), education level (elementary school, high school or university degree) and per capita family income in Brazilian reals.

The cardiovascular risk variables evaluated were abdominal obesity (waist circumference >102 cm for men, >88 cm for women), hypertension, diabetes, smoking, hypercholesterolemia (LDL ≥130 mg/dL), hypertriglyceridemia (>150 mg/dL), reduced glomerular filtration rate (<60 mL/min), and pulse wave velocity (m/s).

To estimate the risk of a first episode of stroke or AMI (fatal / non-fatal) or cardiovascular death among the study participants over a 10-year period, the risk estimator of cardiovascular atherosclerotic disease (ASCVD) was used, which was considered the dependent variable in this study. Developed by an American Heart Association task force in 2013, this score was generated using data obtained from cohorts that included African-American and white individuals, aged 40-79 years, with no previous history of cardiovascular disease, and who were prospectively followed for a minimum of 12 years.

Statistical methods were implemented to obtain and validate the internal logarithmic equations for specific risk estimates according to sex and race. The variables included to estimate the risk were age, total cholesterol, HDL cholesterol and systolic pressure, as well as a diagnosis of diabetes and smoking habit. Individuals with a risk estimated at ≥7.5% are considered to be at high risk, while those <7.5% are considered to be at low risk for stroke, AMI or cardiovascular death over the next 10 years.[33]

Detailed information on laboratory testing procedures and the methodology used to measure pulse wave velocity can be found in previous publications.[34,35]

Hypertension was determined by the arithmetic mean of the last two measurements when SBP was ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or if the subject used antihypertensive medication. Diabetes was defined by a previous clinical diagnosis, use of antidiabetic medications, fasting glucose ≥126 mg/dl, glycated hemoglobin ≥ 6.5% or postprandial glucose ≥ 200 mg /dL.[34]

The coefficient of variation (standard deviation/mean x 100) of the three SBP measurements obtained for each individual was calculated at baseline of the study and divided into quartiles. Sociodemographic and cardiovascular risk variables, as well as ASCVD risk, were stratified according to each quartile, and expressed as means and standard deviation, or percentages.

Blood pressure measurement

A validated oscillometer device, Omron HEM 705CPINT, was used to measure arterial blood pressure.[35]

Measurements were performed in sitting position, with an empty bladder, and without eating, drinking, smoking or exercising for at least 30 minutes before the measurement. Cuff size was selected according to arm circumference. The brachial artery was detected by palpation between the triceps and biceps, with the cuff placed 2 cm above the cubital fossae, centered over the brachial artery. Three measurements were obtained at one-minute intervals, preferably on the left arm, while the participant was seated comfortably without crossing their legs. Every effort was made to obtain accurate readings and minimize measurement errors, and training included test-retest protocols to

ensure that similar conditions would be used for all participants. The Kappa correlation coefficient for SBP and diastolic blood pressure were 0.88 (95%CI: 0.82-0.91) and 0.89 (95%CI: 0.83-0.92), respectively.[7]

Statistical analysis

Categorical variables were described as frequencies (percentages). The Shapiro-Wilk test was used to test normality of data distribution. Continuous variables were described as mean and standard deviation (SD) or median and interquartile range (IQR; 25th to 75th percentile), according to data distribution. Categorical variables were described by proportions and compared using Pearson's chi-squared test. The ANOVA test was used to compare the means, and the Kruskal-Wallis test for the medians. To estimate associations between risk of cardiovascular disease and BPV, bivariate analysis was performed (Pearson's chi-squared test and chi-square test for trend). Lastly, multivariate analysis was performed by logistic regression. Covariates were tested as potential effect modifiers; when not confirmed in the model as such, they were tested as potential confounders. Confounding variables were identified when a variance of 10% or more was detected with respect to odds ratio (OR) values corresponding to associations between BPV and cardiovascular risk. To analyze the variables as potential effect modifiers, the backward procedure was adopted in the logistic regression model, which allowed the estimation of OR and respective 95% confidence intervals (95% CI). To assess the effect modification, the likelihood-ratio test was used, comparing the complete model with the reduced model - without the product term (s). The level of significance admitted in the study was 5%. For the statistical analysis of the data, the STATA (Stata Corporation, College Station, Texas, USA), version 14.0® software was used.

Results

A total of 14,357 individuals were included in the analysis, 7,884 of whom were females and 6,473 were males, with a mean age of 51.7 years. Table 1 lists the sociodemographic and clinical characteristics of the studied individuals, stratified according to the quartiles determined for the BP coefficient of variation at baseline. Females predominated in both the overall population (54.1%), as well as in all quartiles. With respect to self-reported race/skin color, white was the most predominant across all quartiles. Most participants had university degree or higher in each quartile. Income level gradually increased across the quartiles, with the highest level seen in the fourth quartile (p=0.010).

Individuals of the fourth quartile of BPV presented significantly higher median age (p<0.001), higher median LDL cholesterol levels (p<0.001), blood glucose (p=0.001) and glycated hemoglobin (p<0.001) in comparison to the first quartile. The prevalence of diabetes and reduced glomerular filtration rate were significantly higher among individuals in the last quartile (p=0.001 and p=0.004, respectively). The median pulse wave velocity was also significantly higher among individuals in this quartile (p<0.001). The prevalence of high risk of atherosclerotic cardiovascular disease was significantly higher in the last quartile compared to the first (p<0.001).

Table 2 describes the prevalence of an elevated risk of developing atherosclerotic cardiovascular disease among the quartiles of SBP coefficient of variation according to sex, which was considered as a modifier of effect in the association between BPV and cardiovascular risk. In general, men presented a significantly higher prevalence of high ASCVD risk than women (p <0.001). As BPV increased in both sexes, the prevalence of high risk was also higher, with the largest differences seen in the last quartile of variability (Table 2).

Table 3 details the final model of the multivariate analysis assessing the association between high atherosclerotic cardiovascular risk and BPV according to sex. Comparisons among the quartiles revealed a significantly higher overall cardiovascular risk for men classified in the penultimate and last BPV coefficient quartiles (OR=1.20; 95%CI: 1.02 - 1.40; OR=1.46; 95%CI: 1.25 -1.71, respectively), and for women in the last quartile (OR=1.27; 95%CI: 1.03 - 1.57), after adjusting for confounders (abdominal obesity, income and education level), including mean SBP.

Discussion

In the data collected from individuals included the ELSA-Brasil, within-visit BPV was found to be associated with a high risk of developing atherosclerotic cardiovascular disease, and was related to markers of cardiovascular risk, such as hypercholesterolemia, diabetes, reduced glomerular filtration rate and high pulse wave velocity. The prevalence of high cardiovascular risk progressively increased with BPV, and was observed to be significantly higher among men compared to women in all quartiles evaluated. Regardless of mean SBP, a higher blood pressure coefficient of variation was significantly associated with cardiovascular risk for men in the two highest quartiles, and in the last quartile for women.

The prognostic value of long-term BPV, both through ABPM and casual blood pressure measurements, has been proven in previous studies.[9,11,16,18] A recently published Korean cohort study[36] demonstrated an association of SBP, blood glucose, total cholesterol and body mass index variability with mortality and cardiovascular events.[36]

The prognostic value of short-term (24-hour) BPV, as measured by ABPM, has been extensively demonstrated regarding target organ damage and cardiovascular outcomes in cross-sectional and longitudinal studies.[11,14,15,23,14] However, there is less evidence regarding BPV in a single consultation,[12,17,28] highlighting the need for further confirmation in terms of clinical implications. Compared to other studies that evaluated this issue, our results corroborated findings reported by Grassi et al.,[12] who described the relationship between within-visit BPV and cardiovascular risk factors, such as advanced age, hypercholesterolemia and the presence of diabetes, which were significantly more prevalent in the last quartile of the SBP coefficient of variation. In contrast to the results reported by Grassi et al., we demonstrated a positive relationship between elevated cardiovascular risk and BPV among both sexes, which was shown to be stronger in men. Another study[13] involving a smaller-sized population in Turkey evaluated BPV by ABPM and SBP coefficient of variation,

Table 1 – Sociodemographic and clinical characteristics of participants in the ELSA-Brasil baseline according to blood pressure variability quartiles

Variables	Systolic blood pressure variability (%)				p-value
	1st quartile (0 – 1.78)	2nd quartile (1.79 – 2.88)	3rd quartile (2.89 – 4.34)	4th quartile (>4.34)	
Sex (Male=6,473; Female=7,883)					
Female, n (%)	52.7	54.1	54.5	56.3	0.018[p]
Age (years), median (IQR)	50 (44 - 57)	50 (44 - 57)	51 (45 -58)	53 (45 - 59)	<0.001[k]
Skin color, n (%)					
Black/brown	46.4	44.3	43.4	41.6	
White	50.5	52.4	52.2	54.6	
Asian	2.0	2.3	3.0	2.5	
Indigenous	1.1	0.9	0.8	1.2	0.003[p]
Schooling, n (%)					
Low	9.3	8.2	9.6	10.1	
Medium	30.8	30.6	30.1	30.8	
Superior	59.9	61.2	60.3	59.1	0.151[p]
Income, median (IQR)	1348.6 (691.5 - 2074.8)	1348.6 (726.1 - 2074.8)	1410.9 (726.1 - 2282.3)	1452.3.1 (726.1 - 2351.5)	0.010[k]
SBP (mm Hg), mean (SD)	120.2±16.7	120.4±16.5	121.2±17.0	123.6/±17.9	<0.001[a]
DBP (mm Hg), mean (SD)	76.3±10.5	76.3±10.6	76.4±10.6	77.1±10.8	0.001[a]
LDL cholesterol (mg / dL), median (IQR)	127 (107 - 150)	129 (108 - 151)	130 (109 - 154)	130 (109 - 154)	<0.001[k]
HDL cholesterol (mg / dL), median (IQR)	54 (46 - 65)	54 (46 - 65)	55 (47 - 65)	55 (47 - 65)	0.020[k]
Triglycerides (mg / dL), median (IQR)	113 (81 - 163)	114 (80 - 165)	113 (81 - 166)	115 (83 - 167)	0.470[k]
Blood glucose (mg / dL), median (IQR)	104 (98 - 113)	105 (98 - 113)	105 (98 - 113)	105 (99 - 115)	0.001[k]
Glycated hemoglobin (%), median (IQR)	5.3 (4.9 - 5.7)	5.5 (4.9 - 5.8)	5.3 (4.9 - 5.7)	5.3 (5.0 - 5.8)	<0.001[k]
Waist circumference (cm), median (IQR)	90.5 (82.4 - 99.4)	90.2 (81.5 - 99.7)	90.2 (81.6 - 98.6)	89.8 (81.9 - 98.2)	0.034[k]
GFR <60ml / min, (%)	3.6	3.9	4.0	5.2	0.004[p]
Diabetes mellitus, (%)	17.4	17.7	18.1	20.7	0.001[p]
Smoking, (%)	42.1	41.2	42.7	43.7	0.157[p]
Pulse wave velocity (m / s), median (IQR)	8.9 (8.0 - 10.0)	9.0 (8.1 - 10.0)	9.0 (8.1 - 10.2)	9.1 (8.1 - 10.3)	<0.001[k]
High cardiovascular risk (%)	23.3	23.7	25.7	30.5	<0.001[p]

** Categorical variables expressed as number (%). Comparisons were made by Pearson's χ2 ([p]), ANOVA ([a]) or Kruskal-Wallis test ([k]); IQR: interquartile range; SD: standard deviation; GFR: glomerular filtration rate; SBP: systolic blood pressure; DBP: diastolic blood pressure.*

observing an independent association between risk and BPV, with no differences between sexes though.

We additionally found a higher prevalence of reduced glomerular filtration rate and higher pulse wave velocity among individuals with higher BPV. Despite the fact that casual blood pressure measurements were used to assess short-term BPV, we did identify a link with reduced glomerular filtration rate, an early risk marker of chronic kidney disease, which was similar to results from another study conducted in a Korean population.[28] Interestingly, the association observed herein between within-visit BPV and elevated pulse wave velocity, an important marker of stiffness in large arteries, corroborated results only seen in studies employing ABPM.[37,38]

The assessment of BPV can be influenced by the choice of method and the time interval considered between measurements.[12] A review of the literature highlighted possible pathophysiological mechanisms involved in the association observed between short-term BPV in a single visit and high cardiovascular risk, including increased central sympathetic activity, decreased arterial and cardiopulmonary reflexes,

Table 2 – Prevalence of high cardiovascular risk (≥ 7.5%) according to quartile of blood pressure variability and sex

Systolic blood pressure variation quartiles	Prevalence of high cardiovascular risk				p-value*
	Sex				
	Male		Female		
	n (%)	Prevalence (95CI%)	n (%)	Prevalence (95%CI)	
1st	1648 (25.9)	36.2 (33.8 – 38.5)	1896 (24.3)	12.1 (10.7 – 13.6)	<0.001
2nd	1601 (25.1)	37.1 (34.7 – 39.5)	1937 (24.9)	12.7 (11.3 – 14.2)	<0.001
3rd	1598 (25.0)	41.0 (38.6 – 43.5)	1951 (25.0)	13.2 (11.7 – 14.7)	<0.001
4th	1550 (24.0)	48.3 (45.8 – 50.8)	2011 (25.8)	17.1 (15.4 – 18.7)	<0.001
p value		<0.001		<0.001	

** X2 trend test*

Table 3 – Final model of the association between high cardiovascular risk and blood pressure variability between men and women

Systolic blood pressure variation quartiles	Sex			
	Male		Female	
	Crude OR (95CI%)	*Adjusted OR (95CI%)	Crude OR (95CI%)	*Adjusted OR (95CI%)
1st	1.0	1.0	1.0	1.0
2nd	1.04 (0.90 – 1.20)	1.03 (0.89 – 1.21)	1.06 (0.87 – 1.28)	1.08 (0.86 – 1.35)
3rd	1.23 (1.06 – 1.41)	1.20 (1.02 – 1.40)	1.10 (0.91 – 1.33)	1.09 (0.87 -1.36)
4th	1.65 (1.43 – 1.90)	1.46 (1.25 – 1.71)	1.49 (1.24 – 1.78)	1.27 (1.03 – 1.57)

** Adjusted for average systolic pressure, abdominal obesity, income and education*

increased blood viscosity, decreased arterial compliance, changes in serum insulin levels, angiotensin II, bradykinins, endothelin and nitric oxide, in addition to emotional and behavioral factors.[26]

The findings here suggest that BPV in a single visit can be considered an important marker of cardiovascular risk, and that the evaluation of this parameter can help physicians to identify patients who should be monitored more closely, as well as those that may even require more intensive treatment. It is important to note that the population studied consisted mainly of normotensive individuals (64.2%), which reinforces the notion that blood pressure is a continuous risk variable and that the assessment of BPV is important not only among hypertensive patients.[10,17] Our results further reinforce the clinical importance of monitoring BPV, in addition to obtaining isolated measures in a single visit, given the possibility of identifying individuals with high cardiovascular risk.[28]

Our results are strengthened by the size of the population evaluated, the use of a simple, low-cost, reproducible and efficient method to assess BPV, and in determining the association between BPV and cardiovascular risk. With regard to limitations, a convenience sample was employed without randomization, and the cross-sectional nature of this study prevents us from determining whether cardiovascular risk lead to development BPV, or vice-versa. Although it is not feasible to generalize our results to the overall population, it is notable that our sample is highly representative of urban populations from large Brazilian capitals, with similar sociodemographic characteristics as those found in other major centers throughout the country. From a future perspective, we highlight the possibility of assessing the association between SBP variability in a single visit and fatal and non-fatal cardiovascular events among the ELSA-Brasil participants in the context of the ongoing research project. The authors further suggest that future studies should be conducted, such as randomized clinical trials using different classes of antihypertensive drugs, in an attempt to determine the impact of these treatments on BPV, as well as establish associations with cardiovascular outcomes and mortality.

Conclusion

The higher values of within-visit variability of SBP found in ELSA-Brasil participants at baseline were associated with higher cardiovascular risk, especially among males, regardless of mean SBP.

Author Contributions

Conception and design of the research: Zarife AS, Mill JG, Lotufo P, Griep RH, Fonseca MJM, Almeida MC, Matos SMA; Acquisition of data: Almeida MC, Matos SMA; Analysis and interpretation of the data: Zarife AS, Fraga-Maia H, Brito LL, Almeida MC, Aras R; Statistical analysis: Zarife AS, Fraga-Maia H, Brito LL, Almeida MC; Obtaining financing: Mill JG,

Lotufo P, Griep RH, Fonseca MJM, Matos SMA; Writing of the manuscript: Zarife AS, Fraga-Maia H, Mill JG, Lotufo P, Griep RH, Fonseca MJM, Brito LL, Aras R, Matos SMA; Critical revision of the manuscript for important intellectual contente: Zarife AS, Fraga-Maia H, Mill JG, Lotufo P, Griep RH, Fonseca MJM, Brito LL, Almeida MC, Aras R, Matos SMA.

Potential Conflict of Interest

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

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Study Association

This article is part of the thesis of doctoral submitted by André Sant'Anna Zarife, from Universidade Federal da Bahia.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Instituto de Saúde Coletiva/Universidade Federal da Bahia under the protocol number 027-06/CEP-ISC. 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. NCD Risk Factor Collaboration (NCD-RisC). 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. doi: 10.1016/S0140-6736(16)31919-5.
2. GBD 2017 Causes of Death Collaborators. Global, Regional, and National Age-Sex-Specific Mortality for 282 Causes of Death in 195 Countries and Territories, 1980-2017: A Systematic Analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392(10159):1736-88. doi: 10.1016/S0140-6736(18)32203-7.
3. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R; Prospective Studies Collaboration. Age-specific Relevance of Usual Blood Pressure to Vascular Mortality: A Meta-Analysis of Individual Data for One Million Adults in 61 Prospective Studies. Lancet. 2002;360(9349):1903-13. doi: 10.1016/s0140-6736(02)11911-8.
4. Magalhães LB, Amorim AM, Rezende EP. Conceito e aspectos epidemiológicos da hipertensão arterial. Rev Bras Hipertens. 2018;25(1):6-12.
5. Andrade SSA, Stopa SR, Brito AS, Chueri PS, Szwarcwald CL, Malta DC. Prevalência de hipertensão arterial autorreferida na população brasileira: análise da Pesquisa Nacional de Saúde, 2013. Epidemiol Serv Saúde. 2015;24(2):297-304. doi: 10.5123/S1679-49742015000200012.
6. Picon RV, Fuchs FD, Moreira LB, Riegel G, Fuchs SC. Trends in Prevalence of Hypertension in Brazil: A Systematic Review with Meta-Analysis. PLoS One. 2012;7(10):e48255. doi: 10.1371/journal.pone.0048255.
7. Chor D, Ribeiro ALP, Carvalho MS, Duncan BB, Lotufo PA, Nobre AA, et al. Prevalence, Awareness, Treatment and Influence of Socioeconomic Variables on Control of High Blood Pressure: Results of the ELSA-Brasil Study. PLoS One. 2015;10(6):e0127382. doi: 10.1371/journal.pone.0127382.
8. Rothwell PM. Limitations of the Usual Blood-Pressure Hypothesis and Importance of Variability, Instability, and Episodic Hypertension. Lancet. 2010;375(9718):938-48. doi: 10.1016/S0140-6736(10)60309-1.
9. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, et al. Prognostic Significance of Visit-to-Visit Variability, Maximum Systolic Blood Pressure, and Episodic Hypertension. Lancet. 2010;375(9718):895-905. doi: 10.1016/S0140-6736(10)60308-X.
10. Mancia G, Bombelli M, Facchetti R, Madotto F, Corrao G, Trevano FQ, et al. Long-Term Prognostic Value of Blood Pressure Variability in the General Population: Results of the Pressioni Arteriose Monitorate e Loro Associazioni Study. Hypertension. 2007;49(6):1265-70. doi: 10.1161/HYPERTENSIONAHA.107.088708.
11. Wan EY, Fung CS, Yu EY, Fong DY, Chen JY, Lam CL. Association of Visit-to-Visit Variability of Systolic Blood Pressure With Cardiovascular Disease and Mortality in Primary Care Chinese Patients With Type 2 Diabetes-A Retrospective Population-Based Cohort Study. Diabetes Care. 2017;40(2):270-9. doi: 10.2337/dc16-1617.
12. Grassi G, Seravalle G, Maloberti A, Facchetti R, Cuspidi C, Bombelli M et al. Within-Visit BP Variability, Cardiovascular Risk Factors, and BP Control in Central and Eastern Europe: Findings from the BP-CARE study. J Hypertens. 2015;33(11):2250-6. doi: 10.1097/HJH.0000000000000700.
13. Celik M, Yuksel UC, Yildirim E, Gursoy E, Koklu M, Yasar S, et al. The Relationship Between Blood Pressure Variability and Pooled Cohort Risk Assessment Equations 10-year Cardiovascular Risk Score. Blood Press Monit. 2016;21(5):282-7. doi: 10.1097/MBP.0000000000000200.
14. Sega R, Corrao G, Bombelli M, Beltrame L, Facchetti R, Grassi G, et al. Blood Pressure Variability and Organ Damage in a General Population: Results from the PAMELA Study (Pressioni Arteriose Monitorate E Loro Associazioni). Hypertension. 2002;39(2 Pt 2):710-4. doi: 10.1161/hy0202.104376.
15. Leoncini G, Viazzi F, Storace G, Deferrari G, Pontremoli R. Blood Pressure Variability and Multiple Organ Damage in Primary Hypertension. J Hum Hypertens. 2013;27(11):663-70. doi: 10.1038/jhh.2013.45.
16. Mancia G, Facchetti R, Parati G, Zanchetti A. Visit-to-Visit Blood Pressure Variability, Carotid Atherosclerosis, and Cardiovascular Events in the European Lacidipine Study on Atherosclerosis. Circulation. 2012;126(5):569-78. doi: 10.1161/CIRCULATIONAHA.112.107565.
17. Ribeiro AH, Lotufo PA, Fujita A, Goulart AC, Chor D, Mill JG, et al. Association Between Short-Term Systolic Blood Pressure Variability and Carotid Intima-Media Thickness in ELSA-Brasil Baseline. Am J Hypertens. 2017;30(10):954-960. doi: 10.1093/ajh/hpx076.
18. Yano Y, Fujimoto S, Kramer H, Sato Y, Konta T, Iseki K, et al. Long-Term Blood Pressure Variability, New-Onset Diabetes Mellitus, and New-Onset Chronic Kidney Disease in the Japanese General Population. Hypertension. 2015;66(1):30-6. doi: 10.1161/HYPERTENSIONAHA.115.05472.
19. Sarafidis PA, Ruilope LM, Loutradis C, Gorostidi M, de la Sierra A, de la Cruz JJ, et al. Blood Pressure Variability Increases with Advancing Chronic Kidney Disease Stage: A Cross-Sectional Analysis of 16 546 Hypertensive Patients. J Hypertens. 2018;36(5):1076-85. doi: 10.1097/HJH.0000000000001670.
20. Stevens SL, Wood S, Koshiaris C, Law K, Glasziou P, Stevens RJ, et al. Blood Pressure Variability and Cardiovascular Disease: Systematic Review and Meta-Analysis. BMJ. 2016;354:i4098. doi: 10.1136/bmj.i4098.

21. Juhanoja EP, Niiranen TJ, Johansson JK, Puukka PJ, Thijs L, Asayama K, et al. Outcome-Driven Thresholds for Increased Home Blood Pressure Variability. Hypertension. 2017;69(4):599-607. doi: 10.1161/HYPERTENSIONAHA.116.08603.

22. Kostis JB, Sedjro JE, Cabrera J, Cosgrove NM, Pantazopoulos JS, Kostis WJ, et al. Visit-to-Visit Blood Pressure Variability and Cardiovascular Death in the Systolic Hypertension in the Elderly Program. J Clin Hypertens. 2014;16(1):34-40. doi: 10.1111/jch.12230.

23. Manios E, Tsagalis G, Tsivgoulis G, Barlas G, Koroboki E, Michas F, et al. Time Rate of Blood Pressure Variation is Associated with Impaired Renal Function in Hypertensive Patients. J Hypertens. 2009;27(11):2244-8. doi: 10.1097/HJH.0b013e328330a94f.

24. Morano A, Ravera A, Agosta L, Sappa M, Falcone Y, Fonte G, et al. Extent of, and Variables Associated with, Blood Pressure Variability Among Older Subjects. Aging Clin Exp Res. 2018;30(11):1327-33. doi: 10.1007/s40520-018-0917-x.

25. Irigoyen MC, Angelis K, Santos F, Dartora DR, Rodrigues B, Consolim-Colombo FM. Hypertension, Blood Pressure Variability, and Target Organ Lesion. Curr Hypertens Rep. 2016;18(4):31. doi: 10.1007/s11906-016-0642-9.

26. Parati G, Ochoa JE, Lombardi C, Bilo G. Assessment and Management of Blood-Pressure Variability. Nat Rev Cardiol. 2013;10(3):143-55. doi: 10.1038/nrcardio.2013.1.

27. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, et al. Effects of Beta Blockers and Calcium-Channel Blockers on Within-Individual Variability in Blood Pressure and Risk of Stroke. Lancet Neurol. 2010;9(5):469-80. doi: 10.1016/S1474-4422(10)70066-1.

28. Shin JH, Shin J, Kim BK, Lim YH, Park HC, Choi SI, et al. Within-Visit Blood Pressure Variability: Relevant Factors in the General Population. J Hum Hypertens. 2013;27(5):328-34. doi: 10.1038/jhh.2012.39.

29. Muntner P, Levitan EB, Reynolds K, Mann DM, Tonelli M, Oparil S, et al. Within-Visit Variability of Blood Pressure and All-Cause and Cardiovascular Mortality Among US Adults. J Clin Hypertens. 2012;14(3):165-71. doi: 10.1111/j.1751-7176.2011.00581.x.

30. Schutte R, Thijs L, Liu YP, Asayama K, Jin Y, Odili A, et al. Within-Subject Blood Pressure Level--Not Variability--Predicts Fatal and Nonfatal Outcomes in a General Population. Hypertension. 2012;60(5):1138-47. doi: 10.1161/HYPERTENSIONAHA.112.202143.

31. Aquino EM, Barreto SM, Bensenor IM, Carvalho MS, Chor D, Duncan BB, et al. Brazilian Longitudinal Study of Adult Health (ELSA-Brasil): Objectives and Design. Am J Epidemiol. 2012;175(4):315-24. doi: 10.1093/aje/kwr294.

32. Aquino EM, Araujo MJ, Almeida Mda C, Conceição P, Andrade CR, Cade NV, et al. Participants Recruitment in ELSA-Brasil (Brazilian Longitudinal Study for Adult Health). Rev Saude Publica. 2013;47 Suppl 2:10-8. doi: 10.1590/s0034-8910.2013047003953.

33. ACC Guidelines Committee. Reply: 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk. J Am Coll Cardiol. 2014;63(25 Pt A):2886. doi: 10.1016/j.jacc.2014.04.003.

34. Schmidt MI, Duncan BB, Mill JG, Lotufo PA, Chor D, Barreto SM, et al. Cohort Profile: Longitudinal Study of Adult Health (ELSA-Brasil). Int J Epidemiol. 2015;44(1):68-75. doi: 10.1093/ije/dyu027.

35. Mill JG, Pinto K, Griep RH, Goulart A, Foppa M, Lotufo PA, et al. Medical Assessments and Measurements in ELSA-Brasil. Rev Saude Publica. 2013;47 Suppl 2:54-62. Portuguese. doi: 10.1590/s0034-8910.2013047003851.

36. Kim MK, Han K, Park YM, Kwon HS, Kang G, Yoon KH, et al. Associations of Variability in Blood Pressure, Glucose and Cholesterol Concentrations, and Body Mass Index With Mortality and Cardiovascular Outcomes in the General Population. Circulation. 2018;138(23):2627-37. doi: 10.1161/CIRCULATIONAHA.118.034978.

37. Tedla YG, Yano Y, Carnethon M, Greenland P. Association Between Long-Term Blood Pressure Variability and 10-Year Progression in Arterial Stiffness: The Multiethnic Study of Atherosclerosis. Hypertension. 2017;69(1):118-27. doi: 10.1161/HYPERTENSIONAHA.116.08427.

38. Boardman H, Lewandowski AJ, Lazdam M, Kenworthy Y, Whitworth P, Zwager CL, et al. Aortic Stiffness and Blood Pressure Variability in Young People: A Multimodality Investigation of Central and Peripheral Vasculature. J Hypertens. 2017;35(3):513-22. doi: 10.1097/HJH.0000000000001192.

Short Editorial

Blood Pressure Variability and Cardiovascular Risk in ELSA-Brasil: A Potential Surrogate Marker for Predicting Mortality and Cardiovascular Outcomes?

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Short Editorial related to the article: Within-Visit Blood Pressure Variability and Cardiovascular Risk in ELSA-Brasil Study Participants

Blood pressure (BP) homeostasis is a crucial element in the protection of cardiovascular events. Many national and international guidelines[1-4] have proposed target values for blood pressure, and these recommendations consider slightly different goals according to hypertension stages, risk stratification and presence of renal or cardiovascular diseases, and target organ lesion. However, these values are based on office measures, ambulatory blood pressure monitoring (ABPM) and home blood pressure monitoring (HBPM), not accounting for blood pressure variability (BPV). BP intraindividual variability is an independent risk factor for cardiovascular diseases regardless of mean BP.[5-7] Fluctuations in physiological measures of blood pressure do not occur randomly and can contribute or be predictors of cardiovascular outcomes.

Most studies evaluated BPV on short (24h), medium (>2 days) or long-term (weekly, monthly, or annually).[8,9] Short-term BPV can be associated with increased cardiovascular risk.[10,11]

In the study by Zarife et al.,[12] the authors used baseline data from 14,357 participants of ELSA-Brasil without prior history of cardiovascular disease.

BPV was quantified in a single visit at baseline by the coefficient of variation of three standardized systolic blood pressure measurements using a validated oscilometer (Omron HEM 705CPINT) and correlated with ASCVD risk. BPV was divided into quartiles, and the highest quartile was associated with a significantly higher cardiovascular risk in both men and women. Males had a higher cardiovascular risk than females in all quartiles, with the greatest difference observed in the fourth BPV quartile. In addition, comparing quartiles by sex showed a significantly higher risk for men in the third and fourth quartiles and the fourth quartile for women. BPV was also associated with higher pulse-wave velocity, lower glomerular filtration rate, and hypercholesterolemia. No studies have reported cardiovascular risk assessment and BPV in a single visit. The results from ELSA-Brasil, in this large prospective cohort suggest that this can be a marker of cardiovascular disease risk and help identify patients needing closer monitoring or more intensive therapy. It is worthy to note that the majority of participants of ELSA-Brasil were normotensive individuals (64%), reinforcing the concept that blood pressure is a continuous measure of risk and that BPV can be important not only for those with hypertension but can be assessed in subjects with normal blood pressure measures. Further steps should be assessing single visit BPV and cardiovascular outcomes in ELSA-Brasil.

References

1. Barroso WKS, Rodrigues CIS, Bortolotto LA, Mota-Gomes MA, Brandão AA, Feitosa ADM, et al. Diretrizes Brasileiras de Hipertensão Arterial – 2020. Arq Bras Cardiol. 2021; 116(3):516-658.doi: 10.36660/abc.20201238.
2. Précoma DB, Oliveira GMM, Simão AF, Dutra OP, Coelho OR, Izar MCO, et al. Atualização da Diretriz de Prevenção Cardiovascular da Sociedade Brasileira de Cardiologia – 2019. Arq Bras Cardiol. 2019; 113(4):787-891. doi: 10.5935/abc.20190204.
3. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bach M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur J Prev Cardiol.2022;29(1):5-115 doi:10.1093/eurjpc/zwab154.
4. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, et al. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. Hypertension 2020;75(6):1334-57. doi: 10.1161/HYPERTENSIONAHA.120.15026.
5. Gosmanova EO, Mikkelsen MK, Molnar MZ, Lu JL, Yessayan LT, KalantarZadeh K, Kovesdy CP. Association of systolic blood pressure variability with mortality, coronary heart disease, stroke, and renal disease. J Am Coll Cardiol. 2016;68(13):1375–86. doi: 10.1016/j.jacc.2016.06.054
6. Muntner P, Shimbo D, Tonelli M, Reynolds K, Arnett DK, Oparil S. The relationship between visit-to-visit variability in systolic blood pressure and all-cause mortality in the general population: findings from NHANES

Keywords

Blood Pressure; Homeostasis; Cardiovascular Diseases; Risk Factors; Hypertension.

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III, 1988 to 1994. Hypertension.2011;57(2):160-6. doi:10.1161/HYPERTENSIONAHA.110.162255

7. Choi S, Shin J, Choi SY, Sung KC, Ihm SH, Kim KI, Kim YM. Impact of visit-to-visit variability in systolic blood pressure on cardiovascular outcomes in Korean National Health Insurance Service-National Sample Cohort. Am J Hypertens. 2017;30(6):577–86. doi: 10.1093/ajh/hpw157.

8. Irigoyen MC, Angelis K, Santos F, Dartora DR, Rodrigues B, Consolim-Colombo FM. Hypertension, Blood Pressure Variability, and Target Organ Lesion. Curr Hypertens Rep. 2016;18:31. doi: 10.1007/s11906-016-0642-9.

9. Parati G, Ochoa JE, Lombardi C, Bilo G. Assessment and Management of Blood-Pressure Variability. Nat Rev Cardiol. 2013;10(3):143-55. doi: 10.1038/ nrcardio.2013.1.

10. Grassi G, Seravalle G, Maloberti A, Facchetti R, Cuspidi C, Bombelli M, et al. Within-visit BP variability, cardiovascular risk factors, and BP control in central and eastern Europe: findings from the BP-CARE study. J Hypertens. 2015;33(11):2250-6. doi: 10.1097/HJH.0000000000000700.

11. Celik M, Yuksel UC, Yildirim E, Gursoy E, Koklu M, Yasar S, et al. The Relationship between blood pressure variability and pooled cohort risk assessment equations 10-year cardiovascular risk score. Blood Press Monit. 2016;21(5):282-7. doi: 10.1097/MBP.0000000000000200.

12. Zarife AS, Fraga-Maia H, Mill JG, Lotufo P, Griep RH, Fonseca MJM, et al. Within-visit blood pressure variability and cardiovascular risk in Elsa-Brasil Study Participants. Arq Bras Cardiol. 2022; 119(4):505-511.

Original Article

Left Atrial Thrombus and Dense Spontaneous Contrast in Direct Oral Anticoagulant Therapy of Atrial Fibrillation: Insights from a Reference Center

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Abstract

Background: **In the treatment of atrial fibrillation (AF), the most frequently sustained arrhythmia, with catheter ablation (CA) or electrical cardioversion (ECV), the periprocedural period is one of the most critical phases. Currently, the use of new direct action oral anticoagulants (DOAC) is increasingly frequent; however, in the real world, there are still few data on studies on the thrombus incidence in the left atrium (TrLA) or dense spontaneous contrast (DSC) on transesophageal echocardiogram (TEE).**

Objective: **To evaluate the prevalence of events and association with risk factors in patients using DOACs. Primary objective: to analyze the prevalence of thrombus in the LA by TEE in patients using DOAC undergoing ECV/CA. Second, evaluate the association of comorbidities with the presence of thrombi and DSC.**

Methods: **Retrospective cohort, single-center study with patients followed at the Arrhythmia Outpatient Unit (InCor-HCFMUSP). Patients indicated for procedures and using DOACs were selected, and their clinical/echocardiographic data were analyzed. A significance level of 5% was considered.**

Results: **354 patients were included, a total of 400 procedures, from March 2012-March 2018. Thrombus in the LA was found in 11 patients (2.8%), associated with advanced age (p=0.007) and higher CHA2DS2-VASc (p<0.001) score. DSC in the LA before TEE was found in 29 patients (7.3%), with lower LVEF (p<0.038) and greater LA dimension (p<0.0001).**

Conclusion: **The incidence of LA thrombus and DSC in patients using DOC in the context of AF ECV/CA, although small, is not negligible. Patients with higher CHA2DS2-VASc scores, especially older and with larger LA diameter, are more prone to these echocardiographic findings.**

Keywords: **Atrial Fibrillation; Electric Countershock; Transesophageal Echocardiography.**

Introduction

Atrial fibrillation (AF) is the most frequent sustained arrhythmia in clinical practice, with a prevalence of around 1% in the general population.[1] One of the critical phases in the treatment of AF refers to the periprocedural period of catheter ablation (CA) or electrical cardioversion (ECV), where the risk of a thromboembolic event needs to be minimized with the use of oral anticoagulation. In the past, only warfarin was available, with a periprocedural incidence rate of thromboembolic events ranging between 0.5 and 1.6%.[2,3] The prevalence of thrombus in the left atrium varies between 0.6% and 6.4% in patients under warfarin treatment.[4-6] Currently, new direct-acting oral anticoagulants (DOACs) are increasingly frequent; however, in real-world conditions, there are still few studies with data regarding thrombus incidence or dense spontaneous contrast at TEE in the left atrium.[7-9]

Methods

Study Population

Retrospective cohort, single-center study with patients followed at the Arrhythmia Outpatient Unit of Instituto do Coração – Hospital das Clínicas da Faculdade de Medicina, Universidade de São Paulo (InCor-HCFMUSP).

Medical records of patients aged ≥ 18 years with a diagnosis of persistent AF, who underwent ECV and/or CA under the use of DOACs at least 3 weeks before the procedure, were included consecutively over 6 years (2012-2018). All patients underwent a transesophageal echocardiogram

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(TEE) during the same hospitalization. The choice and dose of DOAC (rivaroxaban, dabigatran or apixaban) were established by the attending physician, with the assessment of creatinine clearance during clinical follow-up. Patients who had undergone the procedure (cardioversion or ablation) more than 6 months before could be included again. Patients were constantly using DOAC, suspended only on the day of the procedure.

Clinical data such as age, comorbidities, the CHA2DS2-VASc score, structural TEE data - left atrial (LA) dimension, and left ventricular ejection fraction (LVEF) were analyzed. The diameter dimension was chosen for calculation instead of the left atrium indexed volume, as this measurement is not standard in the exam. The TEE team defined the presence of thrombi and/or dense spontaneous contrast.

Objectives

The primary objective was to analyze the prevalence of thrombus in the left atrium, employing TEE, in patients using DOAC for at least 3 weeks who were undergoing ECV and/or ablation. The secondary objective was to evaluate the association of comorbidities with the presence of thrombi and spontaneous contrast.

This study is part of a larger register ("Registro Institucional com o uso dos Anticoagulantes de ação direta em pacientes com Fibrilação atrial não-valvar" [Institutional register for the use of direct-acting oral anticoagulants in patients with non-valvar atrial fibrillation] – CAAE # 57417716.6.0000.0068) approved by the Institutional Research Ethics Committee of the Hospital das Clínicas, University of São Paulo - CEP-HCFMUSP, approval number 1,637,837. The Ethics Committee agreed that informed consent was not required, as the study was retrospective, based on data from the institution's medical records. All data were anonymized before being collected.

Statistical analysis

The collected data were described as means and standard deviation values for continuous variables of normal distribution. Categorical variables were described in absolute numbers and percentages. The normality of the variables was tested using the Kolmogorov-Smirnov test. Statistical tests were performed, according to the type of variable (qualitative/quantitative) and the normality of distribution, using unpaired Student's t-test, chi-square test and multivariate logistic regression. P-values less than 0.05 were considered statistically significant. The statistical analysis was performed using the SSPS software (version 22.0).

Results

Three hundred fifty-four patients were included from March 2012 to March 2018. A total of 400 procedures, 122 cardioversions and 278 ablations, were performed. Among the comorbidities observed, most patients had essential hypertension, and about 33% were over 60 years old (Table 1). There was a difference between the groups taking different DOACs, with a higher presence of the previous stroke in patients who used dabigatran. (Table 2)

There was the presence of thrombus in the left atrium in 11 patients (Table 3), out of a total of 400 procedures (2.8%), and an association with advanced age (p=0.007) and higher CHA2DS2-VASc (p<0.001) was observed. (Table 4)

Dense spontaneous contrast in the left atrium in the procedure prior to TEE was observed in 29 patients (7.3%). Comparing the data of this group with those without the presence of contrast, a lower LVEF (p<0.038) and a greater LA dimension (p<0.0001) were observed. (Table 4)

Combined findings (thrombus and contrast) occurred in 39 (9.8%) patients. In this group, older age (p = 0.007), greater LA dimension (p <0.001) and higher CHA2DS2-VASc (p <0.001) were observed. (Table 4)

When combining the influence of each factor by multiple logistic regression, there is a greater risk in patients with previous stroke (OR: 4.8), patients with HF (OR: 2.9), the elderly (OR: 1.04) and those with larger atrial size (OR: 1.11). An additional analysis was carried out between patients who used DOAC in a single daily dose and those who used the medication twice daily, with no difference between groups regarding thrombus, contrast, or combined situations. (Table 05)

Table 1 – Population characteristics

Male (n) (%)		**288 (72)**
Age (SD)		59.9 (11.4)
LVEF % (SD)		59.6 (8.5)
LA mm (SD)		43.3 (6.2)
CHA2DS2-VASc (SD)		1.68 (0.25)
Hypertension (n) (%)		214 (53.6)
Stroke (n) (%)		27 (6.8)
Heart failure (n) (%)		48 (12.0)
Diabetes (n) (%)		61 (15.3)
Vasculopathy (n) (%)		26 (6.5)
DOACs	Apixaban (n) (%)	79 (19.8)
	Dabigatran (n) (%)	99 (24.8)
	Rivaroxaban (n) (%)	222 (55.5)
Dosage – Single dose (n) (%)		222 (55.5)
Thrombus (n) (%)		11 (2.8)
Spontaneous contrast (n) (%)		29 (7.3)
Thrombus and contrast (n) (%)		39 (9.8)
Procedure	Ablation (n) (%)	278 (69.5)
	Cardioversion (n) (%)	122 (30.5)

DOAC: Direct-acting oral anticoagulants; LA: left atrium; LVEF: Left ventricular ejection fraction.

Table 2 – Characteristics of patients according to DOACs

	DOACs			p
	Apixaban n: 79	Dabigatran n: 99	Rivaroxaban n: 222	
Male (%)	61 (77.2)	66 (66.7)	161 (72.5)	0.29
HF (%)	5 (6.3)	15 (15.2)	28 (12.6)	0.18
Stroke (%)	5 (6.3)	13 (13.1)	9 (4.1)	**0.011**
Hypertension (%)	35 (44.3)	58 (58.6)	121 (54.5)	0.15
Diabetes (%)	8 (10.1)	18 (18.2)	35 (15.8)	0.32
Vasculopathy (%)	5 (6.3)	4 (4)	17 (7.7)	0.48
Thrombus (%)	4 (5.1)	3 (3.0)	4 (1.8)	0.31
Spontaneus contrast (%)	2 (2.5)	13 (13.1)	14 (6.3)	**0.018**

Chi-square test. HF: heart failure ; DOAC: Direct-acting oral anticoagulant.

Table 3 – Type and dosage of DOAC in patients with LA thrombus

Patient N°	Sex	Age	CHA2DS2-VASc	Rhythm in TEE	DOAC
1	Female	72	2	Irregular	Rivaroxaban 20mg
2	Female	67	3	Irregular	Dabigatran 150mg
3	Male	74	3	Irregular	Rivaroxaban 20mg
4	Male	66	1	Irregular	Apixaban 2,5mg
5	Male	64	1	Irregular	Apixaban 5mg
6	Male	67	5	Irregular	Rivaroxaban 20mg
7	Female	83	4	Irregular	Dabigatran 110mg
8	Male	58	3	Irregular	Apixaban 5mg
9	Female	78	6	Irregular	Dabigatran 110mg
10	Female	75	3	Irregular	Rivaroxaban 20mg
11	Male	56	2	Irregular	Apixaban 5mg

TEE: transesophageal echocardiogram; DOAC: Direct-acting oral anticoagulant.

Discussion

In the current literature, the prevalence of thrombus in the LA among patients who were adequately anticoagulated with warfarin before TEE ranges from 0.3% to 7.7%, compared with 2.75% among other series with the use of DOACs.[10-14]

Our study demonstrated that, even in patients using DOACs, a rate of thrombus or dense spontaneous contrast in the LA (9,8%) was found in patients who underwent periprocedural elective ECV and/or CA. A minimum period of 3 weeks of previous use of DOACs was used, which we considered reasonable based on the literature, and there was no statistical difference compared to 4 weeks.

In a subgroup analysis of the RE-LY study, the rate of thrombi in the left atrium in patients before cardioversion was 1.5% for patients using dabigatran. In the ARISTOTLE study, TEE records were available in 86 patients using apixaban; none had a thrombus in the LA. In the ROCKET-AF study, TEE data were not collected to assess the prevalence of thrombus in the LA.[15-17]

Frenkel et al. retrospectively analyzed data from 388 TEE patients before AF or atrial flutter ECV using continuous DOAC or warfarin therapy for 4 weeks. Without statistical significance, the prevalence of thrombus in the LA was 4.4% in the DOAC group and 2.9% in the warfarin group.[18] Al Rawahi et al., analyzing data from 401 patients who underwent ablation or ECV, found a thrombus in the left atrium in 11.2% of the sample. When we separated patients who used only DOACs, the presence of thrombus in those who used dabigatran, rivaroxaban

Table 4 – Analysis of risk factors in relation to the presence of thrombus and spontaneous contrast

	Thrombus		p	Spontaneous contrast		p	Thrombus and contrast		p
	Yes	No		Yes	No		Yes	No	
Male (%)	6 (2.1)	282 (97.9)	0.19	18 (6.3)	270 (93.8)	0.22	24 (8.3)	264 (91.7)	0.13
Age (SD)	69.1 (8.2)	59.6 (11.4)	**0.007**	61.7 (7.2)	59.7 (11.7)	0.19	63.5 (8.1)	59.5 (11.7)	**0.036**
HF (%)	2 (4.2)	46 (95.8)	0.52	6 (12.5)	42 (87.5)	0.14	8 (16.7)	40 (83.3)	0.09
Stroke (%)	3 (11.1)	24 (88.9)	**0.006**	6 (22.2)	21 (77.8)	**0.002**	9 (33.3)	18 (66.7)	**<0.001**
Hypertension (%)	8 (3.7)	206 (96.3)	0.20	17 (7.9)	197 (92.1)	0.57	25 (11.7)	189 (88.3)	0.16
Diabetes (%)	1 (1.6)	60 (90.4)	0.56	7 (11.5)	54 (88.5)	0.17	8 (13.1)	53 (86.9)	0.34
Vasculopathy (%)	1 (3.8)	25 (96.2)	0.72	0 (0)	26 (100)	0.14	1 (3.8)	25 (96.2)	0.29
Dosage-twice a day (%)	7 (3.9)	171 (96.1)	0.20	15 (8.4)	163 (91.6)	0.42	22 (12.4)	156 (87.6)	0.12
LA (SD)	43.6 (4.4)	43.3 (6.2)	0.86	47.8 (5.6)	43.0 (6.1)	**<0.001**	46.6 (5.6)	43.0 (6.1)	**<0.001**
LVEF (SD)	59.8 (10.0)	59.6 (8.5)	0.94	56.5 (9.0)	59.9 (8.4)	**0.038**	57.2 (9.3)	59.9 (8.4)	0.06
CHA_2DS_2-VASc (SD)	3.0 (1.5)	1.64 (1.3)	**0.001**	2.1 (1.4)	1.6 (1.4)	0.06	2.4 (1.5)	1.6 (1.3)	**0.001**

Chi-square test. LA: left atrium; LVEF: left ventricle ejection fraction; HF: heart failure.

Table 5 – Multivariate analysis of risk factors

	OR	CI (95%)		p
		Inferior	Superior	
Male	0.61	0.27	1.36	0.23
HF	2.90	1.15	7.31	**0.024**
Hypertension	1.43	0.67	3.07	0.36
Age (years)	1.04	1.00	1.08	**0.045**
Stroke	4.80	1.84	12.51	**0.001**
Dosage (twice)	1.72	0.83	3.56	0.14
LA (mm)	1.11	1.04	1.17	**0.001**

Multiple logistic regression. HF: heart failure; LA: left atrium; CI: confidence interval.

and apixaban was 5%, 4% and 9%, respectively.[19] Michael Wu et al., analyzing 609 patients using DOAC with an average anticoagulation time of 12 weeks, found 17 patients (2.8%) with thrombus in the LA and 15 patients (2.5%) with spontaneous contrast in the TEE, numbers comparable to our findings.[20]

Despite the theoretical advantages of treating AF patients with DOACs over warfarin, concerning dosage issues and subtherapeutic anticoagulation, the prevalence of thrombus in the LA is not negligible, even with the patient correctly using DOAC for at least 3 weeks. Our rates are comparable to those previously reported among patients using anticoagulation therapy with warfarin, which vary widely, as previously mentioned.

Currently, the indication to perform elective TEE before ablation and/or cardioversion of AF/atrial flutter is still controversial. According to the 2017 HRS / EHRA / ECAS Consensus on AF Catheter Ablation, 50% of the writing group members performed routine TEE, while the remaining group performed TEE only if patients had significant risk factors for LA thrombus or had not been on therapeutic anticoagulation for at least four weeks. However, it is generally accepted that the presence of a thrombus detected by TEE is a contraindication to AF catheter ablation and cardioversion.

There are several potential reasons for the presence of these findings, despite effective anticoagulation with DOAC, which include underlying factors of the patient, including severe atrial myopathy, which makes the thrombus refractory; poor adherence; inadequate dosage

due to fluctuations in drug clearance (e.g., changes in renal function) and inadequate serum levels of the drug due to the incorrect mode of administration (e.g., not taking rivaroxaban with food, leading to reduced bioavailability). Of note, there was no difference between patients according to DOAC dosage regimen (bid versus qd). Another factor that can influence are drug interactions; however, in our study, there was no report of the concomitant use of drugs with high interactions already described in the literature, such as, for example, rifampicin, antiretrovirals and/or antifungals.

Our study suggests that TEE screening should be performed in patients with high CHA2DS2-VASc scores, previous ischemic stroke, and LA dimension greater than 45 mm, despite uninterrupted therapy with DOAC, a hypothesis that should be tested in future randomized clinical trials.

Limitations

Our study has several limitations. First, this is a cross-sectional observational study. Although we excluded patients with a documented history of missed doses of DOAC in the 3 weeks prior to TEE from our study sample, it was not possible to guarantee full adherence in all individuals. In addition, we did not systematically check whether patients were taking DOAC correctly (for example, administering rivaroxaban with meal). However, our study design reflects real-world practice. Another limitation refers to the fact that it was not possible to rule out whether part of these patients already had thrombi previously in the LA that were not dissolved at the time of TEE during elective cardioversion and/or CA, since they did not undergo TEE before the first prescription of the DOAC. Finally, given the absence of patients using edoxaban in our institution, it is not possible to extrapolate these observations to this drug.

Conclusion

The incidence of LA thrombus and spontaneous contrast in patients using DOAC in the context of AF ECV and/or ablation, although small, is not negligible. Patients with a higher CHA2DS2-VASc score (especially older ones) and a larger LA diameter are more prone to these echocardiographic findings.

Author Contributions

Conception and design of the research and Analysis and interpretation of the data: Marques T, Darrieux F, Gouvêa F, Garambone L, Lima APL, Lage JGB, Sacilotto L, Coimbra AL, Pinheiro M, Olivetti N, Lara S, Hardy C, Athayde G, Hachul D, Pisani C, Wu TC, Scanavacca M; Acquisition of data: Marques T, Darrieux F, Gouvêa F, Garambone L, Lima APL, Lage JGB; Statistical analysis: Marques T; Writing of the manuscript: Marques T, Darrieux F, Gouvêa F, Garambone L, Lima APL, Lage JGB, Hardy C, Hachul D, Scanavacca M; Critical revision of the manuscript for intellectual content: Marques T, Darrieux F, Sacilotto L, Coimbra AL, Pinheiro M, Olivetti N, Lara S, Athayde G, Pisani C, Wu TC, Scanavacca M.

Potential Conflict of Interest

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

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Study Association

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

References

1. January CT, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland JC Jr, et al. 2014 AHA/ACC/HRS Guideline for the Management of Patients with Atrial Fibrillation: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. J Am Coll Cardiol. 2014;64(21):e1-76. doi: 10.1016/j.jacc.2014.03.022.
2. Stellbrink C, Nixdorff U, Hofmann T, Lehmacher W, Daniel WG, Hanrath P, Geller C, et al. Safety and Efficacy of Enoxaparin Compared with Unfractionated Heparin and Oral Anticoagulants for Prevention of Thromboembolic Complications in Cardioversion of Nonvalvular Atrial Fibrillation: The Anticoagulation in Cardioversion using Enoxaparin (ACE) Trial. Circulation. 2004;109(8):997-1003. doi: 10.1161/01.CIR.0000120509.64740.DC.
3. Klein AL, Grimm RA, Murray RD, Apperson-Hansen C, Asinger RW, Black IW, et al. Use of Transesophageal Echocardiography to Guide Cardioversion in Patients with Atrial Fibrillation. N Engl J Med. 2001;344(19):1411-20. doi: 10.1056/NEJM200105103441901.
4. Scherr D, Dalal D, Chilukuri K, Dong J, Spragg D, Henrikson CA, et al. Incidence and Predictors of Left Atrial Thrombus Prior to Catheter Ablation of Atrial Fibrillation. J Cardiovasc Electrophysiol. 2009;20(4):379-84. doi: 10.1111/j.1540-8167.2008.01336.x.
5. McCready JW, Nunn L, Lambiase PD, Ahsan SY, Segal OR, Rowland E, et al. Incidence of Left Atrial Thrombus Prior to Atrial Fibrillation Ablation: Is Pre-Procedural Transoesophageal Echocardiography Mandatory? Europace. 2010;12(7):927-32. doi: 10.1093/europace/euq074.
6. Puwanant S, Varr BC, Shrestha K, Hussain SK, Tang WH, Gabriel RS, et al. Role of the CHADS2 Score in the Evaluation of Thromboembolic Risk in Patients with Atrial Fibrillation Undergoing Transesophageal Echocardiography Before Pulmonary Vein Isolation. J Am Coll Cardiol. 2009;54(22):2032-9. doi: 10.1016/j.jacc.2009.07.037.
7. Nishikii-Tachibana M, Murakoshi N, Seo Y, Xu D, Yamamoto M, Ishizu T, et al. Prevalence and Clinical Determinants of Left Atrial Appendage Thrombus in Patients with Atrial Fibrillation Before Pulmonary Vein Isolation. Am J Cardiol. 2015;116(9):1368-73. doi: 10.1016/j.amjcard.2015.07.055.
8. Schmidt H, von der Recke G, Illien S, Lewalter T, Schimpf R, Wolpert C, et al. Prevalence of Left Atrial Chamber and Appendage Thrombi in Patients with Atrial Flutter and its Clinical Significance. J Am Coll Cardiol. 2001;38(3):778-84. doi: 10.1016/s0735-1097(01)01463-2.
9. Wallace TW, Atwater BD, Daubert JP, Voora D, Crowley AL, Bahnson TD, et al. Prevalence and Clinical Characteristics Associated with Left Atrial Appendage Thrombus in Fully Anticoagulated Patients Undergoing Catheter-Directed Atrial Fibrillation Ablation. J Cardiovasc Electrophysiol. 2010;21(8):849-52. doi: 10.1111/j.1540-8167.2010.01729.x.
10. Zhan Y, Joza J, Al Rawahi M, Barbosa RS, Samuel M, Bernier M, et alV. Assessment and Management of the Left Atrial Appendage Thrombus

in Patients With Nonvalvular Atrial Fibrillation. Can J Cardiol. 2018 Mar;34(3):252-261. doi: 10.1016/j.cjca.2017.12.008. Epub 2017 Dec 15. PMID: 29395705.

11. Zoppo F, Brandolino G, Berton A, Frigato N, Michieletto M, Zanocco A, et al. Predictors of left atrium appendage clot detection despite on-target warfarin prevention for atrial fibrillation. J Interv Card Electrophysiol 2012;35:151-8. doi: 10.1007/s10840-012-9707-0.

12. Alqarawi W, Birnie DH, Spence S, Ramirez D, Redpath CJ, Lemery R, et al. Prevalence of left atrial appendage thrombus detected by transoesophageal echocardiography before catheter ablation of atrial fibrillation in patients anticoagulated with non-vitamin K antagonist oral anticoagulants. Europace 2019;21:48-53. doi: 10.1093/europace/euy129.

13. Fukuda S, Watanabe H, Shimada K, Aikawa M, Kono Y, Jissho S, et al. Left atrial thrombus and prognosis after anticoagulation therapy in patients with atrial fibrillation. J Cardiol 2011;58:266-77. doi: 10.1016/j.jjcc.2011.06.009.

14. Wu X, Wang C, Zhang C, Zhang Y, Ding F, Yan J. Computed tomography for detecting left atrial thrombus: a meta-analysis. Arch Med Sci 2012;8:943-51. doi: 10.5114/aoms.2012.32400.

15. Nagarakanti R, Ezekowitz MD, Oldgren J, Yang S, Chernik M, Aikens TH, et al. Dabigatran versus warfarin in patients with atrial fibrillation: an analysis of patients undergoing cardioversion. Circulation 2011;123:131-6. doi: 10.1161/CIRCULATIONAHA.110.977546.

16. Flaker G, Lopes RD, Al-Khatib SM, Hermosillo AG, Hohnloser SH, Tinga B, et al. Efficacy and safety of apixaban in patients after cardioversion for atrial fibrillation: insights from the ARISTOTLE Trial (Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation). J Am Coll Cardiol 2014;63:1082-7. doi: 10.1016/j.jacc.2013.09.062.

17. Piccini JP, Stevens SR, Lokhnygina Y, Patel MR, Halperin JL, Singer DE, et al. Outcomes after cardioversion and atrial fibrillation ablation in patients treated with rivaroxaban and warfarin in the ROCKET AF trial. J Am Coll Cardiol 2013;61:1998-2006. doi: 10.1016/j.jacc.2013.02.025.

18. Frenkel D, D'Amato SA, Al-Kazaz M, Markowitz SM, Liu CF, Thomas G, et al. Prevalence of left atrial thrombus detection by transesophageal echocardiography: a comparison of continuous non-vitamin k antagonist oral anticoagulant versus warfarin therapy in patients undergoing catheter ablation for atrial fibrillation. JACC Clin Electrophysiol 2016;2:295-303. doi: 10.1016/j.jacep.2016.01.004.

19. Al Rawahi M, Samuel M, Galatas C, Joza J, Lima PY, Barbosa R, et al. Incidence and Predictors of Intracardiac Thrombus on Pre-electrophysiological Procedure Transesophageal Echocardiography. CJC Open. 2019;1(5):231-237. doi:10.1016/j.cjco.2019.06.004.

20. Wu M, Gabriels J, Khan M, Shaban N, D'Amato SA, Liu CF, et al. Left atrial thrombus and dense spontaneous echocardiographic contrast in patients on continuous direct oral anticoagulant therapy undergoing catheter ablation of atrial fibrillation: Comparison of dabigatran, rivaroxaban, and apixaban. Heart Rhythm. 2018;15(4):496-502. doi:10.1016/j.hrthm.2017.12.005.

Rhythm Control Interventions in Patients with Atrial Fibrillation – Insights on Preprocedural Anticoagulation and Utility of Left Atrial Imaging

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Short Editorial related to the article: Left Atrial Thrombus and Dense Spontaneous Contrast in Direct Oral Anticoagulant Therapy of Atrial Fibrillation: Insights from a Reference Center

The most common sustained arrhythmia in clinical practice is atrial fibrillation (AF),[1,2] affecting 2-4% of the adult population worldwide. It is even more frequent with aging, with almost 10% prevalence in individuals older than 80.[1] Current estimates state that one in every three adults aged 55 years will develop AF during their lifetime, leading to substantial healthcare and economic burden.[2] Clinical issues relate primarily to thromboembolic events (TE) and arrhythmic symptoms, both central targets while managing patients with AF.[1,2]

Overall, atrial fibrillation confers a 2-5-fold escalated risk of TE, which is not evenly distributed, depending on unique modifiers.[2] Important risk factors abridged in the CHA_2DS_2-VASc score – Congestive heart failure, Hypertension, Age ≥75 years, Diabetes, Stroke/TIA, Vascular disease, Age 65-74 years, Sex (female) – may predict stroke risk, consistently mitigated in almost 70% by proper anticoagulation. Vitamin K antagonists (VKAs) were the only oral anticoagulants available for over half a century. From 2009 to 2013, pivotal randomized controlled trials acquainted the scientific community with the new/direct oral anticoagulants (DOACs).[3-6] These drugs not only held similar efficacy to VKA in preventing thromboembolic events but also had a better safety profile against major bleeding – notably intracranial hemorrhage – and a more predictable pharmacokinetic and pharmacodynamic profile, ruling out the need for routine laboratory monitoring.[7] However, the applicability of DOACs in off-label backgrounds, including stroke prevention during rhythm control interventions, remained unclear for many years.

Encompassing treatments such as cardioversion, antiarrhythmics and catheter ablation, the rhythm control strategy comprises efforts to restore and maintain sinus rhythm in patients with AF.[2] This approach has formal indications for reducing symptoms and improving quality of life after failure or intolerance to class I or III antiarrhythmic drugs.[2] Nowadays, there is a trend toward an early indication of rhythm control procedures, trying to avoid atrial remodeling and postpone AF progression.[1,2] AF cardioversion and catheter ablation may precipitate TE events by dislodgement of pre-existing thrombi or different *de novo* thrombus formation mechanisms, such as atrial stunning and adherence to the ablation's equipment thrombogenic surface or ablation sites with endothelial disruption.[2,8] Hence, the presence of cardiac *thrombi* contraindicates cardioversion and ablation procedures.[9] In AF lasting more than 48 hours, the periprocedural thromboembolic risk may reach 5-7% without adequate prophylaxis.[8,10]

Most AF-related thromboemboli stem from the left atrial (LA) appendage.[8,10] However, the reported prevalence of LA-thrombi varies significantly, from 0.6% to 27%, depending on population characteristics and treatment status.[8,10] VKAs, within adequate time in the therapeutic range (INR 2.0-3.0) for at least three weeks before sinus rhythm restoration, effectively decrease the rates of stroke and thromboembolism.[2] Sub-analyses of the randomized controlled trials RE-LY, ROCKET-AF and ARISTOTLE demonstrated that DOACs were also successful in this setting.[2,7] Current guidelines, thus, recommend therapeutic oral anticoagulation with VKAs/DOACS for ≥3 weeks before any rhythm control attempt.[1,2,11-13] If that is unfeasible, for urgency or practical reasons, pre-procedural screening for LA thrombus with transoesophageal echocardiography (TOE) may be performed.[2,13] However, the preprocedural anticoagulation period suggested in the guidelines was arbitrarily based on the assumed time needed for endothelialization or resolution of pre-existing AF thrombus.[2] Moreover, these endorsements relied on trials examining periprocedural thromboembolic complications. Existing literature on LA thrombi prevalence in individuals receiving guideline-directed anticoagulation is scarce.[9] Most observational studies reporting data on real-world experience are limited, lacking comparison between the diverse OACs or different posology in the same study and observance for confounders, like proper anticoagulation (sufficient period and adequate time within therapeutic range) before TOE.

The work of Marques et al.[14] in ABC's current edition added relevant insights into this field. The authors investigated the presence of left atrial thrombi and dense spontaneous contrast (DSC) in a retrospective unicentric cohort that included 354 patients undergoing TOE before direct current cardioversion or AF catheter ablation. All patients received ≥3 weeks of DOACs (Dabigatran 99, Rivaroxaban 222, and Apixaban 79). In this cohort, LA thrombi were present in 2.8% and DSC in 7.3% of the

Keywords

Atrial Fibrillation; Electric Countershock; Transesophageal Echocardiography; Thrombosis.

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patients.[14] LA thrombi and DSC were more frequent in individuals with more advanced age and higher CHA_2DS_2-VASc scores, and those with left atrial enlargement and reduced left ventricular function.[14] There was no statistically significant difference in LA thrombi, and DSC rates between the three tested DOACs.[14] These reported data aligned with a recent meta-analysis including 14,653 individuals that found a non-negligible 3% prevalence of LA thrombus in anticoagulated patients with atrial fibrillation or atrial flutter, with increased odds for patients with non-paroxysmal atrial fibrillation and a CHA_2DS_2-VASc score ≥3, irrespective of the OAC used.[9,14]

In essence, continued oral anticoagulation yields low periprocedural stroke rates, which are similar to all available OACs.[2] However, along with the existing knowledge, Marques *et al*. demonstrated that, despite adequate anticoagulation, some patients may still present LA thrombi and DSC,[14] suggesting the need for more individualized and risk-based use of TOE to improve the safety of rhythm control interventions in patients with AF.

References

1. Magalhães LP, Figueiredo MJO, Cintra FD, Saad EB, Kuniyoshi RR, Menezes Lorga Filho A, et al. Executive Summary of the II Brazilian Guidelines for Atrial Fibrillation. Arq Bras Cardiol. 2016;107(6):501-8. doi: 10.5935/abc.20160190.
2. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, et al. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): The Task Force for the diagnosis and management of atrial fibrillation of the European Society of Cardiology (ESC) Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. Eur Heart J. 2021;42(5):373-498. doi: 10.1093/eurheartj/ehaa612.
3. Camm AJ. The RE-LY study: Randomized Evaluation of Long-term anticoagulant therapY: dabigatran vs. warfarin. Eur Heart J. 2009;30(21):2554-5. DOI: 10.1093/eurheartj/ehp384
4. Investigators RAS. Rivaroxaban-once daily, oral, direct factor Xa inhibition compared with vitamin K antagonism for prevention of stroke and Embolism Trial in Atrial Fibrillation: rationale and design of the ROCKET AF study. Am Heart J. 2010;159(3):340-7.e1. DOI: 10.1016/j.ahj.2009.11.025
5. Granger CB, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M, et al. Apixaban versus warfarin in patients with atrial fibrillation. N Engl J Med. 2011;365(11):981-92. DOI: 10.1056/NEJMoa1107039
6. Giugliano RP, Ruff CT, Braunwald E, Murphy SA, Wiviott SD, Halperin JL, et al. Edoxaban versus Warfarin in Patients with Atrial Fibrillation. N Engl J Med. 2013;369(22):2)(3-104. doi: 10.1056/NEJMoa1310907.
7. Coppola G, Manno G, Mignano A, Luparelli M, Zarcone A, Novo G, et al. Management of Direct Oral Anticoagulants in Patients with Atrial Fibrillation Undergoing Cardioversion. Medicina (Kaunas). 2019;55(10):660. doi: 10.3390/medicina55100660.
8. Zhan Y, Joza J, Al Rawahi M, Barbosa RS, Samuel M, Bernier M, et al. Assessment and Management of the Left Atrial Appendage Thrombus in Patients With Nonvalvular Atrial Fibrillation. Can J Cardiol. 2018;34(3):252-61. doi: 10.1016/j.cjca.2017.12.008.
9. Lurie A, Wang J, Hinnegan KJ, McIntyre WF, Belley-Côté EP, Amit G, et al. Prevalence of Left Atrial Thrombus in Anticoagulated Patients With Atrial Fibrillation. J Am Coll Cardiol. 2021;77(23):2875-86. doi: 10.1016/j.jacc.2021.04.036.
10. Kawabata M, Goya M, Sasaki T, Maeda S, Shirai Y, Nishimura T, et al. Left Atrial Appendage Thrombi Formation in Japanese Non-Valvular Atrial Fibrillation Patients During Anticoagulation Therapy - Warfarin vs. Direct Oral Anticoagulants. Circ J. 2017;81(5):645-51. DOI: 10.1253/ circj.CJ-16-1089
11. Andrade JG, Verma A, Mitchell LB, Parkash R, Leblanc K, Atzema C, et al. 2018 Focused Update of the Canadian Cardiovascular Society Guidelines for the Management of Atrial Fibrillation. Can J Cardiol. 2018;34(11):1371-92. doi: 10.1016/j.cjca.2018.08.026.
12. Calkins H, Hindricks G, Cappato R, Kim YH, Saad EB, Aguinaga L, et al. 2017 HRS/EHRA/ECAS/APHRS/SOLAECE expert consensus statement on catheter and surgical ablation of atrial fibrillation: Executive summary. J Arrhythm. 2017;33(5):369-409. doi: 10.1016/j.joa.2017.08.001
13. January CT, Wann LS, Calkins H, Chen LY, Cigarroa JE, Cleveland JC, et al. 2019 AHA/ACC/HRS Focused Update of the 2014 AHA/ ACC/HRS Guideline for the Management of Patients With Atrial Fibrillation: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society in Collaboration With the Society of Thoracic Surgeons. Circulation. 2019;140(2):e125-e51. doi: 10.1161/ CIR.0000000000000665
14. Marques T, Darrieux F, Gouvea F, Carambone L, Lindoso AP, Lage J, et al. Trombo atrial esquerdo e contraste espontâneo denso no uso de anticoagulante oral de ação direta em fibrilação atrial. Arq Bras Cardiol.2022; ahead print PP-00.

Long-Term Ventricular Pacing Dependency and Pacemaker Implantation Predictors after Transcatheter Aortic Valve Replacement – A 1-Year Follow-Up

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Abstract

Background: **Conduction disturbances (CD) are the most frequent complication after transcatheter aortic valve replacement (TAVR), and there continues to be a lack of consensus on their management.**

Objective: **To assess new CD and permanent pacemaker (PPM) implantation after TAVR and to evaluate the ventricular pacing percentage (VP) up to 1 year of follow-up.**

Methods: **Patients who underwent TAVR from October 2014 to November 2019 were enrolled; patients with previous PPM were excluded. Clinical, procedure, ECG, and PPM data were collected up to 1 year after implantation. The significance level adopted in the statistical analysis was 0.05.**

Results: **A total of 340 patients underwent TAVR. The most frequent CD was the new left bundle branch block (LBBB; 32.2%), which 56% resolved after 6 months. Right bundle branch block (RBBB) was the biggest risk factor for advanced atrioventricular block (AVB) [OR=8.46; p<0.001] and PPM implantation [OR=5.18, p<0.001], followed by previous low-grade AVB [OR=2.25; p=0.016 for PPM implantation]. Regarding procedure characteristics, newer generation valves and valve-in-valve procedures were associated with fewer CDs. Overall, 18.5% of patients had a PPM implanted post-TAVR. At first PPM evaluation, patients with advanced AVB had a median percentage of VP of 80% and 83% at one year. Regarding patients with LBBB plus low-grade AVB, median VP was lower (6% at first assessment, p=0.036; 2% at one year, p = 0.065).**

Conclusion: **LBBB was the most frequent CD after TAVR, with more than half being resolved in the first six months. RBBB was the major risk factor for advanced AVB and PPM implantation. Advanced AVB was associated with a higher percentage of VP at 1 year of follow-up.**

Keywords: **Aortic Valve Stenosis; Atrioventriclar Block; Transcatheter Aortic Valve Replacement; Pacemaker,Artificial; Heart Valve Prosthesis Implantation; Cardiac Conduction System Disease.**

Introduction

Transcatheter aortic valve replacement (TAVR) is a well-established procedure to treat patients with symptomatic severe aortic stenosis at increased or prohibitive surgical risk. Increased experience has led to a growing consideration of TAVR as an option to people at lower risk.[1-3] The widespread adoption of TAVR was accompanied by a reduction in the majority of periprocedural complications, except for new conduction disturbances and consequent need for PPM implantation.[1,4,5] New LBBB, with an incidence of about 25% (4% to 65%), is the most frequently documented rhythm disorder after TAVR and probably the most challenging.[1] Although often self-limited, a significant percentage of these patients evolve to advanced AVB or complete heart block, the most serious complications of conduction after-TAVR.[1,2,4,6,7]

Major questions remain about the management of conduction disturbances after TAVR, leading to distinct approaches among different centers. Patients commonly continue to be monitored with telemetry and daily electrocardiogram (ECG) after the procedure, sometimes with backup temporary pacemaker, increasing the hospitalization length and procedural cost.[4,7,8] There are limited data on risk factors for the development of advanced AVB and the need to maintain a temporary pacemaker, which also translates into varying rates of PPM implantation post-TAVR.[1,7]

The aim of the present study was to describe new conduction disturbances and PPM implantation in patients

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undergoing TAVR with either a balloon-expandable or a self-expandable valve prosthesis. We also evaluated the percentage of VP in patients who underwent PPM implantation up to 1 year of follow-up.

Methods

Study population

The present study included a sample of consecutive patients undergoing TAVR at Centro Hospitalar Universitário de São João, E.P.E., a tertiary center in Porto, Portugal, from October 2014 to November 2019 (n = 371). Patients who had PPM previous to valve implantation were excluded (n = 31). The remaining 340 patients were retrospectively analyzed. Clinical, electrocardiographic, echocardiographic, and procedure data were collected at presentation and up to 1 year after implantation, including systematic interrogation of implanted PPM. This study was approved by the institutional ethics committee.

Definitions, data, and ECG collection

Clinical endpoints and definition of conduction disturbances were in accordance with the Valve Academic Research Consortium (VARC)-2 Consensus and the consensus by JACC Scientific Expert Panel, respectively.[1,9] ECGs were systematically obtained at baseline (usually the day before TAVR), immediately after valve implantation (at admission in cardiac care unit) and at least daily until hospital discharge. All patients had continuous electrocardiographic monitoring during hospital stay. Most ECGs in our institution were electronically recorded, and were assessed and reviewed by cardiologists. Clinical, echocardiographic, and procedure data were collected from digital records. Low grade AVB was defined as 1st degree or 2nd degree Mobitz I AVB. Advanced AVB was defined as 2nd degree Mobitz II or 3rd degree AVB.

Procedure

Patients submitted to TAVR with self-expandable (Medtronic CoreValve, Medtronic CoreValve Evolut R, Medtronic CoreValve Evolut Pro, Boston Scientific Acurate Neo, Abbott Portico, and Boston Scientific LOTUS) and with balloon-expandable (Edwards SAPIEN 3) valves were included. All patients had a temporary transvenous pacing catheter placed in the right ventricle. Depending on new-onset conduction disturbances or pre-procedure risk of rhythm disorder, and in accordance with the consensus by JACC Scientific Expert Panel,[1] the temporary pacemaker was removed, either immediately in the catheterization laboratory or later during hospitalization (usually 24 – 48h). For the purpose of this study, the newer generation valve analysis included procedures with SAPIEN 3, CoreValve Evolut Pro and Acurate Neo valves, while the remaining were classified as earlier generation valves.

Permanent pacemaker indication and follow-up

PPM were implanted according to 2018 ACC/AHA/HRS guidelines for bradycardia and cardiac conduction delay and in accordance with JACC Scientific Expert Panel.[1,10] All devices were reviewed on day 1 and 7 after implantation. Intrinsic AV conduction was systematically queried and algorithms to minimize VP were applied (Managed Ventricular Pacing mode or AAI mode with backup VVI pacing in most patients). For the purpose of the study, first PPM evaluation was defined as first ambulatory device evaluation after discharge (median time 3 months after implantation, IQR 3 - 4 months) and one-year evaluation was defined as second ambulatory device evaluation (median time 12 months after implantation, IQR 10 - 12 months). Because some patients were followed up at other medical institutions, data from PPM follow-up was unavailable in 30% and 43% of patients for first PPM and one-year evaluations, respectively.

Statistical analysis

Data are presented as median (interquartile range [IQR]) for continuous variables, and as number and percentages for categorical variables. One-sample Kolmogorov-Smirnov test was performed to evaluate normal distribution. Categorical variables were compared using the chi-square test; odds ratios (OR) are presented when considered relevant. Continuous variables were compared using the Mann-Whitney U test. Differences were considered statistically significant when p value < 0.05. Statistical analysis was performed in IBM SPSS Statistics version 25.

Results

Study population

A total of 340 patients undergoing TAVR between October 2014 and November 2019 were included in our sample, after excluding 31 patients with a previous PPM.

Baseline characteristics of the study sample are summarized in table 1 and table 2. Median age was 81 years (IQR 76 to 85 years) and 57% of the patients were female.

At baseline, 77% of patients were in sinus rhythm and 23% AF; in patients who were in sinus rhythm (SR), most had normal atrioventricular (AV) conduction. Regarding intraventricular (IV) conduction, 60% had no conduction disturbance, and the most frequent disturbance was nonspecific intraventricular conduction delay (NICD; table 2).

Self-expandable CoreValve Evolut R was the most frequently used valve (41% of cases), followed by CoreValve Evolut Pro and Acurate Neo (Table 3). There were 23 valve-in-valve procedures, and 90 patients underwent balloon valve pre-dilation.

Conduction Disturbances Post-TAVR and ECG predictors

After TAVR, 50.9% of the patients exhibited new conduction disturbances (table 4). Regarding AV conduction, 13.6% of patients developed low grade AVB (1st degree or 2nd degree Mobitz I) and 12.4% developed advanced AVB (2nd degree Mobitz II or 3rd degree). Regarding IV conduction, de novo LBBB was the most frequent disturbance (32.2%).

Previous AF was not associated with advanced AVB or PPM implantation. Low-grade AVB, when compared with patients with normal AV conduction, was associated with a higher PPM implantation rate (30.4% vs 16.2%, p=0.016), but not with advanced AVB (Figures 2 and 3).

Concerning IV conduction, previous LBBB did not increase the risk of new advanced AVB or PPM implantation. By contrast, the presence of previous RBBB proved to be a strong risk factor for advanced AVB (7.2% vs 39.6%, p<0.001) and PPM implantation (14.0% vs 45.8%,

Table 1 – Baseline

N	340
Age, yrs (IQR)	81 (76 - 81)
Female (%)	193 (57)
Hypertension (%)	294 (87)
Diabetes (%)	127 (37)
Dyslipidemia (%)	244 (72)
Prior kidney disease (%)	185 (62)
on dialysis (%)	10 (3)
Atrial fibrillation (%)	78 (23)
Preserved LV function (%)	244 (73)
Bicuspid valve (%)	8 (3)
Aortic valve area (IQR)	0,7 cm² (0,6 – 0,9)
Transvalvular pressure gradient (IQR)	46 mmHg (39.5 - 59)
LV ejection fraction (IQR)	60 % (44 - 65)
Severe aortic regurgitation (%)	15 (6)

Table 1 presents the baseline characteristics of study population. Values were presented as median (IQR) or number of cases (%). IQR: interquartile range; yrs: years-old; LV: left ventricle.

Table 2 – Pre-TAVR rhythm characteristics

Rhythm	
Sinus rhythm	262 (77)
Atrial Fibrillation	78 (23)
AV conduction	
Normal AV conduction	207 (79)
1st degree AVB	53 (20)
2nd degree Mobitz I AVB	2 (1)
IV conduction	
LBBB	31 (9)
RBBB	25 (7)
Left anterior fascicular block	24 (7)
Bifascicular block	23 (7)
Nonspecific intraventricular conduction delay	33 (10)

Table 2 summarizes cardiac rhythm, atrioventricular (AV) conduction and intraventricular (IV) conduction of study population before TAVR. AV conduction was considered only in sinus rhythm. Values were presented as number of cases (%). LBBB: left bundle branch block; RBBB: right bundle branch block; AVB: atrioventricular block.

Table 3 – Procedure characteristics

Valve type	
CoreValve Evolut R	140 (41)
CoreValve Evolut Pro	72 (21)
Acurate Neo	44 (13)
SAPIEN 3	33 (10)
Portico	31 (9)
CoreValve	14 (4)
LOTUS	6 (2)
Balloon pre-dilation	90 (27)
Valve-in-valve	23 (7)

Table 3 shows the procedure characteristics of the TAVR sample. Values were presented as number of cases (%).

Table 4 – New conduction disturbances

N	172 (50.9)
AV conduction	
1st degree AVB	42 (12.4)
2nd degree Mobitz I AVB	4 (1.2)
2nd degree Mobitz II AVB	2 (0.6)
3rd degree AVB	40 (11.8)
IV conduction	
Fascicular block	5 (1.5)
LBBB	109 (32.2)
RBBB	1 (0.3)
ABBB	1 (0.3)
NICD	2 (0.6)

Table 4 shows de novo *conduction disturbances after valve implantation. Values were presented as number of cases (%). AV: atrioventricular; AVB: atrioventricular block; LBBB: left bundle branch block; RBBB: right bundle branch block; ABBB: alternating bundle branch block; NICD: nonspecific intraventricular conduction delay.*

p<0.001). Fascicular block and NICD were not associated with advanced AVB or PPM implantation.

Three cases of advanced AVB reverted early after TAVR (less than 24h). Upon hospital discharge, 27.5% of de novo LBBB was resolved. After 6 months of follow-up, the rate of recovery was higher, with 56.1% of the cases reverted to normal intraventricular conduction.

TAVR procedure and rhythm disturbances

The highest proportion of new conduction disturbances was seen with the LOTUS valve (80% of patients), followed by Portico (71%), CoreValve (64%), CoreValve Evolut R (51%), CoreValve Evolut Pro (47%), SAPIEN 3 (42%), and Acurate Neo (39%). Table 5 and Figure 1 summarize the main findings based on procedure characteristics. There was a significant difference between newer and earlier generation valves regarding incidence of new conduction disturbances, advanced AVB and PPM implantation.

Pre-dilation was not associated with development of conduction disorders nor differences in regression of these disturbances upon 6 months of follow-up. When comparing balloon-expandable with self-expandable valves, no statistically significant difference was found.

Valve-in-valve procedures were associated with fewer changes in conduction, with only 17.4% of patients developing conduction delays [OR=0.19 (95% CI 0.06-0.58)] and only 8.7% requiring PPM implantation, despite a similar rate of pre-TAVR AV and IV conduction disturbances.

An additional analysis was also conducted, including only newer generation valves. In this group, no difference was found in new conduction disturbances and advanced AVB, but a statistically significant difference was found in PPM implantation in favor of Acurate Neo (p=0.032).

PPM implantation and follow-up

Overall, 18.5% (N = 63) had a PPM implanted after TAVR, 81% dual-chamber devices, and no major complications occurred during admission. The main reason for pacemaker implantation was advanced AVB (60.3%), followed by LBBB with low-grade AVB (22.2%), isolated LBBB (4.8%), and alternating bundle branch block (ABBB, 4.8%).

Upon first PPM evaluation, patients with advanced AVB had a median percentage of VP of 80%, with 44.4% of patients presenting >90% of VP and 14.8% <1% of VP; one year after TAVR the median percentage of VP was 83%, almost half of patients (46.2%) with VP >90% and 19.2% with VP under one percent.

Regarding patients with LBBB plus low-grade AVB, median VP upon first assessment was 6% (44.4% had < 1% of VP) and 11.1% had >90% of VP; PM evaluation at one year showed a

Table 5 – TAVR procedure and rhythm disturbances

Procedure	New rhythm disturbances	p-value	Advanced AVB	p-value	PPM implantation	p-value
Newer vs earlier generation valves		**0.023**		**0.027**		**0.015**
Newer generation	43.6%		7.4%		12.8%	
Earlier generation	56.1%		15.2%		23.0%	
Balloon- vs self-expandable valves		0.323		0.616		0.676
Balloon-expandable	42.4%		9.1%		21.2%	
Self-expandable	51.5%		12.1%		18.2%	
Pre-dilation		0.320		0.545		0.245
No pre-dilation	52.2%		12.4%		20.0%	
Pre-dilation	46.1%		10.0%		14.4%	
Valve-in-valve		**0.001**		0.253		0.209
Native valve	53.0%		12.3%		19.2%	
Valve-in-valve	17.4%		4.3%		8.7%	
Newer generation valves		0.656		0.302		**0.032**
SAPIEN 3	42.4%		9.1%		21.2%	
CoreValve Evolut Pro	47.2%		9.7%		15.3%	
Acurate Neo	38.6%		2.3%		2.3%	

Table 5 summarizes the association of procedure characteristics with new rhythm disturbances, advanced atrioventricular block (AVB), and permanent pacemaker (PPM) implantation. Data were presented in percentage and significative p-values in bold. New rhythm disturbances included any de novo *atrioventricular or intraventricular conduction disturbance that appeared after transcatheter aortic valve implantion.*

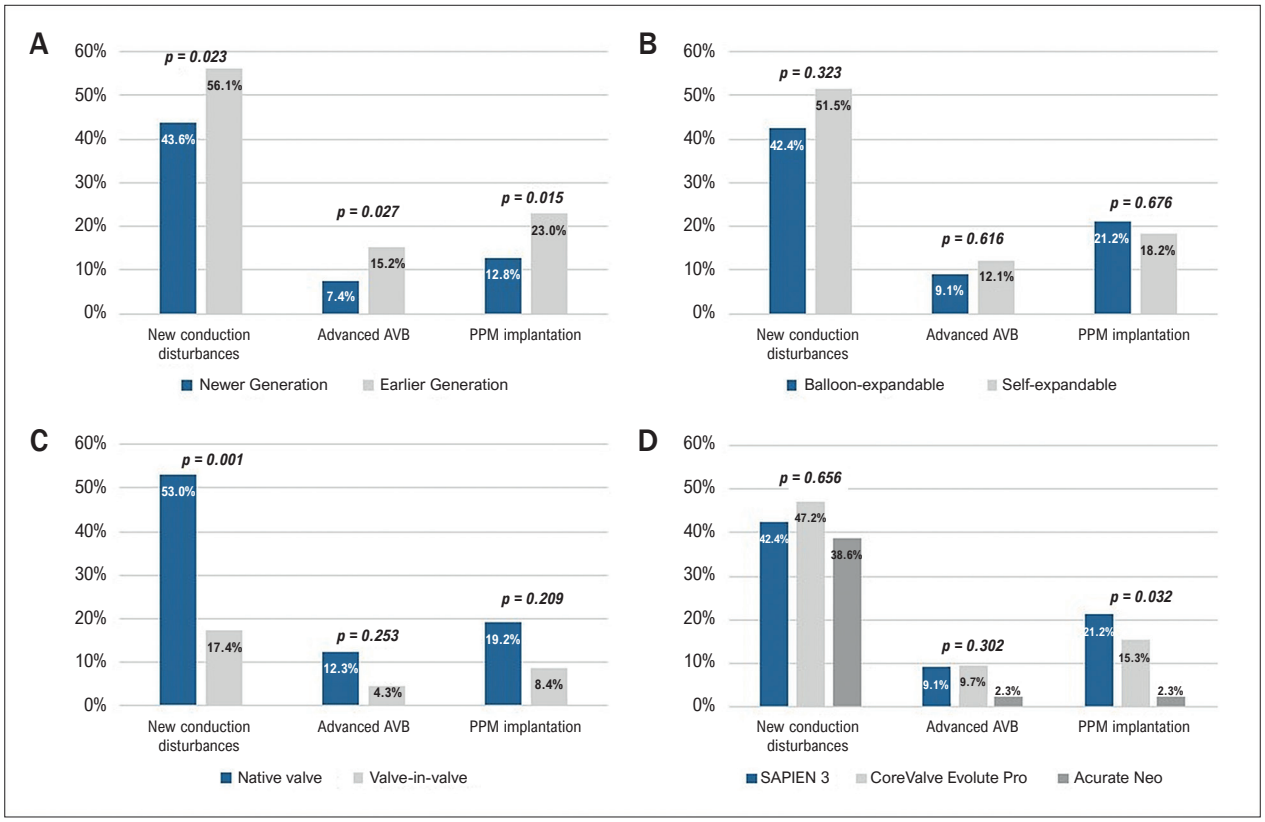

Figure 1 – *TAVR procedure and rhythm disturbances. Figure 1 displayed the association of procedure characteristics with new rhythm disturbances, advanced AVB, and PPM implantation concerning valve generation (A), balloon- or self-expandable valve (B), valve-in-valve implantation (C), or newer generation valve model (D). AVB: atrioventricular block; PPM: permanent pacemaker.*

median VP of 2%, half of patients with VP under one percent. The difference in VP between patients with advanced AVB and patients with LBBB plus low-grade AVB is statistically significant in the first evaluation (p = 0.036). After one year of PPM implantation, patients with LBBB plus low-grade AVB tended to have lower VP (p = 0.062) and lesser patients with VP >40% (33.3% vs 73.1%, p = 0.065).

In patients with isolated LBBB or ABBB, median VP was 9% and 13% at the first evaluation, and 20% and 15% after one year, respectively.

The forest plots in Figures 2 and 3 summarize the main characteristics associated with new-onset advanced AVB and PPM implantation in our sample.

Discussion

Conduction disturbances after TAVR continue to be challenging, and an effort should be made to recognize patients at risk for high-degree conduction defects and PPM implantation.

In the present study study, among 340 patients without previous PPM, half exhibited new conduction disturbances after TAVR, and 18.5% of patients had a PPM implanted. In accordance with literature, de novo LBBB was the most frequent conduction disturbance observed post-procedure,[1] occurring in one-third of the patients.

Several studies have identified pre-existing conduction disturbances (namely first-degree AV block, RBBB, LBBB, and fascicular block) as risk factors for PPM implantation after TAVR.[1,2,5,11,12] The role of first-degree AV block as a risk factor for conduction disturbance has proven to be controversial in recent studies.[1,5,11-13] In our sample, a significant relation between previous low-grade AVB and PPM implantation (OR of 2.25) was found, but not with advanced AVB. This can most likely be explained by the fact that one of the main reasons to implant a PPM in our center was low-grade AVB plus LBBB (22.2% of PPM implantations).

RBBB was the only disturbance in pre-TAVR ECG that was associated with a significant increase in the risk of both advanced AVB and PPM implantation, with an approximately eightfold increased risk of advanced AVB and five times more risk of PPM implantation. This is in agreement with several other reports that identified RBBB as the most important risk factor for advanced AVB / complete heart block and need for PPM following TAVR.[1,7,12-14] In fact, Watanabe et al. demonstrated that patients with pre-existing RBBB, without PPM, had a higher risk for cardiac death after discharge, hypothesizing this could be due to the development of high-grade AVB.[15]

LBBB and left anterior fascicular block are other controversial risk factors for PPM implantation.[12,16] Our findings were not consistent with that hypothesis, showing no relation with more advanced AVB nor PPM implantation.

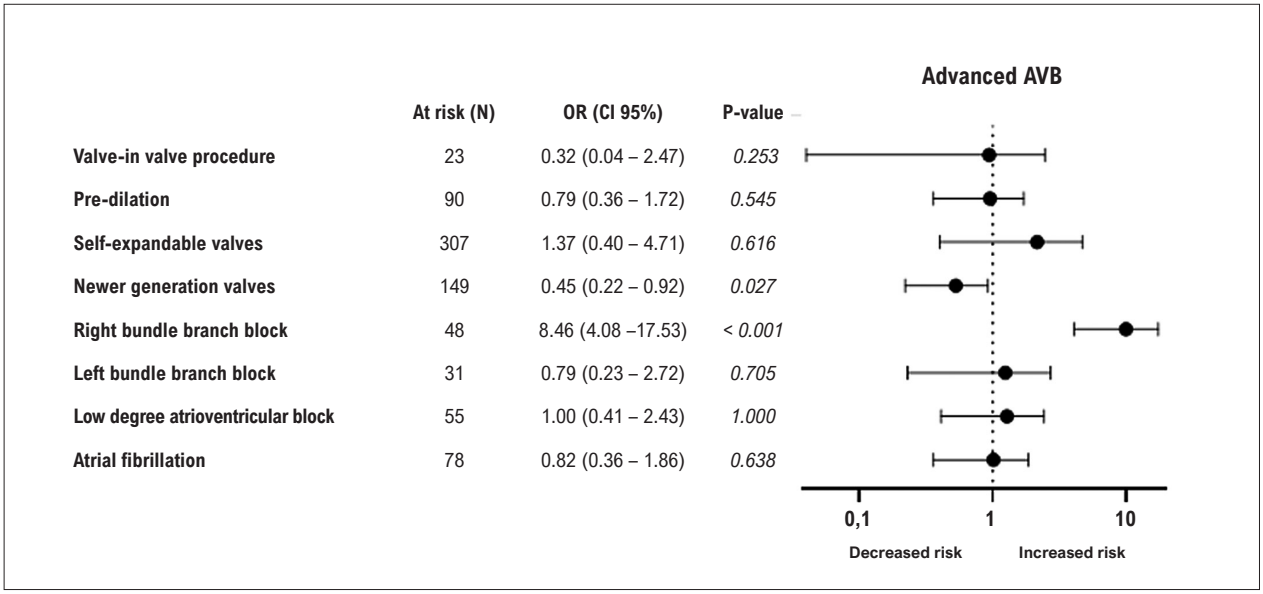

Figure 2 – *Predictors of advanced AVB. Figure 2 showed a forest plot that compiled the main possible predictors of advanced AVB. Chi-square test was used to analyze the difference between groups. AVB: atrioventricular block.*

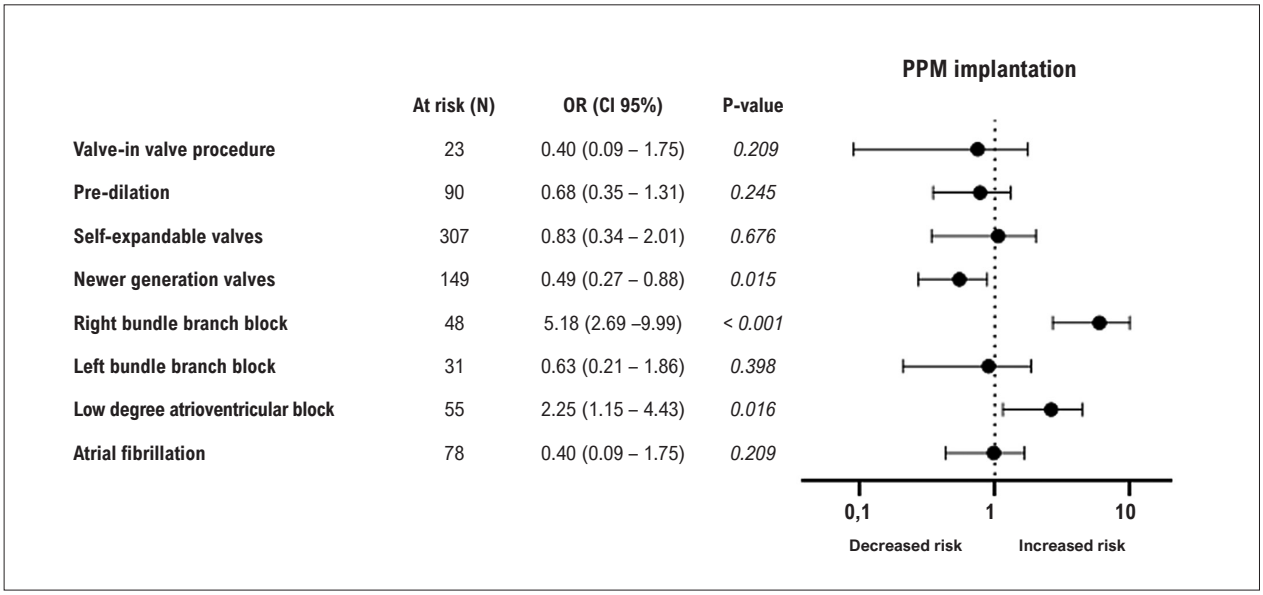

Figure 3 – *Predictors of PPM implantation. Figure 3 displayed a forest plot that summarized the main possible predictors of PPM implantation. Chi-square test was used to analyze the difference between groups. PPM: permanent pacemaker.*

Procedure characteristics are also implicated in the occurrence of peri-TAVR conduction complications. Several earlier reports suggested higher rates of rhythm disorders with native valve pre-dilation and self-expandable valves,[1,17-19] although this was not observed in our sample, as has been suggested by more contemporary data.[20,21] Valve-in-valve procedures were associated with less de novo conduction disturbances (OR = 0.19), and this difference was not explained by statistically significant differences in pre-TAVR AV or IV conduction, which runs in line with previously published data.[22]

As proposed in a systematic review,[23] newer generation valves were associated with a significantly lower incidence of new conduction disturbances, advanced AVB, and PPM implantation. An additional analysis was conducted, including only newer generation valves, finding a statistically significant difference in PPM implantation in favor of Acurate Neo, possibly explained by a lower radial force causing less mechanical injury.[24] Regarding new-onset conduction disturbances, only three cases of advanced AVB (7%) reverted during hospitalization, all during the first 24

hours; these were discharged and presented no advanced AVB during follow-up. Regarding LBBB, in accordance with published data,[2,6,13,25] a higher percentage of cases were reverted, with more than a quarter being resolved before hospital discharge and more than half after 6 months of follow-up.

De novo LBBB remains the most challenging rhythm disorder to handle post-TAVR. According to previous reports, some patients with new-onset LBBB will develop advanced AVB,[2,7,26] but a significant proportion will partially or completely normalize their ECG.[1,5,6,8] Although current data do not support systematic implantation of PPM in these patients, some studies have suggested a higher risk of delayed advanced AVB during follow-up in patients with long QRS (over 150 - 160 ms), particularly when associated with a long PR interval (more than 240 ms). According to the recent JACC Scientific Expert Panel's consensus, it may be reasonable to implant PPM in patients with LBBB and a PR interval over 240 ms or LBBB with QRS duration more than 150 - 160 ms.[1] The 2020 ACC expert consensus also considers the possibility of electrophysiological study and recommends ambulatory rhythm monitoring for at least 14 days after hospital discharge with a monitor capable of communicating episodes of advanced AVB, allowing prompt activation of emergency medical services.[5]

This study conducted an independent analysis in patients with de novo PPM, showing that patients who had a PPM due to advanced AVB had a higher percentage of VP than patients receiving a PPM for other indications, with 44.4% and 46.2% presenting more than 90% of VP uopn first PPM evaluation and one year after implantation, respectively; these results are consistent with a recently published study from Italy.[27] In the subgroup of patients implanting PPM due to LBBB plus low-grade AVB, the median VP was very low (2% at one year), with half having less than 1% of VP and only 33.3% more than 40%. Despite this lower percentage of VP, one cannot exclude pacing use during brief paroxysmal episodes of extreme bradycardia or advanced AVB. These results enhance the knowledge regarding PPM long-term dependency in post-TAVR patients, highlighting a more accurate selection of LBBB patients that benefit from PPM implantation and strengthening the importance of ambulatory rhythm monitoring in new-LBBB patients to promptly recognize advanced AVB events. On the other hand, high VP observed in patients with advanced AVB reinforces the rationale of implanting more physiological modes of pacing like His bundle pacing or biventricular pacing in these patients.

Limitations

The present study was a single-center retrospective observational study, which was its major limitation. Although ECGs were all assessed by cardiologists, there was no Core Lab responsible for ECG revision. PR and QRS interval durations were not recorded.

Conclusions

This study showed that LBBB was the most frequent de novo conduction disturbance after TAVR, with more than half of the cases being resolved in the first 6 months. Previous RBBB and low-grade AVB were significantly associated with a higher rate of PPM implantation post-TAVR, with a fivefold increase of risk in patients with RBBB. Unlike native valve pre-dilation and self-expandable valves, valve-in-valve procedures were related to significantly less conduction disturbances, and the Acurate Neo valve was associated with less PPM implantation. Regarding PPM follow-up, patients who had a PPM due to advanced AVB presented a significantly higher percentage of VP than did patients receiving it for other reasons, such as LBBB plus low-grade AVB. Altogether, this report highlights the importance of further evidence to more accurately select patients with LBBB that benefit from PPM implantation and those who do not, strengthening the ambulatory close monitoring strategy to promptly recognize advanced AVB events in these patients. Furthermore, results in advanced AVB patients reinforce the rationale of implanting more physiological modes of pacing in this group.

Author contributions

Conception and design of the research: Pinto RA, Proença T, Carvalho MM, Pestana G, Lebreiro A, Adão L, Macedo F; Acquisition of data: Pinto RA, Proença T, Carvalho MM; Analysis and interpretation of the data: Pinto RA, Proença T; Statistical analysis: Pinto RA; Writing of the manuscript: Pinto RA, Proença T, Pestana G; Critical revision of the manuscript for intellectual content: Carvalho MM, Pestana G, Lebreiro A, Adão L, Macedo F.

Potential Conflict of Interest

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

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Study Association

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Ethics approval and consent to participate

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

References

1. Rodes-Cabau J, Ellenbogen KA, Krahn AD,Latib A, Mack M, Mittal S, et al. Management of Conduction Disturbances Associated With Transcatheter Aortic Valve Replacement: JACC Scientific Expert Panel. J Am Coll Cardiol. 2019;74(8):1086-106. doi: 10.1016/j.jacc.2019.07.014.

2. Auffret V, Puri R, Urena M, Chamandi C, Rodrigues-Gabella T, Phillipon F, et al. Conduction Disturbances After Transcatheter Aortic Valve Replacement: Current Status and Future Perspectives. Circulation.2017;136(11):1049-69. doi: 10.1161/CIRCULATIONAHA.117.028352

3. Siontis GCM, Overtchouk P, Cahill TJ, Modine T, Prendergast B, Praz F et al. Transcatheter aortic valve implantation vs. surgical aortic valve replacement for treatment of symptomatic severe aortic stenosis: an updated meta-analysis. Eur Heart J. 2019;40(38):3143-53. doi: 10.1093/eurheartj/ehz275

4. Bagur R, Rodes-Cabau J, Gurvitch R, Dumont É, Velianou JL, Manazzoni J, et al. Need for permanent pacemaker as a complication of transcatheter aortic valve implantation and surgical aortic valve replacement in elderly patients with severe aortic stenosis and similar baseline electrocardiographic findings. JACC Cardiovasc Interv. 2012;5(5):540-51. doi: 10.1016/j.jcin.2012.03.004

5. Lilly SM, Deshmukh AJ, Epstein AE, Ricciardi MJ, Sheenivas S, Vilagapudi P,et al. 2020 ACC Expert Consensus Decision Pathway on Management of Conduction Disturbances in Patients Undergoing Transcatheter Aortic Valve Replacement.J Am Coll Cardiol.2020;76(20):2391-411. doi: 10.1016/j.jcin.2012.03.004

6. Rodes-Cabau J, Urena M, Nombela-Franco L,Amat-Santos I, Kleiman N, Munozz-Garcia M, Atienza F, et al. Arrhythmic Burden as Determined by Ambulatory Continuous Cardiac Monitoring in Patients With New-Onset Persistent Left Bundle Branch Block Following Transcatheter Aortic Valve Replacement: The MARE Study. JACC Cardiovasc Interv. 2018;11(15):1495-505. doi: 10.1016/j.jcin.2018.04.016

7. Toggweiler S, Stortecky S, Holy E, Zuk K, Cuculi F, Nietlispach F, et al. The Electrocardiogram After Transcatheter Aortic Valve Replacement Determines the Risk for Post-Procedural High-Degree AV Block and the Need for Telemetry Monitoring. JACC Cardiovasc Interv. 2016;9(12):1269-76. doi: 10.1016/j.jcin.2016.03.024

8. Jorgensen TH, De Backer O, Gerds TA, Bieliauskas G, Svendsen JH, Sondergaard L. Immediate Post-Procedural 12-Lead Electrocardiography as Predictor of Late Conduction Defects After Transcatheter Aortic Valve Replacement. JACC Cardiovasc Interv. 2018;11(15):1509-18. doi: 10.1016/j.jcin.2018.04.011.

9. Kappetein AP, Head SJ, Genereux P, Piazza N, van Mieghem NM.Updated standardized endpoint definitions for transcatheter aortic valve implantation: the Valve Academic Research Consortium-2 consensus document. Eur Heart J. 2012;33(19):2403-18. doi: 10.1093/eurheartj/ehs255.

10. Kusumoto FM, Schoenfeld MH, Barrett C, Edgerton JR, Ellenbogen KA, Gold MR, et al. 2018 ACC/AHA/HRS Guideline on the Evaluation and Management of Patients With Bradycardia and Cardiac Conduction Delay: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society. Circulation. 2019;140(8):e382-e482. doi: 10.1161/CIR.0000000000000628

11. Mangieri A, Lanzillo G, Bertoldi L, Jabbour RJ, Regazzoli D, Ancona MB, et al. Predictors of Advanced Conduction Disturbances Requiring a Late (>/=48 H) Permanent Pacemaker Following Transcatheter Aortic Valve Replacement. JACC Cardiovasc Interv. 2018;11(15):1519-26. doi: 10.1016/j.jcin.2018.06.014.

12. Siontis GC, Juni P, Pilgrim T, Stortecky S, Büllesfeld L, Meier B,et al. Predictors of permanent pacemaker implantation in patients with severe aortic stenosis undergoing TAVR: a meta-analysis. J Am Coll Cardiol.2014;64(2):129-40. doi: 10.1016/j.jacc.2014.04.033.

13. Nazif TM, Dizon JM, Hahn RT, Xu K, Babaliaros V, Douglas PS, et al. Predictors and clinical outcomes of permanent pacemaker implantation after transcatheter aortic valve replacement: the PARTNER (Placement of AoRtic TraNscathetER Valves) trial and registry. JACC Cardiovasc Interv. 2015;8(1 Pt A):60-9. doi: 10.1016/j.jcin.2014.07.022.

14. Mack MJ, Leon MB, Thourani VH, Makkar R, Kodali SK, Russo M, et al. Transcatheter Aortic-Valve Replacement with a Balloon-Expandable Valve in Low-Risk Patients. N Engl J Med. 2019;18(12):1695-705. doi: 10.1056/NEJMoa1814052.

15. Watanabe Y, Kozuma K, Hioki H, Kawashima H, Nara Y, Kataoka A, et al. Pre-Existing Right Bundle Branch Block Increases Risk for Death After Transcatheter Aortic Valve Replacement With a Balloon-Expandable Valve. JACC Cardiovasc Interv. 2016;9(21):2210-6. doi: 10.1016/j.jcin.2016.08.035.

16. Fischer Q, Himbert D, Webb JG, Eltchaninoff H, Muñoz-García AJ, Tamburino C,et al. Impact of Preexisting Left Bundle Branch Block in Transcatheter Aortic Valve Replacement Recipients. Circ Cardiovasc Interv. 2018 Nov;11(11):e006927. doi: 10.1161/CIRCINTERVENTIONS.118.006927

17. Campelo-Parada F, Nombela-Franco L, Urena M, Regueiro A, Jiménez-Quevedo P, Del Trigo M, et al. Timing of Onset and Outcome of New Conduction Abnormalities Following Transcatheter Aortic Valve Implantation: Role of Balloon Aortic Valvuloplasty. Rev Esp Cardiol. (Engl Ed) 2018; 71(3):162-9. doi: 10.1016/j.rec.2017.04.010.

18. Gensas CS, Caixeta A, Siqueira D, Sarmento -Leite G, Mangione J, et al. Predictors of permanent pacemaker requirement after transcatheter aortic valve implantation: insights from a Brazilian registry. Int J Cardiol .2014;175(2):248-52. doi: 10.1016/j.ijcard.2014.05.020.

19. Banerjee K, Kandregula K, Sankaramangalam K, Anumandla A, Kumar A, Parikh P, et al. Meta-analysis of the Impact of Avoiding Balloon Predilation in Transcatheter Aortic Valve Implantation. Am J Cardiol. 2018; 122(3):477-82. doi: 10.1016/j.amjcard.2018.04.025:

20. Thiele H, Kurz T, Feistritzer HJ, Stachel G, Hartung P, Eitel I,et al. Comparison of newer generation self-expandable vs. balloon-expandable valves in transcatheter aortic valve implantation: the randomized SOLVE-TAVI trial. Eur Heart J. 2020;41(20):1890-9. doi: 10.1093/eurheartj/ehaa036.

21. Pagnesi M, Kim WK, Conradi L. Impact of Predilatation Prior to Transcatheter Aortic Valve Implantation With the Self-Expanding Acurate neo Device (from the Multicenter NEOPRO Registry).Am J Cardiol.2020;125(9):1369-77. doi: 10.1016/j.amjcard.2020.02.003

22. Ferrari E, Stortecky S, Heg D, Muller O, Nietlispach F, Tueller D, et al. The hospital results and 1-year outcomes of transcatheter aortic valve-in-valve procedures and transcatheter aortic valve implantations .in the native valves: the results from the Swiss-TAVI Registry. Eur J Cardiothorac Surg. 2019; 56(1):55-63. doi: 10.1093/ejcts/ezy471

23. van Rosendael PJ, Delgado V, Bax JJ. Pacemaker implantation rate after transcatheter aortic valve implantation with early and new-generation devices: a systematic review. Eur Heart J. 2018;39(21):2003-13. doi: 10.1093/eurheartj/ehx785.

24. Okuno T, Lanz J, Pilgrim T. ACURATE neo: How Is This TAVR Valve Doing to Fit into an Increasingly Crowded Field? Curr Cardiol Rep .2020; 22(10):107. doi: 10.1007/s11886-020-01364-4.

25. Houthuizen P, van der Boon RM, Urena M, Van Mieghem N, Brueren GB, Poels TT, et al. Occurrence, fate and consequences of ventricular conduction abnormalities after transcatheter aortic valve implantation. EuroIntervention. 2014; 9(10):1142-50. doi: 10.4244/EIJV9I10A194

26. Akin I, Kische S, Paranskaya L, Schneider H, Rehders TC, Trautwauin U, et al. Predictive factors for pacemaker requirement after transcatheter aortic valve implantation. BMC Cardiovasc Disord. 2012;12:87. Doi:10.1186/1471-2261-1287

27. Baldi E, Compagnone M, Errigo D, Ferlini M, Ziachi M, Castagno D, et al. Long-term percentage of ventricular pacing in patients requiring pacemaker implantation after transcatheter aortic valve replacement: A multicenter 10-year experience. Heart Rhythm.2020;17(11):1897-903.doi:10.1016/j.hrthm.2020.05.040

Conduction Disturbances Associated with Transcatheter Aortic Valve Implantation: Challenge for another 20 Years?

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Short Editorial related to the article: Long-Term Ventricular Pacing Dependency and Pacemaker Implantation Predictors after Transcatheter Aortic Valve Replacement – A 1-Year Follow-Up

Transcatheter aortic valve implantation (TAVI) is a well-established procedure for treating severe aortic stenosis in elderly patients, regardless of the surgical risk.[1] Since its introduction two decades ago, there have been major technological advances in devices, which, combined with new implantation techniques, have brought significant reductions in periprocedural complication rates, leading to their greater adoption worldwide. However, the incidence of conduction disorders showed a modest reduction, remaining the most frequent complication after TAVI,[2-4] which contributes to the increase in hospital stay, costs and the worsening of short and long-term clinical outcomes.[4,5] In addition, the approach to conduction disorders still varies greatly between centers, especially regarding the management of new left bundle branch block (LBBB), post-procedure advanced atrioventricular block (AVB) and previous right bundle branch block (RBBB), translated into variable rates of permanent pacemaker (PM) implantation.[3] Among patients who received PM after TAVI, there is also great variability regarding their dependence (ventricular pacing) at follow-up.

In this journal edition, Pinto et al.[6] evaluated the incidence of conduction disorders, predictors and the rate of PM dependence in a population of 340 consecutive patients undergoing TAVI.[6] Conduction disorders occurred in more than 50% of post-procedure patients, with LBBB being the most frequent (32%), showing spontaneous resolution in 56% of them after 6 months. The overall PM implant rate was 18.5%, with prior RBBB being its main predictor. Among the patients who required PM, the main reasons were advanced AVB (60.3%), followed by LBBB with low-degree AVB (22%). Interestingly, there was a wide variation in the percentage of ventricular pacing among patients who received PM, being 83% in patients with advanced AVB (Advanced AVB and Mobitz Type II) and only 2% in those implanted with LBBB and low-degree AVB (first-degree AVB and Mobitz type I).[2-4,7]However, some aspects of this study deserve reflection.

Keywords

TAVI; Pacemaker; Conduction Disturbances.

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First, pre-procedure assessment is essential to identify risk factors for conduction disorders and assist the operator's strategy. As demonstrated by Pinto et al.[6] the presence of previous RBBB (~10% of patients)[8] is the main risk factor for PM implantation after TAVI, increasing its incidence by 3 to 4 times,[8,9] it is also a predictor of post-procedure mortality.[9] In the present study, previous LBBB and first-degree AVB were not correlated with a greater need for PM, unlike other authors who, in a larger number of patients, have shown that LBBB can have an impact on the need for PM in the first 30 days, but not in the follow-up after 30 days, despite no impact on mortality.[8,10]

A second important aspect is a procedure in which some modifiable aspects can also influence the rates of conduction disorders. For example, half of the conduction disturbances occur before valve implantation, mainly during predilation, suggesting some correlation with balloon valvuloplasty,[11] as also evidenced in the present study. In addition, new generation valves and techniques for higher implantantion in the annulus have significantly reduced PM rates to <10%.[7,8,12] In fact, Pinto et al.[6] showed a reduction of almost 50% with new generation prostheses and in the presence of dysfunctional surgical bioprosthesis (valve-in-valve).[13]

At the end of the procedure, a 12-lead electrocardiogram (ECG) should be performed to determine the management of any conduction disturbances and continuous monitoring for 12-24 hours.[2] As seen by Pinto et al.[6] the main conduction disorder is LBBB, with 10-15% progressing to PM in the first year,[9] reinforcing the importance of outpatient monitoring of these cases. Therefore, serial ECG evaluation is recommended, and in cases of increased PR or QRS intervals > 20 ms, especially in the presence of PR>240ms and QRS>150ms, prophylactic PM implantation may be indicated by the risk of sudden death and advanced AVB.[2] In the study by Pinto et al.[6] 22% of the PM indications were for LBBB combined with first-degree AVB. However, this group had only 2% of ventricular pacing at one year, demonstrating the difficulty in managing this type of patient as well as the real need for a PM in certain circumstances since, despite low stimulation, it has occurred during paroxysmal episodes of advanced AVB or extreme bradycardia that are life-threatening.

Despite two decades of technological advancement and improvement in TAVI results, conduction disturbances remain the most frequent complication. Several studies in recent years have contributed to identify risk factors, allowing a reduction in PM rates and has helped in the management of these patients. Although the study by Pinto et al.[6] presents some limitations (retrospective, observational and unicentric), it reinforces previous

RBBB as the main risk factor for the need of PM, and it brings a reflection on its indication for patients with LBBB and first-degree AVB, where prospective studies such as PROMOTE (clinicaltrials.org.*NCT: 04139616)* will evaluate specific algorithms for the management of conduction disorders after TAVI. In such patients, perhaps the electrophysiological assessment of the conduction system, even in the periprocedural period, may help in the management and the more precise indication of PM.[14]

References

1. Tarasoutchi F, Montera MW, Ramos AIO, Sampaio RO, Rosa VEE, Accorsi TAD, et al. Update of the Brazilian Guidelines for Valvular Heart Disease - 2020. Arq Bras Cardiol. 2020;115(4):720-75. doi: 10.36660/abc.20201047.
2. Rodés-Cabau J, Ellenbogen KA, Krahn AD, Latib A, Mack M, Mittal S, et al. Management of Conduction Disturbances Associated With Transcatheter Aortic Valve Replacement: JACC Scientific Expert Panel. J Am Coll Cardiol. 2019;74(8):1086-106. doi: 10.1016/j.jacc.2019.07.014.
3. van Rosendael PJ, Delgado V, Bax JJ. Pacemaker Implantation Rate After Transcatheter Aortic Valve Implantation with Early and New-generation Devices: A Systematic Review. Eur Heart J. 2018;39(21):2003-13. doi: 10.1093/eurheartj/ehx785.
4. Auffret V, Lefevre T, Van Belle E, Eltchaninoff H, Iung B, Koning R, et al. Temporal Trends in Transcatheter Aortic Valve Replacement in France: FRANCE 2 to FRANCE TAVI. J Am Coll Cardiol. 2017;70(1):42-55. doi: 10.1016/j.jacc.2017.04.053.
5. Faroux L, Chen S, Muntané-Carol G, Regueiro A, Philippon F, Sondergaard L, et al. Clinical Impact of Conduction Disturbances in Transcatheter Aortic Valve Replacement Recipients: A Systematic Review and Meta-analysis. Eur Heart J. 2020;41(29):2771-81. doi: 10.1093/eurheartj/ehz924.
6. Pinto RA, Proença T, Carvalho MM, Pestana G, Lebreiro A, Adão L, et al. Long-Term Ventricular Pacing Dependency and Pacemaker Implantation Predictors after Transcatheter Aortic Valve Replacement - A 1-Year Follow-Up. Arq Bras Cardiol. 2022; 119(4):522-530.
7. Thiele H, Kurz T, Feistritzer HJ, Stachel G, Hartung P, Eitel I, et al. Comparison of Newer Generation Self-expandable vs. Balloon-expandable Valves in Transcatheter Aortic Valve Implantation: The Randomized SOLVE-TAVI Trial. Eur Heart J. 2020;41(20):1890-9. doi: 10.1093/eurheartj/ehaa036.
8. Mangieri A, Lanzillo G, Bertoldi L, Jabbour RJ, Regazzoli D, Ancona MB, et al. Predictors of Advanced Conduction Disturbances Requiring a Late (≥48 H) Permanent Pacemaker Following Transcatheter Aortic Valve Replacement. JACC Cardiovasc Interv. 2018;11(15):1519-26. doi: 10.1016/j.jcin.2018.06.014.
9. Auffret V, Webb JG, Eltchaninoff H, Muñoz-García AJ, Himbert D, Tamburino C, et al. Clinical Impact of Baseline Right Bundle Branch Block in Patients Undergoing Transcatheter Aortic Valve Replacement. JACC Cardiovasc Interv. 2017;10(15):1564-74. doi: 10.1016/j.jcin.2017.05.030.
10. Fischer Q, Himbert D, Webb JG, Eltchaninoff H, Muñoz-García AJ, Tamburino C, et al. Impact of Preexisting Left Bundle Branch Block in Transcatheter Aortic Valve Replacement Recipients. Circ Cardiovasc Interv. 2018;11(11):e006927. doi: 10.1161/CIRCINTERVENTIONS.118.006927.
11. Campelo-Parada F, Nombela-Franco L, Urena M, Regueiro A, Jiménez-Quevedo P, Del Trigo M, et al. Timing of Onset and Outcome of New Conduction Abnormalities Following Transcatheter Aortic Valve Implantation: Role of Balloon Aortic Valvuloplasty. Rev Esp Cardiol (Engl Ed). 2018;71(3):162-9. doi: 10.1016/j.rec.2017.04.010.
12. Mendiz OA, Noč M, Fava CM, Jaikel LAG, Sztejfman M, Pleskovič A, et al. Impact of Cusp-Overlap View for TAVR with Self-Expandable Valves on 30-Day Conduction Disturbances. J Interv Cardiol. 2021;2021:9991528. doi: 10.1155/2021/9991528.
13. Paradis JM, Del Trigo M, Puri R, Rodés-Cabau J. Transcatheter Valve-in-Valve and Valve-in-Ring for Treating Aortic and Mitral Surgical Prosthetic Dysfunction. J Am Coll Cardiol. 2015;66(18):2019-37. doi: 10.1016/j.jacc.2015.09.015.
14. Badertscher P, Knecht S, Spies F, Auberson C, Salis M, Jeger RV, et al. Value of Periprocedural Electrophysiology Testing During Transcatheter Aortic Valve Replacement for Risk Stratification of Patients With New-Onset Left Bundle-Branch Block. J Am Heart Assoc. 2022;11(15):e026239. doi: 10.1161/JAHA.122.026239.

Profile of IL-6 and TNF in Foam Cell Formation: An Improved Method Using Fluorescein Isothiocyanate (FITC) Probe

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Abstract

Background: The formation of foam cells occurs due to the increase in low-density plasma lipoprotein (LDL) and dysregulation of inflammation, which is important for the development of atherosclerosis.

Objective: To evaluate the profile of tumor necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6) in the existing foam cell formation method, optimizing this protocol.

Methods: The LDL was isolated, oxidized, and labeled with a Fluorescein isothiocyanate (FITC) probe. Foam cells were generated from THP-1 human monocyte-derived cells and incubated in the absence (control) or presence of FITC-ox-LDL (10, 50, 100, 150, or 200 μg/mL), for 12, 24, 48, or 72 hours. The accumulated FITC-ox-LDL in the cell was quantified by microscopy. The enzyme-linked immunosorbent assay was evaluated to quantify the IL-6 and TNF-α, with $p < 0.05$ considered significant.

Results: All the FITC-ox-LDL concentrations tested showed a higher fluorescence when compared to the control, showing a greater accumulation of lipoprotein in cells. The higher the concentration of FITC-ox-LDL, the greater the production of TNF-α and IL-6. The production of IL-6 by foam cells was detected up to the value of 150 μg/mL of the maximum stimulus for LDL. Concentrations above 50 μg/mL LDL stimulated greater release of TNF-α compared to control.

Conclusions: Our model contributes to the understanding of the release of IL-6 and TNF-α in response to different concentrations of ox-LDL, using an optimized method for the formation of foam cells.

Keywords: Atherosclerosis; Inflammation; Foam Cells; Lipids; Plaque, Atherosclerotic; Isotiocianatos, Fluoresceina.

Introduction

Atherosclerosis is one of the most important causes of morbidity and mortality worldwide, and is detected by the accumulation of lipids in macrophages that in this stage are known as foam cells in the sub-endothelial space of the arterial wall.[1] Foam cell formation occurs by the increase of plasma low-density lipoprotein (LDL), which undergoes various physiological processes mediated by oxidation, acetylation, and denaturation. These modifications are physiological stimuli that favor the Internalization of lipid particles by macrophages generating the foam cell.[2] Alternative cell types present in the neointima, such as smooth muscle and endothelial cells, can also internalize lipid droplets and transdifferentiate to a state similar to foam cells from macrophages, contributing to the formation of atherosclerotic plaque.[3,4]

Macrophages can contribute to the development of atherosclerosis, displaying high heterogeneity[5] due to its resulting phenotype. This phenotype can be classified as M1 and M2. M1 macrophages are characterized as pro-inflammatory and have a high expression of pro-inflammatory proteins that contribute to the formation of atherosclerotic plaque. M2 macrophages play a preventive role by reducing the size and improving the stability of the plaque, as it has an anti-inflammatory profile.[5,6]

Stimulating the pro-inflammatory profile is important in the process of foam cell formation, given that inflammatory mechanisms can act both as precursors in the lipid-centric formation as well as promote atherogenesis via cholesterol absorption and a decrease in cholesterol efflux.[2] Although hyperlipidemia stimulates atherogenesis by providing more lipids for foam cell formation, some induced inflammatory mediators increase lipid oxidation, such as tumor necrosis factor alpha (TNF-α) and Interleukin-6 (IL-6).[7] IL-6 is a pleiotropic cytokine that exhibits pro and anti-inflammatory

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properties, depending on the type of target cell. An increase in IL-6 in atherosclerosis results in effects on multiple cells involved in lipid processing and plaque formation, such as the activation of endothelial cells, smooth muscle cell proliferation, and accumulation of macrophage lipids.[8] There is now strong evidence for the role of macrophage-derived TNF-α in the development of atherosclerosis and increased vascular inflammation.[9] Therefore, investigating the physiopathology of foam cell formation is useful in developing new therapeutic interventions for atherosclerosis.[10]

The most commonly used techniques for studying foam cell formation are ox-LDL-labeled quantification inside the macrophages or using non-specific stains such as oil. The present study aimed to evaluate the profile of TNF-α and IL-6 in the existing foam cell formation method, thereby optimizing this protocol. The presence of these inflammatory mediators act as markers of the formation of pro-inflammatory foam cells, the beginning of the formation of atherosclerotic plaque.

Materials and methods

Chemicals and reagents

This study used RPMI 1640, Fetal Bovine Serum (FBS) (Vitrocell Embriolife, Campinas, SP, BR), PMSF (phenyl-methyl-sulfonyl-fluoride), Phorbol 12-myristate 13-acetate (PMA), Fluorescein Isothiocyanate (FITC), 4′,6-Diamidine-2′-phenylindole dihydrochloride (DAPI), benzamidine, gentamicin chloramphenicol, aprotinin, Thiazolyl Blue Tetrazolium Bromide (MTT), which were purchased from Sigma-Aldrich, St. Louis, MO, USA, Amplex Red Cholesterol Assay Kit (Catalog no. A12216, Invitrogen, Molecular Probes, Eugene, OR); IL-6 and TNF-α R&D Systems, 614 McKinley Pl NE, Minneapolis, MN, USA.

LDL isolation

The present study was approved by the Human Research Ethics Committee at the Universidade Federal de São Carlos - UFSCar (#2.243.706) and the participants provided their written consent. Blood was collected from 10 normolipidemic volunteers (men and women, aged 18 to 45 years), and plasma was obtained after centrifugation at 1,000 g for 15 min in the presence of K_2EDTA 0.1mL for each 5 mL of blood. Next, benzamidine (2 mM), gentamicin (0.5%), chloramphenicol (0.25%), PMSF (phenyl-methyl-sulfonyl-fluoride) (0.5 mM), and aprotinin (5μl/mL) (all acquired from Sigma-Aldrich, St. Louis, MO, USA) were added to the plasma pool, as described in previous report.[11] The plasma density was raised to 1.021 g/mL by KBr (the plasma volume is multiplied by factor 0.3265, and the amount is then obtained in grams of solid KBr). After, 2.5 mL of plasma was added to the polypropylene tube (4 mL), and the tube was completed with a KBr solution of d = 1.006. The LDL was isolated by ultracentrifugation (337 g for 4h at 4°C) in a SW60TI fixed-angle rotor (Beckman Coulter, Beckman). The yellow-orange LDL fraction remained in the infranatant. The LDL fraction was collected by suction, using a 1 mL syringe. The collected LDL was dialyzed in the dark at 4ºC in 2 L of PBS, pH 7.4, with four PBS exchanges for 24 hours. After dialysis, LDL was filtered (0.22 μm) and stored at 4°C. The protein concentration was determined by using the Folin phenol reagent method.[12]

Oxidative modification of LDL

Oxidized LDL (ox-LDL) was obtained by incubating LDL with CuSO4 (5μmol/mL per mg of LDL protein/ 4 h/ 37°C). Oxidation was stopped by adding 20 μmol/mL EDTA. The degree of oxidation was determined by measuring the ferrous oxidation-xylenol orange.[13] After oxidation, the ox-LDL was dialyzed in the dark for 24 h at 4°C and washed 4 times with 2 L of PBS and EDTA (0.3 mM).

Fluorescent labeling of LDL

The oxidized LDL was labeled with Fluorescein isothiocyanate (FITC). All procedures were performed in the dark. LDL (1 mg/mL) and FITC (50 μg/mL) were mixed and incubated at 37°C for 3h. Unbound FITC was removed by dialysis against PBS for 48h at 4°C with eight changes of PBS and filtered through a 0.22 μm filter.[14] FITC-ox-LDL was then stored at 4°C and used for up to two months.

Cell culture

The THP-1 human monocyte-derived cell line was purchased from the *Rio de Janeiro Cell Bank,* Rio de Janeiro, *Brazil,* and was grown at 37°C in a 5% CO_2 atmosphere to a density of 10^6 cells/mL. The growth medium for the THP-1 cells was RPMI Medium 1640 supplemented with 10% Fetal Bovine Serum (FBS) (Gibco BRL), 50 mg/L Gentamicin Sulfate, and 2 mg/L Amphotericin B. The THP-1 cells were used in the experiments for induction in macrophages, using the 100 nM phorbol myristate acetate (PMA, Sigma)[15] and interferon (IFN)-γ (500 U/mL) to induce the M1 phenotype.[16] PMA induces THP-1 cell differentiation through direct interaction with PKCδ, which binds to Thrombomodulin and activates ERK1/2, which in turn increases cell the cycle inhibitor $p21^{Cip1/WAF1}$ expression via NF-kB p65 signaling. In addition, ERK1/2 participates in the phosphorylation of paxillin, cofilin, LIMK1, and PYK2, which mediate cytoskeletal remodeling to promote differentiation.[17] Interferon γ (IFN-γ), through the activator of transcription 1 (STAT1), favors the polarization of M1 macrophages, which produce pro-inflammatory mediators, including TNF-α, IL-6, and IL-1.[18] After this induction, the THP-1 macrophage cells were incubated without FITC-ox-LDL or with 10, 50, 100, 150, or 200 μg/mL, for different times (12, 24, 48 or 72 hours), depending on the experimental purpose.

Cellular uptake of cholesterol

To induce THP-1 monocyte differentiation in macrophages, THP-1 monocytes (10^4 cells/mL) in 96-well plates were treated with 100 nM PMA for 48 hours at 37°C. To identify the best ox-LDL concentration to induce foam cell formation, a concentration-response curve was performed: for 24 hours at 10 μg/mL, 50 μg/mL, 100 μg/mL, 150 μg/mL, and 200 μg/mL FITC-ox-LDL + IFN-γ (500 U/mL). For the temporal analysis,

differentiated cells were incubated in the absence or presence of FITC-ox-LDL (100 μg/mL) + interferon γ (500 U/mL) for 12h, 24h, 48h, and 72h. The cell nucleus was labeled with 1 μg/mL DAPI fluorescent probe (Sigma) for 10 minutes and washed 3 times with PBS. To analyze the fluorescence image, an automated fluorescence microscope system, ImageXpress Micro (Molecular Devices), with 495nm excitation, 525 nm FITC-ox-LDL emission, 340 nm excitation, and 488 nm emission for DAPI was used.

Cholesterol/cholesteryl ester quantitation in cell lysate

Foam cell cholesteryl ester content was quantified by the Amplex Red Cholesterol Assay Kit (Catalog no. A12216, Molecular Probes, Eugene, OR), according to the manufacturer's protocol. For this analysis, the THP-1 cells ($2x10^6$ cells/well) were cultured in 6-well plates; differentiated into macrophages, as described above; and incubated with or without ox-LDL. Foam cells were fixed in 2% paraformaldehyde for 15 min, washed once with PBS, and incubated with a 200 μl/well of absolute ethanol for 30 min at 4°C to extract cellular lipids. Cholesterol content was determined by incubating 50 μl of ethanol-extracted lipids diluted in 1x reaction buffer (0.1 M K_2HPO_4, pH 7.4, 0.05 M NaCl, 5 mM Cholic acid, 0.1% Triton X-100) or undiluted solution with 50 μl of assay solution (total cholesterol) or 50 μl of assay solution lacking cholesterol esterase (free cholesterol), for 30 min at 37°C in the dark and then by measuring the fluorescence (HTS-7000 microplate fluorometer; 530-nm excitation, 590-nm). The value was relativized for the total cellular protein levels. To quantify the total cellular protein levels, the lipid-extracted foam cells were incubated with 0.1% (weight/volume ratio [w/v]) SDS/0.2 M NaOH for 30 min at room temperature to extract the cellular protein. Total cellular protein levels were determined by using the Folin phenol reagent method.[12] Total cholesterol and cholesteryl ester levels were represented as nanograms of total cholesterol or cholesteryl ester per microgram of protein.

Cytokine measurements

Quantification of inflammatory cytokines in foam cell lysate was performed using the enzyme-linked immunosorbent assay (ELISA). IL-6 and TNF-α concentration in the supernatants of macrophages were measured using DuoSet kits (R&D Systems, 614 McKinley Pl NE, Minneapolis, MN, USA). Macrophages that incubated in absence of ox-LDL were defined as the control group (M).

Cell viability

Cell viability was determined by MTT (Thiazolyl Blue Tetrazolium Bromide) (Sigma- Aldrich, St. Louis, MO, USA) (Mosmann, 1983). THP-1 monocytes (10^4 cells/mL) were seeded in 96-well plates and treated with 100 nM PMA for macrophage differentiation, for 48 hours, maintained at 37°C in a humidified incubator containing 5% CO_2. After 48 hours, cells were exposed for 24 hours to 10 μg/mL, 50 μg/mL, 100 μg/mL, 150 μg/mL, and 200 μg/mL of FITC-ox-LDL + interferon γ (500 U/mL). The analysis of cell viability over time was also performed, where the cells were incubated in the absence (Control group - M) or presence of FITC-ox-LDL (FC) (100 μg/mL) + interferon γ (500 U/mL) for 12h, 24h, 48h, and 72h. Later, 5 mg/mL of MTT was added, followed by 4 hours of incubation at 5% CO_2, 37°C. After this time, 100 μL of dimethylsulfoxide (DMSO) was added, and the plate remained on the plate shaker for 10 minutes. The absorbance was measured at 540 nm, using a microplate reader SpectraMax GeminiXS (Molecular Devices, Sunnyvale, CA, USA).

Statistical analysis

The entire study was carried out at least in triplicate, in three independent experiments, according to recommendations for Good Cell Culture Practice (GCCP).[19,20] Data normality was verified by the Kolmogorov-Smirnov test; equality of variance (Levene's test). All values were presented as mean ± standard deviation (SD). To determine the difference between conditions, ANOVA was applied with the Bonferroni post hoc test for multiple comparisons. To determine the difference between two conditions, the unpaired Student's t-test was used (SigmaStat version 3.5; Systat). The significance level adopted in the statistical analysis was 5%.

Results

There was a greater accumulation of ox-LDL labeled with a FITC probe (indicated by the presence of green fluorescence), not only in the perinuclear area, but also distributed throughout the cytosol of most cells, as shown in Figure 1B. As expected, no accumulation of LDL was found in the untreated cells (Figure 1A), which exhibited only blue fluorescence, which labels the cell nucleus.

In Figure 2B, the microscopic fluorescence images show that FITC-ox-LDL was absorbed in all concentrations, showing higher fluorescence when compared to the control, using 24h of incubation. Above 50 μg/mL, the fluorescence was higher when compared to 10 μg/mL, but no difference was observed between them (Figure 2A).

The THP-1 macrophages were incubated with 100 μg / mL of FITC-ox-LDL for 0, 12, 24, 48, and 72 h (Figure 3). It was observed that within 12, 24, 48, and 72h the fluorescence intensity of the cells treated with FITC-ox-LDL increased significantly from the background level (Control group 0), but only after 72h was the fluorescence greater than other times (Figure 3A and 3B).

Figure 4 showed the relative cholesterol, other techniques to confirm the presence of cholesterol in cells, and the survival of the cells in this condition. Quantitatively, when the cell was incubated for 24h in different concentrations, only with 150 and 200 μg/mL of ox-LDL, a greater cholesterol concentration was found when compared to the control that was not incubated with ox-LDL (Figure 4A). This condition did not cause a major change in cell survival (Figure 4C). In Figure 4B, incubations of 100 μg/mL at different times of exposure of ox-LDL showed no difference between times, but all times showed a greater concentration of cholesterol the compared to time 0 (Figure 4B). Macrophages reduced

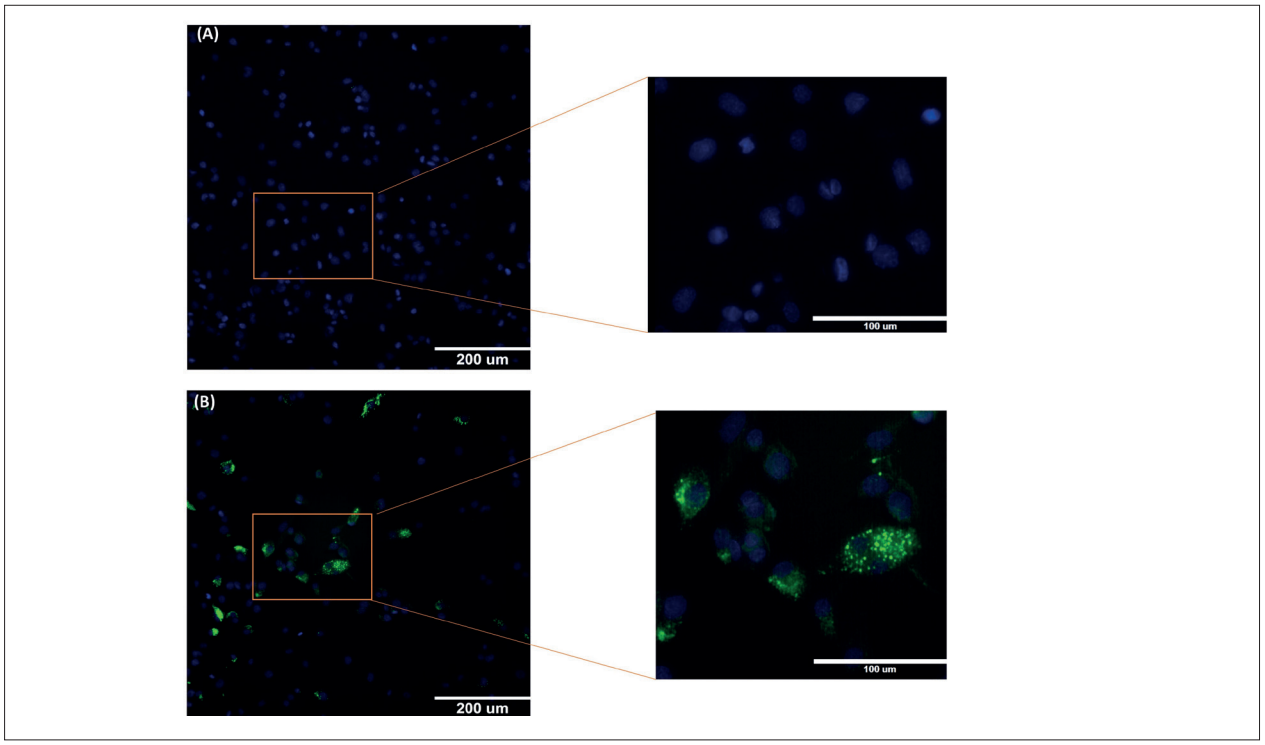

Figure 1 – *Representative images of FITC-ox-LDL uptake in THP-1 macrophages. THP-1 were incubated in the absence (Ctrl group - A or presence B) of indicated concentrations of FITC-ox-LDL for 24h. Cells were then washed, fixed, and examined, using a 546 nm filter set. FITC-ox-LDL uptake was shown in green and the cells´ nucleus was labeled using DAPI (blue).*

their viability over time (24, 48, and 72h) as compared to the time of 12h; however, foam cells showed a reduction in viability only at times of 24 and 48h. By contrast, in 72h, these cells had a greater viability when compared to macrophages in 72h, which was similar to that found for the group of foam cells at 12h (Figure 4D).

In the production of inflammatory cytokines, the time curve shows that both IL-6 and TNF-α were higher in foam cells when compared to macrophages at each time of exposure to ox-LDL (Figure 5A, 5B), but only IL-6 was higher in times 48h and 72h when compared to 12h and 24h (Figure 5A). Considering the ox-LDL concentration curve, IL-6 production was higher in all tested concentrations when compared to cells without exposure to ox-LDL (control group 0) (Figure 5C). In addition, when exposed to 50, 100, and 150 μg/mL, the production of IL-6 was greater when compared to the concentration of 10 μg/mL. The concentration of 200 μg/mL also decreased the release of IL-6, matching the values of concentration of 10 μg/mL. The release of TNF-α was only more expressive at concentrations of 50 to 200 μg / mL (Figure 5D).

Discussion

Our experiments document an optimization of the existing method of oxidized LDL-induced foam cell formation for *in vitro* foam cell formation, from THP-1 macrophages and incubation with FITC-ox-LDL, in addition to the verification of the response of such cytokines as IL6 and TNF-α. With 12h of incubation, foam cell formation takes place with the M1 pro-inflammatory phenotype, that is, with an increase in the concentrations of Il-6 and TNF-α. The characterization of the inflammatory profile of macrophages is important, considering that classically activated pro-inflammatory M1 macrophages stimulate atherogenesis, while M2 macrophages stabilize the atherosclerotic plaque.[6]

Other studies have used the foam cell formation technique, adopting such protocols as Oil Red O staining or labeled LDL with their own probes, together with oxidized LDL complexed with DiL dye (DiL-Ox-LDL). A study conducted by Xu et al.,[21] showed that incubation with DiL-ox-LDL (10 μg/mL) for 4h resulted in a significant increase in ox-LDL uptake in macrophages; however, they did not evaluate the inflammation of these macrophages.[21] Although the foam cell induction protocol using the DiL-ox-LDL probe is more efficient when compared to other techniques, it also has a low yield, that is, a large amount of material is needed to perform it, making it rather costly. In addition to this technique, this work points out that foam cell formation using Oil Red staining and LDL-ox incubation (50 μg/mL) for 24h is not an accurate protocol, since in this protocol neutral lipids (mainly triglycerides) are stained with an orange-red dye,[22] which may cause a low specificity in the technique, as in foam cells there is more cholesterol ester and no neutral lipids. Therefore, the present study sought to optimize the methods using oxidized LDL labeled with an FITC fluorescent probe (FITC-ox-LDL), introducing a simple and practical staining method for foam cell formation from

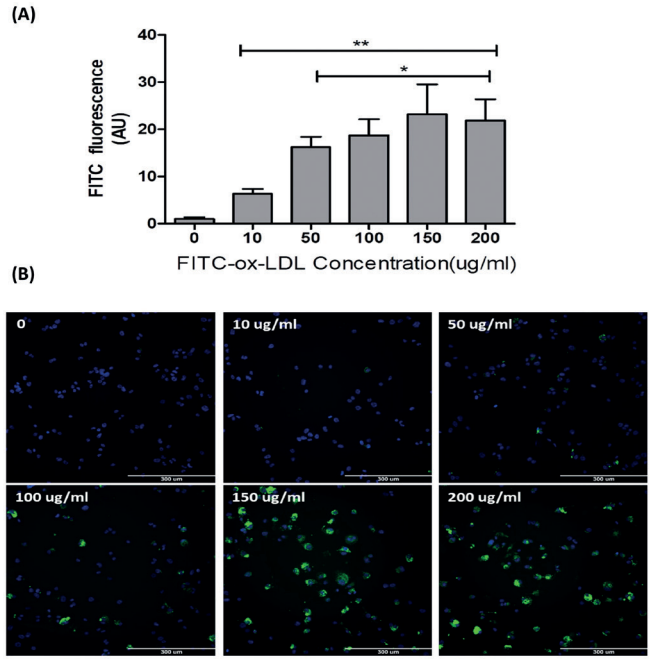

Figure 2 – *Measurement of FITC-ox-LDL concentration in THP-1 cells. A) Concentration-dependent fluorescent cholesterol uptake by THP-1 macrophages in arbitrary unit (AU). B) Representative images of FITC-ox-LDL uptake in THP-1 macrophages. THP-1 were incubated in the absence (Control group: 0) or presence of indicated concentrations of FITC-ox-LDL (10 – 200 µg/mL) for 24 h. FITC-ox-LDL uptake was shown in green, and cell nucleus was labeled using DAPI (blue). Cells were viewed under fluorescence microscope (20× objectives). Values are expressed as mean ± SD. * P < 0.05, compared to cells incubated with 10 µg/mL; ** P < 0.01, compared to cells incubated in absence of FITC-ox-LDL.*

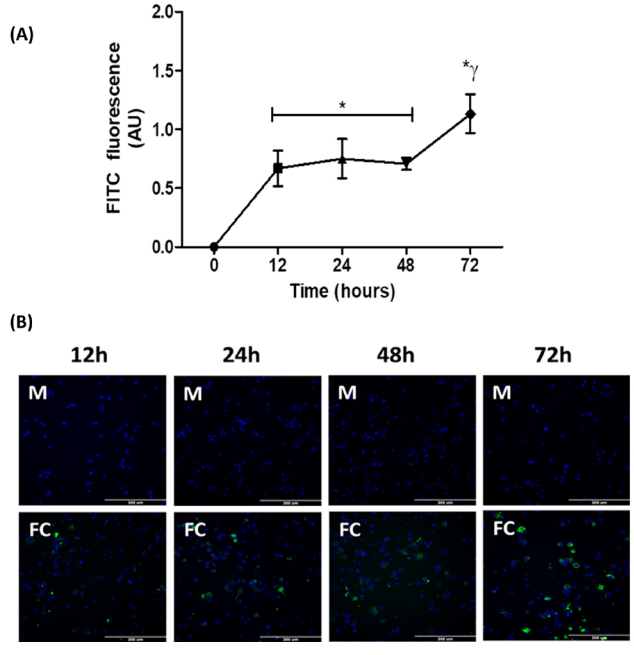

Figure 3 – *Measurement of time-dependent increased uptake of cholesterol by THP-1 macrophages. A) Time-dependent fluorescent cholesterol uptake by THP-1 macrophages in arbitrary unit (AU). B) Representative images of FITC-ox-LDL uptake in THP-1 macrophages. THP-1 were incubated which 100 ug/mL of FITC-ox-LDL for 0h, 12h, 24h, 48h, and 72h. FITC-ox-LDL uptake was shown in green, and cell nucleus was labeled by DAPI (blue). Cells were viewed under fluorescence microscope (20× objectives). M, macrophage; FC, foam cells. Values are expressed as mean ± SD. * P < 0.05, compared to cells incubated in absence of FITC-ox-LDL; γ P < 0.001, compared to cells incubated with the other FITC-ox-LDL concentrations.*

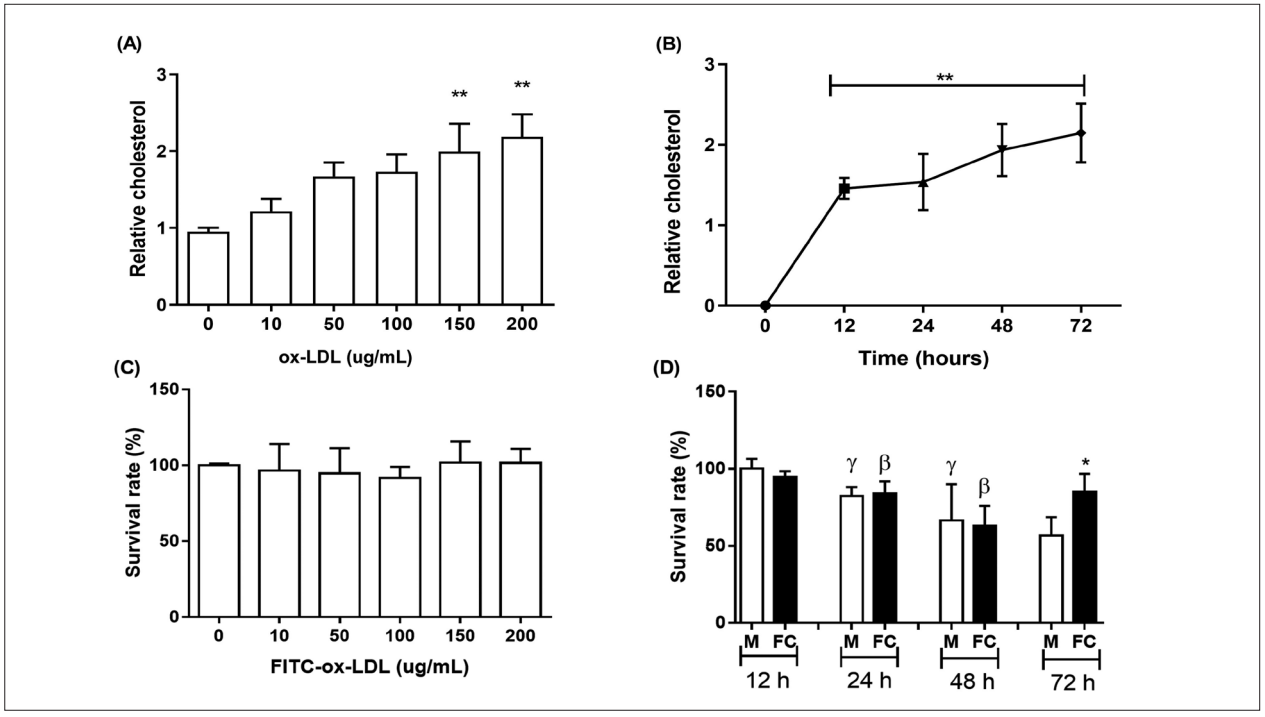

Figure 4 – *Lipid uptake in THP-1 macrophage and cell viability. A) Concentration-dependent increased uptake of cholesterol by THP-1 macrophages (Relative cholesterol - the value was relativized for the total cellular protein levels); B) Time-dependent increased uptake of cholesterol by THP-1 macrophages (Relative cholesterol - the value was relativized for the total cellular protein levels). C) Survival rate in different concentrations of FITC-ox-LDL. D) Survival rate in different exposure time of FITC-ox-LDL. M, macrophage absence of FITC-ox-LDL; FC, foam cells. Values are expressed as mean ± SD. P < 0.05: * M vs FC per Time; ** compared to cells incubated in absence of FITC-ox-LDL (time 0); γ compared to 12h M; β compared to 12h FC.*

macrophages. Using ox-LDL labeling with FITC and the quantification of inflammation in cell formation, a method with the quality of low-cost fluorescent probes was obtained, producing high quality photos.

For foam cell formation, human LDL was isolated by ultracentrifugation, oxidized, and labeled with fluorescein isothiocyanate conjugate (FITC). The use of FITC as a fluorescent probe is widely used because the isothiocyanate group reacts with terminal and primary amino groups in proteins, making it a viable and highly accessible technique.[11,14] Adherent THP-1 cells accumulate numerous lipid droplets (stained with green) upon exposure to a 100 μg/mL concentration of oxidized LDL for 24 hours, as shown in the literature.[23,24] In addition, the macrophage-differentiated THP-1 assumed the morphological appearance of foam cells with fluorescent lipid droplets present along the cytosol and near to the nucleus of most cells. THP-1 monocytes have been extensively used as an *in vitro* model of macrophages, but little care has been given to optimizing foam cell formation from macrophages without checking for inflammation.

The concentration of 100 μg/mL is most commonly used in the literature;[23,24] however, the present study's data show that macrophages derived from THP-1 monocytes are well differentiated in foam cells with 50 μg/mL FITC-ox-LDL for only 12h. Under these conditions, there is an accumulation of cholesterol in the cell with an increased production of pro-inflammatory cytokine drugs, such as IL-6 and TNF-α, without altering the viability of this cell. The pro-inflammatory phenotype is of great importance in foam cell formation, as the components present in ox-LDL can induce diverse biological effects in vitro and *in vivo*, such as monocyte differentiation, activation of endothelial cells, and activation of the immune system. Moreover, there is evidence that its action is due to the activation of TLR4.[25] Therefore, the oxidative process seems to be directly involved in the stimulation of these substances.

In addition to the concentration of LDL in the macrophage cytoplasm, it is important to monitor the production of inflammatory cytokines, as macrophages can contribute to atherogenesis, mainly after its interaction with ox-LDL in the intima layer of the artery, producing cytokines and inflammatory mediators.[7] The increasing expression of inflammatory markers can be caused by the activation of macrophages during the atherosclerotic process, leading to an increase in the uptake of ox-LDL.[2] The results shown in this work demonstrated that IL-6 and TNF-α production increased in macrophages when exposed to different exposure times. IL-6 was released in a higher concentration in the foam cells when compared to its controls; in addition, the longer the exposure time to the ox-LDL, the greater the release of IL-6, so that 48h and 72h had a greater release when compared to 12h and 24h. Considering TNF-α, all times were greater than their controls, but there was no difference between

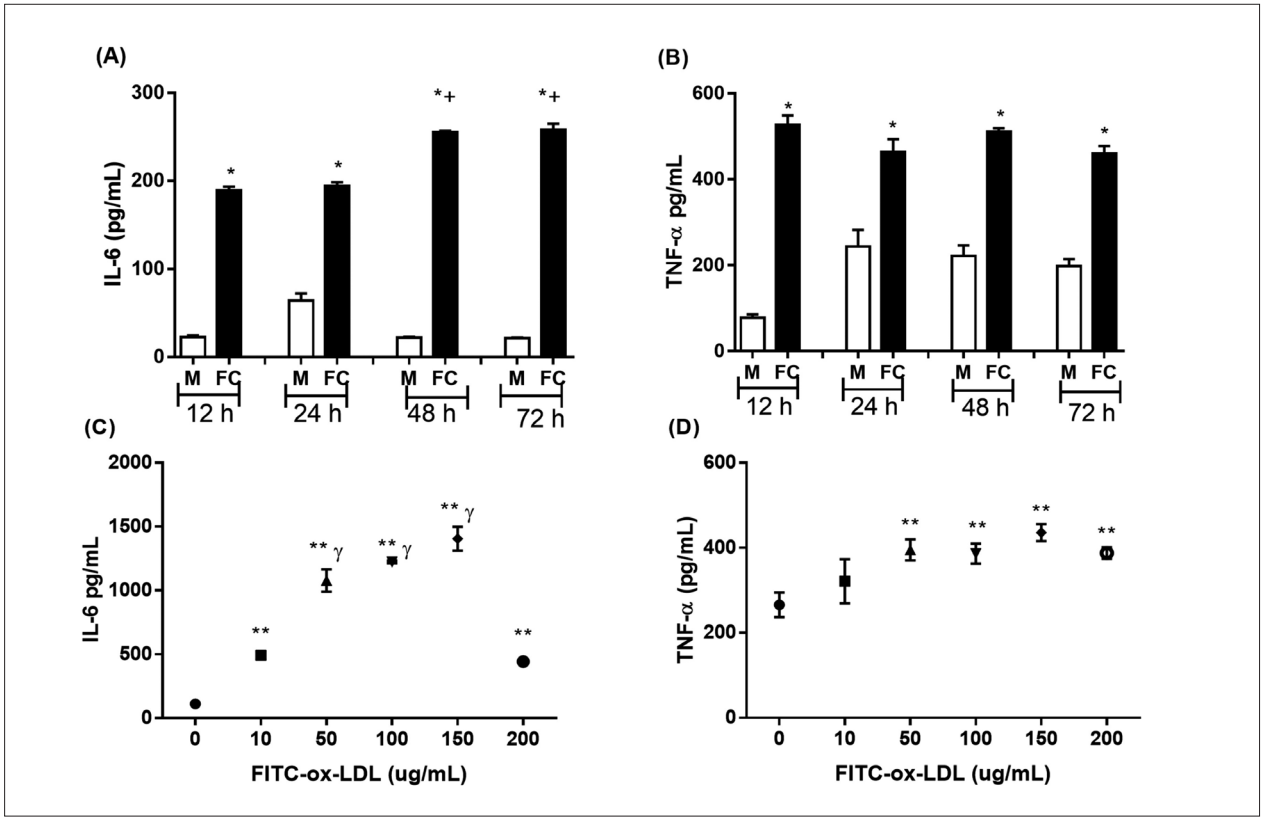

Figure 5 – *Time and concentration effect of ox-LDL in the proinflammatory cytokines of THP-1 cell: A) Interleukin 6 at different times with 100 µg/mL ox-LDL; B) Tumor Necrosis Factor alpha (TNF-α) at different times with 100 µg/mL ox-LDL. C) Interleukin 6 in different concentrations of ox-LDL, treated for 24 hours. D) Tumor Necrosis Factor alpha (TNF-α) in different concentrations of ox-LDL, treated for 24 hours. Values are expressed as mean ± SD. M, macrophage absence of FITC-ox-LDL; FC, foam cells. * P < 0.001, M vs FC per Time (Test T Student); P < 0.05: ** compared to cells incubated in absence of FITC-ox-LDL (time 0); γ compared to cells incubated with 10 µg/mL. + P < 0.01, compared to 12h and 24h.*

exposure times. In an environment with high inflammation, it is of utmost importance to consider the viability of these cells, so that under these experimental conditions, the 72-hour exposure to FITC-ox-LDL reduced cell viability when compared to 12 hours of exposure. Together, these data may suggest that the production of IL-6 and TNF-α could contribute to the upregulation of macrophage phagocytosis, especially if in this microenvironment the macrophages (M1) are in greater quantities, thus promoting an inflammatory process, inducing chronicity, and promoting deleterious effects on tissues.

The foam cells in the atherosclerotic plaque produce proinflammatory cytokines that may contribute to local inflammation. Their inflammatory nature has been supported by *in vitro* studies that show human monocyte-derived M2 macrophages, which normally have an anti-inflammatory phenotype, consume high levels of ox-LDL, and produce proinflammatory factors (IL-6, IL-8, MCP-1), followed by the formation of foam cell, thus taking on a more M1-like proinflammatory phenotype.[2] Macrophages, *in vivo*, are a dynamic cell population with both phenotypic and functional traits that differ significantly one with another, depending on their maturation environment and the nature of the added stimuli.[7] For example, THP-1 cells can be directed to an M1 phenotype using the IFN-γ,[16] as we used in our protocol and which was confirmed by the high release of inflammatory cytokines. Other studies use a very prolonged LDL exposure protocol lasting 48h or more,[26,27] which we show has no viability, given that, with 12h of incubation, the foam cell formation is already obtained, ensuring a high degree of inflammation. In addition, at 48h, the cell viability was reduced by approximately 50%, hindering possible interventions.

Thus, the lack of a uniform protocol that presents inflammatory components greatly affects the interpretation of results and the ability to compare studies. This is because the experimental design does not represent possible phenotypic and/or functional differences in the macrophage populations that are attributable to the use of different maturation protocols, exposure time, and LDL concentration, without evaluating the inflammatory profile.

Conclusion

The present study's results suggest a model that contributes to the understanding of the release of IL-6 and TNF-α in response to different concentrations of ox-LDL using an optimized method for the formation of foam cells. Therefore, the understanding of the phenotypic relationships

of macrophages and inflammatory mechanisms is important for the development of research to fight/attenuate the condition of atherosclerosis.

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Author Contributions

Conception and design of the research: Castro CA, Bagnato VS, Inada NM, Aníbal FF, Rodrigues GJ; Acquisition of data: Castro CA, Buzinari TC, Lino RLB, Selistre-de-Araújo HS, Aníbal FF, Rodrigues GJ; Analysis and interpretation of the data: Castro CA, Buzinari TC, Lino RLB, Rodrigues GJ; Statistical analysis: Castro CA, Buzinari TC, Bagnato VS; Obtaining financing: Bagnato VS, Inada NM, Selistre-de-Araújo HS, Rodrigues GJ; Writing of the manuscript: Castro CA, Lino RLB, Bagnato VS, Inada NM, Aníbal FF; Critical revision of the manuscript for intellectual content: Castro CA, Lino RLB, Bagnato VS, Inada NM, Selistre-de-Araújo HS, Aníbal FF, Rodrigues GJ.

Potential Conflict of Interest

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

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Study Association

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

References

1. Tabas I, García-Cardeña G, Owens GK. Recent insights into the cellular biology of atherosclerosis. J Cell Biol. 2015;209(1):13-22. doi: 10.1083/jcb.201412052.
2. Angelovich TA, Hearps AC, Jaworowski A. Inflammation-induced foam cell formation in chronic inflammatory disease. Immunol Cell Biol.2015;93(8):683-93. doi: 10.1038/icb.2015.26
3. Bao Z, Li L, Geng Y, Yan J, Dai Z, Shao C. Advanced Glycation End Products Induce Vascular Smooth Muscle Cell-Derived Foam Cell Formation and Transdifferentiate to a Macrophage-Like State. Mediators Inflamm. 2020:6850187. doi: 10.1155/2020/6850187.
4. Maguire EM, Pearce SWA, Xiao Q. Foam cell formation: A new target for fighting atherosclerosis and cardiovascular disease. Vascul Pharmacol. 2019;112;54-71. doi: 10.1016/j.vph.2018.08.002.
5. Yang S, Yuan HQ, Hao YM, Ren Z, Qu SL, Liu LS, et al. Macrophage polarization in atherosclerosis. Clin Chim Acta.2020;501:142-6. doi: 10.1016/j.cca.2019.10.034.
6. Volobueva A, Zhang D, Grechko A V, Orekhov AN. ScienceDirect Review article Foam cell formation and cholesterol trafficking and metabolism disturbances in atherosclerosis. Cor Vasa. 2019;61:e48–e55. doi:10.1016/j.crvasa.2018.06.006
7. Valledor AF, Lloberas J, Celada A. Macrophage Foam Cells. In: eLS. Wiley; 2015.p: 1–10. https://doi.org/10.1002/9780470015902.a0020730
8. Reiss AB, Siegart NM, Leon J De, Reiss AB, Siegart NM, Interleukin- JDL, et al. Interleukin-6 in atherosclerosis: atherogenic or atheroprotective? Clin Lipidol. 2017112(1):14-23.doi: 10.1080/17584299.2017.1319787
9. Lu X-T, Zhao Y-X, Zhang Y, Jiang F. Psychological Stress, Vascular Inflammation, and Atherogenesis: Potential Roles of Circulating Cytokines. J Cardiovasc Pharmacol. 2013;62(1):6–12. doi: 10.1097/FJC.0b013e3182858fac
10. Yu XH, Fu YC, Zhang DW, Yin K, Tang CK. Foam cells in atherosclerosis. Clin Chim Acta. 2013;424:245–52. doi: 10.1016/j.cca.2013.06.006.
11. Rios FJO, Ferracini M, Pecenin M, Koga MM, Wang Y, Ketelhuth DFJ, et al. Uptake of oxLDL and IL-10 Production by Macrophages Requires PAFR and CD36 Recruitment into the Same Lipid Rafts. Cignarella A, editor. PLoS One. 2013;8(10): e76893. doi: 10.1371/journal.pone.0076893.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265–75. PMID: 14907713
13. Jiang ZY, Woollard AC, Wolff SP. Lipid hydroperoxide measurement by oxidation of Fe2+ in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. Lipids. 1991;26(10):853–6. PMID: 8613704
14. Bian F, Yang X, Zhou F, Wu PH, Xing S, Xu G, et al. C-reactive protein promotes atherosclerosis by increasing LDL transcytosis across endothelial cells. Br J Pharmacol. 2014;171(10):2671–84. doi: 10.1111/bph.12616
15. Schwende H, Fitzke E, Ambs P, Dieter P. Differences in the state of differentiation of THP-1 cells induced by phorbol ester and 1,25-dihydroxyvitamin D3. J Leukoc Biol. 1996;59(4):555–61. PMID: 8613704
16. Chanput W, Mes JJ, Savelkoul HFJ, Wichers HJ. Characterization of polarized THP-1 macrophages and polarizing ability of LPS and food compounds. Food Funct. 2013;4(2):266–76. doi: 10.1039/c2fo30156c
17. Tsai CS, Lin YW, Huang CY, Shih CM, Tsai YT, Tsao NW, et al. Thrombomodulin regulates monocye differentiation via PKCÎ and ERK1/2 pathway in vitro and in atherosclerotic artery. Sci Rep. 2016;6:1–15. doi: 10.1038/srep38421
18. Liu YC, Zou XB, Chai YF, Yao YM. Macrophage polarization in inflammatory diseases. Int J Biol Sci. 2014;10(5):520–9. doi: 10.7150/ijbs.8879
19. Eskes C, Boström AC, Bowe G, Coecke S, Hartung T, Hendriks G, et al. Good cell culture practices & in vitro toxicology. Toxicol Vitr. 2017;45(April):272–7. doi: 10.1016/j.tiv.2017.04.022
20. Hirsch C, Schildknecht S. In vitro research reproducibility: Keeping up high standards. Front Pharmacol. 2019;10:1484;1-9. doi:10.3389/fphar.2019.01484.
21. Xu S, Huang Y, Xie Y, Lan T, Le K, Red ÁO. Evaluation of foam cell formation in cultured macrophages: an improved method with Oil Red O staining and DiI-oxLDL uptake. 2010;473–81. doi: 10.1007/s10616-010-9290-0
22. Lillie RD AL. Supersaturated solutions of fat stains in dilute isopropanol for demonstration of acute fatty degeneration not shown by Herxheimer's technique. Arch Pathol. 1943.

23. Li XY, Kong LX, Li J, He HX, Zhou Y Da. Kaempferol suppresses lipid accumulation in macrophages through the downregulation of cluster of differentiation 36 and the upregulation of scavenger receptor class B type i and ATP-binding cassette transporters A1 and G1. Int J Mol Med. 2013;31(2):331–8. doi.org/10.3892/ijmm.2012.1204

24. Banka CL, Black AS, Dyer CA, Curtiss LK. THP-1 cells form foam cells in response to coculture with lipoproteins but not platelets. J Lipid Res. 1991 Jan;32(1):35–43. doi:10.1016/S0022-2275(20)42241-2

25. Miller YI, Choi SH, Fang L, Harkewicz R. Toll-Like Receptor-4 and Lipoprotein Accumulation in Macrophages. Vol. 19, Trends Cardiovasc Med. 2009.19:227-32. doi.org/10.1016/j.tcm.2010.02.001

26. Singh V, Rana M, Jain M, Singh N, Naqvi A, Malasoni R, et al. Curcuma oil attenuates accelerated atherosclerosis and macrophage foam-cell formation by modulating genes involved in plaque stability, lipid homeostasis and inflammation. Br J Nutr. 2015;113(1):100–13. doi: 10.1017/S0007114514003195.

27. Zhang XX, Wu C, Wu H, Sheng L, Su Y, Zhang XX, et al. Anti-Hyperlipidemic Effects and Potential Mechanisms of Action of the Caffeoylquinic Acid-Rich Pandanus tectorius Fruit Extract in Hamsters Fed a High Fat-Diet. PLoS One. 2013;8(4).e61922. doi: 10.1371/journal.pone.0061922.

Foam Cells in Atherosclerosis

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Short Editorial related to the article: Profile of IL-6 and TNF in Foam Cell Formation: An Improved Method Using Fluorescein Isothiocyanate (FITC) Probe

In the early stages of atherosclerotic lesions, there is an accumulation of lipids in the tunica intima, which, in this location, undergo modifications such as oxidation and become capable of inducing inflammatory reactions and, consequently, the progression of the pathological process. It is worth noting that there are indications that interactions with components present in this arterial layer are important for fats to be retained and, therefore, subject to the modifications above that bring them pro-inflammatory properties. In this sense, it is interesting to note that while most mammalian species have a "virtual" arterial intima – that is, at light microscopy, the endothelium appears to lie directly on the internal elastic lamina (although observation electron microscopy reveals that this is not the case) and are less susceptible to atherosclerotic disease, species in which there is an amount of tissue that can be seen at light microscopy separating the endothelium from the internal elastic lamina, such as rabbits, pigs, apes and, mainly, human beings (Figure 1), are the ones that are prone to such alteration.

When retained in the intima, lipids initially accumulate in the extracellular space but are also internalized by cells, which assume the appearance called "foam cells" under the microscope. This is because, in the usual histological processing, tissue samples go through a series of baths to dehydrate and degrease them and improve the section quality. With the removal of the fat, intracellular septa are left that give the cytoplasm a lacy pattern, giving the impression of what would correspond in two dimensions to an appearance of foam (figure 2). There are fat staining methods without the usual processing (figure 3), but on the other hand, quality is lost in the histological section. The same cells are also called "xanthomatous," by the Greek "ξανθιά" ("xanthia"), which corresponds to "blond, yellow"; because it has a large amount of fat, macroscopically, the region has this color. Most foam cells are made up of macrophages, cells that have as one of their primary functions to internalize exogenous material that appears in the interstitium, whatever the organ. Specifically, smooth muscle cells and others are also supportive in atherosclerotic lesions in this task.

Castro et al. present in this issue of *Arquivos Brasileiros de Cardiologia* a work in which they study in vitro the stimuli that lead to the transformation of macrophages into foam cells;[1] as they comment, macrophages may have a phenotype classified as M1, with high expression of pro-inflammatory proteins that can contribute to the formation of atherosclerotic plaque, or M2, which play a preventive role, reducing the size of the plaque and improving its stability.

Therefore, verifying its pro-inflammatory profile is more important than just evaluating foam cell formation. For this, they analyzed how the formation of foam cells contributes to the production of two cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). They showed how concentrations of oxidized low-density lipids and incubation time influence the formation of these pro-inflammatory cytokines, thus contributing to the elucidation of cellular mechanisms involved in the pathogenesis of atherosclerosis.

Keywords

Atherosclerosis; Pathology; Foam Cells

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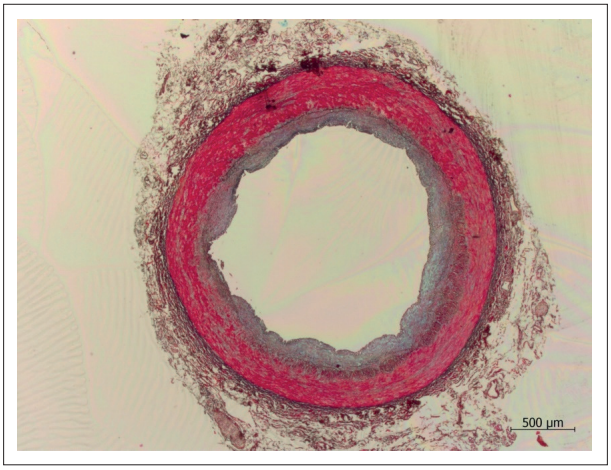

Figure 1 – *Histological section of a normal human coronary artery shows that in the tunica intima, that is, internal to the media (stained red), there is connective tissue. Staining by Movat's pentachrome method; objective magnification: 2.5x.*

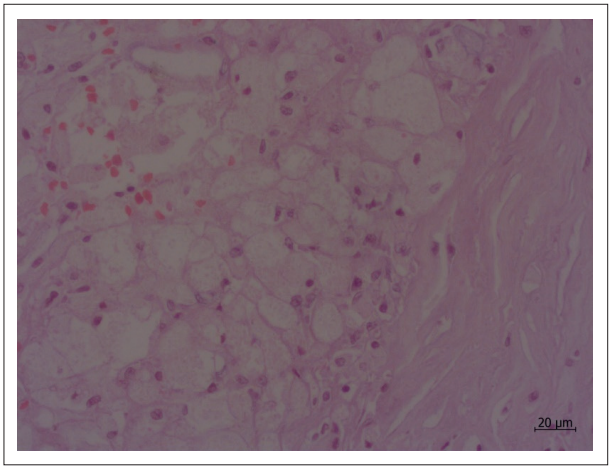

Figure 2 – *Histological section of human coronary artery showing foam cells, characterized by cytoplasm with a lacy appearance. Staining by the hematoxylin & eosin method; objective magnification: 40x.*

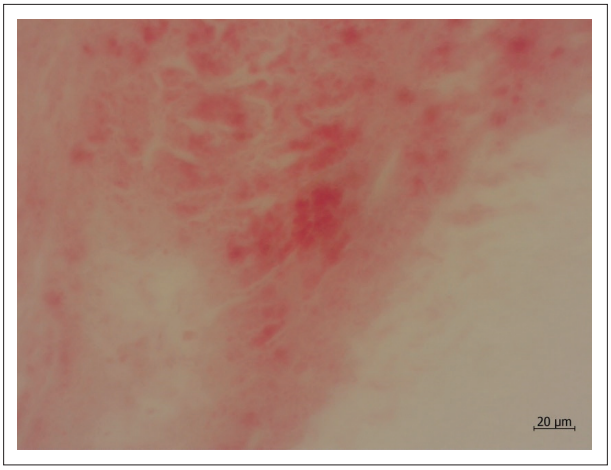

Figure 3 – *Histological section of a human coronary artery sample submitted to freezing (and not to the usual processing) shows lipid deposition, stained in red. Due to the loss of quality in the cut, it is difficult to be precise, but at least part of the fat appears to be located in the intracellular space, possibly forming part of foam cells. Scarlet coloration R; objective magnification: 40x.*

References

1. Castro CA, Buzinari TC, Lino RLB, Araújo HSS, Aníbal FF, Verzola RMM, et al. Profile of IL-6 and TNF in Foam Cell Formation: An Improved Method Using Fluorescein Isothiocyanate (FITC) Probe. Arq Bras Cardiol. 2022; 119(4):533-541.

Can the Serum Endocan Level Be Used as a Biomarker to Predict Subclinical Atherosclerosis in Patients with Prediabetes?

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Abstract

Background: **Patients with prediabetes have an increased risk of atherosclerotic cardiovascular disease; therefore, early detection is important.**

Objective: **The present study aimed to reveal the usability of serum endocan levels as a biomarker in the diagnosis of subclinical atherosclerosis in patients with prediabetes, based on CIMT measurements.**

Methods: **Participants were classified according to the presence (n=42) or absence (n=42) of prediabetes. Serum endocan, fasting blood sugar, fasting insulin, and glycated hemoglobin (HbA1c) values of patients were examined, and CIMT was measured. The level of significance for statistical analysis was 0.05.**

Results: **While serum endocan levels were found to be lower in patients with prediabetes, when compared to the control group (p=0.042), CIMT values were found to be higher (p=0.046). When evaluated by multivariate regression analysis, the serum endocan level was found to be associated with CIMT, regardless of other parameters (p=0.007). A negative correlation was found between plasma fasting insulin and endocan levels (r=-0.320, p=0.001).**

Conclusions: **Carotid intima media thickness was found to be high and the serum endocan level was low in patients with prediabetes. Decreased serum endocan levels in patients with prediabetes may be a contributing factor to atherosclerosis formation mechanisms.**

Keywords: **Atherosclerosis; Carotid Intima-Media Thickness; Prediabetic State.**

Introduction

Prediabetes, defined as levels between normal and diabetic blood sugar, is rapidly increasing around the world. Nearly 38% of the adult population in the United States of America[1] and nearly 50% of the Chinese population have prediabetes.[2] Prediabetes is important because of the increased risk of microvascular and macrovascular complications and progression to type 2 diabetes in a short time. High plasma glucose levels are known to be a major risk factor for atherosclerotic cardiovascular disease.[3] Additionally, insulin resistance may be connected to atherosclerosis due to worse lipid profiles,[4] proinflammatory state,[5] and endothelial dysfunction.[6]

Detection of atherosclerotic cardiovascular disease in the early period is important for follow-up and treatment. Carotid intima media-thickness (CIMT) is used to detect subclinical atherosclerosis in the early stages and was able to predict cardiovascular events.[7-10] Each 0.1 mm increase in CIMT increases the risk of myocardial infarction by 10-15% and stroke by 13-18%.[11] It is very appropriate for use in large-scale population studies, as it is non-invasive and can be obtained with a simple measurement.

In addition to non-invasive methods to determine atherosclerosis development, a variety of biomarkers are known to be included in predictions. Endothelial specific molecule-1 (ESM-1), called endocan, is a proteoglycan released from endothelial cells under the control of inflammatory cytokines. Endocan activates compounds ensuring the necessary substrate for collection, adhesion, and transmigration of leukocytes along activated endothelium.[12] In previous studies, serum endocan levels were found to be

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higher in patients with type 2 diabetes and acute coronary syndrome compared to control groups.[13,14] Studies showed that serum endocan levels were associated with the severity of disease.[10-12]

There are studies assessing serum endocan levels in patients with prediabetes and insulin resistance. However, it is unclear whether changes in serum endocan levels are a cause or a consequence, particularly in atherosclerotic events. When serum endocan levels were compared in these patient groups with control groups, contrary to high values in type 2 diabetes, they were found to be low or unchanged in group with prediabetes.[15,16] Although the tendency toward atherosclerosis increases in both prediabetes and diabetes patients, the differences in serum endocan levels are remarkable. There are studies evaluating endocan levels in atherosclerosis and vascular events in type 2 diabetes patients. However, we could not find any study in the literature that evaluated endocan levels in prediabetes patients over atherosclerosis.

Therefore, the present study sought to reveal the role of serum endocan levels in predicting subclinical atherosclerosis based on prediabetes patients on CIMT.

Methods

The present study complied with the Declaration of Helsinki, and approval was obtained from the hospital research ethics committee Prof Dr Cemil Tascioglu City (approval number 525). An informed consent form was signed by the participants. This cross-sectional study was conducted in the internal medicine outpatient clinic of our tertiary care hospital between June and August 2021. A total of 84 participants over the age of 18 were included in the study, of which 42 were patients with prediabetes and 42 were normoglycemic (BMI, age, and gender are similar).

According to the American Diabetes Association (ADA) criteria, those with fasting blood glucose levels between 100-125 mg/dL (impaired glucose tolerance (IGT)) or HbA1c 5.7-6.4%, or 2nd hour plasma glucose during 75g oral glucose tolerance test (OGTT) levels between 140 and 199 mg/dL (impaired glucose tolerance (IGT)), were included in the group with prediabetes.[17] Normoglycemic participants with lower values were included in the control group. Individuals in the normoglycemic and prediabetes groups were not using any antidiabetic drugs.

Those with a history of myocardial infarction or coronary revascularization, cerebrovascular events, a previous diagnosis of cardiovascular disease or systolic heart failure, severe valve disease, hypertrophic cardiomyopathy, angina pectoris, ST-T wave variations on electrocardiogram, Q waves, left branch block, chronic liver or kidney disease, active malignancy, hypertension, inflammatory diseases, respiratory system disease, peripheral artery disease, smokers, or those who refused to participate were excluded from the study.

The blood pressure of participants was measured, and their BMI was calculated by measuring their height and weight (weight/square of height, kg/m^2). After overnight fasting, blood sugar, insulin HbA1c, lipid levels (high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides), c-reactive protein (CRP), creatinine, and serum endocan were analyzed. Homeostasis model assessment-estimated insulin resistance (HOMA-IR) values were calculated with the formula (fasting blood sugar x fasting insulin level)/405.

Serum endocan measurements

After overnight fasting, 10 ml of venous blood was collected from the participants. The samples were centrifuged for 10 minutes at 1700 rpm. The serum was stored at -80℃ until analysis. Serum endocan levels were measured with an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol (Human Endocan Elisa Kit; lot no:201506, cat. No: E3160Hu, Sunred Biological Technology Shanghai, China). Results are given as ng/L. The measurement interval of the kit is 31-2000 ng/L.

Assessment of carotid intima-media thickness

CIMT was measured using a multifrequency (12 MHz) linear array transponder (Samsung HS50 GE Ultrasound). All measurements were taken on high-resolution B-mode images. For CIMT measurements, patients were placed in the supine position, with their heads turned 45° away from the measurement side. B-mode images of the extension of the distal segment of the right main carotid artery were obtained for three sequential sections of the far wall of the main carotid artery. The distance between the blood intima and media-adventitia interfaces was then measured for each section. CIMT was calculated by taking the average of the measurement values.

Statistical analysis

Statistical analyses were performed using SPSS version 26.0 (SPSS Inc, Chicago, Illinois). Mean and standard deviation were used for continuous variables with normal distribution, and median and interquartile ranges were used for those without normal distribution. Categorical variables are shown as absolute numbers and percentages. Distribution of variables was assessed with the Kolmogorov–Smirnov test. Continuous variables were compared using independent two sample t-test (unpaired) or Mann-Whitney U tests according to their distribution. The chi-square test was used for categorical variables. Pearson or Spearman tests were used for correlation analysis according to whether the variables were parametric or nonparametric. Multivariate linear regression analysis was used to evaluate the CIMT determinants. Normal distribution of all parameters is required for multivariate lineer regression analysis. Normal distribution was obtained by taking the logarithm of serum endocan and triglyceride levels. The statistical significance level was determined as $P < 0.05$.

Reproducibility

Considering that the intra-observer and inter-observer agreement is 0.75, the minimal sample size (assuming Type error 0.05, Type II error 0.20 Power 0.80) is n=13. Considering the possible losses for any reason, 15 people were included in the study.

Power analysis

Power analysis was performed using the G-power program. Based on previous data in the literature, for effect size 0.57, alpha error share 5%, and 80% power to represent the population, the smallest size for each sample group was calculated as 39.

Results

Age, gender, and BMI values of the group with prediabetes and normoglycemia were similar (p>0.05).

Serum endocan levels were significantly lower in the group with prediabetes than in the control group (p=0.042), while CIMT values were higher (p=0.046) (Table 1).

There was a significant correlation between CIMT values and age and triglyceride levels of all participants (Table-2). Multivariate lineer regression analysis of age, endocan, HbA1c, FPI, FPG, and triglyceride values was performed with CIMT. The logarithm of serum endocan and triglyceride values were taken to ensure normal distribution. The serum endocan level proved to be associated with CIMT, regardless of other parameters (p=0.007) (Table 3). While there was no correlation between serum endocan levels and CIMT measurements in the group with prediabetes (r=0.104 p=0.514) (Figure 1), a positive correlation was found in the group without prediabetes (r=0.340, p=0.028) (Figure 2).

Correlations between the parameters in Table 1 and serum endocan levels were examined. Of these parameters, only fasting insulin was correlated with endocan levels. This correlation was negative (r=-0.320, p=0.001) (Figure 3).

Reproducibility

A total of 15 patients were chosen at random for inter- and intra-observer variability analysis. The compatibility of intra- and inter-observer CIMT values was calculated. The intra-class correlation coefficient for intra-observer and inter-observer variability was, respectively: 0.93 (95% CI, 0.87–0.97) and 0.90 (95% CI, 0.85–0.95) for CIMT.

Discussion

The present study attempted to explain the role of endocan levels in predicting subclinical atherosclerosis in patients with prediabetes based on CIMT measurements. Plasma endocan levels were lower in the prediabetes patient group than in the control group. By contrast, CIMT values were higher in patients with prediabetes. In our study, there was no correlation between CIMT values and serum endocan levels. When the groups were evaluated separately, the correlation between CIMT measurements and endocan levels was found in the normoglycemic group but not in the group with prediabetes. However, according to the results of the regression analysis, serum endocan levels significantly explained the CIMT value.

Many studies show that prediabetes can cause cardiovascular disease.[3-6] In addition, the burden of coronary atherosclerosis in patients with prediabetes is higher than in normal people. In particular, the burden of atherosclerosis precedes the clinical symptoms of type 2 diabetes. In our study, CIMT values were high in patients with prediabetes, which is consistent with studies using CIMT as a subclinical atherosclerosis marker.[18,19]

Patients with prediabetes have hyperinsulinemia as a result of insulin resistance, and the results of our study are consistent with this. A negative correlation was confirmed between plasma insulin and endocan levels. It can be said that serum endocan levels are low in patients with prediabetes due to the hyperinsulinemic state.

The relationship between hyperinsulinemia and atherosclerosis has been demonstrated by previous studies. Insulin resistance has elicited great interest in medical and scientific communities because of its association with cardiovascular disease. However, the molecular mechanisms linking insulin resistance to the development and/or progression of atherosclerosis remains enigmatic. Some mechanisms come to the fore regarding this situation. Insulin signaling plays a critical role in activating nitric oxide synthase, which regulates nitric oxide production.[20,21] Nitric oxide is a potent vasodilator and anti-atherogenic agent.[20] Nitric oxide deficiency activates multiple pathways involved in atherogenesis.[22,23] Thus, a defect in insulin signaling not only impairs glucose use, but also causes hypertension and accelerated atherosclerosis. It

Table 1 – Demographic Features and Laboratory Findings in Patients with Prediabetes and in Controls

	Control Group n=42	Patient group with prediabetes n=42	p
Age (year)	47.8±9.7	49.9±8.5	0.112
Sex (F/M)	28/14	30/12	0.814
BMI (kg/m^2)	33.8±4.1	32.2±8.8	0.066
Endocan (ng/L) *	138 (84-300)	120 (65-185)	0.042
FPI (µU/ml)	11.2±5.3	20.1±8.8	<0.001
FPG (mg/dL)	87±5.3	103±9.7	<0.001
2-h PG during 75-g OGTT (mg/dL)	101±19	141±34	<0.001
HOMA-IR	2.4±1.1	5.2±2.3	<0.001
HbA1c (%)	5.5 ±0.3	5.9±0.5	0.039
CRP (mg/L)	4.9± 2.6	5.1±2.9	0.245
Total Cholesterol (mg/dL)	188±32	206±33	0.020
LDL-Cholesterol (mg/dL)	110±31	120±26	0.107
HDL-Cholesterol (mg/dL)	53±11	49±13	0.103
TG (mg/dL)*	108 (79-133)	152 (95-257)	0.002
CIMT (mm)	0.67±0.16	0.74±0.17	0.046

BMI: body mass index; FPI: fasting plasma insulin; FPG: fasting plasma glucose; PG: plasma glucose; OGTT: oral glucose tolerance test; HOMA-IR: homeostasis model assessment-estimated insulin resistance; HbA1c: glycated hemoglobin; CRP: C-reactive protein; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TG: triglycerides; CIMT: carotid intima-media thickness.

Table 2 – Correlations between CIMT and other parameters

	All participants (n=84)		Control Group (n=42)		Patient group with prediabetes (n=42)	
	r	p	r	p	r	p
Endocan (ng/L) *	0.206	0.060	0.340	0.028	0.104	0.514
Age (Year)	0.363	0.001	0.490	0.001	0.215	0.172
BMI (kg/m^2)	-0.015	0.895	-0.009	0.956	0.034	0.833
FPI ((µU/mL)	0.180	0.104	0.360	0.021	0.020	0.900
FPG (mg/dL)	0.195	0.075	0.212	0.178	0.119	0.454
2-h PG (OGTT)	0.166	0.131	0.080	0.485	0.164	0.300
HOMA-IR	0.180	0.102	0.379	0.013	0.004	0.982
HbA1c (%)	0.242	0.080	0.349	0.143	0.199	0.260
CRP (mg/L)	0.077	0.520	0.063	0.694	0.065	0.730
Total-C (mg/dL)	-0.015	0.895	-0.076	0.632	-0.015	0.927
LDL-C (mg/dL)	-0.031	0.781	-0.093	0.557	-0.192	0.223
HDL-C (mg/dL)	-0.111	0.313	0.032	0.839	0.227	0.149
TG (mg/dL)*	0.257	0.018	0.306	0.030	0.342	0.027

*BMI: body mass index; FPI: fasting plasma insulin; FPG: fasting plasma glucose; PG: plasma glucose; OGTT: oral glucose tolerance test; HOMA-IR: homeostasis model assessment-estimated insulin resistance; HbA1c: glycated hemoglobin; CRP: C-reactive protein; Total-C: Total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; TG: triglycerides; CIMT: carotid intima-media thickness.*Spearman correlation test, others: Pearson correlation test.*

Table 3 – Multivariate lineer regression analysis showing CIMT predictors

	Beta	IC 95%		p
		Inferior	Superior	
Age	0.525	0.004	0.016	0.002
FPI	0.324	-0.001	0.016	0.068
Log (TG)	-0.142	-0.381	0.154	0.396
Log (Endocan)	0.435	0.056	0.336	0.007
HbA1c	0.181	-0.053	0.219	0.222
CRP	0.024	-0.019	0.022	0.862

FPI: fasting plasma insulin; Log: logarithm; TG: triglycerides; HbA1c: glycated hemoglobin; CRP: C-reactive protein.

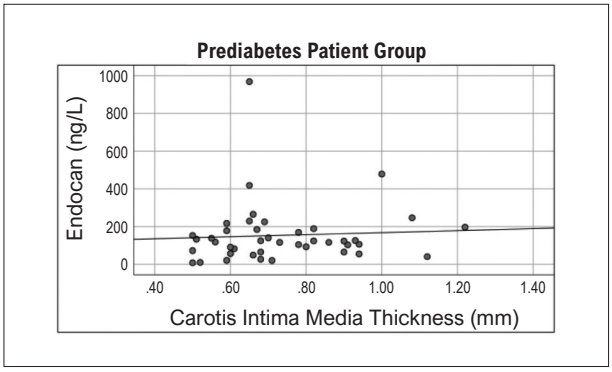

Figure 1 – *Correlation between plasma endocan levels and CIMT values in the patient group with prediabetes. (r=0.104, p=0.514)*

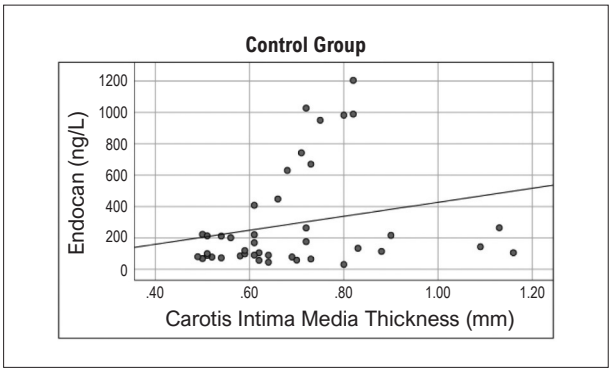

Figure 2 – *Correlation between plasma endocan levels and CIMT values in the control group (r=0.340, p=0.028).*

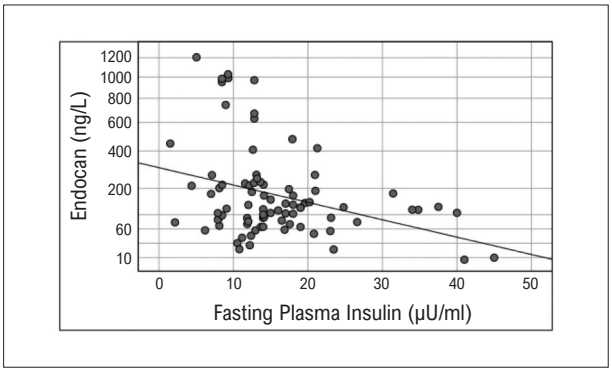

Figure 3 – *Correlation between serum endocan levels and fasting plasma insulin (r=-0.320, p=0.001).*

is difficult to distinguish the effect of insulin resistance from the compensatory hyperinsulinemia that always accompanies insulin resistance. It has been suggested that if the detrimental effect of insulin resistance is a result of diminished insulin action, compensatory hyperinsulinemia may be just an innocent bystander. Conversely, if certain aspects of insulin action are not affected by the decreased potency of insulin, the presence of compensatory hyperinsulinemia may have its own effect. Consequently, compensatory hyperinsulinemia can stimulate or even overstimulate certain aspects of insulin action in various cells and tissues. Thus, the primary critical point in understanding the role of insulin resistance is to determine whether reduced insulin action (effect of insulin resistance) will coexist with normal or even increased insulin action (effect of hyperinsulinemia) within the same tissue and within the same cell. This task was made possible by unraveling the intracellular insulin signaling chain. Hyperinsulinemia is a potent growth factor,[24-28] whose growth-promoting effects are mediated via

the mitogen-activated protein (MAP) kinase pathway.[29] After the interaction between insulin receptor substrate-1 (IRS-1) and src homology 2 domain-containing (SHC) transforming protein, extracellular regulated kinase (ERK) is activated,[30,31] translocates into the nucleus and catalyses phosphorylation of transcription factors that promote cell growth, cell proliferation, and cell differentiation.[30] Thus, this pathway plays an important role in atherogenesis.

In addition to its role in atherosclerotic mechanisms, insulin has been reported to ameliorate the endotoxin-induced systemic inflammatory response by decreasing TNF-α expression and increasing the anti-inflammatory cascade.[26,32] The expression of endocan is differentially regulated by cytokines. TNF-α and interleukin-1 beta (IL-1β) up-regulate and interferon-gamma (IFN-γ) down-regulates the secretion of endocan.[33] The lowering effect of hyperinsulinemia on TNF-α may explain the decrease in serum endocan levels. Janke et al. also showed that endocan is expressed by human adipocytes and that insulin administration reduces endocan production in adipocytes. As a result, it has been suggested that endocan secretion from adipocytes may significantly affect local or systemic endocan levels.[34] In our study, the suppressive effect of insulin on adipocytes may be another effective factor in the low plasma endocan levels in the patient group with prediabetes.

Menon et al. researched the role of endocan during atherosclerotic lesion formation in ApoE null mice and identified high rates of expression in atherosclerotic plaques. In the study, endocan expression was at low levels in quiescent endothelium, while they showed it was up-regulated in activated endothelium.[35] The subjects in our study group were chosen from people without known vascular disease or any other situation causing inflammation. For this reason, there is a high probability that both controls and patients of prediabetes had quiescent endothelium. In this case, it can be said that, in our patient group, the effect of subclinical atherosclerosis on endocan secretion from the endothelium may be limited. It is our opinion that the effect of insulin on TNF-α and adipose tissue is more dominant and causes a decrease in the serum endocan level.

It has been shown that plasma endocan levels increase depending on the severity of the disease in patients with atherosclerosis, vascular inflammation, and acute coronary syndrome. This increase in the serum endocan level has proven to be associated with atherosclerotic heart diseases, but a cut-off value has not been determined.[36,37] This increase in the serum endocan level has been accepted as a predictor of atherosclerosis in many studies. Endocan has been suggested as a functional inhibitor of the lymphocyte function-associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1) interaction, suggesting its anti-inflammatory role, through the inhibition of leukocyte rolling, adhesion, or transmigration.[12] The beneficial effect obtained *in vivo* by blocking adhesion with mAbs in mice and in other animal models clearly demonstrates that LFA-1 and ICAM-1 are involved in acute inflammation,[38] ischemia/reperfusion injury,[39] allograft rejection,[40-42] and antitumor immunity. Therefore, it can be said that endocan is secreted from the endothelium in response to acute inflammation and plays a regulatory role with its anti-inflammatory effect. The present study showed that serum endocan levels were decreased in patients with prediabetes, most likely due to hyperinsulinemia. It can be concluded that endocan plays an inhibitory role on the interaction between LFA-1 and ICAM-1. An increase in ICAM-1 activity is expected with decreasing endocan levels. The increase in ICAM-1 activity may cause vascular inflammation. ICAM-1 is a well-known molecule involved in the pathogenesis of atherosclerotic plaque.[43,44]

In studies with groups without prediabetes or insulin resistance, serum endocan levels were high, possibly in response to inflammation in the atherosclerotic vessel.[35,36] However, our study showed that this response was insufficient and that serum endocan levels decreased in patients with prediabetes and subclinical atherosclerosis, especially due to hyperinsulinemia. Low serum endocan levels may be involved in atherosclerosis formation mechanisms. Comprehensive studies are needed on this subject.

Study limitations

There are some limitations to our study. The main limitation is the low number of patients and the study being performed in a single center. Secondly, only CIMT measurements were used when assessing subclinical atherosclerosis. Finally, another limitation is that we do not know how long our patients had been prediabetic.

Conclusions

Our results show that hyperinsulinemia causes a decrease in endocan levels. However, there is no threshold value to predict atherosclerosis. The decrease in serum endocan values measured periodically in the follow-up of patients with prediabetes may give more information about the development of atherosclerosis. Prospective studies are needed for this purpose.

Author Contributions

Conception and design of the research: Arman Y, Yoldemir S, Tükek T; Acquisition of data: Arman Y, Atici A, Sarikaya R, Yoldemir S, Akarsu M, Kutlu O, Ozturk GZ, Demir P, Özcan M, Bayraktarlı R, Tükek T; Analysis and interpretation of the data: Arman Y, Atici A, Altun O, Sarikaya R, Akarsu M, Kutlu O, Demir P, Özcan M, Bayraktarlı R; Statistical analysis: Arman Y, Atici A; Writing of the manuscript: Arman Y, Altun O, Tükek T; Critical revision of the manuscript for important intellectual content: Arman Y, Tükek T.

Potential Conflict of Interest

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

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Study Association

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References

1. Menke A, Casagrande S, Geiss L, Cowie CC. Prevalence of and Trends in Diabetes Among Adults in the United States, 1988-2012. JAMA. 2015;314(10):1021-9. doi: 10.1001/jama.2015.10029.
2. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global Estimates Of Diabetes Prevalence for 2013 and Projections for 2035. Diabetes Res Clin Pract. 2014;103(2):137-49. doi: 10.1016/j.diabres.2013.11.002.
3. Park C, Guallar E, Linton JA, Lee DC, Jang Y, Son DK, et al. Fasting Glucose Level and the Risk of Incident Atherosclerotic Cardiovascular Diseases. Diabetes Care. 2013;36(7):1988-93. doi: 10.2337/dc12-1577.
4. Holland WL, Knotts TA, Chavez JA, Wang LP, Hoehn KL, Summers SA. Lipid Mediators of Insulin Resistance. Nutr Rev. 2007;65(6 Pt 2):S39-46. doi: 10.1111/j.1753-4887.2007.tb00327.x.
5. Stöhr R, Federici M. Insulin Resistance and Atherosclerosis: Convergence between Metabolic Pathways and Inflammatory Nodes. Biochem J. 2013;454(1):1-11. doi: 10.1042/BJ20130121.
6. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal Relationships between Insulin Resistance and Endothelial Dysfunction: Molecular and Pathophysiological Mechanisms. Circulation. 2006;113(15):1888-904. doi: 10.1161/CIRCULATIONAHA.105.563213.
7. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of Carotid Ultrasound to Identify Subclinical Vascular Disease and Evaluate Cardiovascular Disease Risk: A Consensus Statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr. 2008;21(2):93-111. doi: 10.1016/j.echo.2007.11.011.
8. Santos IS, Goulart AC, Brunoni AR, Kemp AH, Lotufo PA, Bensenor IM. Anxiety and Depressive Symptoms are Associated with Higher Carotid Intima-Media Thickness. Cross-Sectional Analysis from ELSA-Brasil Baseline Data. Atherosclerosis. 2015;240(2):529-34. doi: 10.1016/j.atherosclerosis.2015.04.800.
9. Webb DR, Davies MJ, Gray LJ, Abrams KR, Srinivasan B, Das S, et al. Searching for the Right Outcome? A Systematic Review and Meta-Analysis of Controlled Trials Using Carotid Intima-Media Thickness or Pulse Wave Velocity to Infer Antiatherogenic Properties of Thiazolidinediones. Diabetes Obes Metab. 2010;12(2):124-32. doi: 10.1111/j.1463-1326.2009.01122.x.
10. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of Clinical Cardiovascular Events with Carotid Intima-Media Thickness: A Systematic Review and Meta-Analysis. Circulation. 2007;115(4):459-67. doi: 10.1161/CIRCULATIONAHA.106.628875.
11. Lorenz MW, Polak JF, Kavousi M, Mathiesen EB, Völzke H, Tuomainen TP, et al. Carotid Intima-Media Thickness Progression to Predict Cardiovascular Events in the General Population (the PROG-IMT Collaborative Project): A Meta-Analysis of Individual Participant Data. Lancet. 2012;379(9831):2053-62. doi: 10.1016/S0140-6736(12)60441-3.
12. Béchard D, Scherpereel A, Hammad H, Gentina T, Tsicopoulos A, Aumercier M, et al. Human Endothelial-Cell Specific Molecule-1 Binds Directly to the Integrin CD11a/CD18 (LFA-1) and Blocks Binding to Intercellular Adhesion Molecule-1. J Immunol. 2001;167(6):3099-106. doi: 10.4049/jimmunol.167.6.3099.
13. Klisic A, Kavaric N, Vujcic S, Mihajlovic M, Zeljkovic A, Ivanisevic J, et al. Inverse Association between Serum Endocan Levels and Small LDL and HDL Particles in Patients with Type 2 Diabetes Mellitus. Eur Rev Med Pharmacol Sci. 2020;24(15):8127-35. doi: 10.26355/eurrev_202008_22499.
14. Lv Y, Zhang Y, Shi W, Liu J, Li Y, Zhou Z, et al. The Association between Endocan Levels and Subclinical Atherosclerosis in Patients With Type 2 Diabetes Mellitus. Am J Med Sci. 2017;353(5):433-38. doi: 10.1016/j.amjms.2017.02.004.
15. Belongie KJ, Ferrannini E, Johnson K, Andrade-Gordon P, Hansen MK, Petrie JR. Identification of Novel Biomarkers to Monitor □-cell Function and Enable Early Detection of Type 2 Diabetes Risk. PLoS One. 2017;12(8):e0182932. doi: 10.1371/journal.pone.0182932.
16. Klisic A, Kavaric N, Stanisic V, Vujcic S, Spasojevic-Kalimanovska V, Ninic A, et al. Endocan and a Novel Score for Dyslipidemia, Oxidative Stress and Inflammation (DOI score) are Independently Correlated with Glycated Hemoglobin (HbA1c) in Patients with Prediabetes and Type 2 Diabetes. Arch Med Sci. 2019;16(1):42-50. doi: 10.5114/aoms.2019.87541.
17. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. Diabetes Care. 2021;44(Suppl 1):S15-S33. doi: 10.2337/dc21-S002.
18. Mahat RK, Singh N, Rathore V, Gupta A, Shah RK. Relationship between Atherogenic Indices and Carotid Intima-Media Thickness in Prediabetes: A Cross-Sectional Study from Central India. Med Sci. 2018;6(3):55. doi: 10.3390/medsci6030055.
19. Bhinder HPS, Kambie TK. The Study of Carotid Intima-Media Thickness in Prediabetes and its Correlation with Cardiovascular Risk Factors. J Datta Meghe Inst Med Sci Univ. 2018;13(2):79-82. doi: 10.4103/jdmimsu.jdmimsu_58_18.
20. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-Mediated Skeletal Muscle Vasodilation is Nitric Oxide Dependent. A Novel Action of Insulin to Increase Nitric Oxide Release. J Clin Invest. 1994;94(3):1172-9. doi: 10.1172/JCI117433.
21. Montagnani M, Chen H, Barr VA, Quon MJ. Insulin-Stimulated Activation of eNOS is Independent of Ca2+ but Requires Phosphorylation by Akt at Ser(1179). J Biol Chem. 2001;276(32):30392-8. doi: 10.1074/jbc.M103702200.
22. Brunner H, Cockcroft JR, Deanfield J, Donald A, Ferrannini E, Halcox J, et al. A Statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. J Hypertens. 2005;23(2):233-46. doi: 10.1097/00004872-200502000-00001.
23. Naruse K, Shimizu K, Muramatsu M, Toki Y, Miyazaki Y, Okumura K, et al. Long-Term Inhibition of NO Synthesis Promotes Atherosclerosis in the Hypercholesterolemic Rabbit Thoracic Aorta. PGH2 Does Not Contribute to Impaired Endothelium-Dependent Relaxation. Arterioscler Thromb. 1994;14(5):746-52. doi: 10.1161/01.atv.14.5.746.
24. Balta I, Balta S, Demirkol S, Mikhailidis DP, Celik T, Akhan M, et al. Elevated Serum Levels of Endocan in Patients with Psoriasis Vulgaris: Correlations with Cardiovascular Risk and Activity of Disease. Br J Dermatol. 2013;169(5):1066-70. doi: 10.1111/bjd.12525.
25. Bazzoni F, Beutler B. The Tumor Necrosis Factor Ligand and Receptor Families. N Engl J Med. 1996;334(26):1717-25. doi: 10.1056/NEJM199606273342607.
26. Jeschke MG, Klein D, Bolder U, Einspanier R. Insulin Attenuates the Systemic Inflammatory Response in Endotoxemic rats. Endocrinology. 2004;145(9):4084-93. doi: 10.1210/en.2004-0592.
27. Tokudome T, Horio T, Yoshihara F, Suga S, Kawano Y, Kohno M, et al. Direct Effects of High Glucose and Insulin on Protein Synthesis in Cultured Cardiac Myocytes and DNA and Collagen Synthesis in Cardiac Fibroblasts. Metabolism. 2004;53(6):710-5. doi: 10.1016/j.metabol.2004.01.006.
28. Sasaoka T, Ishiki M, Sawa T, Ishihara H, Takata Y, Imamura T, et al. Comparison of the Insulin and Insulin-Like Growth Factor 1 Mitogenic Intracellular Signaling Pathways. Endocrinology. 1996;137(10):4427-34. doi: 10.1210/endo.137.10.8828504.
29. Wang CC, Goalstone ML, Draznin B. Molecular Mechanisms of Insulin Resistance that Impact Cardiovascular Biology. Diabetes. 2004;53(11):2735-40. doi: 10.2337/diabetes.53.11.2735.
30. Taniguchi CM, Emanuelli B, Kahn CR. Critical Nodes in Signalling Pathways: Insights Into Insulin Action. Nat Rev Mol Cell Biol. 2006;7(2):85-96. doi: 10.1038/nrm1837.
31. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, et al. Insulin Resistance Differentially Affects the PI 3-Kinase- and MAP Kinase-Mediated Signaling in Human Muscle. J Clin Invest. 2000;105(3):311-20. doi: 10.1172/JCI7535.

32. Brix-Christensen V, Andersen SK, Andersen R, Mengel A, Dyhr T, Andersen NT, et al. Acute Hyperinsulinemia Restrains Endotoxin-Induced Systemic Inflammatory Response: An Experimental Study in a Porcine Model. Anesthesiology. 2004;100(4):861-70. doi: 10.1097/00000542-200404000-00016.

33. Lassalle P, Molet S, Janin A, Heyden JV, Tavernier J, Fiers W, et al. ESM-1 is a Novel Human Endothelial Cell-Specific Molecule Expressed in Lung and Regulated by Cytokines. J Biol Chem. 1996;271(34):20458-64. doi: 10.1074/jbc.271.34.20458.

34. Janke J, Engeli S, Gorzelniak K, Feldpausch M, Heintze U, Böhnke J, et al. Adipose Tissue and Circulating Endothelial Cell Specific Molecule-1 in Human Obesity. Horm Metab Res. 2006;38(1):28-33. doi: 10.1055/s-2006-924973.

35. Menon P, Kocher ON, Aird WC. Endothelial Cell Specific Molecule-1 (ESM-1), a Novel Secreted Proteoglycan Stimulates Vascular Smooth Muscle Cell Proliferation and Migration. Circulation. 2011;124:A15455.

36. Lee W, Ku SK, Kim SW, Bae JS. Endocan Elicits Severe Vascular Inflammatory Responses in Vitro and In Vivo. J Cell Physiol. 2014;229(5):620-30. doi: 10.1002/jcp.24485.

37. Kose M, Emet S, Akpinar TS, Kocaaga M, Cakmak R, Akarsu M, et al. Serum Endocan Level and the Severity of Coronary Artery Disease: A Pilot Study. Angiology. 2015;66(8):727-31. doi: 10.1177/0003319714548870.

38. Guérette B, Skuk D, Célestin F, Huard C, Tardif F, Asselin I, et al. Prevention by Anti-LFA-1 of Acute Myoblast Death Following Transplantation. J Immunol. 1997;159(5):2522-31.

39. Tajra LC, Martin X, Margonari J, Blanc-Brunat N, Ishibashi M, Vivier G, Panaye G, et al. In Vivo Effects of Monoclonal Antibodies Against Rat Beta(2) Integrins on Kidney Ischemia-Reperfusion Injury. J Surg Res. 1999;87(1):32-8. doi: 10.1006/jsre.1999.5724.

40. Arai K, Sunamura M, Wada Y, Takahashi M, Kobari M, Kato K, et al. Preventing Effect of Anti-ICAM-1 and Anti-LFA-1 Monoclonal Antibodies on Murine Islet Allograft Rejection. Int J Pancreatol. 1999;26(1):23-31. doi: 10.1385/IJGC:26:1:23.

41. Suzuki J, Isobe M, Izawa A, Takahashi W, Yamazaki S, Okubo Y, et al. Differential Th1 and Th2 cell Regulation of Murine Cardiac Allograft Acceptance by Blocking Cell Adhesion of ICAM-1/LFA-1 and VCAM-1/VLA-4. Transpl Immunol. 1999;7(1):65-72. doi: 10.1016/s0966-3274(99)80021-5.

42. Isobe M, Suzuki J, Yamazaki S, Yazaki Y, Horie S, Okubo Y, et al. Regulation by Differential Development of Th1 and Th2 Cells in Peripheral Tolerance to Cardiac Allograft Induced by Blocking ICAM-1/LFA-1 Adhesion. Circulation. 1997;96(7):2247-53. doi: 10.1161/01.cir.96.7.2247.

43. Gómez Rosso L, Benítez MB, Fornari MC, Berardi V, Lynch S, Schreier L, et al. Alterations in Cell Adhesion Molecules and other Biomarkers of Cardiovascular Disease in Patients with Metabolic Syndrome. Atherosclerosis. 2008;199(2):415-23. doi: 10.1016/j.atherosclerosis.2007.11.007.

44. Poston RN, Haskard DO, Coucher JR, Gall NP, Johnson-Tidey RR. Expression of Intercellular Adhesion Molecule-1 in Atherosclerotic Plaques. Am J Pathol. 1992 Mar;140(3):665-73.

Endothelial Biomarkers and Translational Medicine: Still a Challenge

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Short Editorial related to the article: Can the Serum Endocan Level Be Used as a Biomarker to Predict Subclinical Atherosclerosis in Patients with Prediabetes?

The article "Can the Serum Endocan Level Be Used as a Biomarker to Predict Subclinical Atherosclerosis in Patients with Prediabetes?"[1] addressed a topic of the greatest relevance and originality, the diagnostic investigation of subclinical atherosclerosis in pre-diabetic patients. Therefore, the study evaluated the endothelial cell-specific molecule-1 (ESM-1) endocan concentration in pre-diabetic patients to verify the role of serum endocan levels in detecting subclinical atherosclerosis, aided by measuring the intima-media layer of the carotid arteries thickness (IMT).

Due to the global epidemic of obesity, there is an increasing number of patients diagnosed with type 2 diabetes. However, preceding this morbidity, an even greater contingent of individuals is hidden, those in the pre-diabetic or insulin resistance phase.

It is known that insulin resistance increases cardiovascular risk, as it is related to a worse lipid profile, pro-inflammatory state, and endothelial dysfunction. Expressive endothelial changes occur, increasing from the expression of inflammatory markers.[2]

On the other hand, adding tools that contribute to the early detection of these biomarkers in this population is essential to try to change the disease evolution. Among them, the IMT measurement has become one of the main assessment and diagnosis methods. Moreover, in addition to being an easy-to-perform test, it directly correlates with early endothelial alteration in atherosclerotic disease.[3-6]

Endocan, in turn, is a proteoglycan released by endothelial cells from inflammatory cytokines, which would regulate the inflammatory process. It is closely linked to endothelial injury. It has been shown that serum endocan levels would be higher in diabetic patients and acute coronary syndrome.[7,8]

However, intriguing results indicated that the serum levels of endocan would be low or unchanged in pre-diabetic patients,[9,10] in contrast to the IMT values, which would be higher. In addition, this population appeared to have no correlation between IMT values and serum endocan levels. When evaluating pre-diabetic and normoglycemic groups of patients, there was a correlation between IMT and serum endocan in normoglycemic patients but not in pre-diabetic patients.

Thus, serum endocan levels would be low in pre-diabetic patients. This result would probably occur due to the hyperinsulinemic state of the patients. It is difficult to distinguish the effect of insulin resistance from compensatory hyperinsulinemia.[1] Hyperinsulinemia signaling would act in the regulation of nitric oxide production.[11,12] In addition to its role in atherosclerotic mechanisms, it would attenuate the systemic inflammatory response induced by endotoxins, decreasing the expression of TNF-α and increasing the anti-inflammatory cascade.[13,14] The reducing effect of hyperinsulinemia on TNF-α levels would explain the decrease in serum endocan levels, which are secreted by TNF-α and interleukin-1 beta (IL-1β).[15]

In summary, the study showed that endocan levels decreased in pre-diabetic patients, and this result would likely be related to hyperinsulinemia in this population. However, despite serum endocan levels being normal, subclinical atherosclerosis in the group of patients cannot be ruled out since this same population presented changes in IMT. Furthermore, the fact that the body mass index values were similar between the two groups evaluated could have contributed to the above finding.

However, the authors recognize some limitations: the small number of included patients, the work was carried out in a single center, and the lack of knowledge of the time when these patients would be in the pre-diabetic phase could have influenced the results. I would add to this list the dosage of CRPhs. Perhaps this marker could add some information to the study setting.

More robust studies in more diversified populations would be necessary to understand this biomarker better.

For now, the use of endocan proteoglycan in the stratification of cardiovascular risk for patients undergoing primary prevention but at high and intermediate risk may explore this gap not yet fully understood in the arterial endothelium. If so, we will increasingly evolve on the frontiers of translational medicine.

The authors should be congratulated for taking the initiative to explore such a conflicting subject!

Keywords

Atherosclerosis; Prediabetic State; Insulin Resistance; Diabetes Mellitus; Carotid Intima-Media Thickness; Obesity.

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References

1. ArmanY, Atici A, Altun O, Sarikaya R, Yoldemir A, Akarsu M, et al. O nível de endocan sérico pode ser usadocomo biomarcador para prever aterosclerose subclínica em pacientes pré-diabéticos. Can the Serum Endocan Level Be Used as a Biomarker to Predict Subclinical Atherosclerosis in Patients with Prediabetes? Arq Bras Cardiol. 2022; 119(4):544-550.
2. Park C, Guallar E, Linton JA, Lee DC, Jang Y, Son DK, et al. Fasting Glucose Level and the Risk of Incident Atherosclerotic Cardiovascular Diseases. Diabetes Care. 2013;36(7):1988-93. doi: 10.2337/dc12-1577.
3. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of Carotid Ultrasound to Identify Subclinical Vascular Disease and Evaluate Cardiovascular Disease Risk: A Consensus Statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr. 2008;21(2):93-111. doi: 10.1016/j.echo.2007.11.011.
4. Santos IS, Goulart AC, Brunoni AR, Kemp AH, Lotufo PA, Bensenor IM. Anxiety and Depressive Symptoms are Associated with Higher Carotid Intima-Media Thickness. Cross-Sectional Analysis from ELSA-Brasil Baseline Data. Atherosclerosis. 2015;240(2):529-34. doi: 10.1016/j.atherosclerosis.2015.04.800.
5. Webb DR, Davies MJ, Gray LJ, Abrams KR, Srinivasan B, Das S, et al. Searching for the Right Outcome? A Systematic Review and Meta-Analysis of Controlled Trials Using Carotid Intima-Media Thickness or Pulse Wave Velocity to Infer Antiatherogenic Properties of Thiazolidinediones. Diabetes Obes Metab. 2010;12(2):124-32. doi: 10.1111/j.1463-1326.2009.01122.x.
6. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of Clinical Cardiovascular Events with Carotid Intima-Media Thickness: A Systematic Review and Meta-Analysis. Circulation. 2007;115(4):459-67. doi: 10.1161/ CIRCULATION AHA.106.628875.
7. Klisic A, Kavaric N, Vujcic S, Mihajlovic M, Zeljkovic A, Ivanisevic J, et al. Inverse Association between Serum Endocan Levels and Small LDL and HDL Particles in Patients with Type 2 Diabetes Mellitus. Eur Rev Med Pharmacol S Sci. 2020;24(15):8127 35.doi:10.26355/eurrev_202008_22499.
8. Lv Y, Zhang Y, Shi W, Liu J, Li Y, Zhou Z, et al. The Association between Endocan Levels and Subclinical Atherosclerosis in Patients With Type 2 Diabetes Mellitus. Am J Med Sci. 2017;353(5):433-8. doi:10.1016/j.amjms.2017.02.004.
9. Belongie KJ, Ferrannini E, Johnson K, Andrade-Gordon P, Hansen MK, Petrie JR. Identification of Novel Biomarkers to Monitor β-cell Function and Enable Early Detection of Type 2 Diabetes Risk. PLoS One. 2017;12(8):e0182932. doi: 10.1371/journal.pone.0182932.
10. Klisic A, Kavaric N, Stanisic V, Vujcic S, Spasojevic-Kalimanovska V, Ninic A, et al. Endocan and a Novel Score for Dyslipidemia, Oxidative Stress and Inflammation (DOI score) are Independently Correlated with Glycated Hemoglobin (HbA1c) in Patients with Prediabetes and Type 2 Diabetes. Arch Med Sci. 2019;16(1):42-50. doi: 10.5114/aoms.2019.87541.
11. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-Mediated Skeletal Muscle Vasodilation is Nitric Oxide Dependent. A Novel Action of Insulin to Increase Nitric Oxide Release. J Clin Invest. 1994;94(3):1172-9. doi: 10.1172/ JCI117433.
12. Montagnani M, Chen H, Barr VA, Quon MJ. Insulin-Stimulated Activation of eNOS is Independent of Ca2+ but Requires Phosphorylation by Akt at Ser(1179). J Biol Chem.2001;276(32):30392-8. doi:10.1074/jbc.M103702200.
13. Jeschke MG, Klein D, Bolder U, Einspanier R. Insulin Attenuates the Systemic Inflammatory Response in Endotoxemic rats. Endocrinology. 2004;145(9):4084- 93. doi: 10.1210/en.2004-0592.
14. Brix-Christensen V, Andersen SK, Andersen R, Mengel A, Dyhr T, Andersen NT, et al. Acute Hyperinsulinemia Restrains Endotoxin-Induced Systemic Inflammatory Response: An Experimental Study in a Porcine Model. Anesthesiology. 2004;100(4):861-70. doi: 10.1097/00000542-200404000-00016.
15. Lassalle P, Molet S, Janin A, Heyden JV, Tavernier J, Fiers W, et al. ESM-1 is a Novel Human Endothelial Cell-Specific Molecule Expressed in Lung and Regulated by Cytokines. J Biol Chem. 1996;271(34):20458-64. doi: 10.1074/jbc.271.34.20458.

Acute Effects of Energy Drink on Autonomic and Cardiovascular Parameters Recovery in Individuals with Different Cardiorespiratory Fitness: A Randomized, Crossover, Double-Blind and Placebo-Controlled Trial

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Abstract

Background: **It has been suggested that the consumption of energy drinks (ED) may affect cardiovascular activity.**

Objectives: **to investigate the acute effects of ED intake on heart rate variability (HRV) and cardiovascular recovery after moderate aerobic exercise in males with different cardiorespiratory capacities.**

Methods: **This is a randomized, double-blind, crossover, placebo-controlled study. Twenty-eight young adults were split into two groups according to their peak oxygen consumption (VO2peak) values: (1) High VO2 peak (HO) - VO2 peak > 52.15 mL/kg/min, and (2) low VO2 peak (LO) - peak VO2 <52.15 mL/kg/min. Subjects of both groups underwent two exercise protocols in randomized order: moderate aerobic exercise (60% of VO2peak) following the intake of 250 mL of water (placebo protocol) or 250 mL of ED (ED protocol). During the exercise tests, values of cardiorespiratory and HRV parameters were recorded.**

Results: **Significant differences were observed for the LF (normalized units) index between rest and Rec1 in HO energy and LO groups during the ED protocol. For the LF/HF ratio, significant differences were seen between rest and Rec1 in HO and LO during ED protocols.**

Conclusion: **Acute ED intake delayed heart rate recovery after exercise in subjects with low and high cardiorespiratory fitness.**

Keywords: **Energy Drinks; Dietary Supplements; Exercise; Autonomic Nervous System; Cardiovascular system.**

Introduction

Energy drinks (EDs) are widely consumed in the sport environment to improve alertness and performance, and their use is mainly attributed to their caffeine content.[1,2] According to the International Olympic Committee[3] and the International Society of Sports Nutrition,[4] caffeine is considered an ergogenic supplement capable of increasing physical performance during exercise.[3,4] There is conjecture that other ED components (e.g., vitamins and minerals) have synergism with caffeine and taurine, and thereby may potentiate their effects. However, these issues have not been fully elucidated.[5]

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Numerous studies have been performed to better understand the potential effects of EDs on the cardiovascular system.[6] So far, it has been found that a modest consumption of EDs, corresponding to 200mg of caffeine, poses no risk to the cardiovascular health. Nevertheless, the acute consumption of approximately 1,000mL of ED was associated with an increase in adverse cardiovascular effects (e.g., prolonged QT interval and tachycardias).[6,7]

Still, the scientific research literature has highlighted that stimulants may increase the risk of adverse cardiac events during and after exercise.[7] Heart rate (HR) slowing after exercise has been demonstrated to be an important predictor of adverse cardiac events and mortality.[8] Its analysis has been increasingly used as a non-invasive, yet reliable technique to study the adaptation of the autonomic nervous system (ANS) (vagal reactivation) to various conditions.[9]

HR variability (HRV) evaluates the fluctuations in the intervals between consecutive heartbeats (RR intervals), which reflects the ANS function.[9] Physically active and healthy subjects show rapid HR recovery from exercise, which allows adequate ANS adaptation and low cardiovascular risk.[10] Thus, the use of

compounds that delay the post-exercise autonomic recovery can lead to cardiac activity overload, thereby disrupting the autonomic control of HR.[10]

Scientific evidence has shown that the moderate consumption of caffeine alone (e.g., 3-6mg/kg or 300-400mg in a single dose) is permissible to delay HR recovery following exercise.[11,12] Recently, it has been documented that caffeine has greater effects on individuals with a low cardiorespiratory capacity, measured by the maximum oxygen consumption (VO2max), concerning post-exercise HR recovery.[13]

So far, studies that evaluated the effects of EDs on HR recovery have not compared them between populations with different cardiorespiratory profiles.[14-17] A modest dose of approximately 250mL of ED seems to have no effect on HR recovery after exercise in trained individuals.[14-16] Even so, no study has considered the individuals' cardiorespiratory capacity and, hence, there is still a gap in the literature.

Therefore, this study aimed to evaluate the acute effects of ED intake on HR and cardiovascular recovery after moderate aerobic exercise in young male adults with different cardiorespiratory capacities. Participants were divided according to their peak oxygen consumption (VO2 peak).[18]

Methods

This study was reported according to the Consolidated Standards of Reporting Trials (CONSORT) statement. This is a crossover, randomized, double-blind and placebo-controlled trial. The study was evaluated and approved by UNIFESP ethics committee (registration number: CEP-2200/11). All participants signed an informed consent agreeing to participate in the study. The details of the experimental protocols are registered in the Clinical Trials.gov (first publication – September 28, 2016) (Protocol NCT02917889, https://clinicaltrials.gov/ct2/show/NCT02917889).

Participants

The study was performed with healthy and physically active young adult males recruited via social media. We excluded subjects who were not considered physically active according to the International Physical Activity Questionnaire (IPAQ).[18]

Initial assessment

The individuals were first interviewed to obtain data such as: age (years), body weight (Kg), height (cm), and body mass index (Kg/m^2). Anthropometric measures were taken according to previously published recommendations.[19]

Interventions

The experimental protocol consisted of three phases with an interval of at least 48 hours to allow adequate recovery of the subjects.

The study was performed between 17:30 and 21:30 to standardize circadian variations, in a quiet room with humidity between 60% and 70% and temperature between 23°C and 24°C.[20] The subjects were told to refrain from drinking alcohol or performing exhaustive exercise 24 hours prior to each section and to avoid ingesting caffeinated beverages or foods 24 hours before the experimental procedure. Subjects were advised to wear comfortable clothes that are appropriate to exercise, and to eat a light meal two hours before the procedures.

Following recommendations from the American College of Sports Medicine (ACSM),[21] to avoid dehydration of the participants during the exercise,[22] participants were instructed to drink 500 mL of water two hours prior to the sessions.

In the first phase of the study, VO2max of each participant was determined. In the second phase, the subjects followed the placebo protocol (250mL water) or the ED protocol (250 mL ED) 15 minutes before the exercise. In the third phase, participants followed the alternative protocol to the previous stage. An independent researcher who did not participate in the study data collection provided the drinks. Both researchers and subjects were blinded to the sequence of interventions.

The ED (250mL) had an energy content of 45 kcal and was composed of 11.2 g of carbohydrates, 80 mg of sodium, 32 mg of caffeine, 400 mg of taurine, 4.6 mg of niacin, 2 mg of pantothenic acid, 0.5 mg of vitamin B6, 0.4 mg of vitamin B12, 240 mg of glucuronolactone, and 20 mg of inositol.[16]

The intensity of aerobic exercise in all stages was prescribed based on the VO2max of each participant. The treadmill test had a total duration of 30 minutes. First, the subjects walked on a treadmill at a speed of 5Km/h for five minutes of warm-up; the speed was increased to the corresponding 60% of VO2max for 25 minutes. Then, the subjects rested in the supine position for 60 minutes (recovery period).

Cardiorespiratory variables

The test to determine the VO2max was performed on a treadmill (TPEE; Inbrasport ATL 2000) using the Bruce protocol.[23] The subjects remained at rest on the treadmill in an orthostatic position for stabilization of baseline cardiovascular values. Then, the stress test was initiated, with progressive increase in the workload by means of increased inclination and speed of the treadmill every three minutes. Verbal encouragement was given in an attempt to obtain maximum physical effort. The test was interrupted because of exhaustion or any clinical and/or electrocardiographic abnormality.

During the test, HR and subjective perception of effort were monitored at the end of each stage by the Borg Scale for perceived pain and effort.[24] For the test to be recognized as maximum, subjects should attain 90% of maximum HR, earlier estimated (220 - age).[25]

The analysis of expired gases was conducted using the Quark PFT commercial system (Comend, Rome, Italy), and the VO2peak was defined as the highest VO2max attained during the test.

The subjects were split into two groups based on the median VO2 peak value:

(1) Higher VO2 peak (HO) group, composed of subjects with peak VO2 > 52.15 mL/Kg/min, and

(2) Lower VO2 peak (LO) group, composed of subjects with peak VO2 <52.15 mL/Kg/min.

Cardiovascular parameters

Cardiovascular parameters were measured with subjects in the supine position. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) measures were taken by auscultation with a stethoscope (Littman Classic II®, St. Paul, USA) and a calibrated aneroid sphygmomanometer (Welch Allyn Tycos®, New York, USA) on the individuals' left arm. HR was measured using a Polar RS800CX® HR monitor. Respiratory rate (RR) was determined by counting the subjects' breaths for one minute, without the subjects being aware of it, so that no change in the breathing pattern occurred. Oxygen saturation (SpO2) was measured by pulse oximetry (PM-50 Mindray®, China).

HRV Analysis

HRV was measured according to the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.[26] The sensor chest strap was worn on the chest the Polar RS800CX heart rate receiver was placed on the left wrist. The HRV pattern was recorded beat by beat. The final 256 consecutive stable RR intervals of each recording were selected. Then, digital and manual filtrations were performed to eliminate artifacts and premature ectopic beats. Only series with an excess of 95% of sinus beats were included in the analysis.

The time-domain index of HRV was determined by the root mean square of successive differences (RMSSD) and standard deviation of the normalized N–N interval (SDNN). The frequency-domain index was evaluated by the high-frequency (HF) (0.15 to 0.4 Hz) and low-frequency components (LF) of the power spectral density (0.04 to 0.15 Hz) in milliseconds squared and absolute units, and ratio LF/HF (ms^2). The Poincaré plot analysis was made using the SD1 (standard deviation of the instantaneous beat-to-beat variability) and SD2 (standard deviation of long-term continuous beat-to-beat variability).

The Kubios HRV® analysis software package was used to compute these indices.

Measurement of parameters

HR, RR, SBP, DBP and SpO2 were recorded at the following time points: rest – 15th minute after ED and placebo ingestion – and during recovery - 1st, 3rd, 5th, 7th, 10th, 20th, 30th, 40th, 50th and 60th minutes after exercise.

The HRV indexes were measured at the following time points: "rest" (15 to 20 minutes of resting after EB or placebo ingestion; and during "recovery": Rec1 (zero to five minutes), Rec2 (five to ten minutes), Rec3 (15 to 20 minutes), Rec4 (25 to 30 minutes), Rec5 (35 to 40 minutes), Rec6 (45 to 50 minutes), and Rec7 (55 to 60 minutes).

Sample size

The sample size was calculated based on a previous study,[22] which gave us the magnitude of the difference, and we calculated the RMSSD index as a reference. We determined a standard deviation of 16.2ms and the magnitude of the difference was 11ms. A minimum sample size of 14 subjects per group was calculated, with an alpha risk of 5% and beta risk of 80%.

Statistical analysis

Data analysis and data reporting were conducted following the recommendations of Laborde *et al*.[27] Data normality was tested by the Shapiro-Wilk test. To compare cardiovascular variables and HRV, we performed the repeated-measures analysis of variance (ANOVA), followed by the Bonferroni post-test for parametric distributions or Friedman followed by Dunn's post-test for non-parametric distributions. P-values <0.05 were considered significant. The analyses were performed using the IBM SPSS Statistics software version 22.0 (SPSS Inc., Chicago, IL, USA).

Randomization and outcome assessment

With the aim of minimizing the selection bias, the subjects and the researchers were uninformed about the sequence of procedures. An investigator who did not participate in the study randomly assigned the participants the interventions. Researchers specialized in the field who did not participate in the collections were invited to assess the outcome. So, the outcome evaluators were blinded, allowing the study to be less susceptible to detection bias. Also, all outcomes were reported in full, decreasing the likelihood of reporting bias.

Results

Thirty-five men were considered eligible for the study; 28 met the inclusion criteria and completed the study (Figure 1).

Table 1 describes the anthropometric characteristics and the responses obtained in the maximum effort test for the groups with the highest VO2peak (HO), and with the lowest VO2peak (LO).

In relation to HRV frequency-domain and HRV indexes, we detected a time effect ($p=0.0001$). No protocol interaction effect was seen for LF (normalized units, n.u.) ($p=0.880$), HF (n.u.) ($p=0.163$) and LF/HF ms^2 ($p=0.086$) indexes. No protocol effect was observed for LF (n.u.) ($p=1.000$), HF (n.u.) ($p=0.675$) and LF/HF ($p=0.531$). For LF (n.u,) index significant differences were achieved between rest and Rec1 in HO and LO in the ED protocols. There were significant differences in HF (n.u.) between rest and Rec1 for HO in the placebo protocol, and HO and LO during the ED protocol. Regarding the LF/HF ratio, significant differences were found between rest and Rec1 in HO and LO during the ED protocol. The response of frequency-domain HRV indexes are shown in Figure 2.

SDNN and SD2 indices showed significant differences in the time effects (SDNN: $p=0.0001$; SD2: $p=0.0001$) and protocol interaction (SDNN: $p<0.0001$; SD2: $p=0.0002$), and only SDNN showed differences between protocols (SDNN: $p=0.015$; SD2 $p=0.061$). Significant differences in SDNN index were seen between rest and Rec1 in the LO group during the control protocol, and in RMSSD between Rest and Rec1 in both placebo and ED protocols.

Regarding RMSSD and SD1 indexes we detected significant differences in time effects (RMSSD: $p<0.0001$; SD1: $p<0.0001$), protocol interaction (RMSSD: $p=0.009$; SD1: $p=0.036$) and between protocols (RMSSD: $p=0.025$; SD1$=0.010$). Significant changes for the time domain were observed between rest and Rec1 for RMSSD index and SD1 index for all protocols. Significant differences for the time domain were found between rest and Rec2 in the HO group

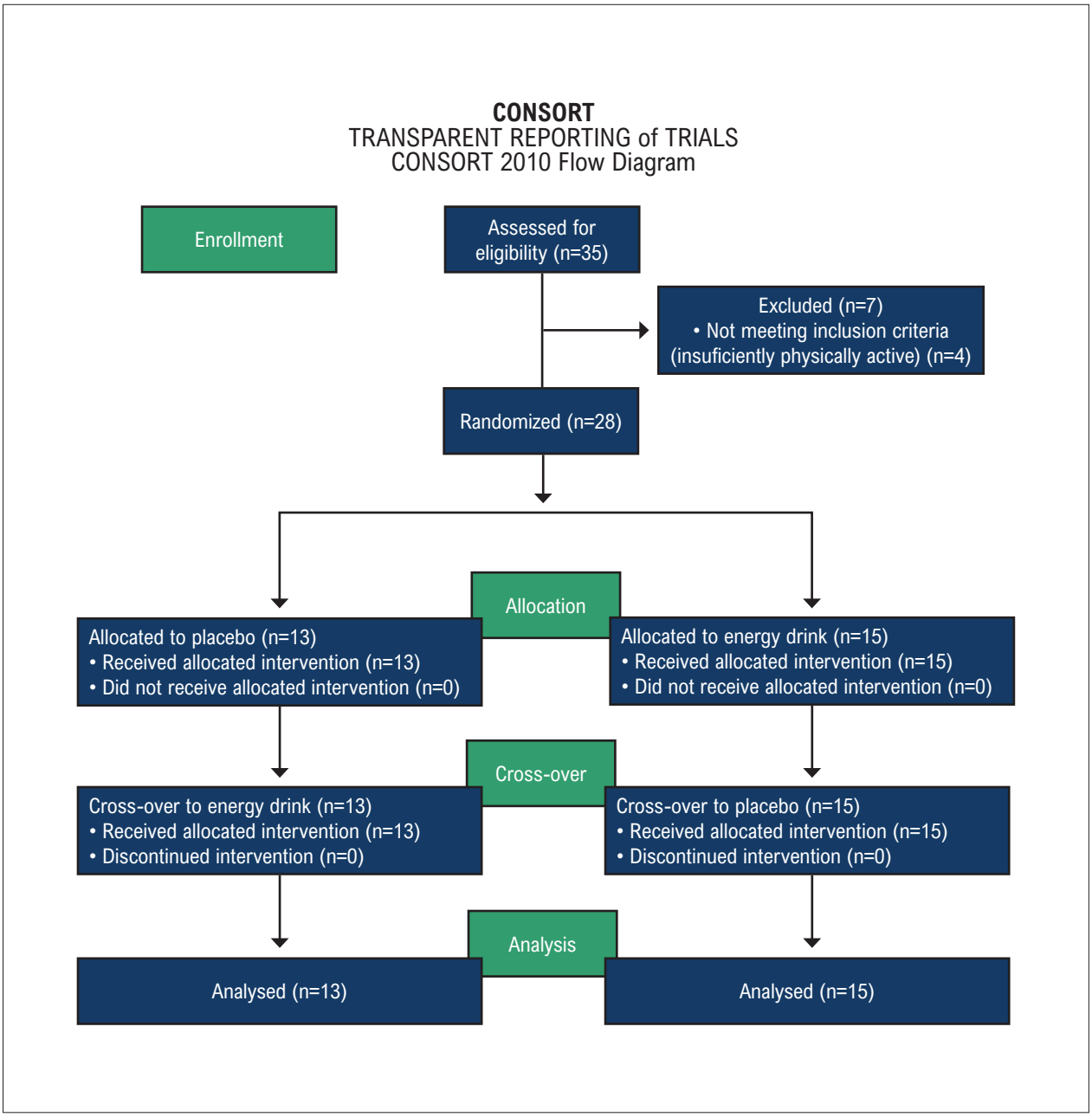

Figure 1 – *The CONSORT flow diagram.*

Table 1 – Anthropometric characteristics and VO2peak values of the study subjects

	High VO2 peak		Low VO2 peak	
	Mean ± SD	Min - Máx	Mean ± SD	Min - Máx
Age (y)	22.93 ± 2.62	[18 - 26]	25.29 ± 3.07	[21 - 29]
Height (m)	1.78 ± 0.08	[1.68 - 1.94]	1.81 ± 12.52	[1.65 – 1.93]
Mass (kg)	77.55 ± 6.92	[60 - 96]	89.48 ± 12.52	[63.30 – 107.50]
BMI (kg/m²)	24.46 ± 2.56	[20.05 - 29.41]	27.12 ± 3.07	[19.94 - 27.70]
$VO2_{peak}$ (ml/kg/min)	60.14 ± 6.43	[52.40 - 77.77]	41.76 ± 10.14	[23.03 – 29.94]

Y: years; m: meters; kg: kilogram; BMI: body mass index.

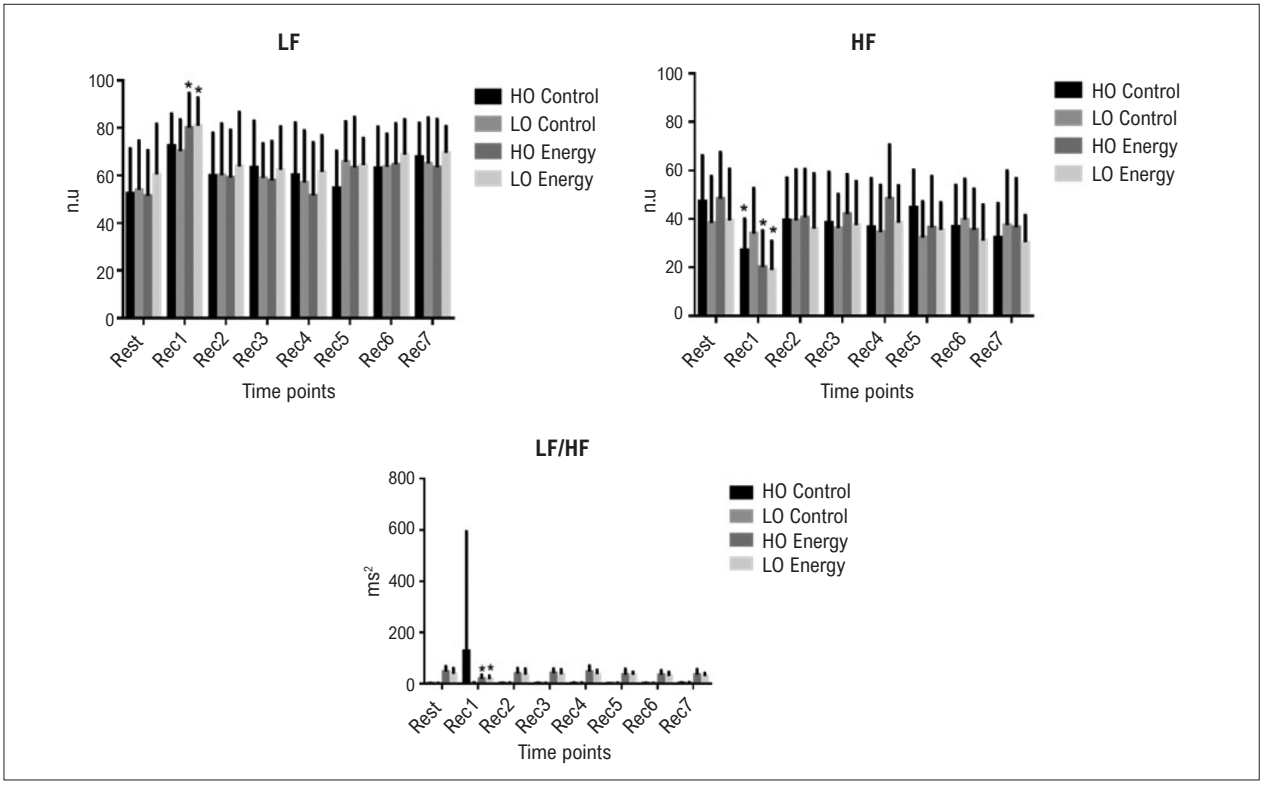

Figure 2 – *Response of the frequency-domain heart rate variability indices at rest and during recovery from exercise in the groups of subjects with high VO2 peak (HO) and low VO2 peak (LO) receiving energy drink (energy) or placebo (Control).*

in the control protocol and in the LO group in the ED protocol for SD1 index. Figure 3 displays the response of time-domain HRV indexes at rest and during recovery from exercise.

In relation to cardiorespiratory parameters, we observed a time effect (p=0.0001) for HR, RF, SBP, DBP (p=0.0001) and no effect was observed in SpO2 (p=0.188). No significant protocol interaction effect was seen for SBP, DBP, RF or SpO2 (SBP: p=0.424; DBP: p=0.259; RF: p=0.340; SpO2: p=0.346), but a significant effect was seen for HR (p<0.0001). Significant differences were achieved between protocols for SBP, DBP and HR (SBP: p=0.001; DBP: p=0.014; HR: p=0.011) and no difference was found for RF and SpO2 (RF: p=0.132; SpO2: p=0.083). Significant differences in HR and SBP were seen in the time domain between rest and Rec1 for all protocols. Figure 4 displays the response of cardiorespiratory parameters at rest and during recovery from exercise.

Discussion

Our study was undertaken to evaluate the impact of ED on HRV and cardiovascular recovery after exercise in individuals with different cardiorespiratory fitness. As key findings, we reported that ED before exercise did not influence SBP, DBP, SpO2 or RF in the post-exercise recovery, and delayed the LF and LF/HF recovery following effort.

Constituents such as caffeine, taurine, glucuronolactone, B vitamins, guarana, ginseng, ginkgo biloba, l-carnitine, sugars, antioxidants, and trace elements are usually found in EDs.[28] Caffeine stimulates the central nervous system via the activation of the sympathetic adrenal-medullary system, raising blood pressure in situations of psychological[29] and physiological stress, for instance physical exercise.[30,31]

Cardiovascular adjustments are required to maintain adequate perfusion to other organs.[32] When the exercise begins, the central command resets the levels of the arterial baroreflex, resulting in lessened parasympathetic conduction, and light reduction in the ANS activity because of the venous return in this first phase.[33]

The upsurge in reflexive amplitude via the early increase in HR is caused by the increased load on pulmonary baroreceptors, which allows the parasympathetic nervous system to cut its cardiac activity. As the workload increases, the central command increases and readapts the arterial baroreflex. Therefore, there is a depression of the parasympathetic reflex response, increase in the sympathetic nervous system, increasing HR and cardiac contraction strength.[34]

There are reports in the scientific literature that indicate an intimate connection between ED and changes in the cardiovascular system. ED depresses the parasympathetic nervous system and/or increases the sympathetic nervous system in obese young people,[35] increases SBP,[36] changes nonlinear HRV in young adults,[37] and delays HR and HRV post-exercise recovery when mixed with alcohol.

Recently, our group reported that ED is unable to postpone the HR recovery after exercise.[15] In the cited study, 29 healthy

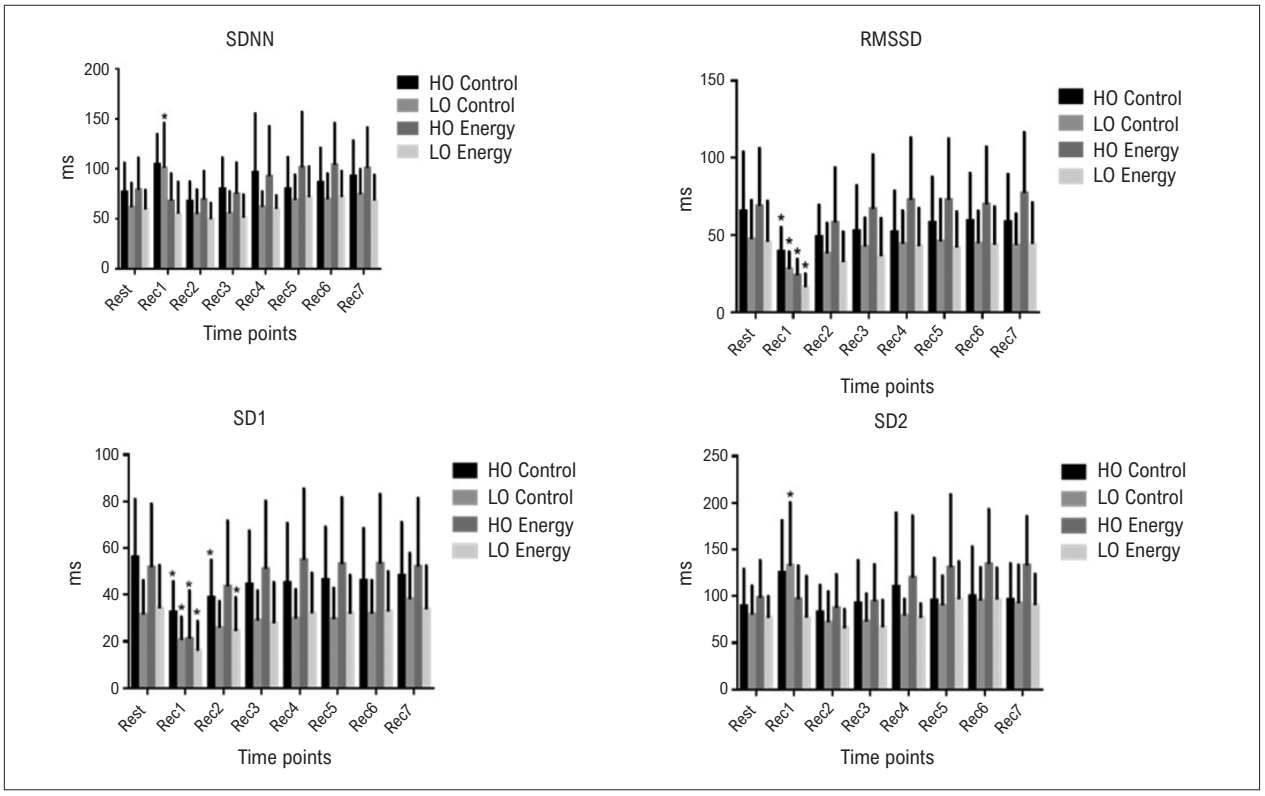

Figure 3 – *Time-domain heart rate variability indexes at rest and during recovery from exercise in the groups of subjects with high VO2 peak (HO) and low VO2 peak (LO) receiving energy drink (energy) or placebo (Control)*

men between 18 and 30 years old performed aerobic exercise after consuming ED or placebo. There was an important reduction in HRV in the initial five minutes after exercise in both protocols. So, the main conclusion was that ED was unable to influence post-exercise HR recovery.[15] In another study with similar protocols, An *et al.*[16] found no significant fluctuations in these parameters between the interventions, suggesting no significant effect of ED.

In another randomized, crossover, placebo-controlled clinical trial, 15 (eight men) young adults who were physically active were evaluated for the effects of ED.[17] After fasting for eight hours, they consumed standardized ED (2mg/kg of caffeine) or placebo with a similar taste.[17] After submaximal aerobic exercise for 30 minutes, these individuals were induced to fatigue by pedaling 10 minutes at 80% of the ventilatory threshold. Resting HR was higher when subjects consumed ED, when compared to placebo (ED: 65+10bpm vs. Placebo: 58+8bpm, p=0.02), but HRV indices (RMSSD, SDNN, PNN50, HF, LF and LF/HF) were unchanged.[17]

In the double-blind, crossover, counterbalanced and placebo-controlled study by Clark et al.[14] 17 (10 women) young adults were exposed to a graded test of exhaustion on an exercise bike after ingestion of 140mg of caffeine or placebo. HRV data were recorded before, during and after 15 minutes of physical exercise. Substantial increases in HF and RMSSD indices were detected in the ED group during exercise. A sub analysis between genders demonstrated changes in the initial RMSSD values and in the amount of decline. The consumption of ED was able to sex-dependently affect cardiac autonomic responses during low-, moderate- and high-intensity exercise. However, in the post-exercise, no differences were found in HR recovery after ED ingestion.

It is crucial to emphasize that these research studies did not take into account the cardiorespiratory capacity of the subjects. A more recent study[13] evaluated the impact of caffeine on post-exercise HR recovery in men with different VO2. The authors split young male adults into two groups based on their VO2: (1) high VO2 (HO): 16 volunteers, peak VO2> 42.46 mL/kg/min; and (2) low VO2 (LO): 16 individuals, VO2 <42.46 mL/kg/min). The subjects participated in two intervention protocols, with ingestion of capsules containing 300mg of starch (placebo protocol) or 300 mg of caffeine (caffeine protocol). After the ingestion of caffeine or placebo, participants rested for 15 minutes, and then were submitted to 30 minutes of exercise on a treadmill at 60% of VO2 peak. HRV indices in the time and frequency domains disclosed significant changes for the RMSSD and SDNN indices in the recovery between groups (p<0.001). Remarkable adjustments were observed (rest *versus* recovery) from the 0 to the 5th minute of recovery from exercise for the LO group in the placebo protocol and from the 5th minute to the 10th minute of recovery for the LO in the caffeine protocol. In our study, significant deviations were detected only in the first five minutes of recovery in the HO individuals in both protocols. These data corroborate that caffeine delays parasympathetic recovery from exercise in individuals with lower cardiorespiratory capacity.[13]

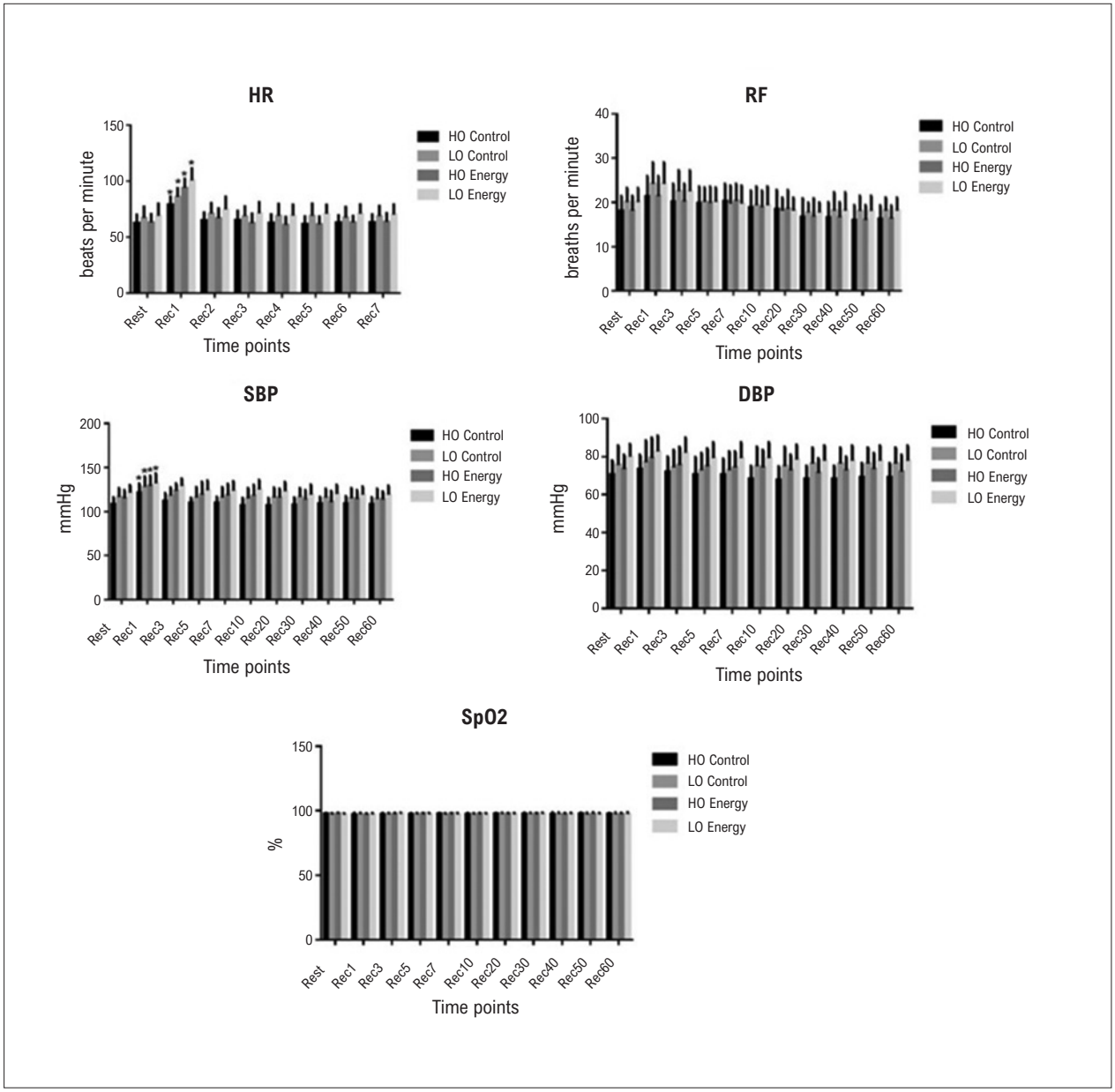

Figure 4 – *Cardiorespiratory parameters at rest and during recovery from exercise in the groups of subjects with high VO2 peak (HO) and low VO2 peak (LO) receiving energy drink (energy) or placebo (Control). HR: high frequency; RF: Respiratory frequency.; SBP: systolic blood pressure; DBP: diastolic blood pressure.*

Concerning the cardiorespiratory parameters, no significant changes were found that would suggest different effects of ED in individuals with different cardiorespiratory capacities. These findings corroborate the study conducted by An et al.,[16] where no significant changes were revealed in HR and blood pressure during recovery after maximum exercise, after ingestion of ED in different concentrations (1.25 to 2.5 mg/Kg).

It has been suggested that the effect of ED on the cardiovascular system may be dose-related. In the study by Shah et al.,[35] consumption of ED in high doses (32 ounces, equivalent to 946.3 mL) resulted in a significant and prolonged increase in the QTc interval, SBP and DBP as compared with placebo in healthy young people.

Regarding parameters that reflect the respiratory component, for instance SpO2 and RF, no significant differences were found in our study. In both protocols, all individuals showed adequate values of these variables, as would be expected for healthy subjects without recognized cardiopulmonary diseases.[11]

Finally, considering that we detected slightly delayed HR recovery in both groups that ingested ED, our data draws attention to subjects with cardiovascular and metabolic diseases who consume EDs (as a supplement) before exercise.

Strengths and limitations of the study

One of the strengths of this study concerns is methodology. Although we did not assess plasma catecholamine concentrations or sympathetic nerve activity, we evaluated HRV, a simple, reliable, non-invasive method and a significant quantitative marker for estimating autonomic HR modulation.[9] The sample was comprised of healthy young people, with the aim of avoiding the influence of sex hormones. For this reason, our results cannot be applied to females or subjects taking medications that could that affect the ANS. Nonetheless, the study design and performance of rigorous procedures to avoid selection, detection, attrition and reporting biases support our results. Our study provides relevant information about the mechanisms linked to the impact of ED on post-exercise recovery.

Conclusions

Acute ED intake delayed HR recovery following exercise in subjects with low and high cardiorespiratory fitness.

Author Contributions

Conception and design of the research: Porto AA, Ferreira C, Valenti VE; Acquisition of data: Porto AA, Gonzaga LA, Vanderlei LCM; Analysis and interpretation of the data: Benjamim CJR, Vanderlei LCM; Statistical analysis: Gonzaga LA; Writing of the manuscript: Porto AA, Gonzaga LA, Benjamim CJR, Bueno Jr. CR, Garner DM, Valenti VE; Critical revision of the manuscript for intellectual content: Bueno Jr. CR, Garner DM, Vanderlei LCM, Ferreira C, Valenti VE.

Potential Conflict of Interest

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

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Study Association

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

References

1. van Dam RM, Hu FB, Willett WC. Coffee, Caffeine, and Health. N Engl J Med. 2020;383(4):369-78. doi: 10.1056/NEJMra1816604.
2. Salinero JJ, Lara B, Abian-Vicen J, Gonzalez-Millán C, Areces F, Gallo-Salazar C, et al. The Use of Energy Drinks in Sport: Perceived Ergogenicity and Side Effects in Male and Female Athletes. Br J Nutr. 2014;112(9):1494-502. doi: 10.1017/S0007114514002189.
3. Maughan RJ, Burke LM, Dvorak J, Larson-Meyer DE, Peeling P, Phillips SM, et al. IOC Consensus Statement: Dietary Supplements and the High-performance Athlete. Br J Sports Med. 2018;52(7):439-55. doi: 10.1136/bjsports-2018-099027.
4. Guest NS, VanDusseldorp TA, Nelson MT, Grgic J, Schoenfeld BJ, Jenkins NDM, et al. International Society of Sports Nutrition Position Stand: Caffeine and Exercise Performance. J Int Soc Sports Nutr. 2021;18(1):1. doi: 10.1186/s12970-020-00383-4.
5. Childs E. Influence of Energy Drink Ingredients on Mood and Cognitive Performance. Nutr Rev. 2014;72(Suppl 1):48-59. doi: 10.1111/nure.12148.
6. Shah SA, Szeto AH, Farewell R, Shek A, Fan D, Quach KN, et al. Impact of High Volume Energy Drink Consumption on Electrocardiographic and Blood Pressure Parameters: A Randomized Trial. J Am Heart Assoc. 2019;8(11):e011318. doi: 10.1161/JAHA.118.011318.
7. Somers KR, Svatikova A. Cardiovascular and Autonomic Responses to Energy Drinks-Clinical Implications. J Clin Med. 2020;9(2):431. doi: 10.3390/jcm9020431.
8. Cole CR, Blackstone EH, Pashkow FJ, Snader CE, Lauer MS. Heart-rate Recovery Immediately After Exercise as a Predictor of Mortality. N Engl J Med. 1999;341(18):1351-7. doi: 10.1056/NEJM199910283411804.
9. Vanderlei LC, Pastre CM, Hoshi RA, Carvalho TD, Godoy MF. Basic Notions of Heart Rate Variability and its Clinical Applicability. Rev Bras Cir Cardiovasc. 2009;24(2):205-17. doi: 10.1590/s0102-76382009000200018.
10. Peçanha T, Bartels R, Brito LC, Paula-Ribeiro M, Oliveira RS, Goldberger JJ. Methods of Assessment of the Post-exercise Cardiac Autonomic Recovery: A Methodological Review. Int J Cardiol. 2017;227:795-802. doi: 10.1016/j.ijcard.2016.10.057.
11. Gonzaga LA, Vanderlei LCM, Gomes RL, Valenti VE. Caffeine Affects Autonomic Control of Heart Rate and Blood Pressure Recovery After Aerobic Exercise in Young Adults: A Crossover Study. Sci Rep. 2017;7(1):14091. doi: 10.1038/s41598-017-14540-4.
12. Benjamim CJR, Kliszczewicz B, Garner DM, Cavalcante TCF, da Silva AAM, Santana MDR, et al. Is Caffeine Recommended Before Exercise? A Systematic Review To Investigate Its Impact On Cardiac Autonomic Control Via Heart Rate And Its Variability. J Am Coll Nutr. 2020;39(6):563-73. doi: 10.1080/07315724.2019.1705201.
13. Gonzaga LA, Vanderlei LCM, Gomes RL, Garner DM, Valenti VE. Involvement of Cardiorespiratory Capacity on the Acute Effects of Caffeine on Autonomic Recovery. Medicina. 2019;55(5):196. doi: 10.3390/medicina55050196.
14. Clark NW, Herring CH, Goldstein ER, Stout JR, Wells AJ, Fukuda DH. Heart Rate Variability Behavior during Exercise and Short-Term Recovery Following Energy Drink Consumption in Men and Women. Nutrients. 2020;12(8):2372. doi: 10.3390/nu12082372.
15. Porto AA, Valenti VE, Amaral JAT, Benjamim CJR, Garner DM, Ferreira C. Energy Drink before Exercise Did Not Affect Autonomic Recovery Following Moderate Aerobic Exercise: A Crossover, Randomized and Controlled Trial. J Am Coll Nutr. 2021;40(3):280-6. doi: 10.1080/07315724.2020.1768175.
16. An SM, Park JS, Kim SH. Effect of Energy Drink dose on Exercise Capacity, Heart Rate Recovery and Heart Rate Variability after High-Intensity Exercise. J Exerc Nutrition Biochem. 2014; 18: 31-39.
17. Nelson MT, Biltz GR, Dengel DR. Cardiovascular and Ride Time-to-exhaustion Effects of an Energy Drink. J Int Soc Sports Nutr. 2014;11(1):2. doi: 10.1186/1550-2783-11-2.
18. Pardini R, Matsudo S, Araujo T, Matsudo V, Andrade E, Braggion G, et al. Validation of the International Physical Activity Questionaire (IPAQ version 6): Pilot Study in Brazilian Young Adults. Braz J Sci Mov. 2001;9:45-51. doi: 10.18511/rbcm.v9i3.393.
19. Lohman TG RA, Martorelli R. Antropometric Standardization Reference Manual. Champaign: Human Kinetics Books; 1998.
20. Black N, D'Souza A, Wang Y, Piggins H, Dobrzynski H, Morris G, et al. Circadian Rhythm of Cardiac Electrophysiology, Arrhythmogenesis, and the Underlying Mechanisms. Heart Rhythm. 2019;16(2):298-307. doi: 10.1016/j.hrthm.2018.08.026.

21. Rodriguez NR, Di Marco NM, Langley S. American College of Sports Medicine Position Stand. Nutrition and Athletic Performance. Med Sci Sports Exerc. 2009;41(3):709-31. doi: 10.1249/MSS.0b013e31890eb86.

22. Moreno IL, Pastre CM, Ferreira C, Abreu LC, Valenti VE, Vanderlei LC. Effects of an Isotonic Beverage on Autonomic Regulation During and After Exercise. J Int Soc Sports Nutr. 2013;10(1):2. doi: 10.1186/1550-2783-10-2.

23. Novitsky S, Segal KR, Chatr-Aryamontri B, Guvakov D, Katch VL. Validity of a New Portable Indirect Calorimeter: The AeroSport TEEM 100. Eur J Appl Physiol Occup Physiol. 1995;70(5):462-7. doi: 10.1007/BF00618499.

24. Billat VL, Morton RH, Blondel N, Berthoin S, Bocquet V, Koralsztein JP, et al. Oxygen Kinetics and Modelling of Time to Exhaustion Whilst Running at Various Velocities at Maximal Oxygen Uptake. Eur J Appl Physiol. 2000;82(3):178-87. doi: 10.1007/s004210050670.

25. Barroso WKS, Rodrigues CIS, Bortolotto LA, Mota-Gomes MA, Brandão AA, Feitosa ADM, et al. Brazilian Guidelines of Hypertension - 2020. Arq Bras Cardiol. 2021;116(3):516-658. doi: 10.36660/abc.20201238.

26. Heart Rate Variability: Standards of Measurement, Physiological Interpretation and Clinical Use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation. 1996;93(5):1043-65.

27. Laborde S, Mosley E, Thayer JF. Heart Rate Variability and Cardiac Vagal Tone in Psychophysiological Research - Recommendations for Experiment Planning, Data Analysis, and Data Reporting. Front Psychol. 2017;8:213. doi: 10.3389/fpsyg.2017.00213.

28. Higgins JP, Tuttle TD, Higgins CL. Energy Beverages: Content and Safety. Mayo Clin Proc. 2010;85(11):1033-41. doi: 10.4065/mcp.2010.0381.

29. Lane JD, Adcock RA, Williams RB, Kuhn CM. Caffeine Effects on Cardiovascular and Neuroendocrine Responses to Acute Psychosocial Stress and Their Relationship to Level of Habitual Caffeine Consumption. Psychosom Med. 1990;52(3):320-36. doi: 10.1097/00006842-199005000-00006.

30. Sung BH, Lovallo WR, Pincomb GA, Wilson MF. Effects of Caffeine on Blood Pressure Response During Exercise in Normotensive Healthy Young Men. Am J Cardiol. 1990;65(13):909-13. doi: 10.1016/0002-9149(90)91435-9.

31. Mal'chikova LS, Elizarova EP. Taurine and the adenosine cyclic monophosphate levels in the heart. Kardiologiia. 1981;21(1):85-9.

32. Michael S, Graham KS, Davis GMO. Cardiac Autonomic Responses during Exercise and Post-exercise Recovery Using Heart Rate Variability and Systolic Time Intervals-A Review. Front Physiol. 2017;8:301. doi: 10.3389/fphys.2017.00301.

33. Wasserman K. Coupling of External to Cellular Respiration During Exercise: The Wisdom of the Body Revisited. Am J Physiol. 1994;266(4 Pt 1):519-39. doi: 10.1152/ajpendo.1994.266.4.E519.

34. Alsunni A, Majeed F, Yar T, AlRahim A, Alhawaj AF, Alzaki M. Effects of Energy Drink Consumption on Corrected QT Interval and Heart Rate Variability in Young Obese Saudi Male University Students. Ann Saudi Med. 2015;35(4):282-7. doi: 10.5144/0256-4947.2015.282.

35. Shah S, Lacey C, Riddock I, Lee M, Dargush AE. Impact of Energy Drinks on Electrocardiographic and Blood Pressure Parameters: A Meta-Analysis of Clinical Studies. Circulation 2013, 127(AP324). doi: 10.1161/circ.127.suppl_12.AP324.

36. Caliskan SG, Bilgin MD. Non-Linear Analysis of Heart Rate Variability for Evaluating the Acute Effects of Caffeinated Beverages in Young Adults. Cardiol Young. 2020;30(7):1018-23. doi: 10.1017/S1047951120001481.

37. Brothers RM, Christmas KM, Patik JC, Bhella PS. Heart Rate, Blood Pressure and Repolarization Effects of an Energy Drink as Compared to Coffee. Clin Physiol Funct Imaging. 2017;37(6):675-81. doi: 10.1111/cpf.12357.

The Action of the Energy Drink on the Recovery Heart Rate is Independent of the Functional Capacity

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Short Editorial related to the article: Acute Effects of Energy Drink on Autonomic and Cardiovascular Parameters Recovery in Individuals with Different Cardiorespiratory Fitness: A Randomized, Crossover, Double-Blind and Placebo-Controlled Trial

The consumption of caffeinated energy drinks (ED) has increased considerably in recent years.[1] Increased ability to concentrate, gain in work performance and increased performance in physical activity are some of the reasons that lead to the search for the drink.[2]

During the pandemic, with changes in routine imposed by the need for social isolation, some works reported an increase in ED intake. There was also greater consumption in the young population motivated by factors such as better performance in sports and the ability to concentrate.[3,4]

Caffeine doses of up to 400 mg/day or up to 200 mg in a single dose are considered safe from a cardiovascular point of view.[5] However, substances such as taurine, guarana, vitamins, and minerals are often added that can potentiate the effect of the ED and, consequently, increase the risk of adverse events.[6]

Fletcher et al.[7] published in 2017 that the intake of 32 oz. (946 ml) of an ED containing 320 mg of caffeine led to a statistically significant increase in QTc interval and systolic blood pressure when compared to caffeine intake alone in the same amount (320 mg).[7] Other studies have shown adverse events such as atrial fibrillation, ventricular fibrillation and ST-segment elevation related to ED consumption.[8]

The magnitude of the heart rate (HR) drops in the first minute of the post-exercise test recovery phase reflects the ability of the parasympathetic autonomic nervous system to reactivate after exercise. This parameter is an important predictor of cardiovascular risk and prognosis.[9] Heart rate variability (HRV) is also an important means of non-invasively assessing the functioning of the autonomic nervous system. Previous studies have analyzed the effect of ED and caffeine on HR and HRV recovery after physical exercise. In some studies using 300-400 mg of caffeine before exercise, there was a delay in parasympathetic reactivation in the recovery phase.[10,11] Such findings, however, are still divergent in the literature.[12]

In previous work by the group, Porto et al.[13] analyzed the effect of the ED before physical activity and found no differences in the autonomic control of HR in the recovery phase after submaximal aerobic exercise.[13]

In this most recent work, Porto et al.[14] evaluated the impact of ED on HRV and HR recovery after exercise in individuals with different cardiorespiratory capacities. Despite using a protocol similar to the previous study, this time, the group found an impact of the ED in both those with high and low cardiorespiratory capacity.[14]

Regarding the methodology, we consider that the work has strong points, that were the use of a randomized, crossover and double-blind protocol, which contributed to the reduction of biases. An important parameter that could have been analyzed would be the HR at the peak of the physical effort and its comparison with the HR at the end of the first minute of recovery. This indicator would reinforce the HRV findings in the assessment of vagal reactivation and its prognostic factor.[9]

It is important to report that the study used a 250 ml ED containing 32 mg of caffeine. This volume is below that described in previous studies showing arrhythmogenic effects of the ED and with a dose of caffeine well below the maximum considered safe. Currently, several soluble energy compounds and capsules use doses of 100 to 200 mg of caffeine associated with other substances that enhance their effect.

Another important point is that caffeine may be tolerable after prolonged use.[15] This can lead to higher consumption and, consequently, greater potential for side effects over time.

Excessive intake of ED can cause several adverse effects from a cardiovascular point of view. Its more frequent consumption, in addition to the increase in consumption in young people, deserves attention, especially when associated with other substances. Future work evaluating energy substances available in capsules or soluble containing higher doses of caffeine, their effects on sedentary people and the impact on women would be of great importance.

Keywords

Energy Drinks; Dietary Supplements; Autonomc Nervous System; Cardiovascular System; Exercise

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References

1. Global energy drinks market: insights, market size, share, growth, trends analysis and forecast to 2021. AIM Market Insight. April 2015. Available at: https://www.researchandmarkets.com/research/mbbjvv/global_energy
2. McLellan TM, Caldwell JA, Lieberman HR. A review of caffeine's effects on cognitive, physical and occupational performance. Neurosci Biobehav Rev. 2016;71:294-312. doi:10.1016/j.neubiorev.2016.09.001
3. Mattioli AV, Sabatini S. Changes in energy drink consumption during the COVID-19 quarantine. Clin Nutr ESPEN. 2021 Oct;45:516-517. doi: 10.1016/j.clnesp.2021.06.034.
4. Majori S, Pilati S, Gazzani D, Paiano J, Ferrari S, Sannino A, et al. Energy drink and ginseng consumption by Italian university students: a cross-sectional study. J Prev Med Hyg. 2018;59(1):E63-E74. doi:10.15167/2421-4248/jpmh2018.59.1.813
5. EFSA Panel on Dietetic Products, Nutrition and Allergies. Scientific Opinion on the safety of caffeine. EFSA J 2015; 13(5):4102. doi.org/10.2903/j.efsa.2015.4102
6. Shah SA, Szeto AH, Farewell R, Shek A, Fan D, Quach KN, et al. Impact of High Volume Energy Drink Consumption on Electrocardiographic and Blood Pressure Parameters: A Randomized Trial. J Am Heart Assoc. 2019;8(11):e011318. doi:10.1161/JAHA.118.011318
7. Fletcher EA, Lacey CS, Aaron M, Kolasa M, Occiano A, Shah SA. Randomized Controlled Trial of High-Volume Energy Drink Versus Caffeine Consumption on ECG and Hemodynamic Parameters. J Am Heart Assoc. 2017 Apr 26;6(5):e004448. doi: 10.1161/JAHA.116.004448.
8. Goldfarb M, Tellier C, Thanassoulis G. Review of published cases of adverse cardiovascular events after ingestion of energy drinks. Am J Cardiol. 2014;113(1):168-72. doi: 10.1016/j.amjcard.2013.08.058.
9. Cole CR, Blackstone EH, Pashkow FJ, Snader CE, Lauer MS. Heart-rate Recovery Immediately After Exercise as a Predictor of Mortality. N Engl J Med. 1999;341(18):1351-7. doi: 10.1056/NEJM199910283411804.
10. Gonzaga LA, Vanderlei LCM, Gomes RL, Valenti VE. Caffeine Affects Autonomic Control of Heart Rate and Blood Pressure Recovery After Aerobic Exercise in Young Adults: A Crossover Study. Sci Rep. 2017;7(1):14091. doi: 10.1038/s41598-017-14540-4.
11. Bunsawat K, White DW, Kappus RM, Baynard T. Caffeine delays autonomic recovery following acute exercise. Eur J Prev Cardiol. 2015;22(11):1473-9. doi:10.1177/2047487314554867
12. Benjamim CJR, Kliszczewicz B, Garner DM, Cavalcante TCF, da Silva AAM, Santana MDR, et al. Is Caffeine Recommended Before Exercise? A Systematic Review To Investigate Its Impact On Cardiac Autonomic Control Via Heart Rate And Its Variability. J Am Coll Nutr. 2020;39(6):563-73. doi: 10.1080/07315724.2019.1705201.
13. Porto AA, Valenti VE, Tonon do Amaral JA, Benjamim CJR, Garner DM, et al. Energy Drink before Exercise Did Not Affect Autonomic Recovery Following Moderate Aerobic Exercise: A Crossover, Randomized and Controlled Trial. J Am Coll Nutr. 2021;40(3):280-6. doi:10.1080/07315724.2020.1768175
14. Porto AA, Gonzaga LA, Benjamin CJ, Bueno Jr CR, Vanderlei LCM, Ferreira C, et al. Acute Effects of Energy Drink on Autonomic and Cardiovascular Parameters Recovery in Individuals with Different Cardiorespiratory Fitness: A Randomized, Crossover, Double-Blind and Placebo-Controlled Trial. Arq Bras Cardiol. 2022; 119(4):553-561.
15. Beaumont R, Cordery P, Funnell M, Mears S, James L, Watson P. Chronic ingestion of a low dose of caffeine induces tolerance to the performance benefits of caffeine. J Sports Sci. 2017;35(19):1920-7. doi:10.1080/02640414.2016.1241421

Association between Atrioventricular Block and Mortality in Primary Care Patients: The CODE Study

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Abstract

Background: **Atrioventricular block (AVB) describes an impairment of conduction from the atria to the ventricles. Although the clinical course of AVB has been evaluated, the findings are from high-income countries and, therefore, cannot be extrapolated to the Latinx population.**

Objective: **Evaluate the association between AVB and mortality.**

Methods: **Patients from the CODE (Clinical Outcomes in Digital Electrocardiology) study, older than 16 years who underwent digital electrocardiogram (ECG) from 2010 to 2017 were included. ECGs were reported by cardiologists and by automated software. To assess the relationship between AVB and mortality, the log-normal model and the Kaplan-Meier curves were used with two-tailed p-values < 0.05 considered statistically significant.**

Results: **The study included 1,557,901 patients; 40.2% were men, and mean age was 51.7 (standard deviation ± 17.6) years. In a mean follow-up of 3.7 years, the mortality rate was 3.35%. The AVB prevalence was 1.38% (21,538). Patients with first-, second-, and third-degree AVB were associated with 24% (relative survival rate [RS] = 0.76; 95% confidence interval [CI]: 0.71-0.81; p < 0.001), 55% (RS = 0.45; 95% CI: 0.27-0.77; p = 0.01), and 64% (RS = 0.36; 95% CI: 0.26-0.49; p < 0.001) lower survival rate when compared to the control group, respectively. Patients with 2:1 AVB had 79% (RS = 0.21; 95% CI: 0.08-0.52; p = 0.005) lower survival rate than the control group. Only Mobitz type I was not associated with higher mortality (p = 0.27).**

Conclusion: **AVB was an independent risk factor for overall mortality, with the exception of Mobitz type I.**

Keywords: **Cardiovascular Diseases/complications; Atrioventricular Block/physiopathology; Atrioventricular Block/complications, Mortality; Diagnostic Imaging; Electrocardiography/methods.**

Introduction

The atrioventricular (AV) node is responsible for the electrical connection between the atria and ventricles.[1] The presence of delay or interruption in AV conduction is called atrioventricular block (AVB),[2] which is classified into three degrees, according to the electrocardiogram (ECG) presentation.[3] There are several known causes of AVB, including ischemic heart disease, degenerative conduction system disease, congenital heart disease, connective tissue disease, inflammatory diseases, medications, and increased autonomic tonus.[4]

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AVB prevalence varies between 0.6% to 6.04% in the literature, depending on the population studied and the degree of AVB.[5,6] The prevalence is usually higher in the elderly and in men.[5] First-degree AVB is the most common, and can be frequently found in outcome patients.[4]

The clinical course of first-degree AVB has been evaluated in studies from community-based samples, such as the Framingham cohort.[4] Patients with first-degree AVB have a higher risk of atrial fibrillation,[7] death, stroke, or hospitalization for heart failure.[8] It is also described that, in patients with acute myocardial infarction, high-degree AVB is associated with an increased risk of morbidity and mortality.[9]

Nonetheless, there is no prospective study on the prognostic value of all degrees of AVB in a general population, which limits the understanding of the significance of these abnormalities in an outpatient setting. Indeed, previous studies from our group showed that ECG abnormalities that are considered prognostically important, such as pre-excitation syndrome, have no prognostic impact in a community setting.[10] In contrast, the risk of mortality for a patient with right bundle branch block (BBB) is almost as high as that of a patient

with left BBB,[11] even though the latter is considered a much stronger marker of risk in general cardiology practice. The CODE (Clinical Outcomes in Digital Electrocardiology) study is a large database that comprises all ECGs performed mostly at primary health care facilities by the Telehealth Network of Minas Gerais, Brazil, from 2010 to 2017.[12] The ECG database was linked to the Brazilian Mortality Information System and can provide epidemiological information in a population that is representative of the general population. Thus, in the present study, we aim to describe the prevalence and risk factors of AVB and, mainly, to evaluate the association between AVB and overall mortality in this large primary care Brazilian cohort.

Methods

Study design

We conducted a retrospective study using a database of digital ECGs from the Telehealth Network of Minas Gerais (TNMG),[13] The CODE dataset,[12,14] which comprises all valid ECGs performed in patients over 16 years old by the TNMG from 2010 to 2017, was analyzed. Exams without valid tracings or with technical problems were excluded. In patients who underwent more than one ECG, only the first exam was analyzed.

Data collection

Clinical data were collected using a standardized questionnaire, which included age, sex, and self-reported comorbidities, such as: hypertension, diabetes, smoking, Chagas disease, previous myocardial infarction, and chronic obstructive pulmonary disease.

ECGs were performed by the local primary care professional, using digital electrocardiographs by Tecnologia Eletrônica Brasileira, model ECGPC (São Paulo, Brazil) or Micromed Biotecnologia, model ErgoPC 13 (Brasilia, Brazil).

Specific software, developed in-house, was capable of capturing ECG tracing, uploading the ECG and the patient's clinical history, and sending them to the TNMG analysis center through the internet. The clinical information, ECG tracings, and reports were stored in a specific database. The ECG reports were generated in a free text model by cardiologists and, also, automatically interpreted and coded into Glasgow and Minnesota codes by the Glasgow 12-lead ECG analysis program (release 28.4.1, issued on June 16, 2009).[15]

Definition of atrioventricular block

The medical reports were performed by a team of 14 trained cardiologists using standardized criteria. Each ECG was interpreted by only one cardiologist. The electrocardiographic diagnosis of AVB was divided into: first-degree AVB, second-degree Mobitz type I AVB, second-degree Mobitz type II, 2:1 AVB, high-degree AVB, and third-degree AVB[3] (Table 1). In this study, we did not include Mobitz type II because of the low prevalence (7 cases) and high-degree AVB (6 cases) was grouped into third-degree AVB for the analysis.

ECG medical reports were generated as an unorganized free text. In order to recognize AVB diagnosis among more than a million reports, hierarchical free-text machine learning was used. First, the text was preprocessed by removing stop words and generating n-grams. Then, we used the classification model called Lazy Associative Classifier,[16] which was built with a 2800-sample dictionary manually created by specialists based on text from real diagnoses. The final report was obtained by imputing the Lazy Associative Classifier results to a decision tree for class disambiguation. The decision tree was trained using the original dataset. The classification model was tested on 4557 medical reports manually labeled by 2 cardiologists with 99% accuracy, 100% positive predictive value, and 99% sensibility.[17]

Electrocardiographic diagnosis of AVB was considered automatically when there was agreement between the cardiologist report and the automatic report from Glasgow or Minnesota code. In the cases where there were discordances between the medical report and one of the automatic programs, manual revision of 9038 ECGs was carried out by trained staff. Cases where AVB were diagnosed by only one of the automatic systems were not considered (Figure 1). The control group was composed of patients without any type of AVB.

Table 1 – Definition and classification of atrioventricular block[3]

Type of AVB	Definition
First-degree	P waves associated with 1:1 atrioventricular conduction and a PR interval > 200 ms
Second-degree Mobitz type I	P waves with a constant rate (< 100 bpm) with a periodic single nonconducted P wave associated with P waves before and after the nonconducted P wave with inconstant PR intervals
Second-degree Mobitz type II	P waves with a constant rate (< 100 bpm) with a periodic single nonconducted P wave associated with other P waves before and after the nonconducted P wave with constant PR intervals (excluding 2:1 atrioventricular block)
2:1	P waves with a constant (or near constant rate because of ventriculophasic sinus arrhythmia) rate (< 100 bpm) where every other P wave conducts to the ventricles
High-degree	≥ 2 consecutive P waves at a constant physiologic rate that do not conduct to the ventricles with evidence of some atrioventricular conduction
Third-degree	No evidence of atrioventricular conduction

AVB: atrioventricular block.

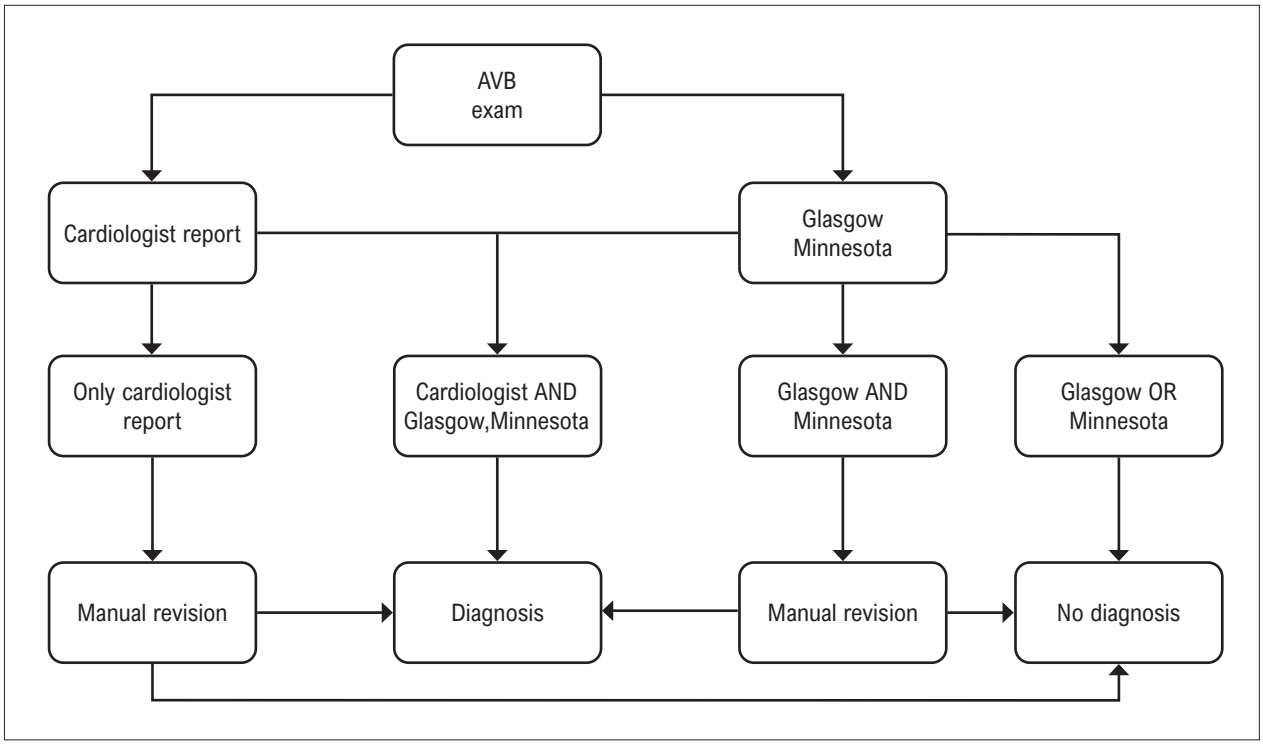

Figure 1 – *Diagram for atrioventricular block diagnosis in the ECG database. AVB: atrioventricular block.*

Probabilistic linkage

The electronic cohort was obtained linking data from the ECG exams (name, sex, date of birth, city of residence) and those from the Brazilian Mortality Information System,[12] using standard probabilistic linkage methods (FRIL: fine-grained record linkage software, v.2.1.5, Atlanta, GA).[12,18]

Statistical analysis

R program (version 3.4.3, Vienna, Austria) was used for statistical analysis. Categorical data were reported as counts and percentages; continuous variables were reported as mean and standard deviation (SD). The endpoint was all-cause mortality, including all International Classification of Diseases codes in the medical certification of cause of the death. The Shapiro-Wilk test was used to verify the normality of the data. The Kaplan-Meier method was used to estimate the survival curves for all causes of death. We used the likelihood ratio test (LRT) to adjust data for the best parametric model, since the proportional assumption for the Cox regression model was violated. In the LRT, the generalized model, represented by the generalized gamma regression model, was compared with the other models of interest (Weibull and log-normal). We chose to work with the log-normal model, since the log-likelihood of this model was higher and the residual analysis indicated that log-normal distribution was a better choice for this data. Relative survival rate (RS) was used as the measure of association, with a confidence interval of 95%. RS < 1 means higher risk of mortality, and RS > 1 means lower risk. Two-tailed p-values < 0.05 were considered statistically significant. This study was approved by the Research Ethics Committee of the Federal University of Minas Gerais.

Results

A total of 1,557,901 patients were included; 40.23% were men, and mean age was 51.67 (SD ± 17.58) years. In a mean follow-up of 3.7 years, the mortality rate was 3.35%. The prevalence of AVB was 1.38% (21,538); 1.32% (20,644) corresponding to first-degree AVB, 0.02% (273) to second-degree AVB, and 0.04% (621) to third-degree AVB. Among these 273 cases of second-degree AVB, 212 were Mobitz type I, and 61 were 2:1. The clinical conditions of all patients are described in Table 2.

After adjustment for sex, age, and clinical conditions, patients with first-, second-, and third-degree AVB were associated with 24%, 55%, and 64% lower survival rate when compared to the control group, respectively (Figure 2). In the survival analysis divided by subtype of AVB, only the second-degree Mobitz type I was not associated with higher mortality. Patients with 2:1 AVB had 79% lower survival rate than the control group, while third-degree AVB had 64% (Table 3; Figure 2).

Discussion

In this large electronic cohort with more than one million patients, AVB was associated with higher risk of overall mortality. Regarding the type of AVB, only Mobitz type I did not have an increased risk of mortality, compared to the control group.

Table 2 – Dados basais dos pacientes, de acordo com a presença de bloqueio atrioventricular e respectivo grau

	Without AVB n = 1,536,363	First-degree AVB n = 20,644	Adjusted OR*	Second-degree AVB n = 273	Adjusted OR*	Third-degree AVB n = 621	Adjusted OR*
Age (years)	51.5 (17.5)	64.9 (16.9)	-	61.7 (19.8)	-	66.6 (17.5)	-
Male sex	615.097 (40)	11.176 (54.1)	-	164 (60.1)	-	286 (46.1)	-
Hypertension	492.488 (32.1)	9370 (45.4)	1.23 (1.19-1.26)	100 (36.6)	0.89 (0.69-1.15)	298 (48.0)	1.18 (1.01-1.39)
Diabetes	100.844 (6.6)	1826 (8.8)	1.10 (1.05-1.15)	18 (6.6)	0.87 (0.52-1.36)	55 (8.9)	1.05 (0.78-1.37)
Current smoking	107.346 (7.0)	1384 (6.7)	0.90 (0.85-0.95)	20 (7.3)	0.93 (0.57-1.43)	51 (8.2)	1.21 (0.90-1.60)
Chagas disease	33.134 (2.2)	1336 (6.5)	2.76 (2.60-2.92)	35 (12.8)	6.04 (4.16-8.50)	81 (13.0)	5.75 (4.52-7.23)
Myocardial infarction	11.286 (0.7)	304 (1.5)	1.48 (1.31-1.66)	0 (0.0)	-	11 (1.8)	1.80 (0.93-3.10)
COPD	11.029 (0.7)	231 (1.1)	1.14 (1.00-1.30)	0 (0.0)	-	4 (0.6)	0.64 (0.20-1.49)

*Data are presented as mean (standard deviation) or number (%). AVB: atrioventricular block; COPD: chronic obstructive pulmonary disease; OR: odds ratio. *Age, sex, hypertension, diabetes, current smoking, Chagas disease, and chronic obstructive pulmonary disease.*

Table 3 – Prognostic value of patients with subtypes of atrioventricular block

	RS (95% CI)		
Type of AVB	Model 1: Unadjusted	Model 2: Adjusted for age and sex	Model 3: Adjusted for clinical variables*
First-degree	0,24 (0,23-0,26)	0,73 (0,69-0,78)	0,76 (0,71-0,81)
Mobitz I	0,26 (0,13-0,50)	0,63 (0,33-1,20)	0,65 (0,34-1,24)
2:1	0,05 (0,02-0,13)	0,20 (0,08-0,50)	0,21 (0,09-0,52)
Third-degree	0,11 (0,08-0,15)	0,34 (0,25-0,46)	0,36 (0,26-0,49)

*AVB: atrioventricular block; CI: confidence interval; RS: relative survival rate. *Age, sex, hypertension, diabetes, current smoking, Chagas disease and chronic obstructive pulmonary disease.*

In patients with structural heart disease, first-degree AVB has been described as a risk factor for adverse outcome.[19,20] On the other hand, previous longitudinal studies in the general population that mainly included young and middle-age men found that prolonged PR interval has a benign course.[21-23] We should highlight that this data came from a specific population with limited surveillance and a relatively low sample of patients with AVB. More recently, a publication from the Framingham cohort[4] changed this paradigm. After 20 years of follow up, PR prolongation was associated with increased risk of atrial fibrillation, pacemaker implantation, and death[4]. A large Danish ECG study with 288,181 patients confirmed the higher risk for atrial fibrillation associated with the presence of the first AVB.[24]

In our population, a 24% reduction in the survival rate of patients with PR > 200 ms, after adjustment for age, sex and clinical conditions were found, contrary to a previous study in the Finnish population.[25] Some differences between these cohorts must be pointed out. The Brazilian cohort was older (mean age 51.7 versus 44 years), and it also included elderly patients. We analyzed about 1.5 million ECG versus 10,000. Chagas disease was relatively prevalent and it had a strong association with the presence of AVB, regardless of the degree. The social differences between both countries might also have contributed. Access to public health services and population education are completely unequal in low- and middle-income countries and may have a prognostic impact on the population.[26]

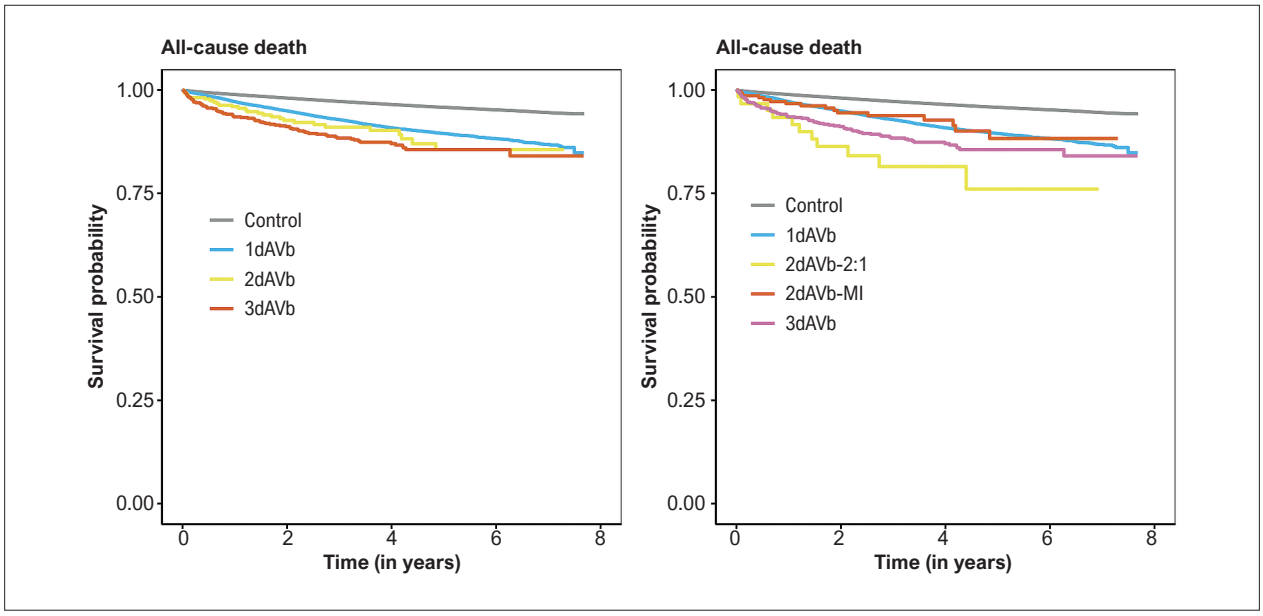

Figure 2 – *Kaplan-Meier survival curves, according to the subtype of atrioventricular block. 1dAVb: first-degree atrioventricular block; 2dAVb: second-degree atrioventricular block; 3dAVb: third-degree atrioventricular block; MI: Mobitz type I.*

It is well established that irreversible Mobitz type II, high and third-degree AVB are indications for permanent pacing, even in asymptomatic patients.[3] Their association with mortality is expected,[9] since AV conduction injury is more severe, and heart disease is often related.[3] The prognosis in 2:1 AVB is intimately related to the site of the AVB: nodal or infranodal.[3] In the present study, 2:1 AVB in the 12-lead ECG was associated with a 79% reduction in relative survival, probably indicating an infranodal block. Mobitz type I AVB, on the other hand, was not associated with higher mortality in our cohort.

Mobitz type I AVB frequently has a benign prognosis, especially in young patients without cardiac disease.[27] It can be a vagal mediated AVB that does not have an anatomical involvement of AV conduction,[28] and it does not, therefore, progress to a high-degree AVB. In older patients, the natural history can be different, and they might benefit from a permanent pacemaker.[29] We did not perform a sub-analysis in elderly patients, and the presence of symptoms is unknown.

Patients with cardiovascular emergencies often seek health assistance in primary care units, especially in small and remote counties. Tele-electrocardiography services play an important role in this setting, mainly for recognizing potentially life-threatening ECG abnormalities that are misdiagnosed by the local physician.[30] In our service, second-degree AVB was statistically higher in the ECGs assigned as elective than in those with emergency priority.[30] Patients' outcomes could change with early referral to the hospital and consequent pacemaker implantation.[31] Hospitalization data was not available for our entire cohort and, therefore, was not included in this paper. Nonetheless, further work in this field is planned to evaluate patients' journey in our healthcare system from the ECG diagnosis of AVB.

Limitations

Our study has limitations. The clinical data was self-reported and, thus, might have been underreported. The Lazy Associative Classifier software used to classify ECG reports has good accuracy, sensibility, and positive predictive value, but it may make errors. In order to minimize this problem, we included the Glasgow and Minnesota automatic classification in the diagnostic algorithm. Furthermore, manual revision of more than 9,000 ECGs was conducted to confirm the presence of AVB. The probabilistic linkage also has some issues, such as less than perfect sensitivity and the possibility of false pairs. Therefore, we defined a high cutoff point for true pairs and conducted manual revision for the doubtful cases. We still do not have information on symptoms or hospitalization data, but data from pacemaker procedures in each group will soon be available for analysis, and future work on this topic has been planned.

Nevertheless, our study brings new data on AVB prognosis, as it evaluates a Latinx population from a primary care centers with more than one million patients. Our findings are consistent and might be a useful tool to direct public health policies and funding resources.

Conclusion

The presence of AVB was associated with an increased risk of overall mortality in the TNMG population. In patients with second and third-degree AVB, only those with Mobitz type I did not have a higher risk of mortality.

Author Contributions

Conception and design of the research: Paixão GMM, Quadros AB, Cabral DPR, Coelho RR, Ribeiro AL; Acquisition of data: Oliveira DM, Nascimento JS, Gomes PR; Analysis and interpretation of the data: Paixão GMM, Lima EM, Oliveira DM, Nascimento JS, Gomes PR, Ribeiro AL; Statistical analysis: Lima EM; Obtaining financing: Ribeiro AL; Writing of the manuscript: Paixão GMM, Quadros AB, Cabral DPR, Coelho RR, Ribeiro AL; Critical revision of the manuscript for intellectual content: Paixão GMM, Ribeiro AL.

Potential Conflict of Interest

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

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Study Association

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais under the protocol number 68496317.7.00005149. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013.

References

1. Pastore CA, Pinho JA, Pinho C, Samesima N, Pereira-Filho HG, Kruse JCL, et al. III Diretrizes da Sociedade Brasileira de Cardiologia sobre análise e emissão de laudos eletrocardiográficos. Arq Bras Cardiol. 2016 Apr;106(4 Suppl 1):1-23. doi: 10.5935/abc.20160054.
2. Barra SNC, Providência R, Paiva L, Nascimento J, Marques AL. A review on advanced atrioventricular block in young or middle-aged adults. Pacing Clin Electrophysiol.2012;35(11):1395-405. doi/10.1111/j.1540-8159.2012.03489
3. Kusumoto FM, Schoenfeld MH, Barrett C, Edgerton JR, Ellenbogen KA, Gold MR, et al. 2018 ACC/AHA/HRS guideline on the evaluation and management of patients with bradycardia and cardiac conduction delay: Executive summary: A report of the American college of cardiology/American heart association task force on clinical practice guidelines, and the heart rhythm society. J Am Coll Cardiol. 2019 Aug 20;74(7):932–87. Doi: doi: 10.1016/j.jacc.2018.10.043.
4. Cheng S, Keyes MJ, Larson MG, McCabe EL, Newton-Cheh C, Levy D, et al. Long-term outcomes in individuals with prolonged PR interval or first-degree atrioventricular block. JAMA.2009;301(24):2571-7. doi.org/10.1001/jama.2009.888
5. Silva M, Palhares D, Ribeiro L, Gomes P, Macfarlane P, Ribeiro A, et al. Prevalence of major and minor electrocardiographic abnormalities in one million primary care Latinos. J Electrocardiol. 2021;64:36-41. doi:10.1016/j.jelectrocard.2020.11.013.
6. Giuliano ICB, Barcellos Jr, von Wangenheim A, Coutinho MS. Emissão de laudos eletrocardiográficos a distância: experiência da rede catarinense de telemedicina. Arq Bras Cardiol.2012;99(5):1023-30. Doi.org/10.1590/S0066-782X2012005000094
7. Kottkamp H, Schreiber D. The Substrate in "Early Persistent" Atrial Fibrillation: Arrhythmia Induced, Risk Factor Induced, or From a Specific Fibrotic Atrial Cardiomyopathy? JACC Clin Electrophysiol.2016;2(2):140-2 http://dx.doi.org/10.1016/j.jacep.2016.02.010
8. Holmqvist F, Daubert JP. First-degree AV block-an entirely benign finding or a potentially curable cause of cardiac disease? Ann Noninvasive Electrocardiol. 2013;18(3):215–24. https://onlinelibrary.wiley.com/doi/10.1111/anec.12062
9. Alnsasra H, Ben-Avraham B, Gottlieb S, Ben-Avraham M, Kronowski R, Iakobishvili Z, et al. High-grade atrioventricular block in patients with acute myocardial infarction. Insights from a contemporary multi-center survey. J Electrocardiol.2018;51(3):386-91. Doi:http://dx.doi.org/10.1016/j.jelectrocard.2018.03.003
10. Paixão GMM, Lima EM, Batista LM, Santos LF, Araujo SLO, Araujo RM, et al. Ventricular pre-excitation in primary care patients: Evaluation of the risk of mortality. J Cardiovasc Electrophysiol.2021;32(5):1290-5. doi: 10.1111/jce.14977
11. Paixão GMM, Lima EM, Gomes PR, Ferreira MPF, Oliveira DM, Ribeiro MH, et al. Evaluation of mortality in bundle branch block patients from an electronic cohort: Clinical Outcomes in Digital Electrocardiography (CODE) study. J Electrocardiol.2019;57S:S56-S60. doi: 10.1016/j.jelectrocard.2019.09.004.
12. Ribeiro ALP, Paixão GMM, Gomes PR, Ribeiro MH, Ribeiro AH, Canazart JA, et al. Tele-electrocardiography and bigdata: The CODE (Clinical Outcomes in Digital Electrocardiography) study. J Electrocardiol.2019;57S:S75–8. doi.org/10.1016/j.eletrocard.2019.09.008
13. Alkmim MB, Figueira RM, Marcolino MS, Cardoso CS, Pena de Abreu M, Cunha LR, et al. Improving patient access to specialized health care: the Telehealth Network of Minas Gerais, Brazil. Bull World Health Organ. 2012;90(5):373–8. doi.org/10.2471/BLT.11.099408
14. Paixão GMM, Silva LGS, Gomes PR, Lima EM, Ferreira MPF, Oliveira DM, et al. Evaluation of Mortality in Atrial Fibrillation: Clinical Outcomes in Digital Electrocardiography (CODE) Study. Glob Heart [Internet]. 2020 Jul 28;15(1):48. http://dx.doi.org/10.5334/gh.772
15. Macfarlane PW, Latif S. Automated serial ECG comparison based on the Minnesota code. J Electrocardiol.1996;29(Suppl):29-34. DOI: 10.1016/s0022-0736(96)80016-1
16. Veloso A, Meira W, Gonçalves M, Zaki M. Multi-label Lazy Associative Classification. In: Knowledge Discovery in Databases: PKDD 2007. Springer Berlin:Springer Heidelberg;2007,p.605-12. doi.org/10.1007/978-3-540-74976-9_64
17. Pedrosa JAO, Oliveira D, Meira W Jr, Ribeiro A. Automated classification of cardiology diagnoses based on textual medical reports. In: Anais do VIII Symposium on Knowledge Discovery, Mining and Learning. Porto Alegre: Sociedade Brasileira de Computação; 2020.p:185-92. ISSN:2763-8944.
18. igowski A, Chaves RBM, Coeli CM, Ribeiro ALP, Tura BR, Kuschnir MCC, et al. Acurácia do relacionamento probabilístico na avaliação da alta complexidade em cardiologia. Rev Saúde Pública.2011;45(2):269-75. doi: 10.1590/s0034-89102011005000012.
19. Crisel RK, Farzaneh-Far R, Na B, Whooley MA. First-degree atrioventricular block is associated with heart failure and death in persons with stable coronary artery disease: data from the Heart

and Soul Study. Eur Heart J .2011;32(15):1875-80. http://dx.doi.org/10.1093/eurheartj/ehr139

20. Nikolaidou T, Ghosh JM, Clark AL. Outcomes related to first-degree atrioventricular block and therapeutic implications in patients with heart failure. JACC Clin Electrophysiol.2016;2(2):181-92.http://dx.doi.org/10.1016/j.jacep.2016.02.012

21. Erikssen J, Otterstad JE. Natural course of a prolonged PR interval and the relation between PR and incidence of coronary heart disease. A 7-year follow-up study of 1832 apparently healthy men aged 40-59 years. Clin Cardiol.1984;7(1):6-13. doi.org/10.1002/clc.4960070104

22. Mymin D, Mathewson FA, Tate RB, Manfreda J. The natural history of primary first-degree atrioventricular heart block. N Engl J Med. 1986;315(19):1183–7. Doi.org/10.1056/NEJM198611063151902

23. Rose G, Baxter PJ, Reid DD, McCartney P. Prevalence and prognosis of electrocardiographic findings in middle-aged men. Br Heart J. 1978;40(6):636–43. doi.org/10.1136/hrt.40.6.636

24. Nielsen JB, Pietersen A, Graff C, Lind B, Struijk JJ, Olesen MS, et al. Risk of atrial fibrillation as a function of the electrocardiographic PR interval: results from the Copenhagen ECG Study. Heart Rhythm.2013;10(9):1249-56. doi.org/10.1016/j.hrthm.2013.04.012

25. Aro AL, Anttonen O, Kerola T, Junttila MJ, Tikkanen JT, Rissanen HA, et al. Prognostic significance of prolonged PR interval in the general population. Eur Heart J [Internet]. 2014 Jan;35(2):123–9. Available from: http://dx.doi.org/10.1093/eurheartj/eht176

26. Ferreira JP, Rossignol P, Dewan P, Lamiral Z, White WB, Pitt B, et al. Income level and inequality as complement to geographical differences in cardiovascular trials. Am Heart J.2019;218:66-74. Doi.org/10.1016/j.ahj.2019.08.019

27. Strasberg B, Amat-Y-Leon F, Dhingra RC, Palileo E, Swiryn S, Bauernfeind R, et al. Natural history of chronic second-degree atrioventricular nodal block. Circulation.1981;63(5):1043-9. http://dx.doi.org/10.1161/01.cir.63.5.1043

28. Alboni P, Holz A, Brignole M. Vagally mediated atrioventricular block: pathophysiology and diagnosis. Heart.2013;99(13):904-8. doi.org/10.1136/heartjnl-2012-303220.

29. Coumbe AG, Naksuk N, Newell MC, Somasundaram PE, Benditt DG, Adabag S. Long-term follow-up of older patients with Mobitz type I second degree atrioventricular block. Heart [Internet]. 2013 Mar;99(5):334–8. doi.org/10.1136/heartjnl-2012-302770

30. Marcolino MS, Santos TMM, Stefanelli FC, Oliveira JA, Silva MVRS, Andrade Jr DF,et al. Cardiovascular emergencies in primary care: an observational retrospective study of a large-scale telecardiology service. Sao Paulo Med J.2017;135(5):481–7. http://dx.doi.org/10.1590/1516-3180.2017.0090110617

31. Cunnington MS, Plummer CJ, McDiarmid AK, Mc Comb JM. The patient journey from symptom onset to pacemaker implantation. QJM.2008;101(12):955-60. http://dx.doi.org/10.1093/qjmed/hcn122

First-Degree Atrioventricular Block: A Finding Not Always Benign!

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Short Editorial related to the article: Association between Atrioventricular Block and Mortality in Primary Care Patients: The CODE Study

First-degree atrioventricular block (AVB) is characterized by sinus rhythm, AV conduction 1:1 and PR interval > 200ms. The prevalence varies according to age group, relatively rare in the population < 60 years (1%), with an increase to 6% in individuals > 60 years. The reported prevalence in the general population ranges from 2 to 14%.[1] In most cases (75%), it is due to a proximal or nodal block that tends to improve conduction with a reduction in the PR interval with maneuvers that lead to an increase in adrenergic tone and/or atropine infusion.[2]

Usually considered a benign finding, PR interval prolongation or first-degree AV block has its prognosis more recently questioned due to emerging evidence that it is the independent factor in the increased risk of atrial fibrillation (AF), cardiac pacemaker implantation[3] and all-cause mortality. In the Framingham cohort,[4] the presence of first-degree AV block is considered a risk factor for the development of AF, a fact confirmed in subsequent studies in other community-based cohort with the demonstration of the association between PR prolongation and heart failure and/or AF.[5]

The relationship between first-degree AVB and the unfavorable outcome was also observed in patients with structural heart disease in a cohort described by Higuchi et al. in 414 patients with hypertrophic cardiomyopathy (HCM). Approximately 1/4 of the cohort demonstrated PR interval prolongation ≥200ms, which was associated in multivariate analyzes with HCM-related death (adjusted RR 2.41;95%CI, 1.27–4.58), and the potentially lethal arrythmic endpoint of sudden death or life-threatening arrhythmic events (adjusted RR 2.60;95% CI, 1.28–5.2).[6]

This fact is compounded by the recognition in recent years of atrial cardiomyopathy, with prognostic implications, especially in patients with AF. One of the etiological factors, inflammation, the basis for several pathological processes, has its role increasingly defined in atrial remodeling, which can be a consequence or reflection of systemic and metabolic diseases such as hypertension, diabetes, renal failure, sleep apnea and obesity in addition to local processes such as atrial wall stretch, myocardial infarction and genetic factors.[7,8] The inflammatory reaction that involves oxidative stress, alterations in calcium regulation, production of pro-inflammatory cytokines, proliferation of fibroblasts and myofibroblasts as well as extracellular matrix and apoptosis causes atrial fibrosis, revealed on the electrocardiogram by the prolongation of the PR or AVB interval and increased in the atrial diameter and volume on echocardiography.[9]

In order to assess the prognostic factor of all AVBs in a Latino population, Paixão et al. from the CODE (Clinical Outcomes in Digital Electrocardiology) study evaluated the association between AVB and overall mortality in a Brazilian cohort of primary care, with 1,557,901 patients, with a mean follow-up of 3.7 years, based on a database with electrocardiograms performed mostly in primary health units. Of these, 40% were men, and the mean age was 51. The prevalence of AVB was 1.38%, the majority of the first degree (1.32% - 20,644), with 0.02% (273) and 0.04% (621) of the second and third degree, respectively. Patients with first, second and third degree AVB was associated with 24% (RS= 0.76; 95% CI: 0.71 to 0.81; p < 0.001), 55% (RS = 0.45; 95% CI: 0.27 to 0.77; p = 0.01) and 64% (RS = 0.36; 95% CI: 0.26 to 0.49; p < 0.001) lower survival rate when compared to the control group, respectively, and only Mobitz I AVB (212 patients), in the analysis of survival divided by AVB subtype, were not associated with higher mortality, unlike patients with AVB 2:1 (61 patients), with a 79% lower survival rate than the control group. Beside worst prognosis, with the lowest survival, in patients with second-degree (except Mobitz I) and third-degree AVB, the study reaffirmed the reduction in survival in patients with first-degree AVB.[10] It is worth mentioning that the mean age was similar to other studies (56 years old) that showed similar outcomes concerning first-degree AVB in a systematic review and meta-analysis carried out by Kwok et al.[1] with 400,750 patients in which they observed an increase in the relative risk of 1, 24 (95% CI 1.02-1.51) for mortality, 1.39 (95% CI 1.18-1.65) for heart failure and 1.45 (95% CI 1.23-1.71) for AF. Interestingly, there was no increase in cardiovascular mortality in this meta-analysis, data not evaluated by the CODE study. Another particularity in the Brazilian cohort is the relatively frequent presence of Chagas disease, a frequent cause of AVB.

With current evidence, the first-degree AVB should be viewed more carefully, and the electrocardiogram, despite all the advances in cardiology, with increasingly detailed and specific diagnostic imaging tests, remains a simple, available, useful, and fundamental tool in our routine.

Keywords

Atrioventricular Block; Mortality; Heart Failure.

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References

1. Kwok CS, Rashid M, Beynon R, Barker D, Patwala A, Morley-Davies A, et al. Prolonged PR Interval, First-degree Heart Block and Adverse Cardiovascular Outcomes: A Systematic Review and Meta-analysis. Heart. 2016;102(9):672-80. doi: 10.1136/heartjnl-2015-308956.
2. Kusumoto FM, Schoenfeld MH, Barrett C, Edgerton JR, Ellenbogen KA, Gold MR, et al. 2018 ACC/AHA/HRS Guideline on the Evaluation and Management of Patients with Bradycardia and Cardiac Conduction Delay: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society. Heart Rhythm. 2019;16(9):128-226. doi: 10.1016/j.hrthm.2018.10.037.
3. Lewalter T, Pürerfellner H, Ungar A, Rieger G, Mangoni L, Duru F. "First-degree AV block-a benign entity?" Insertable Cardiac Monitor in Patients with 1st-degree AV Block Reveals Presence or Progression to Higher Grade Block or Bradycardia Requiring Pacemaker Implant. J Interv Card Electrophysiol. 2018;52(3):303-6. doi: 10.1007/s10840-018-0439-7.
4. Schnabel RB, Sullivan LM, Levy D, Pencina MJ, Massaro JM, D'Agostino RB Sr, et al. Development of a Risk Score for Atrial Fibrillation (Framingham Heart Study): A Community-based Cohort Study. Lancet. 2009;373(9665):739-45. doi: 10.1016/S0140-6736(09)60443-8.
5. Magnani JW, Wang N, Nelson KP, Connelly S, Deo R, Rodondi N, et al. Electrocardiographic PR Interval and Adverse Outcomes in Older Adults: The Health, Aging, and Body Composition study. Circ Arrhythm Electrophysiol. 2013;6(1):84-90. doi: 10.1161/CIRCEP.112.975342.
6. Higuchi S, Minami Y, Shoda M, Shirotani S, Saito C, Haruki S, et al. Prognostic Implication of First-Degree Atrioventricular Block in Patients With Hypertrophic Cardiomyopathy. J Am Heart Assoc. 2020;9(6):e015064. doi: 10.1161/JAHA.119.015064.
7. Harada M, Nattel S. Implications of Inflammation and Fibrosis in Atrial Fibrillation Pathophysiology. Card Electrophysiol Clin. 2021;13(1):25-35. doi: 10.1016/j.ccep.2020.11.002.
8. Vyas V, Hunter RJ, Longhi MP, Finlay MC. Inflammation and Adiposity: New Frontiers in Atrial Fibrillation. Europace. 2020;22(11):1609-18. doi: 10.1093/europace/euaa214.
9. Szilágyi J, Sághy L. Atrial Remodeling in Atrial Fibrillation. Comorbidities and Markers of Disease Progression Predict Catheter Ablation Outcome. Curr Cardiol Rev. 2021;17(2):217-29. doi: 10.2174/1573403X16666200721153620.
10. Paixão GMM, Lima EM, Quadros AB, Cabral DPR, Coelho RR, Oliveira DM, et al. Association between Atrioventricular Block and Mortality in Primary Care Patients: The CODE Study. Arq Bras Cardiol. 2022; 119(4):564-571.

Original Article

Resistance Exercise Training Mitigates Left Ventricular Dysfunctions in Pulmonary Artery Hypertension Model

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Abstract

Background: The right ventricular hypertrophy and dilation observed in pulmonary artery hypertension (PAH) damages the left ventricle (LV) dynamics by flattening the interventricular septum.

Objective: To investigate whether low- to moderate-intensity resistance exercise training (RT) is beneficial to LV and cardiomyocyte contractile functions in rats during the development of monocrotaline (MCT)-induced PAH.

Methods: Male Wistar rats (Body weight: ~ 200 g) were used. To assess the time to potential heart failure onset (i.e., end point), rats were divided into sedentary hypertension until failure (SHF, n=6) and exercise hypertension until failure (EHF, n=6) groups. To test RT effects, rats were divided into sedentary control (SC, n = 7), sedentary hypertension (SH, n=7), and exercise hypertension (EH, n=7) groups. PAH was induced by two MCT injections (20 mg/kg, with 7 days interval). Exercise groups were submitted to an RT protocol (Ladder climbing; 55-65% of carrying maximal load), 5 times/week. Statistical significance was assumed at $P < 0.05$.

Results: RT prolonged the end point (~25 %), enhanced the physical effort tolerance (~ 55%), and mitigated the LV and cardiomyocyte contractility dysfunctions promoted by MCT by preserving the ejection fraction and fractional shortening, the amplitude of shortening, and the velocities of contraction and relaxation in cardiomyocytes. RT also prevented increases in left ventricle fibrosis and type I collagen caused by MCT, and maintained the type III collagen and myocyte dimensions reduced by MCT.

Conclusion: Low- to moderate-intensity RT benefits LV and cardiomyocyte contractile functions in rats during the development of MCT-induced PAH.

Keywords: Heart Failure; Pulmonary Hypertension; Rats; Physical Conditioning, Animal/methods; Myocytes, Cardiac; Ventricular Dysfunction, Left; Exercise.

Introduction

Increases in the pulmonary vasculature resistance, mainly caused by endothelial dysfunction, leads to pulmonary arterial hypertension (PAH).[1] The chronic pulmonary vasculature resistance overloads the right ventricle (RV), resulting in pathological remodeling,[2] and dysfunction because of hypertrophy and dilation.[1] Such remodeling affects the left ventricle (LV) dynamics because of the direct ventricular interaction. In this framework, the left ventricle dynamics are damaged by the interventricular septum flattening,[3,4] as it faces impaired early diastolic filling, reduced end-diastolic volume, and adverse remodeling.[3,5,6] Therefore, PAH patients exhibit reduced stroke volume[3] and physical effort tolerance, which negatively impacts their quality of life and survival.[7]

Pharmacological therapies aim to reduce pulmonary artery pressure and the overload to the RV, thereby maintaining the cardiac function.[8] It has been demonstrated that patients with PAH may maintain the cardiac function by non-pharmacological means, such as practicing regular physical exercise.[9,10] In the experimental model of monocrotaline (MCT)-induced severe PAH, for example, previous and early aerobic exercise have been shown to promote cardiovascular benefits, such as mitigation of right ventricular hypertrophy, dysfunction, and adverse remodeling.[11-16] Our research group[17,18] recently reported that voluntary running (i.e. intermittent high-intensity exercise) postpones the onset of heart failure, and lightens RV e adverse remodeling and myocyte dysfunction (i.e. myocyte contractility and intracellular Ca^{2+} cycling deterioration) in this model. Furthermore, our study also demonstrated that moderate-intensity continuous aerobic exercise prevents

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right ventricle adverse remodeling and myocyte contractility and Ca^{2+} cycling impairments.[19]

The use of low- to moderate-intensity resistance exercise training (RT) has been recommended to compose exercise programs to promote health and prevent cardiovascular diseases,[20,21] including those related to left ventricular dysfunction.[22] Regarding PAH, combined exercise interventions, including aerobic, resistance, and specific inspiratory muscle training proved safe for these patients and yielded significant improvements in muscle power, exercise capacity, and survival.[23-25] Nevertheless, while aerobic exercise has been demonstrated to prevent left ventricular systolic and diastolic dysfunction in both baseline and isovolumic conditions[26] in MCT-induced PAH, the impact of RT in the left ventricular dysfunction in this model is unknown.

While the animal models have supported the discovery of new therapies and the understanding of PAH pathophysiology, the model of MCT lung injury in rodents using the injection of 60 mg/kg body mass induces severe PAH in a subacute process, which is limited in order to simulate human chronic PAH.[27] In this sense, Whang et al.[28] demonstrated that 40 mg/kg MCT divided into two injections of 20 mg/kg with an interval of seven days better mimics chronic PAH with those common changes in the structure and function of pulmonary arteries and RV observed in humans. Therefore, the present study applied this model in rats to test whether low- to moderate-intensity RT might prove beneficial to LV and myocyte contractile functions during the development of MCT-induced PAH. The hypothesis of this study is that low- to moderate-intensity RT is beneficial to LV and myocyte contractile functions in rats during the development of MCT-induced PAH.

Methods

Experimental design and PAH induction

After the definition of the sample size,[29] thirty-three male Wistar rats [Body weight: ~200 g] were obtained from the animal laboratory at the Federal University of Viçosa, MG, Brazil. The animals were housed in transparent polycarbonate cages, kept in a room with a controlled temperature (~22 °C) and ~60% relative humidity, under a 12/12 h light/dark cycles, and had free access to water and commercial chow.

To assess the time to the onset of potential heart failure, 12 animals (~200 g) were divided into two groups, by using simple randomization: sedentary hypertension until failure (SHF, n = 6) and exercise hypertension until failure (EHF, n = 6). After the MCT injections, rats from the SHF and EHF groups were euthanized when they showed previously validated external clinical signs of potential heart failure onset (e.g., weight loss, dyspnea, piloerection) and could no longer feed properly, climb the ladder (EHF group), or even move in the cage,[30-37] which was considered the end point.

To test whether RT is beneficial during the development of PAH, 21 animals (~200 g) were divided into groups using blocked randomization: sedentary control (SC, n = 7), sedentary hypertension (SH, n = 7), and exercise hypertension (EH, n = 7). Animals from the SH, EH, and SC groups were euthanized at the median end point day (± 1 day) of the SHF animals (i.e., 28 days). The median time to the onset of potential heart failure represented the moment after MCT treatment when more than 50% of the group reached the end point day. The animals in the exercise groups were submitted to RT while those in sedentary groups were maintained in their cages.

To induce PAH, animals from the SHF, EHF, SH, and EH groups received 2 intraperitonial MCT (Sigma-Aldrich, USA) injections of 20 mg/kg, at a 7-day interval, to induce right ventricular failure.[28] Control animals received equivalent volume injections of saline.

Experiments were conducted in accordance with international procedures for animal research (Scientific Procedures; Act 1986). All protocols were reviewed and approved by the Institutional Ethics Committee (protocol number 02/2019).

Resistance training and maximal load test

The animals were familiarized to the RT protocol (adapted from Hornberger and Farrar[38]) for one week before the first MCT or saline injection, with no additional load. RT consisted of climbing a ladder (1.1 m high; 80º inclination) with 2 min resting intervals, with the load based on a maximal carrying load test. The maximal carrying load test was performed before MCT or saline injection (time 0) and on the 14th, 21st, and 28th day after injections. The test consisted of ladder climbing with an initial load of 75% of body weight, which was progressively increased by an additional 15% in the subsequent climbs until the animal could no longer climb.[39] The load was fixed on the rat's tail, and climbs were interspersed with 2 min resting intervals. The maximal carrying load was used as the physical effort tolerance index.

Exercised animals were submitted to a RT program, 5 times/week during the experimental period until the day before euthanasia, totaling twenty exercise sessions. RT load was 55-65% of the maximal carrying load, following the recommendations for patients with cardiovascular diseases.[20] Each training session consisted of 15 climbs, interspersed with 60-second intervals, with the training load adjusted after the maximal carrying load tests (14th and 21st day).

Echocardiography and sample collection

The echocardiographic evaluations were performed on the 28th day after the first MCT injection. The animals were anesthetized (Isoflurane 1.5% and 100% oxygen in a constant flow of 1L/min; Isoflurane, BioChimico, Brazil), and the images were obtained while the animals remained in the lateral decubitus position. Two-dimensional studies with a fast-sampling rate of 120 fps in M-mode were performed using the MyLabTM30 ultrasound system (Esaote, Genoa, Italia) and 11 MHz nominal frequency transducers. The two-dimensional transthoracic echocardiography and M-mode was obtained at a scanning speed of 200 mm adjusted according to the heart rate.[40] To evaluate LV function, the following parameters were assessed: LV ejection fraction (EF) and fractional shortening (FS). To characterize the PAH, the tricuspid annular plane systolic excursion (TAPSE) was determined.

At the median end point day (± 1 day) of the SHF animals, animals from SH, EH, and SC groups were euthanized. After euthanasia, animals from SC, SH, and EH groups had the heart, ventricles, and lungs dissected, weighed, and processed

for analyses of interest, as described below. The right tibia was dissected, and its length was measured.

Histomorphometry

The histological analyses of the LV were performed as previously described.[41,42] Briefly, immediately after collection, fragments of the LV were fixed on the Karnovsky fixator (paraformaldehyde 4% and glutaraldehyde 4% in 0.1M phosphate buffer, pH 7.4) for 24 hours. The fragments were then dehydrated in ethanol, clarified in xylol, and embedded in paraffin. Blocks were cut into 5 μm-thick sections, mounted on histological slides, and stained with Hematoxylin & Eosin to measure the cross-sectional area (CSA), or with Sirius Red to count collagen fibers and/or with Masson's trichrome for cardiac fibrosis count. To avoid repeated analyses of the same histological area, the sections were evaluated in semi-series, using one in every 10 sections. Digital images from Sirius Red stained slides were obtained using a polarized light microscope (Olympus AX-70, Tokyo, Japan) connected to a digital camera (Olympus Q Color-3, Tokyo, Japan), and images of slides stained with Hematoxylin & Eosin and Masson's trichrome were obtained using a light microscope (Olympus AX-70, Tokyo, Japan) connected to a digital camera (Olympus Q Color-3, Tokyo, Japan). The quantification of collagen types and cardiac fibrosis was performed using a specific color identification tool using the Image-pro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA). Myocyte CSA was measured using a specific tool (manual measurement in software image pro-plus 4.5).

Isolation of left ventricle myocytes

The heart was attached to a Langendorff-retrograde perfusion system, and single LV myocytes were isolated as previously described.[18] Briefly, the heart perfused system via aorta with Tyrode solution containing (in mM; Sigma-Aldrich, USA): 130 NaCl, 1.43 MgCl2, 5.4 KCl, 0.75 CaCl2, 5.0 Hepes, 10.0 glucose, 20.0 taurine, and 10.0 creatine, pH 7.4 until for about 5 min. The Tyrode solution was exchanged to Tyrode solution containing EGTA (0.1 mM) for 6 min. The heart was then perfused with Tyrode solution containing 1 mg/ml collagenase type II (Worthington, USA) and 0.1 mg/ml protease (Sigma-Aldrich, USA) for about 12 min. Next, the LV of the digested heart was removed and cut into small fragments, which were placed into a conical flask containing the enzymatic solution (collagenase and protease). The cells were mechanically separated by shaking the flask for 5 min. The dispersed cells were separated from the non-dispersed tissue by filtration through centrifugation. The isolated cells were stored at 5°C until use. Isolated myocytes were used within 2 to 3 hours after isolation. The solutions used in the isolation procedure were oxygenated (O_2 100% - White Martins, Brazil) and maintained at 37ºC.

Single myocyte contractile function

The contractile function of LV myocytes was measured using an edge detection system (Ionoptix, Milton, USA) mounted on an inverted microscope (Nikon Eclipse - TS100, Japan) as previously described.[19] Myocytes were placed in a bath on the stage of an inverted microscope and superfused with Tyrode's solution containing, in mM (Sigma-Aldrich, USA): 137 NaCl, 5.4 KCl, 0.33 NaH_2PO_4, 0.5 $MgCl_2$, 5 HEPES, 5.6 glucose 1.8 $CaCl_2$, pH 7.4 with 5N NaOH, at 37°C. Only myocytes exhibiting a clear, regular striation (sarcomere) pattern, with no spontaneous contraction in the absence of external stimulation, and responding to 1Hz stimulation with a single twitch were tested. Myocytes were stimulated (Myopacer, Ionoptix, Milton, USA) to contract at a progressive stimulation frequency (1, 3, 5 and 7 Hz) using external electrodes, and the resultant cell shortening was measured by analyzing a video image of the cell using Ionoptix camera and software (Ionoptix, Milton, MA, USA). Cell shortening was expressed as % of resting cell length.

The myocyte length and width were obtained from the video image of the cell; and the cell volume was calculated as previously described.[43]

Statistical analysis

The normality of the data was tested using the Shapiro-Wilk test. Data are presented as mean ± SD for continuous variables with normal distribution and median accompanied by the interquartile range for continuous variables without normal distribution. The end point, ventricular remodeling parameters, and contractile parameters of isolated cardiomyocytes showed a non-normal distribution, while exercise parameters, body and organ weight, and cross-sectional area and isolated cell morphometry presented normal distribution. The end point was tested by the Kaplan-Meier curve analysis through the Log-rank test. Maximum carrying load, body weight, LV function, organ weight, and single cell parameters were tested by one-way analysis of variance (ANOVA) or Kruskal-Wallis followed by the Dunn's post hoc test. Maximum carrying load was tested by one-way repeated measures ANOVA. ANOVAs were followed by the pairwise Tukey correction test. Person's Chi-squared test (X^2) was used to assess the proportion of animals that presented intraventricular septum flattening. Statistical significance was assumed at $P < 0.05$. Data description, numbers of rats and myocytes are given in the table and figure legends. All analyses were performed using GraphPad Prism, version 6.01 (San Diego, CA, USA).

Results

Onset of potential heart failure and physical effort tolerance

Figure 1A illustrates that animals from the EHF group performed the resistance exercise protocol during the development of PAH until presenting signs of the onset of potential heart failure. The maximal carrying load increased progressively until day 21, and afterwards it decreased to the initial level on the 35th day after the first MCT injection. All animals from both SHF and EHF groups presented signs of the onset of potential heart failure - end point (Figure 1B); however, animals in the EHF group had a longer median end point time (37 days) than did those in SHF group (28 days), indicating benefits of resistance exercise.

Hypertensive rats from the EH group improved their tolerance to physical effort (Figure 1C) throughout the experiment. The maximum carrying load in the EH group was higher on days 21 and 28 than on day 0. Moreover, these

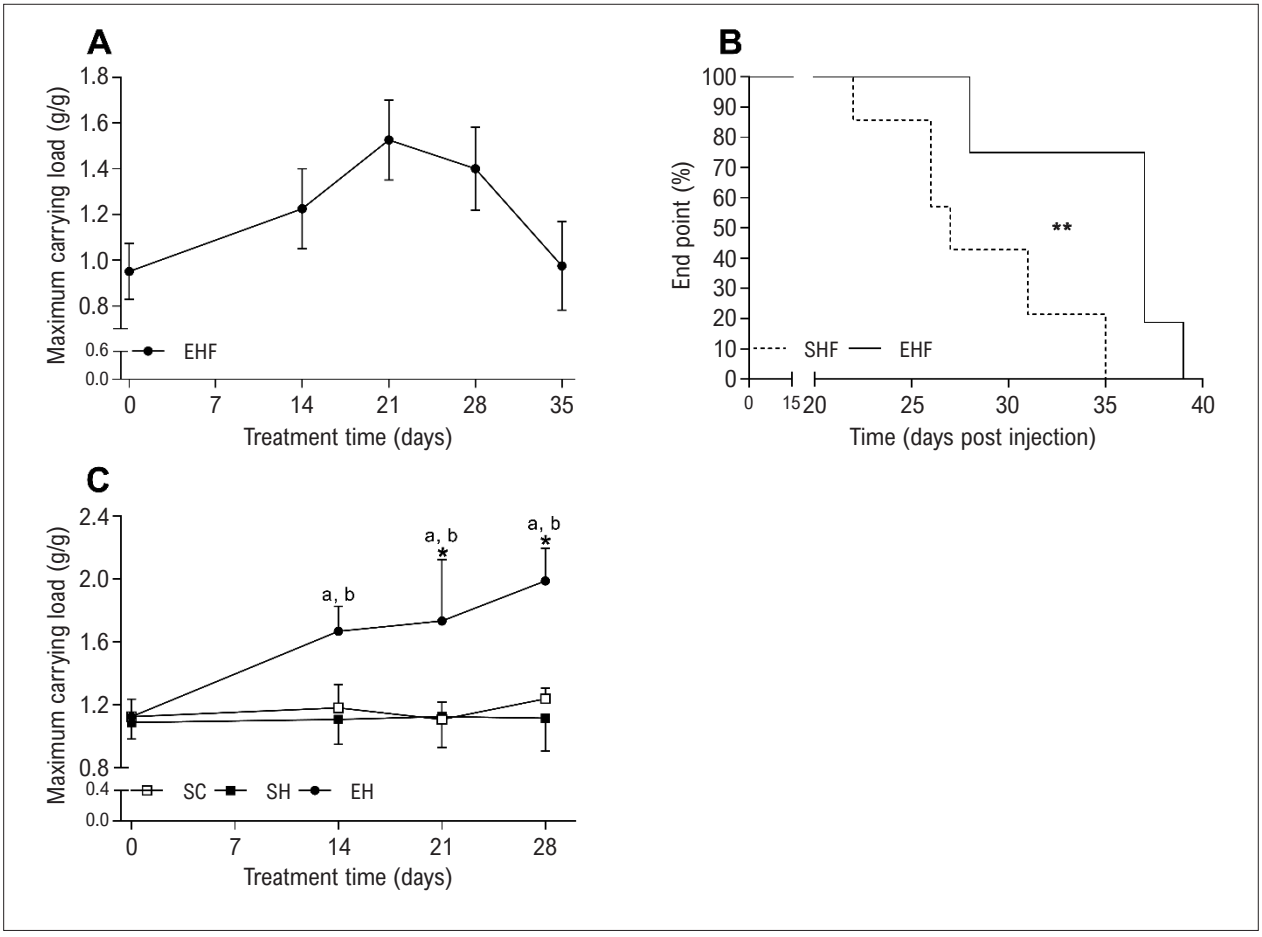

Figure 1 – *Effect of resistance training on the onset of potential heart failure (end point) and physical effort tolerance. (A) Relative maximal carrying load of hypertensive animals until failure, determined by the maximal carrying load normalized to body weight, at pre-injection (day 0) and on the 14th, 21st, 28th, and 35th day after the first monocrotaline injection. (B) End point, measured in days to present signs of the onset of potential heart failure, was significantly shorter in sedentary hypertension until failure (SHF, n = 6) than in exercise hypertension until failure (EHF, n = 6) rats. **P < 0.01, Kaplan-Meier curve analysis by the Log-rank test. (C) Relative maximal carrying load of control, hypertensive sedentary and exercise animals, determined as in panel A. Exercise hypertension (EH, n=7) rats exhibited higher carrying load gain than sedentary control (SC, n=7) and sedentary hypertension (SH, n=7) from the 14th day on. Repeated measures ANOVA followed by Tukey correction test. aP < 0.05 vs. SH; bP < 0.05, vs. SC; *P < 0.05 vs. Before MCT injection.*

animals had a higher maximum carrying load on the 14th, 21st and 28th day, when compared to those in the SC and SH groups.

Left ventricular function and morphology

The echocardiographic evaluation showed a flattening of the interventricular septum, called the D-shaped left ventricle, in animals from the SH and EH groups (Figure 2A), which suggests RV pressure overload, characteristic in PAH. Such a morphological change was greater in the SH than in the SC group, while EH presented intermediate values (Figure 2D). Regarding the left ventricle function, no difference between the groups for ejection fraction (Figure2B) and fractional shortening (Figure 2C) was found. Despite that, it is important to note that 3 out of 7 animals in the SH group presented an ejection fraction < 50%, and 3 out of 7 presented fractional shortening < 25%, indicative of left ventricular failure. By contrast, none of the exercised animals (EH), in the same period, showed an ejection fraction < 50%, and only 1 out of 7 presented fractional shortening < 25%.

The presence of PAH in animals from the SH group was also characterized by the TAPSE values. Animals from the SH group exhibited lower TAPSE values (1.43 ± 0.23) than did those from the SC (2.06 ± 0.17) and EH (2.13 ± 0.36) groups.

Animals from the SH group presented lower body weight than did those from the SC and EH groups (Table 1). Despite no difference between group for heart weight, animals from the SH and EH groups had higher RV weight and right ventricle-to-tibia ratio than did animals in the SC group, which indicates RV hypertrophy. While lung weight and lung weight-to-tibia ratio were higher in the SH and EH groups than in the SC group, no difference between groups for LV weight and LV weight-to-tibia ratio was found. Regarding left ventricle myocyte dimensions, the SH group presented a lower length, width, and volume than did the SC group. The EH group presented intermediate values between the SC and SH groups. Animals of the SH groups exhibited lower CSA when compared to animals in the SC and EH groups. By contrast, there was no difference in the CSA of animals in the EH group when

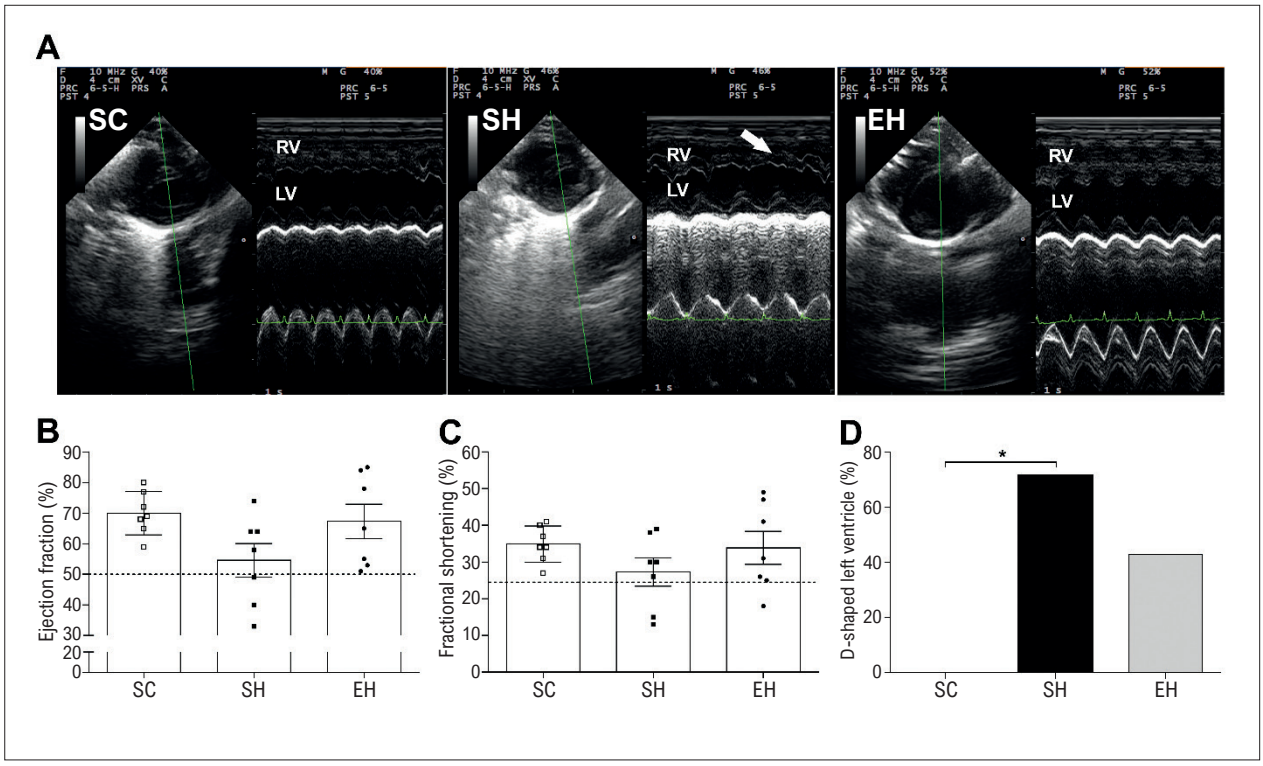

Figure 2 – *Effect of resistance exercise training on left ventricular function assessed on the 28th day after the first monocrotaline injection. (A) Representative echocardiograph images. (B) Ejection fraction. (C) Fractional shortening. (D) D-shaped left ventricle. Values are means ± SD (n = 7 rats in each group). SC: sedentary control; SH: sedentary hypertension; EH: exercise hypertension; RV: right ventricle; LV: left ventricle. Dotted line indicates limits for the classification of impaired function. Panel B and C: One-Way ANOVA followed by the Tukey's post hoc test. Panel D: Pearson' s Chi-squared test (x2 test). *p < 0.05.*

Table 1 – Effect of resistance exercise training on body and organ weights

	SC	SH	EH
Final BW (g)	298.6 ± 19.01	276.3 ± 19.87*	303.7 ± 20.98†
Heart weight (g)	1.23 ± 0.11	1.30 ± 0.18	1.28 ± 0.12
RV weight (g)	0.33 ± 0.04	0.42 ± 0.03*	0.44 ± 0.05*
LV weight (g)	0.74 ± 0.10	0.65 ± 0.07	0.70 ± 0.07
Lung weight (g)	1.65 ± 0.28	2.77 ± 0.41**	2.38 ± 0.33**
Ratio of RV weight to tibia length (g/cm)	0.09 ± 0.01	0.11 ± 0.00*	0.11 ± 0.01*
Ratio of LV weight to tibia length (g/cm)	0.20 ± 0.02	0.17 ± 0.02	0.18 ± 0.02
Ratio of lung weight to tibia length (g/cm)	0.45 ± 0.10	0.73 ± 0.11**	0.63 ± 0.09*
Myocyte length (μm)	132.3 ± 19.09	122.5 ± 19.86**	129.2 ± 21.42
Myocyte width (μm)	46.12 ± 10.08	41.75 ± 9.95*	43.64 ± 9.50
Myocyte volume (pL)	46.24 ± 3.97	38.71 ± 3.18**	42.62 ± 3.61
Myocyte CSA (μm^2)	462.1 ± 21.86	400.5 ± 43.34*	492.2 ± 66.56†

*Data are mean ± SD of 7 rats and 10 cells in each group; SC: sedentary control; SH: sedentary hypertension; EH: exercise hypertension; BW: body weight; RV: right ventricle; LV: left ventricle; *p < 0.05 vs. SC; **p < 0.01 vs. SC; †p < 0.05 vs. SH. One-way ANOVA followed by the Tukey post hoc test.*

compared to animals in the SC group, suggesting a beneficial effect of the resistance exercise program in preventing adverse left ventricle remodeling.

Left ventricular adverse remodeling

Figure 3 shows data on LV collagen fibers and fibrosis. Hypertensive animals (SH and EH) presented a higher percentage of type I collagen compared to animals in the control group (SC) (Figure 3A). However, animals in the EH group had a lower percentage of type I collagen when compared to animals in the SH group, showing the protective effect of RT on the progression of PAH. In addition, animals in the EH group exhibited a higher percentage of type III collagen than those in the sedentary animals (SC and SH) (Figure 3B). Hypertensive animals (SH and EH) had a higher percentage of total collagen, compared to animals in the control group (Figure 3C). Concerning LV fibrosis (Figure 3D), animals in the SH group presented a higher percentage, compared to the those in the SC and EH groups. There was no difference between the percentage of fibrosis in animals from the EH and SC groups, showing a beneficial effect of resistance exercise on the prevention of pathological cardiac remodeling.

Single myocyte contractile function

Under electrical stimulation, myocytes from SH animals showed a positive contraction-frequency relationship at the frequencies of 1, 3, and 5 Hz and a lower magnitude of shortening than those from the SC and EH groups (Table 2). Such a difference lost its statistical difference from 5 to 7Hz, where the contraction-frequency relationship became negative. In addition, the departure velocity (an index of contraction velocity) was slower in cells from the SH group than in those from the SC group over the range of 1-7 Hz. However, when compared to the EH, the lower speed was found only at 1, 3, and 7 Hz. Likewise, the return velocity (an index of relaxation velocity) was slower in the SH group than in the SC group. When compared to the EH, the slower speed was found only at 1 and 3 Hz.

Discussion

The present study examined whether low- to moderate-intensity RT might prove beneficial to LV and myocyte contractile functions in rats during the development of MCT-induced PAH. Our findings demonstrate for the first time that rats treated with MCT (Two MCT injections of 20 mg/kg, at a 7-day interval) climbed the ladder during the development of PAH and progressively increased their tolerance to physical effort. Our study's index of physical effort tolerance, the maximal carrying load, was progressively higher in the EH group compared to the SH and SC groups throughout the experiment. This model of RT was efficient in increasing muscle strength in another rat model of hypertension.[44] The increase in body weight and maximal carrying load observed here suggests a protective effect of RT against skeletal muscle loss and dysfunction. This is an interesting finding since sarcopenia, intolerance to physical effort, and lethargy are reported characteristics of this PAH model.[42,45-47] Muscle power has proven to be improved in PAH patients in response to combined exercise (Aerobic + Resistance) programs.[23-25] Moreover, the increase in muscle strength is important for hypertensive individuals, as it lightens the cardiovascular overload during their daily life activities and has been associated with protection against all-cause mortality.[48]

The RT program used in the present study expanded the time until the animals exhibited the signs of the onset of potential heart failure (i.e., end point). Although there is no study on the effects of the RT model on such an end point in rats with MCT-induced PAH, prolonged end point in rats injected with MCT in response to voluntary running has been reported by our group,[17,18] and extended survival in response to treadmill running has been demonstrated by others,[45,49] more markedly when started at the early stages of the disease. Enhanced survival has also been demonstrated in PAH patients submitted to combined exercise (Aerobic + Resistance) interventions.[23-25]

Our RT regime benefited the LV functional and structural parameters in MCT-injected rats. Regarding left ventricular function, echocardiography showed that 42.86% of sedentary rats injected with MCT (SH group) had an ejection fraction below 50%, and 28.57% presented fractional shortening below 25%, which indicates left ventricular dysfunction. Nevertheless, in exercised animals (EH group) the presence of left ventricular disfunction was lower than in sedentary rats (SH group), thus suggesting a protective role of resistance exercise. These findings run in line with changes caused by the employed RT in the LV tissue. For instance, RT increased the percentage of type III collagen while it reduced the percentage of type I collagen and fibrosis in rats with MCT-induced PAH, thus showing the protective effect of this exercise regime against left ventricular dysfunction and adverse remodeling leading to mitigation of the PAH progression.

The organ parameters showed that MCT-injected sedentary rats (SH group) exhibited higher RV (i.e. RV weight, Fulton's index, and RV weight/tibia length ratio) and lung (i.e. Lung weight, lung weight/tibia length ratio) values than did the control group (SC). Despite no change in whole LV weight and LV to tibia length ratio, single myocyte length, width, and volume were decreased by MCT (SC > SH). However, RT prevented this type of cell dimension reduction (EH = SC), which indicates the maintenance of the left ventricular mass and suggests the protective effect of the applied RT program against the left ventricular adverse remodeling.

Along with reduction in myocyte dimensions, MCT induced single myocyte contractile dysfunction. Myocytes from the SH group had lower shortening and longer contraction and relaxation velocities than did those in the SC group. More importantly, RT mitigated the contractile dysfunction as these cell parameters in the EH group were similar to those in the SC group, thus indicating improvements in the contractile function in myocytes from the EH group relative to those from the SH group. The calcium regulatory proteins (i.e., Ryanodine receptor 2, Phospholamban, and Sarcoplasmic reticulum ATPase 2a) manage the force and time course of cardiomyocyte contraction and are reported to be downregulated in the RV of MCT-treated rats.[15,45] Whether the employed RT regime increases the expression and activity of these proteins warrants further investigations, though such an exercise effect has been demonstrated in normotensive healthy rats.[50,51]

Taken together, the present study's results demonstrate that the RT employed during the development of MCT-induced PAH was beneficial to left ventricle and myocyte contractile structure and function, which resulted in enhanced tolerance to physical effort and time to the onset of potential heart failure in the animals.

Considering resistance exercise recommendations for patients with cardiovascular diseases,[20] the present used low- to moderate-intensity. Considering that high-intensity exercise is reported to promote the highest benefits in patients with heart failure,[52] and that MCT-injected rats exercised with a progressive load until the median end point time of the sedentary rats (28 days), it might be possible to experimentally increase exercise intensity using reward techniques to expand the resistance exercise effects.

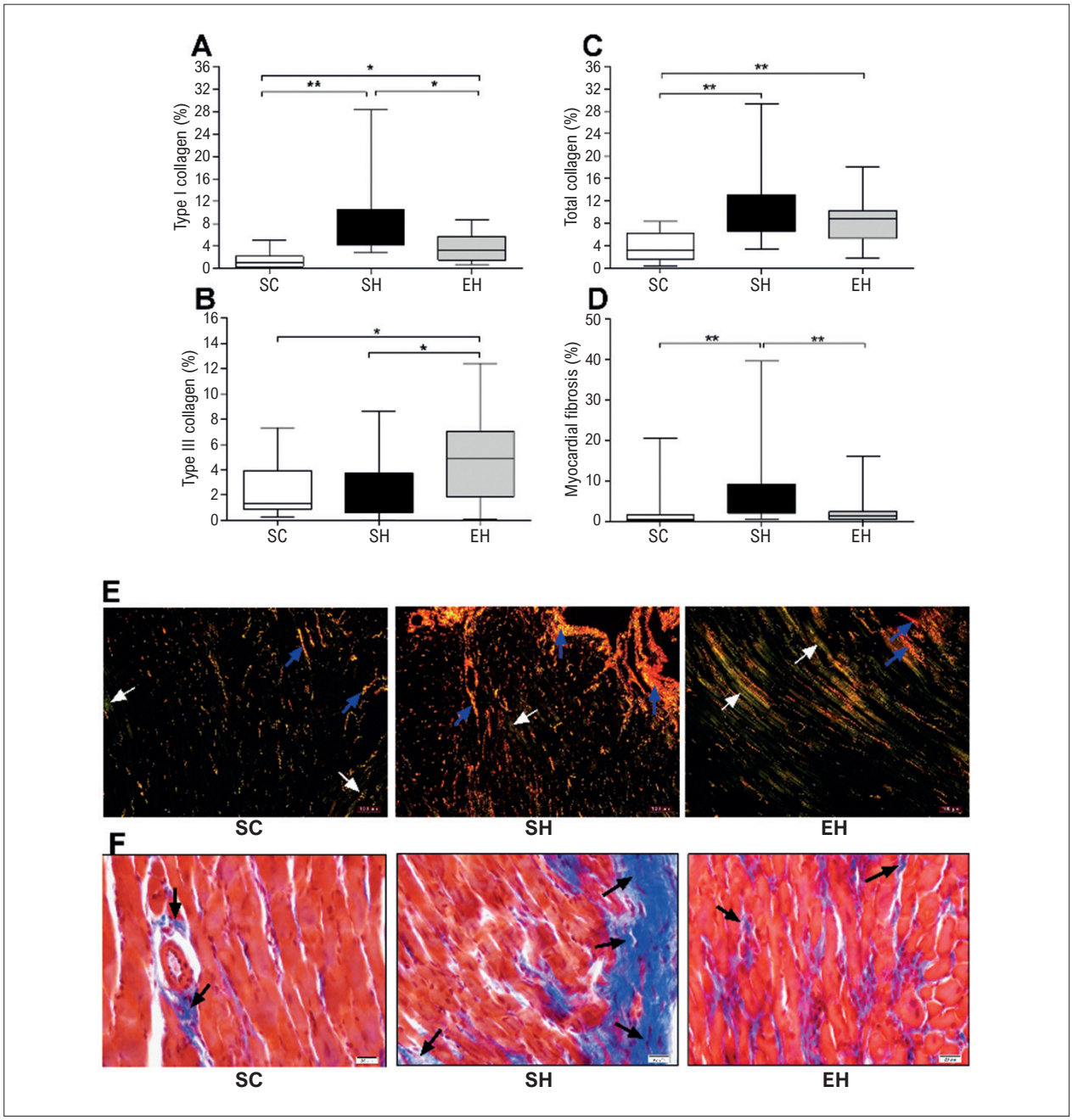

Figure 3 – *Effect of resistance exercise training on left ventricle remodeling. (A) Percentage of type I collagen. (B) Percentage of type III collagen. (C) Percentage of total collagen. (D) Percentage of fibrosis in the LV. (E) Representative photomicrographs of LV tissue stained with Sirius Red; (F) Representative photomicrographs of LV tissue stained with Masson's trichrome. Blue arrow indicates type I collagen; White arrow indicates type III collagen; Black arrow indicates cardiac fibrosis. Values are presented as median accompanied by the interquartile range of 10 images per animal in each group (n = 5 rats in each group). SC: sedentary control; SH: sedentary hypertension; EH: exercise hypertension. Kruskal-Wallis, followed by the Dunn's post hoc test: * P < 0.05, and ** p < 0.01.*

Table 2 – Effect of resistance exercise training on left ventricular myocyte contraction and relaxation

	SC	SH	EH
	Median (IQR 25%-75%)	Median (IQR 25%-75%)	Median (IQR 25%-75%)
Shortening (% r.c.l.)			
S.F. (1 Hz)	7.69 (5.74-9.42)	5.26 (3.23-7.08)*	7.89 (5.80-9.30)†
S.F. (3 Hz)	8.02 (5.47-10.14)	6.08 (3.73-8.29)*	7.70 (6.59-10.11)†
S.F. (5 Hz)	8.16 (6.06-10.15)	6.74 (4.83-8.78)*	8.26 (6.25-10.20)†
S.F. (7 Hz)	7.32 (4.86-9.33)	6.04 (4.37-8.01)	6.95 (5.41-8.90)
Departure velocity			
S.F. (1 Hz)	262.9 (191.8-330.3)	189.2 (106.1-266.8)*	250.7 (179.1-307.0)†
S.F. (3 Hz)	317.7 (222.6-411.2)	250.2 (129.3-332.6)*	288.5 (226.4-416.1)†
S.F. (5 Hz)	365.8 (246.7-473.2)	303.3 (175.5-417)*	342.4 (254.7-467.3)
S.F. (7 Hz)	369.8 (284.4-472.9)	322.2 (209.7-367.9)*	344.9 (293.1-469.5)†
Return velocity			
S.F. (1 Hz)	229.0 (158.2-282.5)	143.6 (76.53-220.7)*	206.5 (148.4-274.1)†
S.F. (3 Hz)	254.6 (177.2-321.3)	191.9 (97.98-254.2)*	241.3 (159.2-323.8)†
S.F. (5 Hz)	273.3 (218.4-354.5)	236.1 (126.9-279.6)*	247.6 (178.4-353.6)
S.F. (7 Hz)	285.2 (226.9-362.6)	234.3 (153.5-293.8)*	260.5 (202.9-356.9)

*Data are presented as median accompanied by the interquartile range (IQR) of 10 cells per animal in each group (n = 7 rats in each group). % r.c.l., percentage of resting cell length; SF: stimulation frequency; SC: sedentary control; SH: sedentary hypertension; EH: exercise hypertension. *p < 0.05 vs. SC; **p < 0.01 vs. SC; †p < 0.05 vs. SH. Kruskal-Wallis, followed by the Dunn's post hoc test.*

Finally, this study has limitations. First, the speed of climbing is not controlled in this model. Second, the duration of the training period is limited by the effects of MCT. Despite that, our results showed positive effects of the resistance exercise program on both the time to the onset of potential heart failure, physical effort tolerance, and LV dysfunction.

Conclusion

Our findings demonstrate that along with the increase in the time to the onset of potential heart failure and in the physical effort tolerance, low- to moderate-intensity resistance exercise mitigates the development of left ventricular dysfunctions in the MCT-induced PAH model. Therefore, low- to moderate-intensity RT is beneficial to left ventricular and myocyte contractile functions in this model. These results are of clinical relevance, as they support the health benefits of resistance exercise to individuals with cardiopulmonary disease, including PAH. We suggest that low- to moderate-intensity resistance exercise should be tested in PAH patients.

Author Contributions

Conception and design of the research: Soares LL, Natali AJ; Acquisition of data: Soares LL, Leite LB, Ervilha LOG, Silva BAF, Freitas MO, Portes AMO, Rezende LMT, Drummond FR, Reis ECC; Analysis and interpretation of the data: Soares LL, Leite LB, Ervilha LOG, Portes AMO, Rezende LMT, Carneiro-Junior MA, Neves MM, Reis ECC, Natali AJ; Statistical analysis: Soares LL; Obtaining financing: Natali AJ; Writing of the manuscript: Soares LL, Natali AJ; Critical revision of the manuscript for important intellectual content: Soares LL, Carneiro-Junior MA, Neves MM, Reis ECC, Natali AJ.

Potential Conflict of Interest

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

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Study Association

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Universidade Federal de Viçosa under the protocol number 02/2019. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013.

References

1. Vaillancourt M, Ruffenach G, Meloche J, Bonet S. Adaptation and remodelling of the pulmonary circulation in pulmonary hypertension. Can J Cardiol.2015;31(4):407-15. Doi: 10.1016/j.cjca.2014.10.023.

2. Lai YC, Potoka KC, Champion HC, Mora AL, Gladwin MT. Pulmonary arterial hypertension: the clinical syndrome. Circ Res.2014;115(1):115-30. Doi: 10.1161/circresaha.115.301146.

3. Gan C, Lankhaar JW, Marcus JT, Westerhof N, Marques KM, Bronzwaer JG,et al. Impaired left ventricular filling due to right-to-left ventricular interaction in patients with pulmonary arterial hypertension. Am j Physiol Heart Circ Physiol.2006;290(4):H1528-H1533. Doi: 10.1152/ajpheart.01031.2005.

4. Puwanant S, Park M, Popovic ZB,WH Wilson Tang, Farha S, George D, et al. Ventricular geometry, strain, and rotational mechanics in pulmonary hypertension. Circulation. 2010;121(2):259-66. Doi: 10.1161/circulationaha.108.844340.

5. Marcus JT, Vonk Noordegraaf A, Roeleveld RJ, Postmus PE, Heethaar RM, Van Rossum AC, A Boonstra A, Impaired left ventricular filling due to right ventricular pressure overload in primary pulmonary hypertension: noninvasive monitoring using MRI. Chest.2001; 119: 1761-5. Doi: 10.1378/chest.119.6.1761.

6. Hardziyenka M, Campian ME, Reesink HJ,Surie S, Bouma BJm Groenink RM, et al. Right ventricular failure following chronic pressure overload is associated with reduction in left ventricular mass: evidence for atrophic remodeling.J Am Coll Cardiol.2011;57(8):921-8. 2011;57(8):921-8.Doi: 10.1016/j.jacc.2010.08.648.

7. McGoon MD, Benza RL, Escribano-Subias P, Jiang X, Miller DP, Peacock AJm et al. Pulmonary arterial hypertension: epidemiology and registries. J Am Coll Cardioil.2013;62(Suppl .1):D51-9. Doi: 10.1016/j.jacc.2013.10.023.

8. Lajoie AC, Bonnet S, Provencher S. Combination therapy in pulmonary arterial hypertension: recent accomplishments and future challenges. Pulm Circ.2017;7(2):312-25. Doi: 10.1177/2045893217710639.

9. Zafrir B. Exercise training and rehabilitation in pulmonary arterial hypertension: rationale and current data evaluation. J Cardiopulm Rehabil Prev.2013;33(5):263-73. Doi: 10.1097/HCR.0b013e3182a0299a.

10. Sahni S, Capozzi B, Iftikhar A, Sgouras V, Ojrzanowski M, Talwar A. Pulmonary rehabilitation and exercise in pulmonary arterial:an underutilized intervention. J Exerc Rehab. 2015;11(2):74-9. Doi: 10.12965/jer.150190.

11. Colombo R, Siqueira R, Becker CU,Fernandes TG, Peres KM, Valença SS, et al. Effects of exercise on monocrotaline-induced changes in right heart function and pulmonary artery remodeling in rats. Can J Physiol Pharmacol.2013;91(1):38-44. Doi: 10.1139/cjpp-2012-0261.

12. Colombo R, Siqueira R, Conzatti A, Fernandes TG, Tavares AM, Araujo AS, et al Cor Pulmonale. J Cardiovasc Pharmacol.2015;66(3):246-53. Doi: 10.1097/fjc.0000000000000272.

13. Moreira-Gonçalves D, Ferreira R, Fonseca H, Padrão Ai, Moreno N, Silva AF, et al. Cardioprotective effects of early and late aerobic exercise training in experimental pulmonary arterial hypertension. Basic Res Cardiol. 2015;110 (6):57. Doi: 10.1007/s00395-015-0514-5.

14. Colombo R, Siqueira R, Conzatti A,Seokin B, Silva J, Tucci PJ, et al. Exercise training contributes to H2O/VEGF signaling in the lung of rats with monocrotaline-induced pulmonary hypertension. Vasc Pharmacol.2016;87:49-59. Doi: 10.1016/j.vph.2016.06.006.

15. Pacagnelli FL, de Almeida Sabela AK, Okoshi K,Mariano TB, Carvalho RF. Preventive aerobic training exerts a cardioprotective effect on rats treated with monocrotaline.Int J Exper Pathol. 2016;97(3):238-47. Doi: 10.1111/iep.12166.

16. Nogueira-Ferreira R, Moreira-Gonçalves D, Silva AF,Duarte JÁ, Leite-Moreira A, Ferreira R, et al. Exercise preconditioning prevents MCT-induced right ventricle remodeling through the regulation of TNF superfamily cytokines. Int J Cardiol.2016;203:858-66. Doi: 10.1016/j.ijcard.2015.11.066.

17. Soares LL, Drummond FR, Rezende LMT,Costa A, Primola Gomes TN, Carneiro Jr MG, et al. Voluntary running counteracts right ventricular adverse remodeling and myocyte contraction impairment in pulmonary arterial hypertension model. Life Sci. 2019; 238:116974. Doi: 10.1016/j.lfs.2019.116974.

18. Natali AJ, Fowler ED, Calaghan SC, White E. Voluntary exercise delays heart failure onset in rats with pulmonary artery hypertension. Am J Physiol Heart Circ Physiol.2015;309(3):H421-4. Doi: 10.1152/ajpheart.00262.2015.

19. Silva FJ, Drummond FR, Fidelis MR. Continuous Aerobic Exercise Prevents Detrimental Remodeling and Right Heart Myocyte Contraction and Calcium Cycling Dysfunction in Pulmonary Artery hypertension. J Cardiovasc Pharmacol.2021;77(1):69-78. Doi: 10.1097/fjc.0000000000000928.

20. Williams MA, Haskell WL, Ades PA, Armsterdam EA, Bittner V, Franklin BA, et al. Resistance exercise in individuals with and without cardiovascular disease: 2007 update: a scientific statement from the American Heart Association Council on Clinical Cardiology and Council on Nutrition, Physical Activity, and Metabolism. Circulation.2007;116(5):572-84. Doi: 10.1161/circulationaha.107.185214.

21. Braith RW, Stewart KJ. Resistance exercise training: its role in the prevention of cardiovascular disease. Circulation. 2006;113(22):2642-50. Doi:10.1161/CIRCULATIONAHA.105.584060.

22. Delagardelle C, Feiereisen P. Strength training for patients with chronic heart failure. Eura Medicophys 2005; 41(1):57-65. PMID:161757.

23. González-Saiz L, Fiuza-Luces C, Sanchis-Gomar F, Santos-Lozano A, Quezada-Loaiza CA, Flox Camacho A. Benefits of skeletal-muscle exercise training in pulmonary arterial hypertension: The WHOLEi+12 trial. Int J Cardiol.2017;231:277-83. Doi: 10.1016/j.ijcard.2016.12.026.

24. Zhang X, Xu D. Effects of exercise rehabilitation training on patients with pulmonary hypertension. Pulm Circ. 2020;10(3):2045894020937129. Doi: 10.1177/2045894020937129.

25. Yan L, Shi W, Liu Z. The benefit of exercise-based rehabilitation programs in patients with pulmonary hypertension: a systematic review and meta-analysis of randomized controlled trials. Pulm Circ.2021;11(2):20458940211007810. Doi: 20458940211007810.

26. Schmidt C, Bovolini JA, Gonçalves N, Vasquez-Novoa F, Andrade MA, Santos M, et al. Exercise preconditioning prevents left ventricular dysfunction and remodeling in monocrotaline-induced pulmonary hypertension. Porto Biomed J.2020;5(5):e081. Doi: 10.1097/j.pbj.0000000000000081.

27. Gomez-Arroyo JG, Farkas L, Alhussaini AA, Farkas D, Kraskaukas D, Voelkel NF,et al. The monocrotaline model of pulmonary hypertension in perspective. Am J Physiol Lung Cell Mol Physiol. 2012;302(4):L363-9. Doi: 10.1152/ajplung.00212.2011.

28. Zhuang W, Lian G, Huang B. Pulmonary arterial hypertension induced by a novel method: Twice-intraperitoneal injection of monocrotaline. Exp Biol Med (Maywood NJ.)2018;243(12):995-1003. Doi: 10.1177/1535370218794128.

29. Charan J, Kantharia ND. How to calculate sample size in animal studies? J Pharmacol. Pharmacoth.2013;4(4):303-6. Doi: 10.4103/0976-500x.119726.

30. Fowler ED, Benoist D, Drinkhill MJ,Stones R, Helmes M, Wust RCI, et al. Decreased creatine kinase is linked to diastolic dysfunction in rats with right heart failure induced by pulmonary artery hypertension. J Mol Cell Cardiol. 2015; 86:1-8. Doi: 10.1016/j.yjmcc.2015.06.016.

31. Buermans HP, Redout EM, Schiel AE, Musters R, Ziudwejk M, Eijk PP, et al. Microarray analysis reveals pivotal divergent mRNA expression profiles early in the development of either compensated ventricular hypertrophy or heart failure. Physiol Genomics. 2005;21(3):314-23. Doi: 10.1152/physiolgenomics.00185.2004.

32. Hardziyenka M, Campian ME, de Bruin-Bon HA, Michel MC, Tan HL. Sequence of echocardiographic changes during development of right

ventricular failure in rat. J Am Soc Echocardiogr.2006;19(10):1272-9. Doi: 10.1016/j.echo.2006.04.036.

33. Lamberts RR, Hamdani N, Soekhoe TW,Boontje NM, Zaremba R, Walker LA, et al. equency-dependent myofilament Ca2+ desensitization in failing rat myocardium. J Physiol.2007;582(Pt2): 695-709. Doi: 10.1113/jphysiol.2007.134486.

34. Henkens IR, Mouchaers KT, Vliegen HW, van der Laarse W, Swenne CA, Maan AC, et al. Early changes in rat hearts with developing pulmonary arterial hypertension can be detected with three-dimensional electrocardiography. Am J Physiol Heart Circ Physiol. 2007;293(2): H1300-1307. Doi:10.1152/ajpheart.01359.2006.

35. de Man FS, Handoko ML, van Ballegoij JJ, Schalij I, Bogaards S, Postmus S, et al. Bisoprolol delays progression towards right heart failure in experimental pulmonary hypertension. Circ Heart Fail. 2012;5:97-105. Doi: 10.1161/circheartfailure.111.964494.

36. Handoko ML, de Man FS, Happé CM, Benson A, Yang Z, Cassan C, et al. Opposite effects of training in rats with stable and progressive pulmonary hypertension. Circulation; 2009;120(1):42-9. Doi:10.1161/circulationaha.108.829713.

37. Benoist D, Stones R, Drinkhill MJ,Benson A, Yang Z, Cassan C, et al. Cardiac arrhythmia mechanisms in rats with heart failure induced by pulmonary hypertension. Am J Physiol Heart Circ Physiol. 2012;302(11):H2381-95. Doi: 10.1152/ajpheart.01084.2011.

38. Hornberger TA Jr, Farrar RP. Physiological hypertrophy of the FHL muscle following 8 weeks of progressive resistance exercise in the rat. Can J Appl Physiol = Rev Can Physiol Appl. 2004; 29:16-31. Doi: 10.1139/h04-002.

39. Sanches IC, Conti FF, Sartori M, Irigoyen MC, De Angelis K. Standardization of resistance exercise training: effects in diabetic ovariectomized rats. Int J Sports Med.2014;35(4):323-9. Doi: 10.1055/s-0033-1351254.

40. Urboniene D, Haber I, Fang YH, Thenapan T, Archer SL. Validation of high-resolution echocardiography and magnetic resonance imaging vs. high-fidelity catheterization in experimental pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol.2010;299(3):L401-L412. Doi: 10.1152/ajplung.00114.2010.

41. Wang Z, Patel JR ,Schreier DA, Moss RL, Chesler NC. Organ-level right ventricular dysfunction with preserved Frank-Starling mechanism in a mouse model of pulmonary arterial hypertension. J Appl J Physiol (Bethesda).1985;124(5):1244-53. Doi: 10.1152/japplphysiol.00725.2017.

42. Cai M, Wang Q, Liu Z, Jia D, Feng R, Tian Z. Effects of different types of exercise on skeletal muscle atrophy, antioxidant capacity and growth factors expression following myocardial infarction. Life Sci. 2018; 213:40-9. Doi: 10.1016/j.lfs.2018.10.015.

43. Satoh H, Delbridge LM, Blatter , Bers DM. Surface:volume relationship in cardiac myocytes studied with confocal microscopy and membrane capacitance measurements: species-dependence and developmental effects. Biophys J.1996;70(3):1494-504. D0i: 10.1016/s0006-3495(96)79711-4.

44. Neves RV, Souza MK, Passos CS, Bacurau RFP,Simoes H, Prestes J, et al. Resistance Training in Spontaneously Hypertensive Rats with Severe Hypertension. Arq Bras Cardiol. 2016; 106(3):201-9. Doi: 10.5935/abc.20160019.

45. Moreira-Goncalves D, Ferreira R, Fonseca H, Padrão AI, Moreno A, Silva AF, et al. Cardioprotective effects of early and late aerobic exercise training in experimental pulmonary arterial hypertension. Basic Res Cardiol.110(6):57. Doi: 10.1007/s00395-015-0514-5.

46. Moreira-Gonçalves D, Padrão AI, Ferreira R, Justino J, Nogueira-Ferreira R, Neuparth MJ, et al. Signaling pathways underlying skeletal muscle wasting in experimental pulmonary arterial hypertension. Biochim Biophis Acta.2015;1852(46):2722-31. Doi: 10.1016/j.bbadis.2015.10.002.

47. Vieira JS, Cunha TF, Paixão NA,Dourado PM, Carrascoza LS, Bacurau AN. Exercise intolerance establishment in pulmonary hypertension: Preventive effect of aerobic exercise training. Life Sci. 2020; 261:118298. Doi: 10.1016/j.lfs.2020.118298.

48. Artero EG, Lee DC, Ruiz JR,Sui X, Ortega FB, Church TS, et al. A prospective study of muscular strength and all-cause mortality in men with hypertension. J Am Coll Cardiol. 2011;57(18):1831-7. Doi: 10.1016/j.jacc.2010.12.025.

49. Souza-Rabbo MP, Silva LF, Auzani JA, Picoral M, Khaper N, Klein AB. Effects of a chronic exercise training protocol on oxidative stress and right ventricular hypertrophy in monocrotaline-treated rats. Clin Exp Pharmacol Physiol. 2008;35((8):944-8. Doi: 10.1111/j.1440-1681.2008.04936.x.

50. Dantas PS, Sakata MM, Perez JD, Watanabe RL, Bizerra FC, Neves VJ, et al. Unraveling the role of high-intensity resistance training on left ventricle proteome: Is there a shift towards maladaptation? Life Sci. 2016;152:156-64. Doi: https://doi.org/10.1016/j.lfs.2016.03.040.

51. Melo SF, Barauna VG, Carneiro Jr MA, Bozi LH, Drumond LR, et al. Resistance training regulates cardiac function through modulation of miRNA-214.Int J Mol Sci. 2015;16(4):6855-67. Doi: 10.3390/ijms16046855.

52. Brown MB, Neves E, Long G, Graber J, Gladish B. High-intensity interval training, but not continuous training, reverses right ventricular hypertrophy and dysfunction in a rat model of pulmonary hypertension. Am J Physiol Reg Integr Comp Physiol. 2017;312(2):R197-210. Doi: 10.1152/ajpregu.00358.2016.

Cardioprotective Effect of Resistance Exercise on Left Ventricular Remodeling Associated with Monocrotaline-Induced Pulmonary Arterial Hypertension

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Short Editorial related to the article: Resistance Exercise Training Mitigates Left Ventricular Dysfunctions in Pulmonary Artery Hypertension Model

Pulmonary arterial hypertension (PAH) is characterized by progressive pulmonary vascular resistance, affecting several arteries and arterioles. These changes are associated with increased right ventricular afterload and remodeling, characterized by severe hypertrophy, initially adaptive and derived from vascular pressure gradient.[1,2] Afterward, these alterations are accompanied by right ventricular dilation and impaired contractile performance, resulting in reduced ejection fraction and ventricular failure.[3,4] Clinically, right ventricular size and function parameters alterations have a recognized association with bad prognosis in pulmonary arterial hypertension.[4]

During the PAH development, when right ventricular filling pressure has been increased, and cardiac output is deteriorating, there are concomitant volumetric and pressoric overload into the right ventricle.[4] These effects make the interventricular septum move to the left side, sustaining a paradoxical movement. Next, left ventricle (LV) ejection fraction and diastolic performance are affected, facing impaired early diastolic filling, reduced end-diastolic volume, and adverse remodeling.[2,4] Therefore, LV dysfunction configures a secondary and important effect from PAH onset.[1,2,5]

In terms of treatment, several pharmacological interventions have been adopted as therapeutic options for PAH. Despite this, PAH condition has been associated with a high prevalence of mortality and morbidity due to cardiac complications. Generally, PAH patients exhibit asthenia, fatigue, dyspnea, and poor scores of effort tolerance and quality of life.[6,7]

Physical exercise training is a potential non-pharmacological tool to be used as a therapeutic option for cardiovascular diseases and complications.[1,7] Several physical training protocols have been used as promissory interventions in PAH experiments. Continuous aerobic exercise protocols promoted beneficial effects in the right ventricle and pulmonary artery remodeling.[8-10] Likewise, high-intensity interval training (HIIT) attenuated right ventricle systolic pressure and remodeling and lowered total pulmonary resistance in a rat model of monocrotaline (MCT)-induced PAH.[11] On the other hand, the potential impacts of exercise training interventions on LV aspects are few clarified in experimental conditions of pulmonary arterial hypertension.

In the current edition of the *Arquivos Brasileiros de Cardiologia*, Soares et al.[12] analyzed the influence of resistance exercise training on LV remodeling and cardiomyocyte performance in rats during the development of monocrotaline (MCT)-induced PAH. In this elegant study, the Authors found that resistance exercise progressively increased tolerance to physical effort during the development of PAH in rats submitted to two injections of MCT (20 mg/kg) interspaced over seven days. Compared to control counterparts, trained PAH-animals exhibited later-onset heart failure signals. Likewise, resistance exercise training improved LV ejection fraction, cardiomyocyte contraction, and relaxation velocities. These improvements were accompanied decreased amount of type I collagen and increased type III collagen in LV samples from trained PAH-animals. Myocardial collagen fibers have distinct biomechanical differences; collagen I fibers confer higher stiffness, while type III collagen is associated with increased susceptibility to mechanical deformation,[13,14] which could be related to better LV contractile performance.

Therefore, low- to moderate-intensity resistance exercise training has adjuvant and cardioprotective effects in controlling LV remodeling secondary to MCT-induced PAH. Based on this, similar interventions may effectively minimize cardiac complications associated with PAH. On the other hand, as exercise training parameters vary and may sustain multiple protocols, it is necessary to better characterize demands relative to speed and intensity, as also discussed by the authors,[12] besides frequency and duration. Further studies will contribute to elucidating the effects of diverse resistance exercise training protocols on cardiopulmonary disorders derived from MCT-induced PAH.

Keywords

Heart; Pulmonary Arterial Hypertension; Exercise; Myocardial Contraction.

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References

1. Vaillancourt M, Ruffenach G, Meloche J, Bonnet S. Adaptation and Remodelling of the Pulmonary Circulation in Pulmonary Hypertension. Can J Cardiol. 2015;31(4):407-15. doi: 10.1016/j.cjca.2014.10.023.
2. Thenappan T, Ormiston ML, Ryan JJ, Archer SL. Pulmonary Arterial Hypertension: Pathogenesis and Clinical Management. BMJ. 2018;360:j5492. doi: 10.1136/bmj.j5492.
3. Gomez-Arroyo JG, Farkas L, Alhussaini AA, Farkas D, Kraskauskas D, Voelkel NF, et al. The Monocrotaline Model of Pulmonary Hypertension in Perspective. Am J Physiol Lung Cell Mol Physiol. 2012;302(4):363-9. doi: 10.1152/ajplung.00212.2011.
4. Kishiki K, Singh A, Narang A, Gomberg-Maitland M, Goyal N, Maffessanti F, et al. Impact of Severe Pulmonary Arterial Hypertension on the Left Heart and Prognostic Implications. J Am Soc Echocardiogr. 2019;32(9):1128-37. doi: 10.1016/j.echo.2019.05.008.
5. Desai AS, Channick RN. Exercise in Patients with Pulmonary Arterial Hypertension. J Cardiopul Rehabil Prev. 2008;28(1):12-6. doi: 10.1097/01.hcr.0000311502.57022.73.
6. Coons JC, Pogue K, Kolodziej AR, Hirsch GA, George MP. Pulmonary Arterial Hypertension: A Pharmacotherapeutic Update. Curr Cardiol Rep. 2019;21(11):141. doi: 10.1007/s11886-019-1235-4.
7. Vecchia LAD, Bussotti M. Exercise Training in Pulmonary Arterial Hypertension. J Thorac Dis. 2018;10(1):508-21. doi: 10.21037/jtd.2018.01.90.
8. Colombo R, Siqueira R, Becker CU, Fernandes TG, Pires KM, Valença SS, et al. Effects of Exercise on Monocrotaline-induced Changes in Right Heart Function and Pulmonary Artery Remodeling in Rats. Can J Physiol Pharmacol. 2013;91(1):38-44. doi: 10.1139/cjpp-2012-0261.
9. Vieira JS, Cunha TF, Paixão NA, Dourado PM, Carrascoza LS, Bacurau AVN, et al. Exercise Intolerance Establishment in Pulmonary Hypertension: Preventive Effect of Aerobic Exercise Training. Life Sci. 2020;261:118298. doi: 10.1016/j.lfs.2020.118298.
10. Silva FJ, Drummond FR, Fidelis MR, Freitas MO, Leal TF, Rezende LMT, et al. Continuous Aerobic Exercise Prevents Detrimental Remodeling and Right Heart Myocyte Contraction and Calcium Cycling Dysfunction in Pulmonary Artery Hypertension. J Cardiovasc Pharmacol. 2021;77(1):69-78. doi: 10.1097/FJC.0000000000000928.
11. Brown MB, Neves E, Long G, Graber J, Gladish B, Wiseman A, et al. High-Intensity Interval Training, but not Continuous Training, Reverses Right Ventricular Hypertrophy and Dysfunction in a Rat Model of Pulmonary Hypertension. Am J Physiol Regul Integr Comp Physiol. 2017;312(2):197-210. doi: 10.1152/ajpregu.00358.2016.
12. Soares LL, Leite LB, Ervilha LOG, Silva BAF, Freitas MO, Pontes AMO, et al. O Treinamento Físico Resistido Atenua as Disfunções Ventriculares Esquerdas em Modelo de Hipertensão Arterial Pulmonar. Arq Bras Cardiol. 2022; 119(4):574-584.
13. Silva DCT, Lima-Leopoldo AP, Leopoldo AS, Campos DHS, Nascimento AF, Oliveira Junior SA, et al. Influence of Term of Exposure to High-fat diet-induced Obesity on Myocardial Collagen Type I and III. Arq Bras Cardiol. 2014;102(2):157-63. doi: 10.5935/abc.20130232.
14. Oliveira SA Jr, Dal Pai M, Guizoni DM, Torres BP, Martinez PF, Campos DHS, et al. Effects of AT1 Receptor Antagonism on Interstitial and Ultrastructural Remodeling of Heart in Response to a Hypercaloric Diet. Physiol Rep. 2019;7(1):e13964. doi: 10.14814/phy2.13964.

Electrocardiographic Evaluation of Normal Newborns in the First Week of Life – Observational Study

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Abstract

Background: The neonatal period is marked by major changes in the cardiovascular system, especially in the first week of life. Unlike the adult population, studies on electrocardiogram (ECG) data in the neonatal period are scarce. This is the first study to describe electrocardiographic changes in a cohort of newborns with normal echocardiograms.

Objectives: To analyze the electrocardiographic patterns of a population of full-term NB, without any cardiac morphological or functional anomalies, and compare the results with the literature.

Methods: In this observational study, echocardiograms and ECG results from 94 newborns divided in three age groups (up to 24 hours, between 25 and 72 hours, and between 73 and 168 hours of life) were evaluated and compared with those reported by Davignon et al. A p-value <0.05 was considered statistically significant.

Results: There were significant differences in T-wave direction in leads V1 (p= 0.04), V2 (p= 0.02), V3 (p= 0.008) and V4 (p= 0.005) between the three age groups. There were differences between our findings and the current literature in most of the parameters.

Conclusion: Term newborns within 24 hours of life showed significantly more positive T waves than older ones. Many differences from the Davignon's ECG parameters were found, particularly in the P, Q, R, S amplitudes, QRS duration, R/S and R+S. These findings indicate that more studies are needed for a definitive interpretation of the ECG in newborns.

Keywords: Electrocardiography; Myocytes, Cardiac; Infant, Newborn.

Introduction

The neonatal period is marked by many hemodynamic and anatomical cardiovascular changes, especially in the first week of life, when the transition of the circulation pattern from fetal to neonatal occurs.[1,2] In the fetus, the placenta is a low-resistance vascular bed and the right ventricle (RV) is the dominant ventricle, responsible for approximately 60% of the cardiac output. The heart works with an almost constant workload, with a high-volume and low-resistance circulation. After the cut of the umbilical cord and the first breath there is a decrease in the pulmonary vascular resistance and an increase in the systemic vascular resistance. Right ventricular pressure and flow drops while the afterload increases as the placenta is removed and the left ventricular (LV) outflow increases by two-fold due to the increased pulmonary blood flow. The *foramen ovale* and *ductus arteriosus* close and the ventricular predominance change from the RV to the LV, with subsequent increase in cardiomyocyte size and number.[1,3]

It is not known whether these circulatory changes in the first days of life can lead to different patterns in the electrocardiogram (ECG). Hemodynamic changes are assessed in the clinical practice through clinical parameters (heart rate, oxygen saturation, respiratory pattern, heart auscultation) and complementary exams (e.g. echocardiogram, serum lactate and sodium bicarbonate). However, unlike the adult population, electrocardiographic studies in the neonatal period are scarce.[4]

The objective of this study was to describe the ECG findings in term neonates without cardiac malformations and normal cardiovascular function during hospital stay, and to compare them with the findings by Davignon et al.[2] This is the first study correlating ECG findings with normal echocardiogram in a cohort of newborns.

Methods

Population

In this observational study, from August 2016 to July 2018, ECG results and echocardiograms of newborns during their

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first seven days of life (168 hours) were evaluated, all born at a tertiary neonatal unit in São Paulo, Brazil.

Ethics

The study was approved by the Institutional Review Board (approval number 272/13/2016; CAPPesq 1.662.356) and conducted in accordance with the Declaration of Helsinki. The Ethics Commission waived the need for a patient's consent form since the ECG and echocardiographic examinations are routine at the neonatal unit.

The inclusion criteria were gestational age (GA) between 37 and 41 weeks and 6 days, and less than 169 hours of postnatal age. Cardiac malformation was excluded using echocardiogram in the first 169 hours of life. All newborns had normal cardiovascular function during hospital stay.

Newborns with major non-cardiac malformations, such as neurological and chromosomal abnormalities, GA less than 37 weeks or equal to or above 42 weeks, or with abnormalities in the echocardiogram such as cardiac malformations (complex heart anomaly, valve dysfunction, major sept defect, aortic coarctation), persistent pulmonary hypertension, functional impairment or with abnormal cardiovascular function during hospitalization were excluded.

Newborns were divided by post-natal age in three groups: up to 24 hours, between 25 and 72 hours and between 73 and 168 hours of life to enable the comparison of our findings with those reported by Davignon et al. [2]

12-Lead ECG

Simultaneous twelve-lead ECG (Philips PageWriter TC20©, Koninklijke Philips, N.V.) was performed and analyzed in all neonates by a single trained not blinded investigator. Solid gel tab electrodes were positioned on the right and left shoulders, right and left iliac crests and V1-V6 as recommended by guidelines[5] (Figure 1). The shoulders and iliac crests were preferred over the right/left arms and legs due to the commonly excessive movement of the newborn, in order to reduce noise and improve ECG signals.[6]

The following parameters were assessed:

- heart rate (bpm, automatically measured by the device),
- frontal plane QRS axis (°),
- P wave amplitude (mm) and duration (ms) and duration of PR interval in DII (ms),
- amplitude of: Q wave in DIII, aVF and V5-V6, R wave in aVR, V1-V2 and V4-V6, and S waves in V1-V2 and V4-V6 (mm),
- R/S ratio in V1 and V6,
- QRS duration, QT and QTc interval (corrected by Bazzet's formula) in V2 (ms), and
- T wave duration (ms) and orientation (+ / -) in all 12 leads.

Echocardiogram

A detailed two-dimensional echocardiography with Doppler was performed in all subjects by the on-call experienced pediatric cardiologist. The equipment used was a Philips CX50 (Koninklijke Philips N.V.), with multifrequency transducers S8-3 and S12-4. M-mode echocardiographic measurements of left atrium, RV, left ventricle, posterior wall, and LV diastolic and systolic diameters were obtained following the American Society of Echocardiography guidelines.[7] LV ejection fraction was obtained by Teichholz method and was considered normal when equal to or greater than 55%.

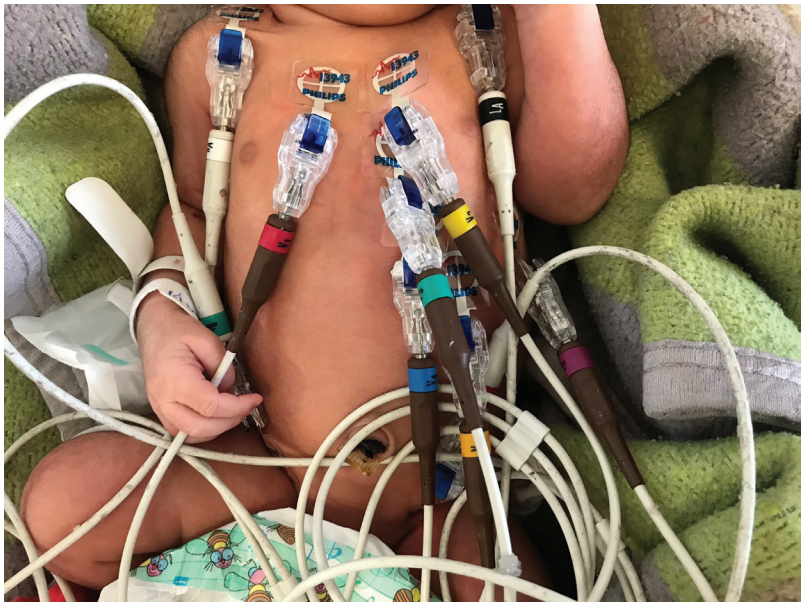

Figure 1 – *Positioning of electrocardiogram electrodes in newborns*

Statistical analysis

Qualitative characteristics of mothers and newborns were described as absolute and relative frequencies. Quantitative characteristics were described using summary measures (mean and standard deviation) for all subjects.[8,9] ECG parameters were described according to maternal diseases such as hypertension (none, primary, gestational, primary + gestational) and diabetes mellitus (none, type 1, type 2, gestational). Based on their birth weight, the newborns were classified as small for GA (SGA), appropriate for GA (AGA), or large for GA (LGA); the values were described as summary measures and compared for the categories of interest using analysis of variance (one-way ANOVA) followed by Bonferroni multiple comparisons when $p<0.05$.[10]

The Kolmogorov-Smirnov data distribution normality test was performed, and the assumption of normality was accepted for most of the parameters evaluated. As this is a weaker assumption of ANOVA, it was conducted for all variables without loss of power in the analyses, since the central limit theorem guarantees the normality of distribution of the mean, even with no normality of the data.

The parameters were described in percentile curves and compared with normal values according to the percentiles reported by Davignon et al.[2] The analyses were performed using the IBM-SPSS for Windows version 22.0 software. The value of $p<0.05$ was considered significant.

Results

During the study period, there were 2,883 live births in the neonatal unit. Of these, 1,916 were full-term newborns. Echocardiograms were performed in 753 babies; 310 of them were full-term newborns, of whom 191 did not have any significant anatomical changes.

ECGs of 113 newborns were performed, 19 of whom were excluded because of major non-cardiac malformations, mainly anomalies of the central nervous system or genetic syndromes. The final series of the present study consisted of 94 patients.

Clinical characteristics of the newborns are presented in Table 1. The percentiles of the ECG parameters studied are in Table 2. In the comparison between-age groups (see Table 3), newborns with less than 24 hours of life had a significantly higher proportion of positive T waves compared to NB in the older groups (25–72 hours and 73–168 hours) in leads V1 ($p = 0.04$), V2 ($p = 0.02$), V3 ($p = 0.008$), and V4 ($p = 0.005$).

When comparing the values found with the estimated values extracted from the study by Davignon et al.[2] (Table 4), we noticed statistically significant differences in several parameters in all age groups (<24 hours, between 25 and 72 hours and between 73 and 168 hours of life), such as amplitude of P, Q, R and S waves, QRS duration and R-S relationship (R/S and R+S).

Table 1 – Clinical characteristics of the newborns evaluated in the study (n=94)

Variables	Number (%)
Age groups (hours of life)	
≤24 hours	11 (12)
25-72 hours	46 (49)
73-168 hours	37 (39)
Classification (body weight)	
SGA	9 (10)
AGA	77 (82)
LGA	8 (8)
Delivery	
Vaginal	31 (33)
Forceps	9 (10)
Cesarean	54 (57)
Gender	
Female	53 (56)
Male	40 (43)
Indeterminate	1 (1)
	average (SD)
GA (weeks)	38.6 (1.1)
Weight at birth (grams)	3,184 (551)

GA: gestational age; SGA: small for GA; AGA: appropriate for GA; LGA: large for GA; SD: standard deviation.

Discussion

Unlike the adult population, electrocardiographic studies in the neonatal period are scarce. In 1979, Davignon et al.[2] published ECG findings of 2,141 children, 549 of them under seven days of life. So far, this is the largest study on newborns, and most guidelines of ECG interpretation in neonates are based on this study. Nevertheless, there is no proof that the newborn studied, in fact, had no cardiac malformation that could influence ECG parameters.

It is expected that ECG changes occur in the first days of life, due to significant circulatory changes in this period. Thus, Davignon et al.[2] divided the newborns in three age groups (<24 hours, between 25 and 72 hours and between 73 and 168 hours of life). In our study, significant differences were found in the direction of the T waves in leads V1, V2, V3 and V4 between the same age groups. The higher proportion of positive T waves in the younger age groups can be explained by the higher pulmonary pressure found in this early phase, leading to an initial repolarization of the RV. With the physiological drop in pulmonary pressure that occurs in the first days of life, a change in ventricular repolarization to the infantile pattern can be expected, leading to a lower proportion of positive T waves in precordial leads (V1 to V4). T wave analysis was not done in Davignon's work. There was no statistical difference in the other electrocardiographic parameters studied.

When comparing our results with the estimated values extracted from the study by Davignon et al.,[2] we observed statistically significant differences in several parameters in all age groups, particularly in wave amplitudes (P, Q, R, S), QRS duration and R-S relationship (R/S and R+S). We did a simple ratio within some ECG parameters between our results and

Table 2 – Percentiles of the electrocardiographic parameters

Parameter	<24 hours of life			24-72 hours of life			73-168 hours of life		
	5%	50%	95%	5%	50%	95%	5%	50%	95%
Heart Rate (bpm)	92.14	122.09	152.04	98.91	122.72	146.53	102.18	131.05	159.92
Ampl P DII (mm)	0.04	0.11	0.18	0.06	0.13	0.21	0.06	0.13	0.21
PR DII (ms)	70.55	92.73	114.91	71.43	99.13	126.83	73.40	98.38	123.36
QT V2 (ms)	227.59	301.82	376.04	206.84	293.48	380.12	202.20	274.05	345.90
QRS axis (°)	54.61	126.36	198.12	61.59	128.75	195.91	58.09	134.44	210.79
Ampl Q DIII (mm)	0.03	0.41	0.79	0.05	0.34	0.64	0.02	0.36	0.70
Ampl Q aVF (mm)	0.00	0.30	0.65	0.00	0.23	0.47	0.00	0.27	0.59
Ampl Q V5 (mm)	0.00	0.10	0.28	0.00	0.04	0.15	0.00	0.10	0.29
Ampl Q V6 (mm)	0.00	0.13	0.32	0.00	0.06	0.18	0.00	0.12	0.31
Ampl R aVR (mm)	0.00	0.33	0.83	0.00	0.33	0.71	0.00	0.25	0.66
Ampl R V1 (mm)	0.15	1.25	2.35	0.42	1.13	1.85	0.38	1.13	1.88
Ampl R V2 (mm)	0.56	1.35	2.14	0.46	1.19	1.92	0.43	1.27	2.11
Ampl R V4 (mm)	0.76	1.74	2.71	0.76	1.57	2.38	0.78	1.57	2.37
Ampl R V5 (mm)	0.35	1.41	2.48	0.51	1.30	2.09	0.49	1.29	2.09
Ampl R V6 (mm)	0.39	1.26	2.12	0.36	1.17	1.98	0.43	1.14	1.85
Ampl S V1 (mm)	0.24	1.00	1.75	0.00	0.97	2.10	0.00	0.67	1.37
Ampl S V2 (mm)	0.57	1.40	2.23	0.15	1.34	2.52	0.12	1.08	2.04
Ampl S V4 (mm)	0.00	1.21	3.23	0.07	0.97	1.87	0.08	0.88	1.67
Ampl S V5 (mm)	0.00	0.77	2.10	0.00	0.75	1.59	0.09	0.62	1.15
Ampl S V6 (mm)	0.00	0.59	1.66	0.00	0.67	1.51	0.08	0.52	0.96
R/S V1	0.24	1.37	2.50	0.00	2.10	5.87	0.00	2.38	5.46
R/S V5	0.00	5.26	18.79	0.00	3.03	8.63	0.00	3.41	11.42
R/S V6	0.00	5.31	17.78	0.00	3.32	9.46	0.00	3.17	8.20
R + S V2 (mm)	-9.16	-0.46	8.25	-11.73	-1.44	8.86	-7.37	1.88	11.13
R + S V4 (mm)	-14.88	5.23	25.33	-3.11	5.98	15.07	-3.29	6.97	17.24
S V2 + R V5 (mm)	1.68	2.81	3.94	1.19	2.64	4.09	1.14	2.38	3.61
S V1 + R V6 (mm)	1.01	2.25	3.50	0.72	2.14	3.55	0.88	1.81	2.73
Dur QRS V5 (ms)	40.00	40.00	40.00	30.60	44.78	58.97	27.41	46.49	65.56

Ampl: amplitude; bpm: beats per minute; Dur: duration; hl: hours of life; mm: millimeters; ms: milliseconds.

Davignon's to emphasize the differences found (mentioned above) – Table 4.

These differences indicate that the parameters of electrocardiographic normality proposed by Davignon et al.[2] may not be the optimal ones for interpreting ECG of Brazilian newborns today.[11] In addition to the possible anthropometric difference between populations (Canada x Brazil), in the Canadian study,[2] there were was no cardiac screening, image examination or follow-up of the newborns. Therefore, no evidence was presented that, in fact, the study population in their study did not have any structural heart disease.

The results obtained in the present study have called into question the applicability of the electrocardiographic parameters of normality reported by Davignon et al.[2] for term newborns with up to seven days of life, to other nationalities and ethnicities.

Limitations

The performance of an ECG on a newborn is fraught with difficulties, since besides dealing with their small chest size to deploy the electrodes, they are also highly agitated. In this way, we decided to have all the ECGs performed by the same physician, to minimize the influence of electrodes positioning. This led to a limited number of newborns studied. It is important to note that it is likely that more differences will be found if a greater number of newborns are studied.

Conclusion

This is the first study correlating ECG findings with normal echocardiogram in a cohort of newborns. Term newborns within 24 hours of life showed significantly more positive T waves than

Table 3 – Electrocardiographic T-wave parameters by age groups

Variable	Hours of life			p
	≤24	25-72	73-168	
T-wave in V1. n (%)				0.04
Positive	5 (45)	8 (17)	2 (5)	
Negative	3 (27)	23 (50)	23 (62)	
Minus-plus	3 (27)	15 (33)	12 (32)	
T-wave in V2. n (%)				0.02
Positive	6 (54)	7 (15)	4 (11)	
Negative	4 (36)	21 (46)	23 (62)	
Minus-plus	1 (9)	18 (39)	10 (27)	
T-wave in V3. n (%)				0.008
Positive	8 (73)	12 (26)	5 (13)	
Negative	2 (18)	15 (33)	20 (54)	
Plus-minus	0 (0)	1 (2)	0 (0)	
Minus-plus	1 (9)	18 (39)	12 (32)	
T-wave in V4. n (%)				0.005
Positive	10 (91)	27 (59)	13 (35)	
Negative	1 (9)	10 (22)	21 (57)	
Indeterminate	0 (0)	2 (4)	0 (0)	
Plus-minus	0 (0)	1 (2)	1 (3)	
Minus-plus	0 (0)	6 (13)	2 (5)	
T-wave in V5. n (%)				0.49
Positive	10 (91)	34 (74)	22 (59)	
Negative	1 (9)	8 (17)	12 (32)	
Indeterminate	0 (0)	1 (2)	1 (3)	
Plus-minus	0 (0)	2 (4)	2 (5)	
Minus-plus	0 (0)	1 (2)	0 (0)	
T-wave in V6. n (%)				0.62
Positive	9 (82)	36 (78)	26 (70)	
Negative	2 (18)	6 (13)	9 (24)	
Indeterminate	0 (0)	1 (2)	0 (0)	
Plus-minus	0 (0)	3 (6)	2 (5)	
Minus-plus	0 (0)	0 (0)	0 (0)	

ANOVA test

Table 4 – Electrocardiographic standards ratio between Pimenta et al. and Davignon et al.[2] for newborn

Variable	Hours of life		
	<24	25-72	73-168
P wave amplitude (DII)	0.36	0.18	0.23
Q wave amplitude			
DIII	3.41	2.83	2.76
aVF	3.33	2.30	3.00
V5	1.10	1.4	1.10
V6	1.13	1.6	2.40
R wave amplitude			
V2	0.22	0.33	0.29
V5	0.41	0.18	0.8
V6	3.15	2.60	2.28
S wave amplitude			
V2	0.33	0.36	0.36
V4	0.25	0.40	0.37
V6	1.68	2.68	1.48
QRS complex duration (V5)	2.53	0.07	0.26
R / S			
V1	0.91	0.86	0.85
V5	0.73	0.85	0.83
V6	0.82	0.90	0.89
R + S (V2)	0.29	0.36	0.34
S in V1 + R (V6)	1.87	1.64	1.39
PR interval (DII)	0.11	0.6	
R wave amplitude			
aVR		1.32	
V1		0.19	
V5	1.41	1.18	
S wave amplitude (V5)		0.21	0.31
R + S waves (V4)		0.21	0.24
S waves in V2 + R (V5)		0.67	0.17

the elders. Many differences from the parameters proposed by Davignon et al.[2] were found and indicate that more studies are needed for a definitive interpretation of the ECG in newborns.

Author Contributions

Conception and design of the research: Pimenta MS, Samesima N, Pastore CA, Krebs VLJ, Carvalho WB; Acquisition of data: Pimenta MS, Krebs VLJ, Leal GN; Analysis and interpretation of the data and Critical revision of the manuscript for important intellectual content: Pimenta MS, Samesima N, Pastore CA, Krebs VLJ, Leal GN, Carvalho WB; Statistical analysis: Samesima N; Obtaining financing: Pastore CA; Writing of the manuscript: Pimenta MS.

Potential Conflict of Interest

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

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References

1. Hastreiter AR, Abella JB. The Electrocardiogram in the Newborn Period. I. The Normal Infant. J Pediatr. 1971;78(1):146-56. doi: 10.1016/s0022-3476(71)80282-2.
2. Davignon A, Rautaharju P, Boisselle E, Soumis F, Mégélas M, Choguette A. Normal ECG Standards for Infants and Children. Pediatr Cardiol. 1980;1:133-152. doi: 10.1007/BF02083144.
3. Bensley JG, De Matteo R, Harding R, Black MJ. The Effects of Preterm Birth and its Antecedents on the Cardiovascular System. Acta Obstet Gynecol Scand. 2016;95(6):652-63. doi: 10.1111/aogs.12880.
4. Rijnbeek PR, Witsenburg M, Schrama E, Hess J, Kors JA. New Normal Limits for the Paediatric Electrocardiogram. Eur Heart J. 2001;22(8):702-11. doi: 10.1053/euhj.2000.2399.
5. Mirvis DM, Goldberger AL. Electrocardiography. In: Braunwald E, Zipes DP, Libby P (editors). Heart Disease. A Textbook of Cardiovascular Medicine. 6th ed. Philadelphia: Saunders, 2001, p. 82-128.
6. Khan GM. A New Electrode Placement Method for Obtaining 12-lead ECGs. Open Heart. 2015;2(1):e000226. doi: 10.1136/openhrt-2014-000226.
7. Lai WW, Geva T, Shirali GS, Frommelt PC, Humes RA, Brook MM, et al. Guidelines and Standards for Performance of a Pediatric Echocardiogram: A Report from the Task Force of the Pediatric Council of the American Society of Echocardiography. J Am Soc Echocardiogr. 2006;19(12):1413-30. doi: 10.1016/j.echo.2006.09.001.
8. Kampmann C, Wiethoff CM, Wenzel A, Stolz G, Betancor M, Wippermann CF, et al. Normal Values of M Mode Echocardiographic Measurements of More than 2000 Healthy Infants and Children in Central Europe. Heart. 2000;83(6):667-72. doi: 10.1136/heart.83.6.667.
9. Kirkwood BR, Sterne JAC. Essential Medical Statistics. 2nd ed. Massachusetts: Blackwell Science, 2006.
10. Neter J, Kutner MH, Nachtsheim CJ, Wasserman W. Applied Linear Statistical Models. 4th ed. Ilinois: Richard D. Irwing, 1996.
11. Pastore CA, Pinho JA, Pinho C, Samesima N, Pereira Filho HG, Kruse JC, et al. III Diretrizes da Sociedade Brasileira de Cardiologia Sobre Análise e Emissão de Laudos Eletrocardiográficos. Arq Bras Cardiol. 2016; 106 (4 Suppl 1): 1-23.

Association of Paraoxonase-1 Genotype and Phenotype with Angiogram Positive Coronary Artery Disease

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Abstract

Background: **It has been shown that increased serum PON1 levels are protective against several disorders. Several single nucleotide polymorphisms (SNPs) of the PON1 gene have been reported to be associated with serum enzyme protein levels and activity.**

Objective: **To investigate the association of SNPs of PON1 and serum paraoxonase activity with coronary artery disease (CAD).**

Methods: **A total of 601 unrelated patients who underwent coronary angiography including those who had >50% stenosis (N=266) and those with <30% stenosis (N=335) were studied. The Paraoxonase gene rs662 and rs840560 SNPs were determined using the ARMS-PCR method and the rs705379 SNP was genotyped using PCR-RFLP analysis. Serum paraoxonase activity was measured using paraoxon as a substrate. A p value of p<0.05 was considered as significant.**

Results: **Serum paraoxonase activity was not significantly different between the study groups. After adjustment for age, sex, hypertension, diabetes mellitus and dyslipidemia, the GG genotype and co-dominant model of rs662 was positively associated with a positive angiogram (respectively, OR=2.424, 95%CI [1.123-5.233], p<0.05, OR=1.663, 95%CI [1.086-2.547]). Serum paraoxonase activity was significantly higher in the G allele and GG variant of rs662, A allele and AA variant of rs854560 and C allele and CC variant of rs705379. The haplotype analysis has shown that the ATC haplotype was significantly more prevalent among the angiogram negative group. The analysis between groups indicated that the A allele of rs662 was significantly associated with lower paraoxonase activity in the positive angiogram group (p=0.019).**

Conclusions: **The presence of the G allele of the rs662 single nucleotide polymorphism is independently associated to increased risk of CAD.**

Keywords: **Coronary Artery Disease; Angiography; Aryldialkylphosphatase.**

***https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases**

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Introduction

The World Health Organization (WHO) reports that 71% of deaths each year are due to non-communicable diseases, of which 43% are due to coronary artery disease (CAD).[1] This was also reported to be approximately 46% in Iran in 2019,[2] where the prevalence of CAD is increasing.[3,4]

Oxidative stress has a key role in the initiation and progression of atherosclerosis,[5] as well as in the pathogenesis of CAD and its related outcomes.[6] It has been shown that high density lipoprotein (HDL) has antioxidant and anti-inflammatory properties, in which paraoxonase 1 (PON1) may play a role by decreasing the production of oxidized low density lipoprotein (LDL) during the process of lipid peroxidation. PON1 is a calcium-dependent esterase, of which serum concentration differs by ethnicity and geographically. It has been shown that a decreased PON1 activity is associated with conditions in which there is oxidative stress, including metabolic syndrome, CAD, Alzheimer and aging, and in this case, increased PON1 levels may be protective.[7] The paraoxonase gene cluster encodes for three distinct members: PON1-PON2 and PON3, which are located on chromosome 7q21.3. More than 160 single nucleotide polymorphisms (SNPs) have been identified for the PON1 gene,[8] of which the rs662, rs854560, rs705379 are reported to be associated with serum enzyme protein levels and activity. The rs662 and rs850560 SNPs are located within coding regions and exert an amino acid substitution,[9,10] whereas the third polymorphism, rs705379, is located on the promoter region.[11] The presence of rs662 results in a glutamine-to-arginine substitution, increases the rate of hydrolysis of paraoxon and chlorpyrifos-oxon. While the rs850560 polymorphism, which results in a leucine-to-methionine amino acid substitution, is also associated with decreased serum PON1.[12] The rs705379 SNP occurs at the binding site of the transcription factor Sp1, and has the greatest effect on the expression of PON1.[11] This polymorphism accounts for approximately 30% of variations in plasma PON1 levels. The C allele of rs705379 is associated with an increased promoter activity, and therefore the expression of the PON1 gene is augmented.[13] Moreover, several studies have shown that this polymorphism is associated with an increased risk of CAD, especially in young people[14-16] and in individuals with type 2 diabetes.[17]

Since CAD is an important disease in relation to mortality and studies have shown that there is an association between PON1 and CAD, it was decided to investigate this association in the Iranian society, especially in the northeast of the country.[18] There are few studies on the association between PON1 genotype, or phenotype and CAD in northeastern Iran[19-21] and serum enzyme activity was not studied along with the polymorphisms. The aim of current study was to assess the association between PON1 polymorphisms and paraoxonase activity with CAD among Iranian adults living in northeastern Iran.

Material and methods

Study design and population

This case-control study was carried out between December 2014 and April 2017; 601 unrelated Iranian patients who underwent elective coronary angiography were recruited. Patients were referred for angiography because of chest pain or equivalent symptoms, such as dyspnea on exertion. Based on the results of the angiography, the patients were divided into two groups: those with obstructive coronary artery disease with coronary stenosis >50% in at least one coronary artery (N=266) (angiogram positive) and patients with non-obstructive coronary artery disease with stenosis <30% in coronary arteries (N=335) (angiogram negative).

Demographic data including sex, age, smoking history, past history of diabetes mellitus (DM), hypertension (HTN), and dyslipidemia were collected from the medical records. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured right before the procedure. Patients with autoimmune disorders, active cancer, thrombophilia or chronic kidney disease were excluded.

Blood samples were taken before the procedure into EDTA tubes for DNA extraction and into tubes with no anticoagulant for paraoxonase activity measurement. Serum was separated by centrifuging the blood for 15 min at 1000 rpm speed (manufacturer's recommended speed) (BEHDAD, Iran) and stored at -80°C.

Genotyping

DNA was extracted from EDTA blood, using a genomic DNA isolation kit (Genet bio, Korea) based on the manufacturer's instructions. DNA Purity and quantification were determined by UV spectrophotometry (Infinite 200PRONanoQuant, Tecan).

Three SNPs of PON1 genes were genotyped. We used the double ARMS-PCR method for the rs662 and rs854560 SNPs and the PCR-RFLP method for rs705379 SNP. Details on the primers used and PCR conditions are detailed in Supplement 1. Gel electrophoresis was performed using 2% agarose in TBE for the three SNPs. To determine the genotypes of rs705379, we used 5 units of Bsh1236I (Thermo Scientific) for 16 hours at 37°C. The 109bp PCR product was cut into 67bp and 42bp fragments and visualized with UV-Trans-illuminator. Sequencing was performed to confirm the accuracy of the genotyping techniques.

Paraoxonase activity

Paraoxonase activity was measured by adding 10μL of serum to 290 μL of Tris-HCl buffer (100mmol/L, pH=8.0) containing 1mmol/L $CaCl_2$ and 1mmol/L paraoxon (D9286, Sigma Chemical Company, Deisenhofen, Germany). The generation of p-nitrophenol was measured at 405 nm and at room temperature using a plate reader (EPOCH, USA) 3 and 6 minutes after adding paraoxon as the substrate. Paraoxonase activity was reported in Units per liter of serum per minute.

Ethical considerations

All participants filled out a written informed consent form. The Ethics Committee at Mashhad University of Medical Sciences approved the study protocol (ID Number: 930834).

Statistical analysis

All data were statistically analyzed using the Statistical Package for Social Sciences software (SPSS Inc., IL, USA). Data normality was checked by the Kolmogorov-Smirnov test. Normally distributed variables were expressed in

Mean ± standard deviation (SD) and variables without a normal distribution were described using median and interquartile range. Categorical variables were reported by number and percentage. For the analysis between groups, the chi-square test was used for categorical data, and the independent sample t-test for quantitative data (for normally distributed data) or Mann-Whitney and Kruskal-Wallis (for non-normally distributed data), respectively. Univariate and multivariate analyses with binary logistic regression were performed to indicate the association between SNPs and positive angiography, being expressed in OR (95%CI). Statistical significance was set at $p < 0.05$. Haplotype analysis was performed using SNPAlyze (demo version, V8.1.1).

Results

Differences in baseline characteristics between the study groups are shown in table 1. The differences between the frequencies of the three genotypes and angiogram positivity are shown in Table 2. After adjusting for age, sex, HTN, DM and dyslipidemia, a recessive model for GG genotype of rs662 was significant between the study populations. Also, a significant result was observed in the co-dominant model for rs662.

The haplotype analysis showed that the "ATC" haplotype showed a significant difference between the two analyzed groups (p=0.017) (Table3).

Table 4 shows the difference between genotypes and paraoxonase activity in the study groups. In total and in both cases and controls, paraoxonase activity was increased in the presence of the G allele in comparison with the presence of the A allele. Moreover, paraoxonase activity was significantly higher in the presence of the A allele of rs850560 in comparison with the presence of the T allele at this locus and the C allele of rs705379 in PON1 promoter. Comparisons between the groups indicated that paraoxonase activity was significantly lower for the AA genotype of the rs662 polymorphism in CAD when compared to the controls (p=0.019).

Discussion

We could not show any significant association between rs850560 and rs705379 polymorphisms and angiographically defined CAD in Iranian adults, whereas the analysis of the rs662 polymorphism showed that homozygosity for the GG variant vs. total AA and AG was associated with a more than a 2-fold higher prevalence in the positive angiogram when compared to the negative angiogram subjects. Moreover, we found that serum paraoxonase activity was associated with all three assessed SNPs in both groups of subjects. It can be said that the paraoxonase enzyme activity was higher in carriers of R alleles of rs662, A allele of rs850560 and C Allele of rs705379. There was no significant relationship between angiogram positivity and serum paraoxonase activity, although a lower mean serum PON1 activity was observed in the angiogram positive patients.

In a meta-analysis on 17 studies carried out in different cities and states of Mexico conducted in 2018, the most frequently associated genotypes with decreased enzyme activity were AT/TT of rs850560 and AA of rs662.[22] These results were compatible with our findings, although this was a national meta-analysis conducted in the Mexico population and, therefore, the ethnicity was different.

Several studies have investigated the relationship between the two coding SNPs (rs662 and rs850560) and CAD. The meta-analysis suggests an association between CAD and PON1. Qinghua Zeng and Juan Zeng suggested that the rs662 polymorphism could be used to identify individuals that are highly susceptible to CAD.[23] In a meta-analysis on 43 studies that assessed 11,000 cases and 13,000 controls, Wheeler et

Table 1 – Baseline characteristics of the study population

Variable		Angiogram negative (N=266)	Angiogram positive (N=335)	p Value
Age (y) (Mean±SD)[1]		55.70±10.96	61.53±8.91	<0.001
Sex (N%)[2]	*Male*	130 (48.9%)	227 (69.8%)	<0.001
	Female	136 (51.1%)	98 (30.2%)	
Positive smoking history (N %)[2]		42 (16.0%)	52 (16.5%)	0.890
HTN history (N %)[2]		121 (45.5%)	192 (59.3%)	0.001
DM history (N %)[2]		70 (26.3%)	130 (40.5%)	<0.001
Dyslipidemia history (N %)[2]		93 (35.1%)	178 (55.3%)	<0.001
BMI (N %)[2]	*Normal (BMI<25)*	87 (37.5%)	104 (35.6%)	0.683
	Overweight (25≤BMI<30)	101 (43.5%)	138 (47.3%)	
	Obese (BMI≥30)	44 (19.0%)	50 (17.1%)	
SBP (Mean ± SD)[1]		121.81±17.37	124.93±15.44	0.035
DBP (Mean ± SD)[1]		76.12±10.42	77.75±8.75	0.063
Serum Paraoxonase activity (U/L) (Median(IQR))[1]		57.60(32.70-105.15)	52.20(30.38-95.18)	0.237

HTN: hypertension; DM: diabetes mellitus; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. [1] Analysis by t-test or Mann-Whitney test, when necessary. [2] Analysis by Chi-square test.

Table 2 – Association between PON1 polymorphisms and CAD

Genotypes			Angiogram negative (N=266)	Angiogram positive (N=335)	Univariate regression	Multivariate regression[1]
rs662	*A*		0.70	0.68	1.093 (0.847-1.410)	1.062 (0.773-1.460)
	G		0.30	0.32		
	AA		112(44.8%)	150(48.1%)	Ref.	Ref.
	AG		121(48.4%)	121(38.8%)	0.747 (0.525-1.061)	0.685 (0.439-1.069)
	GG		17(6.8%)	41(13.1%)	**1.913 (1.022-3.583)***	1.802 (0.827-3.925)
	Dominant model	*AA*	112(44.8%)	150(57.3%)	0.833 (0.632-1.233)	0.818 (0.535-1.250)
		AG+GG	138(55.2%)	162(51.9%)		
	Recessive model	*AA+AG*	234(93.6%)	271(86.9%)	**2.360 (1.274-4.373)****	**2.424 (1.123-5.233)***
		GG	16(6.4%)	41(13.1%)		
	Additive model	*AA*	112(87.5%)	150(78.5%)	**2.041 (1.076-3.871)***	2.080 (0.900-4.810)
		GG	16(12.5%)	41(21.5%)		
	Co-dominant model	*AG*	122(48.8%)	121(38.9%)	**1.508 (1.077-2.113)***	**1.663 (1.086-2.547)***
		AA+GG	128(51.2%)	190(61.1%)		
rs854560	*A*		0.62	0.67	0.944 (0.682-1.309)	0.836 (0.583-1.200)
	T		0.38	0.33		
	AA		88(35.5%)	131(42.3%)	Ref.	Ref.
	AT		131(52.8%)	149(48.1%)	1.053 (0.692-1.603)	0.918 (0.550-1.533)
	TT		29(11.7%)	30(9.7%)	0.299(0.694-1.383)	0.603(0.260-1.399)
	Dominant model	*AA*	86(34.7%)	130(41.9%)	1.004(0.643-1.568)	0.854(0.523-1.393)
		AT+TT	162(65.3%)	180(58.1%)		
	Recessive model	*AA+AT*	220(88.7%)	279(90.0%)	0.770(0.381-1.556)	0.631(0.285-1.398)
		TT	28(11.3%)	31(10.0%)		
	Additive model	*AA*	86(75.4%)	130(81.2%)	0.789(0.374-1.665)	0.601(0.259-1.396)
		TT	28(24.6%)	30(18.8%)		
	Co-dominant model	*AT*	134(54.0%)	149(48.2%)	0.909(0.586-1.412)	0.990 (0.610-1.607)
		AA+TT	114(46.0%)	160(51.8%)		
rs705379	*C*		0.50	0.49	1.055 (0.798-1.394)	0.941(0.669-1.322)
	T		0.50	0.51		
	CC		49(24.1%)	68(21.9%)	Ref.	Ref.
	CT		109(53.7%)	171(55.0%)	0.941(0.574-1.544)	1.079(0.589-1.975)
	TT		45(22.2%)	72(23.2%)	1.098 (0.603-2.000)	0.585(0.418-1.761)
	Dominant model	*CC*	52 (25.6%)	67(21.4%)	1.086 (0.642-1.838)	1.143 (0.645-2.028)
		CT+TT	151(74.4%)	246(78.6%)		
	Recessive model	*CC+CT*	159(78.3%)	242(77.3%)	1.001 (0.600-1.703)	0.820 0.460-1.462)
		TT	44(21.7%)	71(22.7%)		
	Additive model	*CC*	52(54.2%)	67(48.6%)	1.075 (0.561-2.063)	0.939 (0.458-1.924)
		TT	44(45.8%)	71(51.4%)		
	Co-dominant model	*CT*	107(52.7%)	175(55.9%)	0.952 (0.613-1.478)	0.791 (0.486-1.288)
		CC+TT	96(47.3%)	138(44.1%)		

*Analysis was performed using univariate and multivariate regression. *P value ≤ 0.05. **P value ≤ 0.01. [1] Adjusted for age, sex, hypertension, diabetes mellitus, dyslipidemia.*

Table 3 – Difference between haplotype frequencies in the study groups

Haplotypes [1]	Total (N=591)	Angiogram negative (N=266)	Angiogram positive (N=335)	p value
AAC	0,241	0,226	0,248	*0,509*
ATT	0,205	0,200	0,209	*0,781*
AAT	0,150	0,159	0,144	*0,639*
GAC	0,140	0,134	0,147	*0,682*
GAT	0,120	0,110	0,126	*0,549*
ATC	0,095	0,133	0,072	***0,017***
GTT	0,024	0,021	0,026	*0,742*
GTC	0,025	0,017	0,029	*0,437*

[1] Chi-square test used for the analysis.

al. showed there was a significant relationship between the G allele of the rs662 polymorphism and CAD, but there was no association between the rs850560 polymorphism and CAD,[24] similar to our findings. A meta-analysis conducted by Wang et al., of 88 case-control studies in 2011 reported similar results.[25]

Vaisi-Raygani et al., also found a significant relationship between the rs662 polymorphism and CAD in western Iran. Thus, individuals with the G allele have a 1.6-fold chance of having CAD.[26] On the other hand, Ahmad et al. stated that the G allele of rs662 is associated with the risk of developing CAD.[27] Our results are also consistent with another study carried out in the northern Iran, which reported that there was a relationship between rs705379 and CAD. Najafi et al. showed that, while this polymorphism is associated with serum enzyme activity, there was no significant relationship with the occurrence of CAD when compared to the control group.[28]

Table 4 – Analysis between groups of different PON1 genotypes and serum paraoxonase activity

SNPs		Total (N=591)	p value[1]	Angiogram negative (N=266)	p value[2]	Angiogram positive (N=335)	p value[3]	p value[4]
rs662	*A*	45.45 (27.9-78.75)	***<0.001***	46.80 (28.35-87.75)	***<0.001***	45.00 (26.55-67.50)	***<0.001***	***0.019***
	G	94.28 (48.26-148.95)		95.85 (60.19-158.44)		94.28 (48.26(147.15)		*0.527*
	AA	40.05 (25.2-57.60)		41.40 (26.55-58.05)		40.05 (23.40-57.15)		*0.345*
	AG	81.90 (43.76-126.79)	***<0.001*, **, ******	85.63 (44.55-141.08)	***<0.001*, **, ******	80.10 (43.65-122.85)	***<0.001*, **, ******	*0.084*
	GG	129.60 (80.21-175.73)		142.65 (84.60-176.40)		127.35 (62.21-175.28)		*0.933*
rs854560	*A*	60.53 (35.51-117.56)	***<0.001***	65.70 (34.65-119.35)	***0.001***	59.40 (36.90-110.59)	***<0.001***	*0.162*
	T	41.85 (22.95-79.54)		43.00 (26.55-84.71)		42.08 (22.50-70.88)		*0.585*
	AA	67.95 (42.41-129.49)		80.10 (38.40-130.28)		65.48 (43.20(128.81)		*0.228*
	AT	49.50 (28.30-87.75)	***<0.001*, **, ******	57.10 (31.95-95.40)	***<0.001*, *****	47.70 (26.55(83.25)	***<0.001*, **, ******	*0.487*
	TT	29.25 (17.10-46.80)		30.15 (17.55-43.50)		29.25 (17.10-48.15)		*0.888*
rs705379	*C*	65.25 (34.20-122.51)	***<0,001***	78.08 (38.40-133.54)	***0,003***	63.00 (32.85-111.60)	***0.001***	*0.180*
	T	47.70 (27.00-87.75)		45.46 (26.55-91.05)		47.70 (29.25-86.85)		*0.530*
	CC	75.38 (40.50-144.00)		83.93 (44.59-156.83)		72.90 (36.90-127.35)		*0.329*
	CT	54.90 (31.30-98.10)	***<0.001*, **, ******	56.70 (31.46-103.24)	***<0.001*, *****	54.90 (31.16-94.40)	***<0.001*****	*0.299*
	TT	41.40 (22.89-65.93)		37.35 (17.55-73.80)		45.45 (24.98-60.30)		*0.770*

*For statistical analysis, Kruskal–Wallis test was used for comparison between more than 2 groups and Mann-Whitney test for comparison between 2 groups. [1] serum paraoxonase activity status and SNPs in total. [2] serum paraoxonase activity status and SNPs in the angiogram negative group. [3] serum paraoxonase activity status and SNPs in the angiogram positive group. [4] Comparison between serum paraoxonase activity and different genotypes in angiogram positive and negative groups. *Significant difference between dominant homozygous and heterozygous. **Significant difference between two homozygous. ***Significant difference between recessive homozygous and heterozygous.*

Tang et al., evaluated serum PON-1 activity and its related genetic determinants in 3,668 stable subjects (mean age of 63±11) undergoing elective coronary angiography (ECA) without acute coronary syndrome and were prospectively followed for major adverse cardiovascular events for a period of 3 years. Their results showed that the rs854560 and rs662 variants had strong genetic effects on serum PON1 activity but were not associated with the risk of major adverse cardiac events (MACE). They suggested that the genetic effects of this SNP on arylesterase activity are too weak to be observed, especially if a minimum biological threshold of activity change is needed to influence the risk of MACE incidents.[29]

Similarly, the GeneBank study, a prospective study on 1,399 individuals undergoing elective diagnostic coronary angiography with (aged 65±11 years) and without (aged 57±12 years) CAD, showed that the rs662 polymorphism accounts for about 60% of the variations in PON1 activity. Moreover, decreased serum activity of PON1 and its AA genotype were associated with an increase in oxidative stress. This genotype is associated with an increase in mortality and adverse outcomes of cardiovascular events, including myocardial infarction and stroke. Their results showed that the incidence of these adverse effects was significantly lower in individuals with higher PON1 activity.[30]

A study by Ochoa-Martínez et al. found that carriers of the G allele of the rs662 polymorphism had higher levels of CAD biomarkers than carriers of the A allele.[31]

Liu et al., have reported that the rs662 polymorphism is significantly associated with CAD. In line with our findings, this study showed that the carriers of this polymorphism G allele are significantly more exposed to CAD and the activity and concentration of PON1 have been positively associated with the G allele. In addition, the concentration and activity of this enzyme was decreased in patients with CAD compared to the controls,[18] which was not found in our study. There is a paradox, which was previously explained by Aviram et al., who reported that the PON1 active site required for protection against LDL oxidation is different from the one required for its paraoxonase activity, suggesting that the R allele, despite showing higher activity toward paraoxon, is defective as it prevents its antioxidant activity toward LDL due to its active site modulating effect.[32] Thus, carriers of the G allele have reduced capacity to prevent the oxidative modification of LDL and, consequently, are more susceptible to develop CAD than carriers of the A allele.[30]

The rs705379 SNP effect on the PON1 gene promoter is the other determinant of PON1 activity. Brophy et al., have shown that this polymorphism accounted for 22.8% of the inconsistency in PON1 activity. Their results showed that PON1 activity was decreased in 376 white individuals in the presence of the T allele of the rS705379 polymorphism in comparison with presence of the C allele.[33] Several previous studies have reported that the T allele of this polymorphism is associated with an increased risk of CAD in males,[14,15] although we could not confirm this result, which may be due to differences in age, ethnicity, presence of disease, the number of enrolled participants in various studies or difference in control group. Voetsch et al., have shown similar results in a group of 118 young patients (aged <45 years) with a first nonfatal arterial ischemic stroke.[16] Gupta et al. have reported that PON1 activity is reduced in angiographically CAD patients when compared to healthy subjects in a discretely ethnic Indian group in northwest Punjabi who have high incidence of CAD. Moreover, their results showed that the rs662 polymorphisms in the coding regions are all independently associated with CAD.[34] James et al. have reported that there is an association between the T allele of rs705379 and increased risk of CAD in type II diabetic patien.[17]

The presence of the rs662 SNP alone is not enough for atherosclerosis development, because several previous studies have reported that in addition to genotype, enzyme activity and concentrations have important roles, too.[35] Moreover, ethnic differences,[36] dietary and environmental factors,[37] and HDL status[38] can all have an effect on the PON1 phenotype. In addition, a study highlighted the importance of HDL, which is a CAD-related factor, in that PON1 is involved.[39] It has been reported that all three genotypes AA, AG, and GG result in similar mean values of PON1/HDL levels. This result may explain why previous attempts to correlate the rs662 genotype with a predisposition for atherosclerosis failed and opens the road for new PON1 phenotyping methodologies that may provide a better correlation.[40] One limitation of our study was the use of subjects with <30% stenosis as controls. Ideally, the controls would be individuals with minimal angiographically-defined CAD, but these individuals uncommonly require angiography at this age.

Conclusions

We have found that carriers of the G allele of the Q192R polymorphism of the PON1 gene were independently associated with a positive coronary angiogram. Moreover, carriers of G allele rs662, A allele of rs850560 and C allele of rs705379 have increased levels of serum PON1 activity. We could not establish any significant relationship between serum paraoxonase activity and a positive angiogram in a sample from northeastern Iran.

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

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References

1. Helgason D, Helgadottir S, Viktorsson SA, Orrason AW, Ingvarsdottir IL, Geirsson A, et al. Acute Kidney Injury and Outcome Following Aortic Valve Replacement for Aortic Stenosis. Interact Cardiovasc Thorac Surg. 2016;23(2):266-72. doi: 10.1093/icvts/ivw117.
2. Sarrafzadegan N, Mohammmadifard N. Cardiovascular Disease in Iran in the Last 40 Years: Prevalence, Mortality, Morbidity, Challenges and Strategies for Cardiovascular Prevention. Arch Iran Med. 2019;22(4):204-10.
3. Baktashian M, Riaziat MD AR, Moshaveri F, Rouzbahani R. Periodic Health Assessment in Office Workers of Isfahan Insurance Organization, Iran. Journal of Isfahan Medical School. 2012;30(201):1225-33.
4. Esteghamati A, Meysamie A, Khalilzadeh O, Rashidi A, Haghazali M, Asgari F, et al. Third National Surveillance of Risk Factors of Non-Communicable Diseases (SuRFNCD-2007) in Iran: Methods and Results on Prevalence of Diabetes, Hypertension, Obesity, Central Obesity, and Dyslipidemia. BMC Public Health. 2009;9:167. doi: 10.1186/1471-2458-9-167.
5. Alamdari DH, Ghayour-Mobarhan M, Tavallaie S, Parizadeh MR, Moohebati M, Ghafoori F, et al. Prooxidant-antioxidant Balance as a New Risk Factor in Patients with Angiographically Defined Coronary Artery Disease. Clin Biochem. 2008;41(6):375-80. doi: 10.1016/j.clinbiochem.2007.12.008.
6. Ashok BT, Ali R. The Aging Paradox: Free Radical Theory of Aging. Exp Gerontol. 1999;34(3):293-303. doi: 10.1016/s0531-5565(99)00005-4.
7. Martinelli N, Micaglio R, Consoli L, Guarini P, Grison E, Pizzolo F, et al. Low Levels of Serum Paraoxonase Activities are Characteristic of Metabolic Syndrome and May Influence the Metabolic-syndrome-related Risk of Coronary Artery Disease. Exp Diabetes Res. 2012;2012:231502. doi: 10.1155/2012/231502.
8. Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of Paraoxonase (PON1) Activity. Biochem Pharmacol. 2005;69(4):541-50. doi: 10.1016/j.bcp.2004.08.027.
9. Imai Y, Morita H, Kurihara H, Sugiyama T, Kato N, Ebihara A, et al. Evidence for Association Between Paraoxonase Gene Polymorphisms and Atherosclerotic Diseases. Atherosclerosis. 2000;149(2):435-42. doi: 10.1016/s0021-9150(99)00340-8.
10. She ZG, Chen HZ, Yan Y, Li H, Liu DP. The Human Paraoxonase Gene Cluster as a Target in the Treatment of Atherosclerosis. Antioxid Redox Signal. 2012;16(6):597-632. doi: 10.1089/ars.2010.3774.
11. Kim DS, Burt AA, Ranchalis JE, Richter RJ, Marshall JK, Nakayama KS, et al. Dietary Cholesterol Increases Paraoxonase 1 Enzyme Activity. J Lipid Res. 2012;53(11):2450-8. doi: 10.1194/jlr.P030601.
12. Zafiropoulos A, Linardakis M, Jansen EH, Tsatsakis AM, Kafatos A, Tzanakakis GN. Paraoxonase 1 R/Q Alleles are Associated with Differential Accumulation of Saturated Versus 20:5n3 Fatty Acid in Human Adipose Tissue. J Lipid Res. 2010;51(7):1991-2000. doi: 10.1194/jlr.P004960.
13. Furlong CE, Suzuki SM, Stevens RC, Marsillach J, Richter RJ, Jarvik GP, et al. Human PON1, a Biomarker of Risk of Disease and Exposure. Chem Biol Interact. 2010;187(1-3):355-61. doi: 10.1016/j.cbi.2010.03.033.
14. Leviev I, Kalix B, Meynet M-CB, James R. The paraoxonase PON1 promoter polymorphism C (-107) T is associated with increased serum glucose concentrations in non-diabetic patients. Diabetologia. 2001;44(9):1177-83.
15. Leviev I, Poirier O, Nicaud V, Evans A, Kee F, Arveiler D, et al. High Expressor Paraoxonase PON1 Gene Promoter Polymorphisms are Associated with Reduced Risk of Vascular Disease in Younger Coronary Patients. Atherosclerosis. 2002;161(2):463-7. doi: 10.1016/s0021-9150(01)00668-2.
16. Voetsch B, Benke KS, Panhuysen CI, Damasceno BP, Loscalzo J. The Combined Effect of Paraoxonase Promoter and Coding Region Polymorphisms on the Risk of Arterial Ischemic Stroke Among Young Adults. Arch Neurol. 2004;61(3):351-6. doi: 10.1001/archneur.61.3.351.
17. James RW, Leviev I, Ruiz J, Passa P, Froguel P, Garin MC. Promoter Polymorphism T(-107)C of the Paraoxonase PON1 Gene is a Risk Factor for Coronary Heart Disease in Type 2 Diabetic Patients. Diabetes. 2000;49(8):1390-3. doi: 10.2337/diabetes.49.8.1390.
18. Liu T, Zhang X, Zhang J, Liang Z, Cai W, Huang M, et al. Association between PON1 rs662 Polymorphism and Coronary Artery Disease. Eur J Clin Nutr. 2014;68(9):1029-35. doi: 10.1038/ejcn.2014.105.
19. Vaisi-Raygani A, Ghaneialvar H, Rahimi Z, Tavilani H, Pourmotabbed T, Shakiba E, et al. Paraoxonase Arg 192 Allele is an Independent Risk Factor for Three-vessel Stenosis of Coronary Artery Disease. Mol Biol Rep. 2011;38(8):5421-8. doi: 10.1007/s11033-011-0696-3.
20. Shahsavari G, Nouryazdan N, Adibhesami G, Birjandi M. Genetic Associations and Serum Paraoxonase Levels with Atherosclerosis in Western Iranian Patients. Mol Biol Rep. 2020;47(7):5137-44. doi: 10.1007/s11033-020-05585-2.
21. Mahrooz A, Shokri Y, Variji A, Zargari M, Alizadeh A, Mehtarian E. Improved Risk Assessment of Coronary Artery Disease by Substituting Paraoxonase 1 Activity for HDL-C: Novel Cardiometabolic Biomarkers Based on HDL Functionality. Nutr Metab Cardiovasc Dis. 2021;31(4):1166-76. doi: 10.1016/j.numecd.2020.12.026.
22. Moreno-Godínez ME, Galarce-Sosa C, Cahua-Pablo JÁ, Rojas-García AE, Huerta-Beristain G, Alarcón-Romero LDC, et al. Genotypes of Common Polymorphisms in the PON1 Gene Associated with Paraoxonase Activity as Cardiovascular Risk Factor. Arch Med Res. 2018;49(7):486-96. doi: 10.1016/j.arcmed.2019.02.002.
23. Zeng Q, Zeng J. A Meta-analysis on Relationship Between Paraoxonase 1 Polymorphisms and Atherosclerotic Cardiovascular Diseases. Life Sci. 2019;232:116646. doi: 10.1016/j.lfs.2019.116646.
24. Wheeler JG, Keavney BD, Watkins H, Collins R, Danesh J. Four Paraoxonase Gene Polymorphisms in 11212 Cases of Coronary Heart Disease and 12786 Controls: Meta-analysis of 43 Studies. Lancet. 2004;363(9410):689-95. doi: 10.1016/S0140-6736(04)15642-0.
25. Wang M, Lang X, Zou L, Huang S, Xu Z. Four Genetic Polymorphisms of Paraoxonase Gene and Risk of Coronary Heart Disease: A Meta-analysis Based on 88 Case-control Studies. Atherosclerosis. 2011;214(2):377-85. doi: 10.1016/j.atherosclerosis.2010.11.028.

26. Vaisi-Raygani A, Rahimi Z, Tavilani H, Vaisi-Raygani H, Kiani A, Aminian M, et al. Synergism Between Paraoxonase Arg 192 and the Angiotensin Converting Enzyme D Allele is Associated with Severity of Coronary Artery Disease. Mol Biol Rep. 2012;39(3):2723-31. doi: 10.1007/s11033-011-1027-4.

27. Ahmad I, Narang R, Venkatraman A, Das N. Two- and three-locus Haplotypes of the Paraoxonase (PON1) Gene are Associated with Coronary Artery Disease in Asian Indians. Gene. 2012;506(1):242-7. doi: 10.1016/j.gene.2012.06.031.

28. Najafi M, Gohari LH, Firoozrai M. Paraoxonase 1 Gene Promoter Polymorphisms are Associated with the Extent of Stenosis in Coronary Arteries. Thromb Res. 2009;123(3):503-10. doi: 10.1016/j.thromres.2008.03.004.

29. Tang WH, Hartiala J, Fan Y, Wu Y, Stewart AF, Erdmann J, et al. Clinical and Genetic Association of Serum Paraoxonase and Arylesterase Activities with Cardiovascular Risk. Arterioscler Thromb Vasc Biol. 2012;32(11):2803-12. doi: 10.1161/ATVBAHA.112.253930.

30. Bhattacharyya T, Nicholls SJ, Topol EJ, Zhang R, Yang X, Schmitt D, et al. Relationship of Paraoxonase 1 (PON1) Gene Polymorphisms and Functional Activity with Systemic Oxidative Stress and Cardiovascular Risk. JAMA. 2008;299(11):1265-76. doi: 10.1001/jama.299.11.1265.

31. Ochoa-Martínez ÁC, Araiza-Gamboa Y, Varela-Silva JA, Orta-García ST, Carrizales-Yáñez L, Pérez-Maldonado IN. Effect of Gene-environment Interaction (arsenic exposure - PON1 Q192R polymorphism) on Cardiovascular Disease Biomarkers in Mexican Population. Environ Toxicol Pharmacol. 2021;81:103519. doi: 10.1016/j.etap.2020.103519.

32. Aviram M, Billecke S, Sorenson R, Bisgaier C, Newton R, Rosenblat M, et al. Paraoxonase Active Site Required for Protection Against LDL Oxidation Involves its Free Sulfhydryl Group and is Different from that Required for its Arylesterase/paraoxonase Activities: Selective Action of Human Paraoxonase Allozymes Q and R. Arterioscler Thromb Vasc Biol. 1998;18(10):1617-24. doi: 10.1161/01.atv.18.10.1617.

33. Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik GP, Furlong CE. Effects of 5' Regulatory-region Polymorphisms on Paraoxonase-gene (PON1) Expression. Am J Hum Genet. 2001;68(6):1428-36. doi: 10.1086/320600.

34. Gupta N, Singh S, Maturu VN, Sharma YP, Gill KD. Paraoxonase 1 (PON1) Polymorphisms, Haplotypes and Activity in Predicting Cad Risk in North-West Indian Punjabis. PLoS One. 2011;6(5):e17805. doi: 10.1371/journal.pone.0017805.

35. Mackness M, Mackness B. Paraoxonase 1 and Atherosclerosis: Is the Gene or the Protein More Important? Free Radic Biol Med. 2004;37(9):1317-23. doi: 10.1016/j.freeradbiomed.2004.07.034.

36. La Du B. Human Serum Paraoxonase/arylesterase. Amsterdam: Elsevier; 1992.

37. Aviram M, Rosenblat M, Gaitini D, Nitecki S, Hoffman A, Dornfeld L, et al. Pomegranate Juice Consumption for 3 Years by Patients with Carotid Artery Stenosis Reduces Common Carotid Intima-media Thickness, Blood Pressure and LDL Oxidation. Clin Nutr. 2004;23(3):423-33. doi: 10.1016/j.clnu.2003.10.002.

38. Cabana VG, Reardon CA, Feng N, Neath S, Lukens J, Getz GS. Serum Paraoxonase: Effect of the Apolipoprotein Composition of HDL and the Acute Phase Response. J Lipid Res. 2003;44(4):780-92. doi: 10.1194/jlr.M200432-JLR200.

39. Mahrooz A, Mackness M, Bagheri A, Ghaffari-Cherati M, Masoumi P. The Epigenetic Regulation of Paraoxonase 1 (PON1) as an Important Enzyme in HDL Function: The Missing Link Between Environmental and Genetic Regulation. Clin Biochem. 2019;73:1-10. doi: 10.1016/j.clinbiochem.2019.07.010.

40. Gaidukov L, Rosenblat M, Aviram M, Tawfik DS. The 192R/Q Polymorphs of Serum Paraoxonase PON1 Differ in HDL Binding, Lipolactonase Stimulation, and Cholesterol Efflux. J Lipid Res. 2006;47(11):2492-502. doi: 10.1194/jlr.M600297-JLR200.

*Supplemental Materials

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The Paraoxanase 1 (PON1) Gene in the Context of Coronary Artery Disease

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Short Editorial related to the article: Association of Paraoxonase-1 Genotype and Phenotype with Angiogram Positive Coronary Artery Disease

Cardiovascular Diseases (CVD), and more specifically Coronary Artery Disease (CAD), continue to be the most important causes of death from non-communicable diseases in Brazil and in the world.[1,2] Therefore, scientific efforts have been made to identify new biochemical markers and in the elucidation of genotypic risk profiles for CAD and other CVDs in the field of disease-association medical genetics.[3] In this sense, a gene that has been extensively studied is the paraoxanase (PON) gene, which has three gene cluster isoforms: PON1, PON2 and PON3, located on chromosome 7q21.3-22.1.[4] PON1 is the most studied member of the paraoxanases family due to its prominent role in lipoprotein catabolism pathways, being even pointed out as a biochemical marker of the antioxidant capacity of HDL-cholesterol particles.[5,6]

PON1 is a multifunctional calcium-dependent ester hydrolase enzyme that is associated with HDL-cholesterol particles. It has antioxidant and antiatherogenic properties by hydrolyzing oxidized LDL cholesterol and phospholipid peroxidation products. In this way, it provides protection to cell membranes and neutralizes the effects of lipid oxidation, playing an important cardioprotective role.[4,7]

Polymorphisms of paraoxonase enzymes, particularly the PON1 isoform, have been associated with lipid alterations[8] and have been implicated in the pathogenesis of CAD, as demonstrated in some studies,[9,10] although the heterogeneity of results in the literature should be highlighted, as pointed out in extensive meta-analysis studies, who concluded that there was a weak or absent association for the main polymorphisms studied.[11,12] Among which, two polymorphisms in the coding region of the gene stand out, with replacement of the Glutamine (Q) by Arginine (R) at protein position 192 (rs662 or A192G) and Leucine by Methionine at position 55 (rs854560 or A55T) and rs705379 polymorphism (or T[-107]C) in the promoter region of the gene, which have been reported to influence enzyme activity or expression.

Keywords

Coronary Artery Disease/genética; Biomarkers; Genetic Predisposition to Disease; Genetic Association Studies; Aryldialkylphosphatase/genetics; Lipoproteins/blood; Paraoxanase 1 (PON 1); Polymorphism, Single Nucleotide

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In this context, it is worth highlighting the work of Soflaei et al.,[13] who evaluated the association of the mentioned PON1 polymorphisms (rs662, rs854560, rs705379) with CAD in the population of Iran in the northeast region of the country, by comparing patients with angiographically defined CAD (group with positive angiography - obstruction with stenosis coronary artery disease >50% in at least one coronary artery [N=266] and group with negative angiography - non-obstruction with stenosis < 30% in coronary arteries [N=335]). The results obtained indicated a significant association of the G allele (192R isoform of PON1) of the rs662 polymorphism with increased disease risk (GG genotype: OR = 2.424, 95% CI [1.123-5.233]; G allele: OR = 1.663, 95% CI [1.086-2.547]), which is consistent with results from other studies,[9,10] including another study conducted in western Iran,[14] and with meta-analysis findings.[11,12]

The aforementioned study also explored the association of PON1 activity (phenotype) with CAD, not finding any difference between the groups studied, but it confirmed the effect of the polymorphisms evaluated on the level of measured activity of PON1, having observed greater activity of paraoxanase, which would be beneficial, in carriers of the genotypic risk profile (G allele or GG genotype of rs662). This apparently paradoxical finding of risk genotype assessment and PON1 activity profile has been explained, as discussed in the study, by the difference between what is measured as enzyme activity in the biochemical paraoxon hydrolysis assay (paraoxanase activity) and its activity of antioxidant protection in relation to LDL-cholesterol, which involves another active site in the enzyme, so that carriers of the G allele (192R isoform) would actually have a reduced biological activity of PON1 with regard to antioxidant protection, such as evidenced in the study by Aviram et al.,[15] indicating an important aspect to be considered in studies of the activity of this enzyme, due to its multifunctional character.

Currently, genetic association studies have allowed the development of personalized medicine through the application of scientific findings obtained in the construction of panels of genetic tests by groups of diseases, including specific panels for CVD, which are already available to the public in some specialized laboratories. Advances in the level of knowledge related to the effect of genetic variants on molecular mechanisms underlying the pathophysiology of diseases, as has been observed for the paraoxanase genes in CAD, have the potential to foster the development of more accurate genetic panels to impact therapeutic decisions and approaches in genetic counseling.

References

1. Oliveira GM, Brant LC, Polanczyk A, Carisi A, Biolo A, Nascimento BR, et al. Cardiovascular statistics–brazil 2020. *Arq Bras Cardiol*. 2020;115(3):308-439. doi:10.36660/abc.20200812
2. World Health Organization (WHO). Global Health Estimates 2020: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2019. [Accessed April 19 2021] Available from: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-leading-causes-of-death
3. Izar MC, Helfenstein Fonseca FA, Miki Ihara SS, Kasinski N, Won Han S, Lopes IE, et al. Risk Factors, biochemical markers, and genetic polymorphisms in early coronary artery disease. *Arq Bras Cardiol*. 2003;80(4):388-95. doi:10.1590/S0066-782X2003000400003
4. Primo-Parmo SL, Sorenson RC, Teiber J, la Du BN. The Human Serum Paraoxonase/Arylesterase Gene (PON1) Is One Member of a Multigene Family. *Genomics*. 1996;33(3):498-507. doi:10.1006/GENO.1996.0225
5. Garin MCB, Moren X, James RW. Paraoxonase-1 and serum concentrations of HDL-cholesterol and apoA-I. *J Lipid Res*. 2006;47(3):515-20. doi:10.1194/JLR.M500281-JLR200
6. Dullaart RPF, Otvos JD, James RW. Serum paraoxonase-1 activity is more closely related to HDL particle concentration and large HDL particles than to HDL cholesterol in Type 2 diabetic and non-diabetic subjects. *Clin Biochem*. 2014;47(12):1022-7. doi:10.1016/J.CLINBIOCHEM.2014.04.013
7. Mackness MI, Mackness B, Durrington PN, Fogelman AM, Berliner J, Lusis AJ. Paraoxonase and coronary heart disease. *Curr Opin Lipidol*. 1998;9(4):319-24. doi:10.1097/00041433-199808000-00006
8. Scherrer DZ, de Zago VHS, Vieira IC, Parra ES, Panzoldo N, Alexandre F, et al. O SNP p.Q192R da *PON1* não Parece Associar-se a Fatores de Risco para a Aterosclerose Carotídea em Amostra de População Brasileira Normolipidêmica e Assintomática. *Arq Bras Cardiol*. 2015;105(1):45-52. doi:10.5935/ABC.20150053
9. Munshi R, Panchal F, Chaurasia A, Rajadhyaksha G. Association between Paraoxonase 1(PON1) Gene Polymorphisms and PON1 Enzyme Activity in Indian Patients with Coronary Artery Disease (CAD). *Curr Pharmacogenomics Person Med*. 2018;16(3):219-29. doi:10.2174/1875692117666181227112119
10. Liu T, Zhang X, Zhang J, Liang Z, Cai W, Huang M, et al. Association between PON1 rs662 polymorphism and coronary artery disease. *Eur J Clin Nutr. 2014 68(9):1029-35*. 2014;68(9):1029-35. doi:10.1038/ejcn.2014.105
11. Wang M, Lang X, Zou L, Huang S, Xu Z. Four genetic polymorphisms of paraoxonase gene and risk of coronary heart disease: A meta-analysis based on 88 case–control studies. *Atherosclerosis*. 2011;214(2):377-85. doi:10.1016/J.ATHEROSCLEROSIS.2010.11.028
12. Ashiq S, Ashiq K. The Role of Paraoxonase 1 (PON1) Gene Polymorphisms in Coronary Artery Disease: A Systematic Review and Meta-Analysis. *Biochem Genet*. 2021;59(4):919-39. doi:10.1007/S10528-021-10043-0
13. Soflaei SS, Baktashian M, Moghaddam KH, Saberi-Karimian M, Koseri N, Hashemi SM, et al. Association of Paraoxonase-1 Genotype and Phenotype with Angiogram Positive Coronary Artery Disease. Arq Bras Cardiol. 2022; 119(4):593-601.
14. Vaisi-Raygani A, Ghaneialvar H, Rahimi Z, Tavilani H, Rourmotabbed T, Shakiba E, et al. Paraoxonase Arg 192 allele is an independent risk factor for three-vessel stenosis of coronary artery disease. *Mol Biol Rep*. 2011;38(8):5421-8. doi:10.1007/s11033-011-0696-3
15. Aviram M, Billecke S, Sorenson R, Bisgaier C, Newton R, Rosenblat M, et al. Paraoxonase Active Site Required for Protection Against LDL Oxidation Involves Its Free Sulfhydryl Group and Is Different From That Required for Its Arylesterase/Paraoxonase Activities. *Arterioscler Thromb Vasc Biol*. 1998;18(10):1617-24. doi:10.1161/01.ATV.18.10.1617

Review Article

Vascular Aging and Arterial Stiffness

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Abstract

Biological aging occurs as a result of the interaction between genetics, chronological age and external factors. It is the basis for new concepts of vascular aging, whose progression is determined by the difference between biological and chronological age.

From the structural point of view, the effects of vascular aging are more evident in the tunica media of large elastic arteries, marked by increased arterial stiffness, lumen dilation and wall thickness. These effects are described in the continuum of cardiovascular aging (proposed by Dzau in 2010), in which the progressive steps of microvasculature lesions of the heart, kidney and brain are initiated from the aging process. The increase of arterial stiffness can be detected by several non-invasive methods.

Cardiovascular events have been traditionally described using scores that combine conventional risk factors for atherosclerosis. In the classic cardiovascular continuum (Dzau, 2006), to determine the exact contribution of each risk factor is challenging; however, since arterial stiffness reflects both early and cumulative damage of these cardiovascular risk factors, it is an indicator of the actual damage to the arterial wall.

This article provides a general overview of pathophysiological mechanisms, arterial structural changes, and hemodynamic consequences of arterial stiffness; non-invasive methods for the assessment of arterial stiffness and of central blood pressure; the cardiovascular aging continuum, and the application of arterial stiffness in cardiovascular risk stratification.

Keywords

Vascular Stiffness; Hypertension; Heart Disease Risk Factors; Pulse Wave Analysis; Vascular Remodeling.

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Physiopathology of vascular aging

Aging is one of the main risk factors for cardiovascular diseases (CVD) and events, the main cause of death in the world.[1-3] More important than chronological age (passage of time from birth), however, is the quality and velocity of aging and how it is reflected in disease-free years.[3]

Systemic aging reflects not only the chronological age, but also the decline in physiological function (biological age), promoted by chronic exposure to low inflammation – "pro-inflammation", contributing to cellular senescence and pathological aging. Matrix-degrading and pro-inflammatory cellular changes, associated with aging, are the basis for the accelerated vascular aging (AVA), in which biological age surpasses the chronological one, with an exponential increase in the pathogenesis of hypertension and atherosclerosis, predisposing to CVD and early mortality.[3-5]

With aging, physical, mental, and environmental stress increase due to the need for continuous adaptation to life changes. The increase in stress triggers neuroendocrine activation of the renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system (ANS) and endothelin-1 (ET-1). These events are "pro-inflammatory signals" that act on vascular cells promoting the production and secretion of cytokines and chemokines that accumulate on the arterial wall, such as: monocyte chemoattractant protein-1 (MCP-1), transforming growth factor beta (TGF-β), matrix metalloproteinases (MMPs), calpain-1, and milk fat globule-epidermal growth factor 8 (MFG-E8), known as age-associated arterial secretory phenotypes, as well as activation or inactivation of transcription factors (Ets-1, NF-κB, Nrf2 or Sirt1).[2,6,7]

Reactive oxygen species (ROS) are increased in aged arterial wall, nicotinamide adenine dinucleotide phosphate oxidase (NADPH). The levels of the antioxidant proteins copper-zinc superoxide dismutase (Cu/Zn SOD), SOD, and extracellular SOD are negatively regulated during aging. This imbalance, combined with the increase in angiotensin II and ET-1 levels, increases NADPH expression and ROS production, with consequent pro-inflammation, endothelial dysfunction, and stiffening of the aged wall.[2,3,7-11]

Nitric oxide (NO) regulates aging-associated arterial dilation, stiffening and inflammation. In the arterial wall, the expression of both NO synthase and NO are decreased. In addition, NO interacts with ROS to generate peroxynitrite (ONOO –), which reduces NO bioavailability, affecting endothelial relaxation and increasing vasoconstriction and pro-inflammation.[2,3,7–11]

These proinflammatory molecular phenotype alterations eventually lead to cellular and matrix phenotypic changes due to oxidative stress and DNA damage, like replicative senescence (shortened telomeres and inactivation of telomerase) and stress-induced premature senescence (without telomere involvement).[2,3,7]

In arterial cells, mitotic rate is reduced, which is accompanied by cell volume increase and telomere shortening. The angiotensin II signaling cascade leads to intracellular signaling inhibition, functional autophagy, and increased ROS production. At cellular level, vascular cells develop several phenotypes – a subgroup of endothelial cells and vascular smooth muscle cells become senescent, and another subgroup become more proliferative, invasive/migratory, secretory, and rigid.[2,9]

Changes in the extracellular matrix occur, including fibrosis, elastolysis, calcification, amyloidosis and glucose oxidation, increased collagen synthesis and deposition in the arterial walls, mediated by MMPs and TGF-β1, leading to arterial stiffening. Elastolysis occurs due to rupture of interlamellar elastin fibers by MMPs and elastase, resulting in decreased arterial elastic energy storage, complacence, and resilience. Besides, the products of elastolysis seem to be involved in the arterial inflammation and calcification processes. Calcium deposits increase in the arterial wall due to secretion of bone-like substrate (like collagen II). In parallel, there is an overexpression of alkaline phosphatase (pro-calcification molecule) and reduction of anti-calcification molecules (osteonectin and osteopontin). In amyloidosis, increased uncompacted amyloid proteins and fibrils in the arterial wall causes arterial stiffening and calcification. The products of advanced glycation are elevated and contribute to several structural and functional changes in the arterial system, including senescence, pro-inflammation and stiffening.[2,7-9]

At tissue level, pro-inflammation leads to an increase of intima media thickness, endothelial dysfunction, and arterial stiffening and blood pressure. These changes form the basis for the proinflammatory arterial stiffness syndrome.[2,6,7]

Vascular Aging – Arterial Structural Changes

The effects of age are more evident in large elastic arteries. The main changes include the increase of arterial wall stiffness (reduced distensibility), lumen diameter, and intima media thickness.[7,12-14]

The structure of the arterial tree consists of three parts. The aorta, the most elastic segment, is the most proximal and largest portion; the intermediate segment is composed of muscle arteries, and arterioles are the smallest and most distal portion. The arterial tree act as both a conduit (distributing blood from the heart to the capillaries) and cushion (changing pulsatile flow generated by cardiac intermittent contraction to steady flow). Different parts of the arterial tree play different roles; while large elastic arteries act as a cushion, arterioles work as conduits. Differences between predominantly elastic and muscular arteries influence their responses to aging process, to volume and pressure changes and to atherogenic factors.[3,7,12-15]

The tunica media is the main responsible for the distensibility of the vascular wall and consists of elastic fibers, smooth muscle cells, collagen fibers and fundamental substance. Age-dependent change is explained by the "cyclic stress". The succession of cardiac cycles causes structural changes in the arteries because of intermittent cardiac contraction and hemodynamic pressure changes between systole and diastole. This pulsatile stress leads to disorganization of the tunica media of large elastic arteries, through the gradual thinning, division, deterioration, and fragmentation of elastin.[7,9,13,16-19] This elastic material is replaced by collagen, with formation of a rigid matrix, osteogenic differentiation of arterial cells and calcification. The process results in stiffening of the tunica media by transference of the stress from more distensible elastic fibers to less distensible collagen fibers.[7,12,13]

This degeneration is known as "arteriosclerosis", which should be differentiated from "atherosclerosis", that affects the tunica intima, rather than the tunica media, through an endothelial inflammatory process with lipid accumulation (luminal stenosis). Although the two lesions coexist, arteriosclerosis tend to be more diffuse in the elastic arteries, while the atherosclerotic lesions are more located in susceptible elastic and muscular arteries (carotid bifurcation and coronary arteries).[7,12,13]

Structural changes in large arteries due to hypertension are similar to aging-related changes (arteriosclerosis), but have an earlier onset, indicating that hypertension accelerates arterial aging.[7,12,13]

Medium-sized muscular arteries are hardly affected by aging as they are less distensible than elastic arteries and are hence exposed to lower cyclic stretching. In young individuals, arteries are more elastic; with aging, there is a gradual disappearance of elastic uniformity between proximal and distal arterial system, leading to progressive decrease in pulse pressure amplification and negatively affecting the ventricular-arterial interaction.[13,14,16,20]

Lumen dilatation occurs after elastin fracture and degeneration, resulting in weakening of the arterial wall. The arterial wall becomes stiffer with the distension pressure, due to the increase of collagen fibers. Thus, there is a non-linear relationship between tension (pressure) and deformation (diameter), with concavity toward the distension axis, such that distension decreases as force increases. This is essential for an effective functioning of the arteries as conduits, with maintenance of a residual stress, without artery collapse, promoting good blow flow. Wall tension (T), balanced by transmural pressure (P) and radius (r) ($T = P \cdot r$, law of Laplace) has a unique operating point in the pressure-diameter curve. The arterial wall stress is even greater as a consequence of a dilated lumen. Therefore, arterial dilatation and degeneration generates a vicious cycle that contributes to acceleration of vascular aging.[7,12-14,19]

The increase in wall thickness depends on intimal hyperplasia. The possible mechanisms responsible for increased intima–media thickness include atherosclerosis, elevation of local pressure, and biochemical changes with age.[7,12,13]

Risk factors – hypertension, smoking, excess salt consumption, dyslipidemia, diabetes, metabolic syndrome chronic kidney disease (CKD), inflammation, oxidative stress,

fetal and genetic programming, can potentiate the process of arterial aging and the early development of biological features by the vascular system that will lead to the development of CVD.[1,10]

Vascular Aging: Hemodynamic Consequences

Arteries do not have uniform viscoelastic properties and exhibit adaptative mechanisms. While elasticity decreases from the proximal to the distal arteries, stiffness follows the opposite pattern.[12-14,18] Although such heterogeneity made it difficult to develop mathematical models to assess arterial compliance, other models have been constructed to explain hemodynamic characteristics of the arterial tree.[12,14,18]

In the Windkessel model, the arterial system is compared to a fire truck, in which large vessels would represent the air chamber, medium-sized arteries the fire hose and small arterioles the spout. Therefore, the arteries have two well-defined characteristics - the cushioning function (large arteries transforming pulsatile flow into constant flow to the organ) and the conduit function (small arteries and arterioles delivering blood from heart to organs and tissues).[7,12-14,18,19]

The Windkessel model has limitations, as it assumes an infinite pulse wave velocity (PWV). This could not be the case, because both conduit and cushioning functions are not limited to specific arteries, but rather coexist, leading to the heterogeneity of PWV. Besides, there is a progressive loss of the cushioning function from the aorta to the more muscular and rigid peripheral arteries, and a predominant conduit function. This phenomenon, of "wave reflection", leads to an increase in the amplitude of the pressure wave from the heart vessels to the periphery, known as pressure amplification. In addition, the stiffness of medium-sized peripheral arteries is modulated by the vasomotor tone, depending on the endothelial function, the ANS and RAAS.

For this reason, it is better to apply propagative models to the circulatory system. These assume that PWV that travels along an artery has a finite value. The Moens–Korteweg equation: co¼p(Eh/2Rr), where "co" represents wave speed, "E" the Young's modulus in the circumferential direction, "h" the wall thickness, "R" the radius, and "r" the density of fluid] derived the equation: co¼p(V.dP/r.dV), where dV is the change in arterial volume (V) and dP is the change in pressure driving the change in volume. This second equation is currently widely used in the clinical research and demonstrates that the PWV is inversely related to the distensibility of the arterial tube, expressed asdV/V. dP. The PWV provides a straightforward way to quantify arterial stiffness; the stiffer the artery the higher the PWV.[7,12,14,17,18]

Thus, rather than the Windkessel model, a more realistic model of the arterial tree would be a "propagative model" consisting of a simple distensible tube which terminates at the peripheral resistance. However, its elastic properties allow the generation of a pressure wave which travels along the tube, in which conduit and cushioning functions are combined. The proximal end of the tube corresponds to the central aorta, and the distal end to the high-resistance arterioles. The pressure wave generated by cardiac ejection travels along this tube from the proximal end to the distal end, where this forward wave is reflected back.[7,12,14,17,18]

These models make it possible to explain several phenomena that were observed in the real arterial system but not interpretable by the Windkessel model. These include a secondary pressure wave in diastole or late systole, and amplification of the pressure pulse from the proximal aorta to the distal muscular arteries, explaining why arterial stiffness increases central pulse pressure and systolic arterial pressure (SAP). In young individuals and adults with healthy arterial aging, reflected waves generate retrograde waves, which must be superimposed, elevating the pressure during diastole, rather than systole, increasing coronary perfusion.[7,13,14,18,19]

Wave reflections originate in various locations, including bifurcations of conducting arteries and small muscular arteries. Vasoconstriction results in reflection points close to the heart, leading to early aortic wave reflections. The moment when wave reflections arrive at the proximal aorta depends on the PWV of conduit arteries. In addition, increased arterial stiffness, observed in older or hypertensive individuals, promotes an earlier arrival of the reflected wave, which travels rapidly along the arterial tree. Thus, both small and large arteries contribute to early wave reflection, which returns early in systole and superimposes on the forward wave. This process causes an increase in systolic blood pressure (SBP) and reductions in diastolic fluctuations and blood pressure (Figure 1).

A pressure wave that propagates along a viscoelastic tube with numerous branches is progressively amplified from the central conduit towards distal arteries due to wave reflections and higher PWV in a stiffer peripheral artery. As a result, the amplitude of the pressure wave is higher in peripheral arteries than in central arteries, the so-called "amplification phenomenon".[7,12,14,17,19,21]

Non-Invasive Methods For The Assessment Of Arterial Stiffness

Arterial stiffness can be assessed at systemic, local, and regional levels. While systemic assessment of arterial stiffness can only be performed by models of the circulation, regional and local arterial stiffness can be measured directly, by non-invasive methods, with the advantage that the parameters used in these analyses are strongly linked to wall stiffness (Table 1).[7,14,18,19]

Regional determination of arterial stiffness

The aorta is the main vessels used for determination of regional arterial stiffness, since the thoracic and abdominal aorta are the main "cushions" of the arterial tree, and the aortic PWV is an independent predictor of cardiovascular outcomes.[22-32]

The carotid-femoral PWV (cfPWV) is the gold-standard, non-invasive method for arterial stiffness determination. Several studies using cfPWV measures have shown that arterial stiffness is related to cardiovascular events.[7,14,19,29,30,33] cfPWV is determined by transcutaneous measures (tonometry) using the "foot-to-foot" velocity method in right carotid and femoral arteries (Figure 2). The "foot" of the wave is defined at the end of diastole, when the sharp rise of the wavefront begins. cfPWV (m/s) is calculated by the formula cfPWV (m/s) = D (meters) /Δt (seconds). (D) can be calculated as the (1) distance between

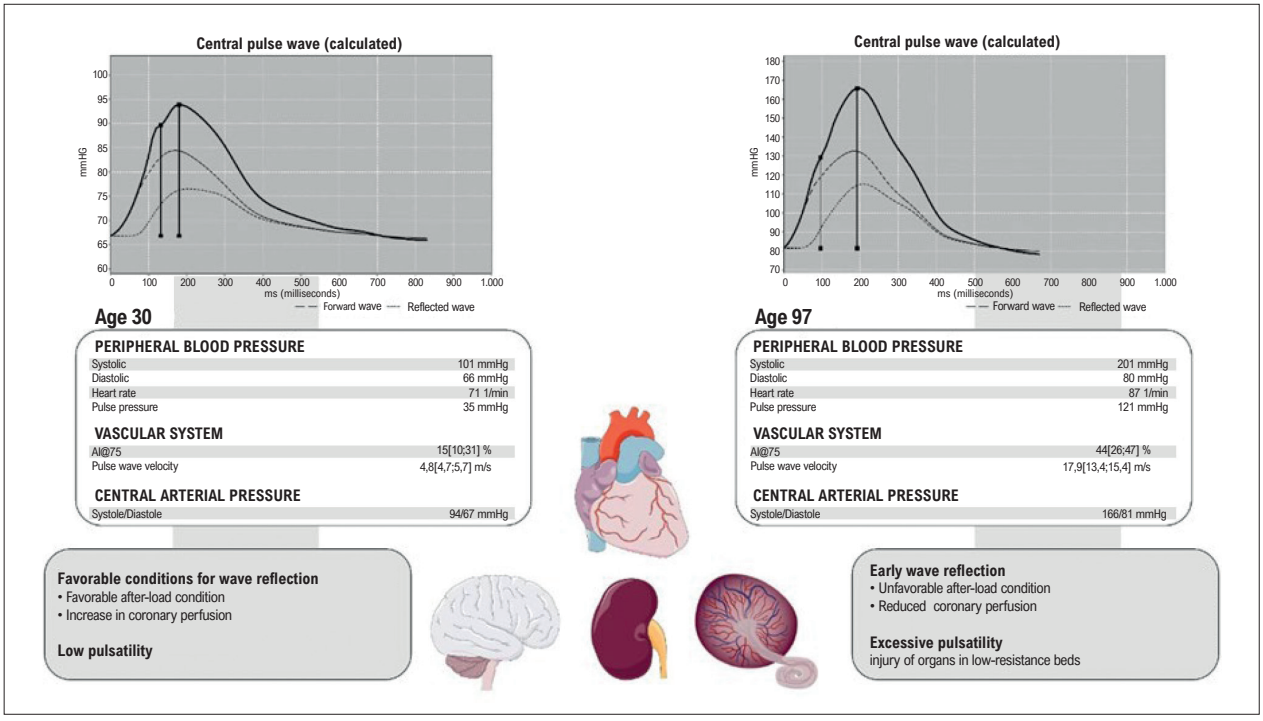

Figure 1 – *Arterial stiffness in large arteries. In a young health individual, a compliant aorta (left): 1) protects excess pulsatility caused by the intermittent left ventricular ejection and 2) exhibits a slow pulse wave velocity (PWV), allowing that reflected waves arrive to the heart during diastole, increasing diastolic coronary perfusion pressure but not after-load. Factors like aging and lifestyle increase aortic wall stiffness, leading to adverse hemodynamic consequences. Aortic stiffness leads to a rise in aortic root impedance, with consequent increase in forward wave amplitude and earlier arrival of the reflected waves to the heart. These hemodynamic changes result in adverse patterns of pulsatile load to the left ventricle during systole and a reduction in perfusion reserve (even in the absence of epicardial coronary disease). This inverse hemodynamic pattern also causes excess pulsatility in the aorta, which is preferably transferred to low-resistance beds, such as the kidney, placenta, and brain. In these organs, microvascular pressure is more directly associated with fluctuations in aortic pressure. AIx@75: augmentation index adjusted at heart rate. Source: the authors.*

two sites (carotid and femoral artery); (2) by subtracting the distance between the carotid site and manubrium sterni from total distance; (3) subtracting the distance between the carotid site and manubrium sterni from the distance between the manubrium sterni and the femoral site. Of all currently used measures, the 80% of the distance from the common carotid to the common femoral artery has been shown to be the most accurate.[3,13,14,19,32,33]

The determination of cfPWV by tonometry has limitations, such as: a) the precise registration of the femoral pressure waveform may be difficult in patients with metabolic syndrome, obesity, and peripheral arterial disease; b) stenosis of the aorta, iliac artery, or proximal femoral artery may attenuate and halt pulse wave progression; and c) abdominal obesity, particularly in men, and large bust size in women can affect the accuracy of distance measurements.[3,13,14,19,32,33]

Therefore, the analysis of PWV from a unique site simplifies the measurement. Several devices have been developed to estimate the PWV in a given arterial pathway from the analysis of brachial pressure wave using an arm cuff. These methods include the determination of the time difference between onset time of electrocardiogram Q wave and onset time of Korotkoff sounds. Arteriograph® provides a single-point estimate of the PWV using a brachial cuff and supra-systolic oscillometry. The Mobil-O-Graph® (Brasil, Dyna Mapa AOP®) uses oscillometric registries obtained by three measurements of the brachial pressure waveform, at mean blood pressure (C1 calibration) or DBP (C2 calibration) to compose the pulse wave by the transfer function (ARCSolver® algorithm). In this last method, both age and blood pressure are used to refine the estimates of PWV.[13,14,19,32,33]

Reference values for cfPWV (tonometry) have been established for healthy individuals and those with cardiovascular risk factors from European countries.[34] Also, reference values for the oscillometric method, of central systolic blood pressure (cSBP), aortic augmentation index (AIx) and PWV for individuals with and without cardiovascular risk factors have been established for the Brazilian population (Table 2).[35]

Despite the importance of PWV estimates in the prediction of cardiovascular events and risk stratification, the method is underused in clinical practice. A European group has proposed a clinical score, the SAGE, to identify patients with priority for PWV estimation, based on easily available variables: systolic blood pressure (S), age (A), fasting plasma glucose (G), and estimated glomerular filtration rate (E) (using the CKD-EPI).[36] The score was applied in the Brazilian population using the oscillometric method and identified that hypertensive patients

Table 1 – Device and methods used for determining regional, local, and systemic arterial stiffness and wave reflections

Year of first publication	Device	Method	Measurement site	Predictive value for CV events (year of first publication) clinical application	Easy clinical application
Regional arterial stiffness					
1984[a]	Complior®	Mechanotransducer	Aorta, cfPWV[b]	Yes (1999)	++
1990[a]	Sphygmocor®	Tonometer	Aorta, cfPWV[b]	Yes (2011)	++
1991	WallTrack®	Echotracking	Aorta, cfPWV[b]	No	+
1994	QKD	ECG +	Aorta, cfPWV[b]	Yes (2005)	++
1997[a]	Cardiovasc. Eng. Inc®	Tonometer	Aorta, cfPWV[b]	Yes (2010)	+
2002	Artlab®	Echotracking	Aorta, cfPWV[b]	No	++
2002	Sistema de ultrassom	Doppler probes	Aorta, cfPWV[b]	Yes (2002)	+
2002	Omron VP-1000®	Pressure cuff	Aorta, baPWV[b]	Yes (2005)	+++
2007	CAVI-Vasera®	ECG + Pressure cuff	Aorta, ctPWV[b]	Yes (2014)	+++
2008	Arteriograph®	Brachial pressure cuff	Aorta, aaPWV[b]	Yes (2013)	++
2009	RMN, ArtFun®	Magnetic resonance imaging	Aorta, aaPWV[b]	Yes (2014)	+
2010	Mobil-O-Graph®	Brachial pressure cuff	Aorta, cfPWV[c]	No	++
2010	Ultrafast®	Echography	Common carotid	No	–
2013	pOpmetre®	Plethysmography	Aorta, dpPWV[b]	No	+++
2017	Withings®	Ballistocardiography	Aorta	No	+++
Local arterial stiffness					
1991	WallTrack®	Echotracking	CCA[d], CFA[d], BA[d].	No	+
1992	NIUS®	Echotracking	RA[d]	No	+/–
2002	Artlab®, Mylab®	Echotracking	CCA[d], CFA, BA	Yes (2014)	++
2017	Ultrasound systems	Echography	CCA[d], CFA, BA	No	+
2009	RMN, ArtFun®	Magnetic resonance imaging	AA[d], DA[d]	No	+
Systemic arterial stiffness					
1989	Area method	Diastolic decay		No	+/–
1995	HDI PW CR-2000®	Modified Windkessel		No	+
1997[a]	Cardiovasc. Eng. Inc®	Tonometry/Doppler/ Echo		Yes (2010)	+/–
2009	MRI, ArtFun®	Magnetic resonance imaging	AA, DA	No	+

[a] Device used in pioneer epidemiological studies that describe the predictive value of aortic stiffness for cardiovascular events; [b] PWV: pulse wave velocity, cf: carotid-femoral, ba: brachial-ankle, ca: cardiac-ankle, aa: aortic arch, ft: finger-toe. [c] Estimated (not measured). [d] All superficial arteries, including those particularly mentioned; Ao: aorta, CCA: common carotid artery, CFA: common femoral artery, BA: brachial artery, RA: radial artery, AA: ascendent aorta, DA: descendent aorta. Source: Adapted from Laurent at al. (2019, p. 143-144)

with SAGE ≥ 8 should be referred for arterial stiffness analysis, due to the high risk of increased PWV.[36-38]

Assessment of local arterial stiffness

Local arterial stiffness can be determined by carotid ultrasound using the high-resolution echo-tracking. The method is highly accurate to determine diameter at diastole and stroke changes in diameter when compared with the classical analysis with other video-image systems. Chest nuclear resonance allows the combined determination of both structure and function of the heart and the aorta, with undoubted accuracy, but at the expense of lower spatial and temporal resolution. However, most of pathophysiological and pharmacological studies used echotracking techniques.[14,19,32,33]

Systemic arterial stiffness

Method based on an electrical circuit using the modified Windkessel model, developed to determine the proximal

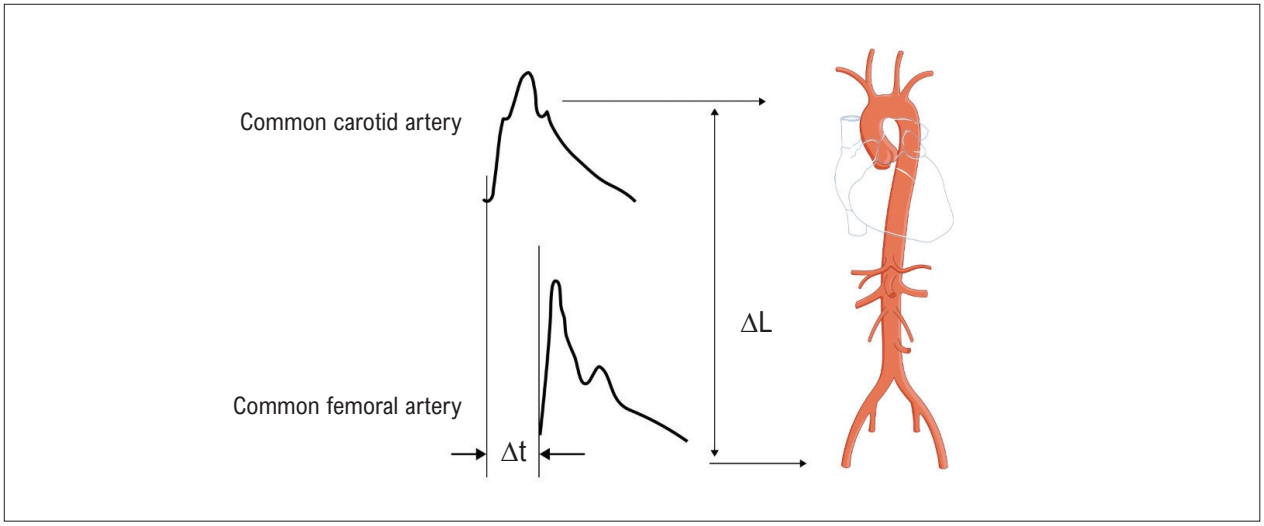

Figure 2 – *Measurement of carotid-femoral pulse wave velocity with the foot-to- foot method. Measurement of carotid-femoral pulse wave velocity with the foot-to-foot method. The waveforms are usually obtained transcutaneously, at the right common carotid artery and the right femoral artery. The time delay (Δt, or transit time) is measured between the feet of the two waveforms (Figure 1). The distance (ΔL) covered by the waves is usually the surface distance between two recording sites, i.e., the common carotid and the femoral artery. Pulse wave velocity (PWV) is calculated as PWV = 0.8 x ΔL (m)/Δt (s). Source: the authors.*

capacitive compliance and distal oscillatory compliance. In addition, systemic arterial compliance can be determined using the "area method", which requires the measurement of the aortic blood flow (velocity obtained from the suprasternal notch) and motor pressure associated with applanation tonometry on the right common carotid artery. Theoretical, technical, and practical limitations make the general application of this method in the clinical setting difficult.[14,19,33]

Central blood pressure

Arterial pressure waveform should be analyzed at the central level (ascending aorta) as it represents the load imposed on the heart, kidney and arterial wall. The most widely used approach is radial artery tonometry, followed by application of a transfer function (SphygmoCor, Atcor, Sydney Australia) to calculate the aortic pressure waveform. The radial artery is sustained by bone tissue, which makes applanation easier.[7,14,19,32,33]

Aortic waveform can be estimated by common carotid artery tonometry, which requires more technical knowledge but does not require the transfer function, as the artery sites are very close, and the waveforms are similar. New methods have been developed to estimate central arterial pressure using the second systolic radial or brachial blood pressure peak. External calibration is necessary, made with brachial SBP and DBP to calibrate the radial artery tonometry, and with mean blood pressure and radial DBP to calibrate the aorta or carotid waveform.[7,14,19,32,33]

The pressure wave is composed by the wavefront, generated by ventricular contraction, and the retrograde wave, generated by wave reflected at bifurcation points. In elastic vessels, PWV is low and the reflected wave travels back towards the aorta root during diastole. In the presence of arterial stiffness, PWV increases, and the reflected wave returns early, adding "augmentation" during systole. This phenomenon can be quantified by the AIx, *i.e.*, the difference between the first and the second systolic peak (P2 – P1), in percentage (Figure 3). Age and PWV are the main determinants of AIx.[7,14,19,32,33]

In peripheral arteries, pressure wave amplitude is greater than in central arteries due to the amplification phenomenon; thus, peripheral SBP and brachial pulse pressure overestimate SBP and central pulse values in young individuals.[39] Pulse wave should be analyzed through central pulse pressure (cPP), central SBP and the AIx.[14,19,32,33] These parameters are independent predictors of all-cause mortality and cardiovascular events.[41,42]

Reference values for cSBP and AIx were defined for the European population[43] by tonometry and for the Brazilian population by the oscillometric method[35] (Table 2).

cSBP, cPP, AIx and PWV cannot be used indistinctly as indicators of arterial stiffness, since they are different determinants. cSBP, cPP and AIx depend on PWV, amplitude of the reflected wave, the reflection point, and the ejection fraction duration and pattern, especially those related to changes in heart rate (HR) and ventricular contractility. Pathophysiological and pharmacological conditions can affect both cPP and AIx without affecting the aortic PWV, suggesting a predominant effect of the reflected wave, HR and ventricular ejection, and no change in aortic stiffness. The influence of age is greater on AIx than on PWV before the age of 50 and greater on PWV than AIx after this age. Therefore, while PWV is a direct measure of arterial stiffness, cSBP and AIx are indirect measures.[7,14,19,32,33]

Arterial stiffness and the cardiovascular continuum

The classical description of the "cardiovascular continuum", published by Dzau et al. (2006)[44] reports the progression of CVD (Figure 4) founded on the atherosclerosis process, which is initiated with the exposure to risk factors (hypertension,

Table 2 – Reference values for central blood pressure, pulse wave velocity, and aortic augmentation index (AIx) for men and women, with and without cardiovascular risk factors

Age groups	Without cardiovascular risk factors		With cardiovascular risk factors	
	Women	Men	Women	Men
cSBP				
<30 years	101 (90; 93; 113; 119)	113 (104; 109; 120; 123)	118 (102; 109; 127; 131)	123 (107; 114; 132; 144)
30-39 years	109 (96; 102; 117; 123)	114 (102; 110; 121; 127)	120 (102; 110; 130; 143)	125 (108; 116; 133; 141)
40-49 years	110 (99; 103; 117; 122)	116 (102; 109; 122; 126)	121 (104; 112; 134; 146)	123 (108; 115; 131; 141)
50-59 years	110 (97; 104; 120; 124)	112 (100; 106; 1 18; 124)	124 (106; 114; 135; 146)	124 (105; 114; 134; 144)
60-69 years	114 (100; 105; 120; 125)	112 (96; 101; 120; 127)	127 (105; 115; 141; 154)	123 (103; 112; 136; 149)
70+ years	113 (100; 103; 121; 126)	116 (94; 104; 125; 129)	131 (108; 118; 146; 165)	125 (102; 111; 140; 156)
cDBP				
<30 years	73 (60; 66; 77; 85)	76 (66; 71; 82; 87)	82 (68; 73; 90; 97)	83 (72; 77; 93; 100)
30-39 years	77 (67; 71; 83; 88)	80 (7 1; 75; 85; 88)	86 (71; 77; 95; 105)	88 (75; 80; 96; 103)
40-49 years	79 (67; 73; 84; 88)	81 (74; 77; 86; 89)	86 (71; 78; 94; 103)	90 (75; 82; 97; 104)
50-59 years	76 (64; 70; 82; 85)	82 (70; 77; 86; 88)	84 (71; 77; 92; 100)	88 (75; 80; 97; 103)
60-69 years	76 (66; 71; 81; 87)	80 (68; 72; 83; 87)	81 (67; 74; 90; 98)	85 (71; 77; 93; 101)
70+ years	76 (60; 70; 79; 83)	79 (60; 70; 84; 90)	81 (66; 72; 89; 97)	82 (68; 74; 91; 98)
cPP				
<30 years	29 (23; 27; 37; 43)	36 (26; 32; 43; 53)	34 (24; 28; 41; 48)	38 (26; 31; 46; 52)
30-39 years	30 (22; 26; 37; 44)	35 (25; 29; 42; 50)	34 (24; 28; 38; 46)	36 (25; 31; 41; 48)
40-49 years	31 (22; 27; 36; 42)	32 (25; 28; 38; 45)	35 (25; 29; 43; 53)	33 (23; 28; 37; 46)
50-59 years	34 (25; 28; 42; 49)	30 (25; 27; 35; 42)	39 (28; 32; 47; 58)	34 (25; 28; 41; 49)
60-69 years	35 (28; 31; 43; 52)	31 (24; 28; 36; 49)	44 (30; 36; 55; 66)	37 (25; 31; 46; 58)
70+ years	39 (28; 34; 45; 52)	37 (19; 27; 41; 51)	50 (33; 41; 63; 77)	42 (28; 34; 52; 66)
PWV				
<30 years	4.9 (4.4;.4.5; 5.0; 5.3)	5.2 (4.9; 5.1; 5.4; 5.7)	5.3 (4.7; 5.0; 5.6; 6.0)	5.5 (5.0; 5.3; 5.8; 6.3)
30-39 years	5.4 (5.0; 5.2; 5.8; 6.1)	5.7 (5.3; 5.5; 5.9; 6.1)	5.8 (5.3; 5.5; 6.2; 6.7)	6.1 (5.5; 5.8; 6.4; 6.7)
40-49 years	6.4 (5.7; 6.0; 6.7; 6.9)	6.5 (5.9; 6.2; 6.8; 7.0)	6.8 (6.0; 6.4; 7.2; 7.7)	6.8 (6.2; 6.4; 7.1; 7.5)
50-59 years	7.5 (6.7; 7.0; 7.8; 8.2)	7.4 (6.9; 7.2; 7.9; 8.0)	7.9 (7.1; 7.5; 8.3; 8.8)	7.9 (7.1; 7.5; 8.3; 8.7)
60-69 years	8.9 (8.1; 8.5; 9.2; 9.4)	8.9 (8.2; 8.6; 9.1; 9.6)	9.3 (8.4; 8.8; 9.8; 10;4)	9.2 (8.4; 8.7; 9.7;10.2)
70+ years	11.3 (10.2;10.4;12.5; 13;2)	11.0 (10.1; 10.6; 11.6; 12.3)	11.8 (10.2; 10.8; 12.9; 14.0)	11.2 (9.9; 10.4; 12.1; 13.2)
AIx				
<30 years	20 (11; 13; 27; 33)	16 (4; 10; 23; 27)	28 (11; 20; 34; 38)	16 (2; 8;23; 30)
30-39 years	22 (12; 16; 28; 34)	14 (1; 7; 18; 24)	26 (11; 18; 32; 37)	15 (3; 9; 2 1; 27)
40-49 years	23 (9; 15; 29; 35)	15 (0; 6; 21; 25)	25 (10; 17; 34; 38)	15 (2; 8; 23; 30)
50-59 years	22 (7; 12; 33; 39)	12 (2; 4; 19; 22)	24 (8; 14; 33; 39)	15 (3; 7; 24; 32)
60-69 years	23 (9; 1 4; 34; 42)	17 (1; 5; 27; 43)	28 (11; 18; 37; 44)	1 7 (3;9; 26; 34)
70+ years	28 (11; 20; 39; 42)	22 (5; 10; 33; 41)	33 (17; 25; 42; 48)	22 (4; 12; 31; 41)

*cSBP: central systolic blood pressure; cDBP: central diastolic blood pressure; cPP: central pulse pressure; PVW: pulse wave velocity; AIx: augmentation index. * Values expressed as 50º (10º. 25º. 75º. 90º) percentage points. †Number of women and men; without CVRF: <30 years (n=50 and 80): 30-39 years (n=134 and 70): 40-49 years (n=114 and 55): 50-59 years (n=121e 67): 60-69 year (n=80 e 38): 70+ years (n=32 e 26). ‡ Number of women and men with CVRF: <30 years (n=94 and 152): 30-39 years (n=240 and 297): 40-49 years (n=418 and 385): 50-59 years (n=827 and 638); 60-69 years (n= 919 and 561): 70+ years (n=671 and 430). § CVRF: cardiovascular risk factors. Source: Adapted from Paiva et al. (2020).*

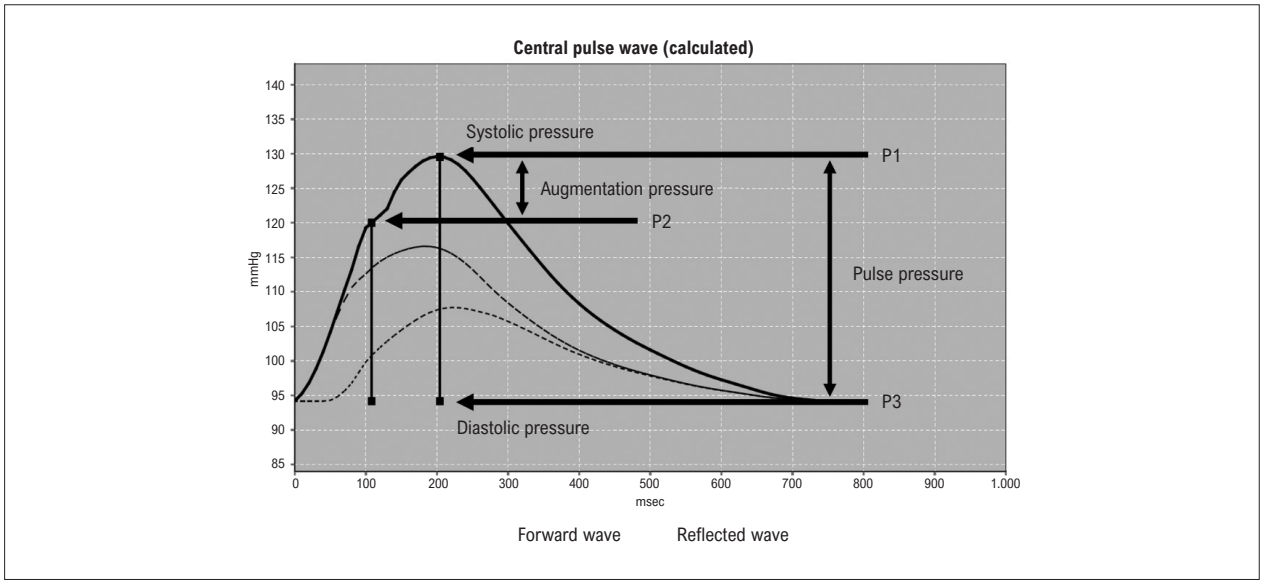

Figure 3 – *Carotid pressure waveform recorded by applanation tonometry. The wave reflection phenomenon can be quantified by the augmentation index (AIx), defined as the difference between the first (P1) and the second (P2) systolic peaks (P2 - P1 = AP, i.e., augmentation pressure), expressed as a percentage of pulse pressure (PP), AIx = AP / PP. Source: the authors.*

diabetes, dyslipidemia, smoking, and obesity), progressing in stages that culminate in the obstruction of coronary arteries, ischemia, and myocardial infarction, end-stage heart disease, heart failure, and death. While this model highlights gene-related pathophysiological aspects, molecules, chemical processes and intracellular mechanisms associated with atherosclerosis, it ignores contributions from cardiovascular aging, derived from physical and mechanical changes of vascular structures.[3,45,46]

In 2010, a novel model was proposed – the Cardiovascular Aging Continuum[46] (Figure 4) – based on the arteriosclerosis process. It initiates with arterial aging, progresses to end-stage cardiac, cerebral and renal microvascular disease, disability and death.[3,7,46]

This new approach highlights the progressive aorta degeneration with deleterious effect to the target organs. It extends the considerations of either obstructive or ischemic arterial disease to the progressive aging-related stiffening of elastic arteries, manifested as increased PWV and AIx.[3,7,46] A 1m/s increase in aortic PWV was associated with a 15% increment in cardiovascular mortality and all-cause mortality.[29] Analysis of cSBP, PWV and AIx revealed that they were better predictors of cardiovascular risk and mortality than peripheral blood pressure.[29,30]

The cardiovascular aging continuum is divided into four stages, as follows (Figure 5).[3,46]

• Stage 1: The heartbeats lead to fracture and fraying of elastic lamellae, with consequent aortic dilation and transfer of mechanical stress to collagen fibers, responsible for arterial stiffness.[3,46]

• Stage 2: Aortic stiffening leads to elevation of SBP, caused by stiffening of proximal aorta and an earlier return of the reflected wave during systole. Consequently, there is increase in ventricular afterload, left ventricular hypertrophy, increased myocardial oxygen consumption and reduction in coronary perfusion.[3,46]

• Stage 3: Intermittent cardiac contractions transfer the pulsatile flow to the stiffened aorta (decreased cushioning capacity) and extend peripherally into the microvasculature, with consequent increase in shear stress, particularly in small arteries of organs with high resting blood flow and low microvascular resistance (the brain, kidney, testicles, liver and placenta).[3,46]

• Stage 4: contractions of the hypertrophied heart occur slowly, so that systole duration is increased, and diastole duration is decrease in any HR. These changes affect coronary blood flow, which fails to supply the demand as both aortic pressure during diastole and diastole period are decreased. The combination of higher supply and decreased coronary perfusion capacity predisposes to ischemia, regardless of coronary narrowing, which is aggravated in atherosclerosis. A vicious cycle is then established: ischemia causes prolongation of ventricular relaxation and ejection time, which, in turn, aggravates ischemia.[3,46]

Although the two "continuums" can be seen independently, they interact in the development of end-stage CVD. They share the same final pathways, that describe complications of myocardial ischemia and progression to end-stage heart disease, as consequence of arterial stiffening and narrowing. The two continuums are combined in Figure 5 to explain the deleterious effects of atherosclerotic disease and of aging, as these progress over years and culminate in the diseases of old age.[46] Cardiac insufficiency is commonly associated with cerebral and renal microvascular disease, causing intellectual deterioration and renal failure.[46]

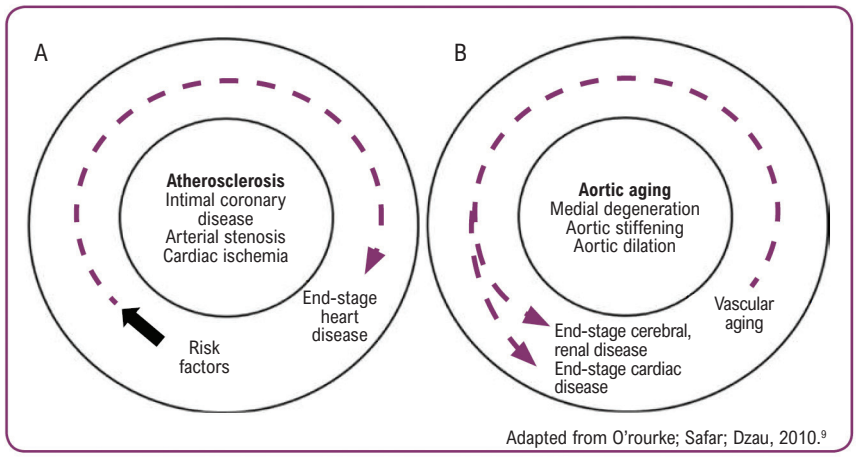

Figure 4 – Comparison between classic cardiovascular continuum (A) and aging cardiovascular continuum (B). Source: Barroso; Barbosa; Mota-Gomes, 2020.

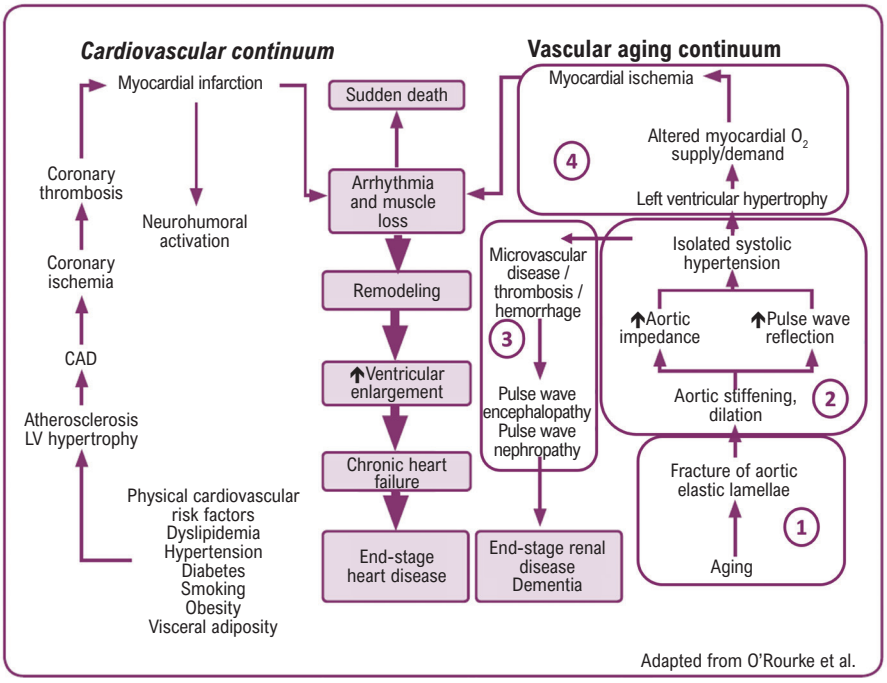

Figure 5 – *Association between classic cardiovascular continuum and aging cardiovascular continuum. LV: left ventricular; CAD: coronary artery disease; VE: ventricular enlargement. Source: Barroso; Barbosa; Mota-Gomes, 2020.*

The brain requires a high blood supply and low arterial resistance, and is susceptible to pulsatile microvascular trauma and hypoperfusion, especially in white matter, which is less vascularized and less perfused than the grey matter. Changes in cerebral perfusion due to an increase in pulsatility lead to microvascular remodeling and low oxygenation, and progressive cognitive decline, dementia, subclinical infarction, and cerebrovascular accident.[7,47-49]

The kidney exhibits the highest flow rate and the lowest vascular resistance as compared to the other organs. For this reason, it is susceptible to trauma due to pulsatile flow, and consequent glomerular damage, albuminuria, and reduction of glomerular filtration rate. CKD also causes stiffening of large arteries because of an imbalance in bone mineral metabolism (increase in osteoprotegerin, fibroblast growth factor and inflammatory cytokines), and increased

vascular calcification. Hyperactivity of the ANS and the RAAS reduces sodium elimination, contributing to arterial stiffening. In individuals with CKD, particularly diabetic patients, PWV increases. The stiffening of large arteries independently predicts a higher risk of cardiovascular events in CKD patients.[7,25,26]

Aging leads to increased vascular stiffening and changes in microcirculation, resulting in the decline of cardiac, cerebral, and renal function. It is possible that microvascular damage may be prevented and/or delayed with therapy aimed at reducing arterial stiffness and wave reflection.[7]

Arterial aging and cardiovascular risk

Part of the residual cardiovascular risk in hypertensive patients has been related to the AVA process. The early detection allows a more effective cardiovascular protection. In the pathophysiology of CVD, there is a bidirectional interaction between AVA and hypertension.[1,10,45]

Classical risk factors are important for selecting, evaluating and guiding lifestyle habits and pharmacological therapy. However, the risk of CVD still represents a challenge; despite prevention and treatment efforts, there is still a need for new pathophysiological models for a better understanding of CV risk and of CVD treatment.[3,45,50]

It has been shown that target organ lesion, like LVH and increased microalbuminuria, represent the boundary between cardiovascular risk factors and cardiovascular events.[45] Besides, arterial stiffness, increased PWV and increased cSBP are independent predictors of cardiovascular events.[29,30] These are examples of an underlying pathological process, since an elevation in PWV can determine the degree of LVH by the increase in the arterial pulse wave reflection, CPP and after-load.[7,19]

Therefore, arterial stiffness is useful to guide clinical investigations in individuals at low and moderate cardiovascular risk.[1,10] These parameters, considered as arterial "biomarkers", can be better predictors than high-sensitive C reactive protein.[32,45] The addition of PWV during risk classification improved risk prediction (13% for CVD in 10 years for intermediate risk).[30] This information, when correctly accessed and used, prevent the misclassification of high-risk patients as low or moderate risk.[45,50]

Perspectives

Vascular aging is responsible for the increase of residual cardiovascular risk and the global CVD burden. Further studies are needed for clinical validation of the cardiovascular outcomes, comparisons between different assessment methods and studies of therapeutical interventions mediated by researchers' network on vascular aging. Continuous education and the wide use of technologies on preventive strategies should be encouraged, aimed at highlighting the role of vascular aging and integrating it into the clinical decision making.[3,50,51]

Science has attempted to improve the understanding and the clinical applicability of biomarkers able to early identify vascular damage. The objective is to improve accuracy in cardiovascular risk stratification in low-to-moderate risk individuals.[32] Analysis of cSBP and arterial stiffness (PWV) is support by strong evidence for early detection of vascular damage, and identification and reclassification of individuals who were initially classified as low/intermediate risk and were later classified as high risk.[30,45] In addition, a PWV ≥ 10m/s can be suggestive of subclinical target-organ damage, and the increase in cSBP is a predictor of arterial hypertension.[7,19,30,52] It is possible that, as new evidence emerges in the context of hypertensive disease and CVD, this method becomes more reliable and safer to be incorporated into clinical practice, aiming to early identify vascular damage.[50] In the realm of precision medicine, this approach allows a tailored clinical practice, with greater assertiveness in the decisions related to classification and treatment of CVD.[50]

Author Contributions

Writing of the manuscript: Oliveira AC, Barroso WKS; Critical revision of the manuscript for important intellectual content: Oliveira AC, Cunha PMGM, Vitorino PVO, Souza ALL, Deus GD, Feitosa A, Barbosa ECD, Gomes MM, Jardim PCBV, Barroso WKS.

Potential Conflict of Interest

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Study Association

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References

1. Hamczyk MR, Nevado RM, Barettino A, Fuster V, Andrés V. Biological Versus Chronological Aging: JACC Focus Seminar. J Am Coll Cardiol. 2020 Mar 3;75(8):919-30. doi: 10.1016/j.jacc.2019.11.062.
2. Wang M, Monticone RE, McGraw KR. Proinflammatory Arterial Stiffness Syndrome: A Signature of Large Arterial Aging. J Vasc Res. 2018;55(4):210-23. doi: 10.1159/000490244.
3. Barroso W, Barbosa E, Mota-Gomes A. Rigidez Arterial e Hemodinâmica Central: Do Endotélio à Camada Média. São Paulo: Athos Mais Editora; 2020.
4. Cunha PG, Boutouyrie P, Nilsson PM, Laurent S. Early Vascular Ageing (EVA): Definitions and Clinical Applicability. Curr Hypertens Rev. 2017;13(1):8-15. doi: 10.2174/1573402113666170413094319.

5. Nilsson PM, Boutouyrie P, Cunha P, Kotsis V, Narkiewicz K, Parati G, et al. Early Vascular Ageing in Translation: From Laboratory Investigations to Clinical Applications in Cardiovascular Prevention. J Hypertens. 2013;31(8):1517-26. doi: 10.1097/HJH.0b013e328361e4bd.

6. Costantino S, Paneni F, Cosentino F. Ageing, Metabolism and Cardiovascular Disease. J Physiol. 2016;594(8):2061–73.

7. Chirinos JA, Segers P, Hughes T, Townsend R. Large-Artery Stiffness in Health and Disease: JACC State-of-the-Art Review. J Am Coll Cardiol. 2019;74(9):1237-63. doi: 10.1016/j.jacc.2019.07.012.

8. Lacolley P, Regnault V, Segers P, Laurent S. Vascular Smooth Muscle Cells and Arterial Stiffening: Relevance in Development, Aging, and Disease. Physiol Rev. 2017;97(4):1555-617. doi: 10.1152/physrev.00003.2017.

9. Lacolley P, Regnault V, Avolio AP. Smooth Muscle Cell and Arterial Aging: Basic and Clinical Aspects. Cardiovasc Res. 2018;114(4):513-28. doi: 10.1093/cvr/cvy009.

10. Laurent S, Boutouyrie P, Cunha PG, Lacolley P, Nilsson PM. Concept of Extremes in Vascular Aging: From Early Vascular Aging to Supernormal Vascular Aging. Hypertension. 2019;74(2):218-28. doi: 10.1161/HYPERTENSIONAHA.119.12655.

11. Michaud M, Balardy L, Moulis G, Gaudin C, Peyrot C, Vellas B, et al. Proinflammatory Cytokines, Aging, and Age-related Diseases. J Am Med Dir Assoc. 2013;14(12):877-82. doi: 10.1016/j.jamda.2013.05.009.

12. Avolio A. Arterial Stiffness. Pulse (Basel). 2013;1(1):14-28. doi: 10.1159/000348620.

13. Hashimoto J, Ito S. Some Mechanical Aspects of Arterial Aging: Physiological Overview Based on Pulse Wave Analysis. Ther Adv Cardiovasc Dis. 2009;3(5):367-78. doi: 10.1177/1753944709338942.

14. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert Consensus Document on Arterial Stiffness: Methodological Issues and Clinical Applications. Eur Heart J. 2006;27(21):2588-605. doi: 10.1093/eurheartj/ehl254.

15. Greenwald SE. Ageing of the Conduit Arteries. J Pathol. 2007;211(2):157-72. doi: 10.1002/path.2101.

16. Lacolley P, Regnault V, Segers P, Laurent S. Vascular Smooth Muscle Cells and Arterial Stiffening: Relevance in Development, Aging, and Disease. Physiol Rev. 2017;97(4):1555-617. doi: 10.1152/physrev.00003.2017.

17. O'Rourke MF, Hashimoto J. Mechanical Factors in Arterial Aging: A Clinical Perspective. J Am Coll Cardiol. 2007;50(1):1-13. doi: 10.1016/j.jacc.2006.12.050.

18. O'Rourke MF. Arterial Aging: Pathophysiological Principles. Vasc Med. 2007;12(4):329-41. doi: 10.1177/1358863X07083392.

19. Laurent S, Hulot J-S, Boutouyrie P. Role of Central Blood Pressure and Arterial Stiffening. Hypertension and Heart Failure. Updates in Hypertension and Cardiovascular Protection. Springer. 2019;135-54.

20. Mikael LR, Paiva AMG, Gomes MM, Sousa ALL, Jardim PCBV, Vitorino PVO, et al. Vascular Aging and Arterial Stiffness. Arq Bras Cardiol. 2017;109(3):253-8. doi: 10.5935/abc.20170091.

21. O'Rourke MF, Adji A. Noninvasive Studies of Central Aortic Pressure. Curr Hypertens Rep. 2012;14(1):8-20. doi: 10.1007/s11906-011-0236-5.

22. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, et al. Arterial Stiffness and Risk of Coronary Heart Disease and Stroke: The Rotterdam Study. Circulation. 2006;113(5):657-63. doi: 10.1161/CIRCULATIONAHA.105.555235.

23. Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, et al. Aortic Stiffness is an Independent Predictor of Primary Coronary Events in Hypertensive Patients: A Longitudinal Study. Hypertension. 2002;39(1):10-5. doi: 10.1161/hy0102.099031.

24. Laurent S, Katsahian S, Fassot C, Tropeano AI, Gautier I, Laloux B, et al. Aortic Stiffness is an Independent Predictor of Fatal Stroke in Essential Hypertension. Stroke. 2003;34(5):1203-6. doi: 10.1161/01.STR.0000065428.03209.64.

25. Safar ME, Blacher J, Pannier B, Guerin AP, Marchais SJ, Guyonvarc'h PM, et al. Central Pulse Pressure and Mortality in End-stage Renal Disease. Hypertension. 2002;39(3):735-8. doi: 10.1161/hy0202.098325.

26. Yannoutsos A, Bahous SA, Safar ME, Blacher J. Clinical Relevance of Aortic Stiffness in End-stage Renal Disease and Diabetes: Implication for Hypertension Management. J Hypertens. 2018;36(6):1237-46. doi: 10.1097/HJH.0000000000001665.

27. Błaszkowska M, Shalimova A, Wolnik B, Orłowska-Kunikowska E, Graff B, Hoffmann M, et al. Subclinical Macroangiopathic Target Organ Damage in Type 1 Diabetes Mellitus Patients. Blood Press. 2020;29(6):344-56. doi: 10.1080/08037051.2020.1770054.

28. Climie RE, van Sloten TT, Bruno RM, Taddei S, Empana JP, Stehouwer CDA, et al. Macrovasculature and Microvasculature at the Crossroads Between Type 2 Diabetes Mellitus and Hypertension. Hypertension. 2019;73(6):1138-49. doi: 10.1161/HYPERTENSIONAHA.118.11769.

29. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of Cardiovascular Events and All-cause Mortality with Arterial Stiffness: A Systematic Review and Meta-analysis. J Am Coll Cardiol. 2010;55(13):1318-27. doi: 10.1016/j.jacc.2009.10.061.

30. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, et al. Aortic Pulse Wave Velocity Improves Cardiovascular Event Prediction: An Individual Participant Meta-analysis of Prospective Observational Data from 17,635 Subjects. J Am Coll Cardiol. 2014;63(7):636-46. doi: 10.1016/j.jacc.2013.09.063.

31. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, et al. Arterial Stiffness and Cardiovascular Events: the Framingham Heart Study. Circulation. 2010;121(4):505-11. doi: 10.1161/CIRCULATIONAHA.109.886655.

32. Vlachopoulos C, Xaplanteris P, Aboyans V, Brodmann M, Cífková R, Cosentino F, et al. The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology Working Group on peripheral circulation: Endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) Society. Atherosclerosis. 2015;241(2):507-32. doi: 10.1016/j.atherosclerosis.2015.05.007.

33. Laurent S, Marais L, Boutouyrie P. The Noninvasive Assessment of Vascular Aging. Can J Cardiol. 2016;32(5):669-79. doi: 10.1016/j.cjca.2016.01.039.

34. Reference Values for Arterial Stiffness' Collaboration. Determinants of Pulse Wave Velocity in Healthy People and in the Presence of Cardiovascular Risk Factors: 'Establishing Normal and Reference Values'. Eur Heart J. 2010;31(19):2338-50. doi: 10.1093/eurheartj/ehq165.

35. Paiva AMG, Mota-Gomes MA, Brandão AA, Silveira FS, Silveira MS, Okawa RTP, et al. Reference Values of Office Central Blood Pressure, Pulse Wave Velocity, and Augmentation Index Recorded by Means of the Mobil-O-Graph PWA Monitor. Hypertens Res. 2020;43(11):1239-48. doi: 10.1038/s41440-020-0490-5.

36. Xaplanteris P, Vlachopoulos C, Protogerou AD, Aznaouridis K, Terentes-Printzios D, Argyris AA, et al. A Clinical Score for Prediction of Elevated Aortic Stiffness: Derivation and Validation in 3943 Hypertensive Patients. J Hypertens. 2019;37(2):339-46. doi: 10.1097/HJH.0000000000001904.

37. Oliveira AC, Barroso WKS, Vitorino PVO, Sousa ALL, Fagundes RR, Deus GD, et al. A SAGE Score Cutoff that Predicts High-pulse Wave Velocity as Measured by Oscillometric Devices in Brazilian Hypertensive Patients. Hypertens Res. 2022;45(2):315-23. doi: 10.1038/s41440-021-00793-0.

38. Tomiyama H, Vlachopoulos C, Xaplanteris P, Nakano H, Shiina K, Ishizu T, et al. Usefulness of the SAGE Score to Predict Elevated Values of Brachial-ankle Pulse Wave Velocity in Japanese Subjects with Hypertension. Hypertens Res. 2020;43(11):1284-92. doi: 10.1038/s41440-020-0472-7.

39. Wilkinson IB, Franklin SS, Hall IR, Tyrrell S, Cockcroft JR. Pressure Amplification Explains why Pulse Pressure is Unrelated to Risk in Young Subjects. Hypertension. 2001;38(6):1461-6. doi: 10.1161/hy1201.097723.

40. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, et al. Aortic Stiffness is an Independent Predictor of All-cause and Cardiovascular Mortality in Hypertensive Patients. Hypertension. 2001;37(5):1236-41. doi: 10.1161/01.hyp.37.5.1236.

41. Weber T, Auer J, O'rourke MF, Kvas E, Lassnig E, Lamm G, et al. Increased Arterial Wave Reflections Predict Severe Cardiovascular Events in Patients Undergoing Percutaneous Coronary Interventions. Eur Heart J. 2005;26(24):2657-63. doi: 10.1093/eurheartj/ehi504.

42. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, et al. Differential Impact of Blood Pressure-lowering Drugs on central Aortic Pressure and Clinical Outcomes: Principal Results of the Conduit Artery Function Evaluation (CAFE) study. Circulation. 2006;113(9):1213-25. doi: 10.1161/CIRCULATIONAHA.105.595496.

43. Herbert A, Cruickshank JK, Laurent S, Boutouyrie P; Reference Values for Arterial Measurements Collaboration. Establishing Reference Values for Central Blood Pressure and its Amplification in a general Healthy Population and According to Cardiovascular Risk Factors. Eur Heart J. 2014;35(44):3122-33. doi: 10.1093/eurheartj/ehu293.

44. Dzau VJ, Antman EM, Black HR, Hayes DL, Manson JE, Plutzky J, et al. The Cardiovascular Disease Continuum Validated: Clinical Evidence of Improved Patient Outcomes: Part I: Pathophysiology and Clinical Trial Evidence (Risk Factors through Stable Coronary Artery Disease). Circulation. 2006;114(25):2850-70. doi: 10.1161/CIRCULATIONAHA.106.655688.

45. Nilsson PM, Boutouyrie P, Laurent S. Vascular Aging: A Tale of EVA and ADAM in Cardiovascular Risk Assessment and Prevention. Hypertension. 2009;54(1):3-10. doi: 10.1161/HYPERTENSIONAHA.109.129114.

46. O'Rourke MF, Safar ME, Dzau V. The Cardiovascular Continuum Extended: Aging Effects on the Aorta and Microvasculature. Vasc Med. 2010;15(6):461-8. doi: 10.1177/1358863X10382946.

47. Singer J, Trollor JN, Baune BT, Sachdev PS, Smith E. Arterial Stiffness, the Brain and Cognition: A Systematic Review. Ageing Res Rev. 2014;15:16-27. doi: 10.1016/j.arr.2014.02.002.

48. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MM. Silent Brain Infarcts and the Risk of Dementia and Cognitive Decline. N Engl J Med. 2003;348(13):1215-22. doi: 10.1056/NEJMoa022066.

49. Mitchell GF, van Buchem MA, Sigurdsson S, Gotal JD, Jonsdottir MK, Kjartansson Ó, et al. Arterial Stiffness, Pressure and Flow Pulsatility and Brain Structure and Function: The Age, Gene/Environment Susceptibility--Reykjavik Study. Brain. 2011;134(11):3398-407. doi: 10.1093/brain/awr253.

50. Barroso WKS, Rodrigues CIS, Bortolotto LA, Mota-Gomes MA, Brandão AA, Feitosa ADM, et al. Brazilian Guidelines of Hypertension - 2020. Arq Bras Cardiol. 2021;116(3):516-658. doi: 10.36660/abc.20201238.

51. Oliveira AC, Barroso WKS. Rigidez Arterial: Um Novo Fator de Risco Cardiovascular. Brazilian Journal of Hypertension. 2021;27(1):13-7. doi: 10.47870/1519-7522/2020270113-7.

52. Boutouyrie P, Chowienczyk P, Humphrey JD, Mitchell GF. Arterial Stiffness and Cardiovascular Risk in Hypertension. Circ Res. 2021;128(7):864-86. doi: 10.1161/CIRCRESAHA.121.318061.

Peek-A-Boo What are You? The Diagnostic Challenge of a Cardiac Mass

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A 49-year-old female was found to have an incidental right atrial mass on a routine transthoracic echocardiography (TTE) (Figure 1). She reported no previous significant medical history, besides a mammoplasty in 2017 complicated with implant rupture in 2019. She did not take any medication, was fit and well, and showed full exercise tolerance. There were no risk factors for venous thromboembolism, and she had no significant family medical history. The patient is a former smoker of 12 pack-years. She had 2 previous pregnancies with no miscarriages. The physical examination was unremarkable.

The differential blood count, general biochemistry, electrocardiogram, and chest radiograph showed no abnormalities.

A CT angiography of the thorax excluded pulmonary embolism and confirmed a hypodense structure with elongated morphology in the area of passage from the inferior vena cava (IVC) to the right atrium, above the confluence of the hepatic veins, with the longest axis measuring about 22mm in the longitudinal plane, with an approximate thickness of 5 to 6 mm, of undetermined etiology.

The cranial computed tomography (CT) scan was normal. The abdominal and pelvic CT scans revealed uterine heterodensity, identifying small infracentimetric focal hypodensities surrounding the endometrium. No systemic emboli or extracardiac tumours were found. A lower-limb ultrasonography excluded deep vein thrombosis. She was assessed by a gynecologist and an ultrasound was performed, which revealed absence of clinical or imaging evidence of gynecological pathologies related to the cardiac finding. A mammography and thyroid ultrasonography were unremarkable.

The transesophageal echocardiography (TEE) (Figure 2) showed a mobile pedunculated mass arising from the IVC, which measured 29×12mm and showed a very irregular contour, without entailing hemodynamic compromise.

The TEE may lead to uncertain information and this is the reason why a cardiac MRI was used synergistically with the echocardiography.[1]

Keywords

Cardiac Mass; Diagnostic Techniques and Procedures; Diagnostic, Imaging/methods; Echocardiography Transesophageal/methods; Resonance Magnetic/methods; Trombosis; Heart Atrial

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A cardiac magnetic resonance imaging (MRI) (Figure 3) showed non-dilated ventricles, with normal global and regional systolic function, as well as absence of areas of infarction, fibrosis or myocardial infiltration. It also showed a very mobile mass, with an irregular and vegetative appearance located inside the right atrium, adjacent to the Eustachian valve, measuring 19x11mm, attached to the wall of the IVC/supra-hepatic vein through a thin pedicle. The mass showed to be isointense relative to the myocardium on T2-weighted sequences and slightly hyperintense on T1-weighted sequences. No apparent vascularization was found in the first-pass perfusion sequence. After gadolinium administration, the mass showed a heterogeneous late enhancement but with absence of early enhancement.

Its location, morphology and signal behavior suggested the most likely diagnostic hypotheses: myxoma with atypical insertion in the IVC, heterotopic liver tissue, hepatocellular tumor with intracardiac extension through the IVC or IVC leiomyosarcoma.[1,2]

In the meantime, also considering the hypothesis of intra-atrial thrombi, the treatment with intravenous (IV) heparin was initiated and the mass was monitored through TTE. However, one week after IV heparin treatment, the mass volume did not change.

The patient remained asymptomatic.

After consultation with the Heart Team, taking into account the size of the mass and the mobile appearance on the echocardiography that seemed to place our patient at high risk for pulmonary embolism, the case assessment led us to choose surgical exploration with a diagnostic and curative purpose.

The patient underwent surgical resection of the mass, which was attached at the junction of the IVC and suprahepatic veins, through a median sternotomy. The surgical inspection of the mass characterized it as having a fibroelastic consistency, whitish and with areas of hemorrhagic aspect (Figure 4). The histological examination unexpectedly showed only thrombotic material with several phases of organization.

The patient had no postoperative complications and was discharged 5 days later with oral anticoagulation treatment with apixaban 5 mg twice a day.

She recovered well. A TTE performed 3 months after the surgery ruled out any relapse of a right atrial thrombus.

A further workup for thrombotic state performed 6 months later revealed normal prothrombin and activated partial thromboplastin times, normal antithrombin III, protein C and S and homocysteine levels. Screening for anticardiolipin antibodies and lupus anticoagulant was negative. The genetic analysis showed normal homozygous state for prothrombin

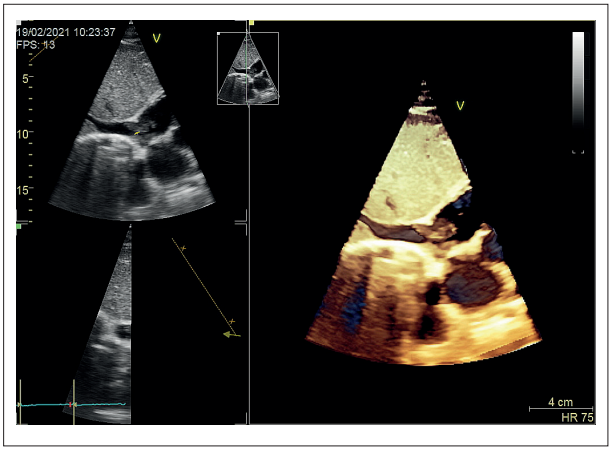

Figure 1 – *Transthoracic echocardiography showing the right atrial mass.*

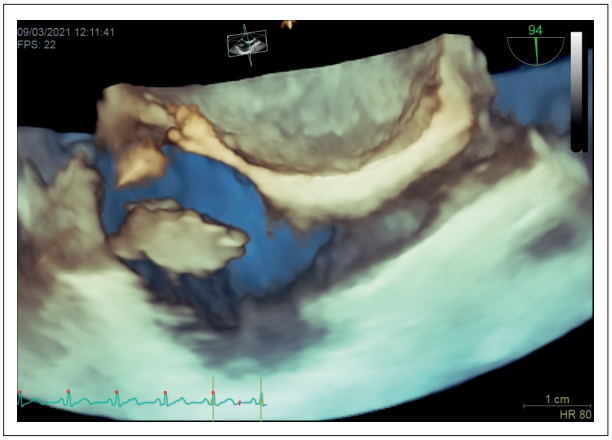

Figure 2 – *Transesophageal echocardiography showing the right atrial mass.*

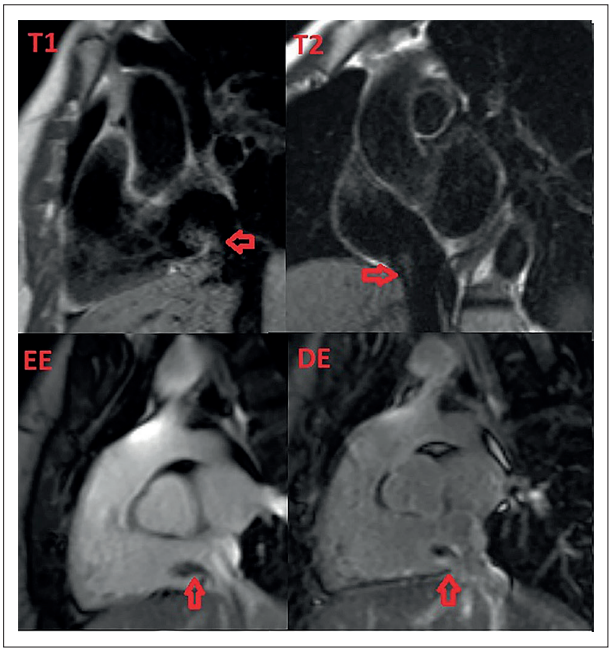

Figure 3 – *Cardiac magnetic resonance imaging showing the right atrial mass.*

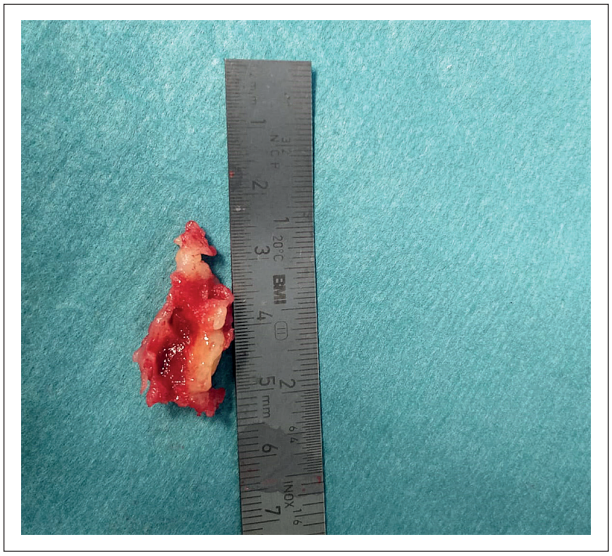

Figure 4 – *Surgical inspection of the cardiac mass.*

and factor V Leiden genes. D-dimer and fibrinogen levels were normal.

We describe here a case of a rare right atrial pedunculated thrombus in a previously healthy, asymptomatic woman without structural heart disease.

In our patient, the preoperative investigations could not differentiate the thrombus from a tumor; consequently, the diagnosis was made postoperatively.

Despite the available advanced and sophisticated diagnostic modalities, differentiating intracardiac masses can still be challenging. Clinical presentation leads to the appropriate conduit of investigations, and histopathology is a confirmatory step.[1,2]

Author Contributions

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

References

1. Aggeli C, Dimitroglou Y, Raftopoulos L, Sarri G, Mavrogeni S, Wong J, et al. Cardiac Masses: the role of cardiovascular imaging in the differential diagnosis.Diagnostics(Basel).2020;10(12):1088. doi:10.3390/diagnostics10121088
2. Tyebally S, Chen D, Bhattacharyya S, Mughrabi A, Hussain Z, Manisty C, et al. Cardiac tumors:JACC CardioOncology State of the Art Review. JACC: CardioOncology. 2020 Jun;2(2):293–311. doi: 10.1016/j.jaccao.2020.05.009

Concomitant Use of Ranolazine and Trimetazidine in Patients with Refractory Angina: An Initial Experience

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Introduction

Refractory angina (RA), an extremely debilitating condition, requires specialized medical treatment with often complex therapeutic adjustments in an attempt to improve symptoms and quality of life as much as possible.[1]

Medical treatment usually comprises a combination of antianginal drugs (AAD). Among them, trimetazidine (T) and ranolazine (R) are an add-on therapy due to their efficacy and safety profile in the treatment of patients with RA.[1-3] However, in the recent "diamond approach",[4] depicting preferred combinations of different classes of AAD for the treatment of patients with angina, does not consider the concomitant use of T and R a useful strategy due to their related mechanism of action.[4] Although no known interaction between both drugs has been described,[5] there is no data on the efficacy and safety of the use of R in patients already on T. Therefore, we aimed to evaluate the effect of the concomitant use of R and T in patients with RA.

Methods

We retrospectively analyzed the clinical records of patients followed in a specialized, outpatient clinic of a tertiary university hospital with diagnosis of RA, defined as disabling angina for at least 3 months caused by coronary insufficiency in the setting of coronary artery disease,[6,7] confirmed by angiography and in patients not eligible for myocardial revascularization.[8] A convenience sampling of patients who were symptomatic after 3 months of at least 3 AAD (including T) were eligible to receive R. This analysis was part of a study approved by the research ethics committee (CAAE:24308213.7.0000.0068). Investigations followed the Declaration of Helsinki. All patients provided written informed consent.

Patients were reassessed monthly for 3 months (baseline visit: V_1; last visit: V_4) regarding their symptoms according to the Canadian Cardiovascular Society (CCS). Resting ECG and laboratory tests were performed on V_1 and V_4. At the physician's discretion, R could be added to the background therapy[9] if a) QT_c < 500 ms; b) glomerular filtration rate > 30 mL.kg^{-1}.min^{-1}; and c) absence of severe hepatic dysfunction. A standard dose of 500 mg twice daily was adopted.

Statistical analysis

Statistical analysis was performed using IBM SPSS, version 20. Variables presented a normal distribution according to Shapiro-Wilk normality test. Therefore, continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were expressed as absolute numbers. For comparison between time points, the paired Student t-test or Wilcoxon signed-rank test were used, as appropriate. Statistical significance was set at a p-value of < 0.05.

Results

This early report evaluated 10 patients (7 men), 61 ± 7 years old, followed for limiting angina CCS 3 (n = 3) or 4 (n = 7), between 2019 and 2020. Table 1 shows their baseline characteristics. On baseline resting ECG, heart rate was 64 ± 7 bpm, and QT_c was 414 ± 16 ms.

All but one patient attended the scheduled visits. At V_2, 4 of the 10 patients presented symptomatic hypotension leading to a change in antihypertensive treatment: antihypertensive drugs had to be either stopped in 2 patients using amlodipine or hydrochlorothiazide or reduced in one patient using losartan. At V_4, a single patient missed his appointment, and, although he was reached by phone, confirming he was clinically stable, this information was not included in the analysis.

Table 2 shows CCS of patients individually at each visit. We observed significant improvement in CCS from V_1 to V_4 (Z= −2.07; p = 0.038). Two patients improved 2 CCS classes, and 3 patients improved 1 class. However, 4 patients exhibited no improvement.

Analysis of the resting ECGs obtained at V_4 disclosed no significant changes in heart rate (61 ± 9 bpm) and QT_c (417 ± 19 ms) compared to baseline (p-values of 0.39 and 0.44, respectively).

Discussion

To our knowledge, this is the first report on the combined use of R (a late Na^+ current inhibitor) with T (a partial free fatty acid oxidation inhibitor) to optimize medical treatment in patients with RA. In this early experience, the combination was safe and well tolerated, and it led to an improvement in CCS.

Because of the persistence of limiting angina despite the association of at least three AAD, including T, the introduction of R in an attempt to better control symptoms was carefully monitored. The only observed adverse event after R was symptomatic hypotension, although R is presumably devoid of any significant hemodynamic effect. The possible explanation

Keywords

Coronary Artery Disease; Refractory Angina; Drug Therapy; Ranolazine/therapeutic use; Trimetazidine/therapeutic use.

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Research Letter

Table 1 – Clinical, electrocardiographic, and laboratory data from V_1 to V_4

Variable	V_1	V_4	p
Cardiovascular risk factors			
Hypertension (n)	6		
Diabetes mellitus (n)	5		
Hyperlipidemia (n)	10		
Smoking (previous or current) (n)	7		
Obesity (n)	3		
Family history of CAD (n)	3		
Past medical history			
CAD diagnostic time, years (mean ± SD)	8.7±6.0		
Acute myocardial infarction (n)	8		
Percutaneous coronary intervention (n)	9		
CABG (n)	4		
Obstructive pattern and LV function			
LVEF (echocardiography), (mean ± SD)	0.56±0.07		
One-vessel disease (n)	2		
Two-vessel disease (n)	3		
Three-vessel disease (n)	5		
Medication			
Aspirin (n)	10		
Clopidogrel (n)	4		
Statin (n)	10		
% maximum dosage (mean ± SD)	100		
β-blockers (n)	9		
% maximum dosage (mean ± SD)	100		
Calcium channel blockers (n)	10		
% maximum dosage (mean ± SD)	80±26		
Long-acting nitrates (n)	10		
% maximum dosage (mean ± SD)	90±23		
Trimetazidine (n)	10		
Ivabradine (n)	2		
Angiotensin-converting-enzyme inhibitors (n)	1		
Angiotensin receptor blockers (n)	4		
% maximum dosage (mean ± SD)	100		
Diuretics, thiazides (n)	4		
Oral antidiabetic drugs (n)	4		
Insulin (n)	2		
Clinical data			
Systolic blood pressure, mmHg (mean ± SD)	122±17	118±17	0.5
Diastolic blood pressure, mmHg (mean ± SD)	75±5	71±9	0.1
Heart rate, bpm (mean ± SD)	65±5	62±10	0.7
ECG			
Heart rate, bpm (mean ± SD)	64±7	61±9	0.39
QT_c, ms (mean ± SD)	414±16	417±19	0.44
Laboratory			
Hemoglobin, g/dL (mean ± SD)	13.4±1.7	13.9±0.9	0.2
Creatinine, mg/dL (mean ± SD)	1.06±0.20	1.08±0.10	0.6
$Hb1_c$, % (mean ± SD)	7.1±2.5	6.7±1.3	0.4
LDL-cholesterol, mg/dL (mean ± SD)	91±43	96±33	0.9
HDL-cholesterol, mg/dL (mean ± SD)	42±9	43±11	0.7
Triglycerides, mg/dL (mean ± SD)	118±27	118±32	0.9
ALT, mg/dL (mean ± SD)	26±8	28±8	0.8
AST, mg/dL (mean ± SD)	20±4	20±6	0.6
Sodium, mmol/L (mean ± SD)	139±3	141±2	0.07
Potassium, mmol/L (mean ± SD)	4.7±0.5	4.8±0.4	0.8

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CABG: coronary artery bypass graft; CAD: coronary artery disease; ECG: electrocardiogram; Hb: hemoglobin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; LV: left ventricular; LVEF: left ventricular ejection fraction; SD: standard deviation; V: visit.

Table 2 – Baseline and V_4 CCS of patients individually

Patient	CCS		p*
	V_1	V_4	0.038
1	3	2	
2	3	1	
3	4	4	
4	4	3	
5	4	4	
6	3	3	
7	4	3	
8	4	-	
9	4	2	
10	4	4	

*CCS: Canadian Cardiovascular Society; V: visit. * Wilcoxon signed-rank test.*

for hypotension comes from the many drug-to-drug interactions ascribed to R. Ranolazine, for instance, may decrease the excretion rate of losartan which could result in a higher serum level.[5] Likewise, the serum concentration of levamlodipine can be increased when it is combined with R. In the CARISA trial,[9] the incidence of hypotension was around 1%, much lower than that observed in our study. The only reported interaction between R and hydrochlorothiazide is an increased risk or severity of QT_c prolongation.

Three months after the introduction of R, we observed significant improvement in CCS and no QT_c prolongation or

laboratory abnormalities, suggesting that the concomitant use of AAD acting both at the cardiac cell level is safe and offers additional angina relief. The central message of this early experience is that, when facing a medical challenge, the clinician must be cautiously audacious. We believe that new strategies, provided they are safe and based on a logical rationale, must be considered, which the aim of bringing hope to so-called "no-option" patients.

Limitations

The findings of this study have to be seen in light of some limitations. Our study is a retrospective analysis of the data of a small sample size, of an open trial, evaluating the subjective endpoint of angina symptoms in patients with RA followed during 3 months. Therefore, although our conclusions are not definite, they generate hypotheses for future trials.

Conclusions

Concomitant use of R and T in patients with RA, during 3 months, improved CCS and was safe, with no evidence of QT_c prolongation or laboratory abnormalities.

Author Contributions

Conception and design of the research: Dourado LOC, Moreno CPD, Grobe SF, Cesar LAM; Acquisition of data: Dourado LOC, Moreno CPD, Grobe SF; Analysis and interpretation of the data: Dourado LOC, Gowdak LHW; Statistical analysis and Writing of the manuscript: Dourado LOC; Critical revision of the manuscript for important intellectual contente: Dourado LOC, Gowdak LHW, Cesar LAM.

Potential Conflict of Interest

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

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References

1. Dourado LO, Poppi NT, Adam EL, Leite TNP, Pereira AC, Krieger JE, et al. The effectiveness of intensive medical treatment in patients initially diagnosed with refractory angina. *Int J Cardiol*. 2015;186:29-31. doi: 10.1016/j.ijcard.2015.03.150.
2. Storey KM, Wang J, Garberich RF, Bnnet NM, Traverse JN, Arndt TL, et al. Long-term (3 Years) outcomes of ranolazine therapy for refractory angina pectoris (from the Ranolazine Refractory Registry). *Am J Cardiol*. 2020;129:1-4. doi: 10.1016/j.amjcard.2020.05.020.
3. Peng S, Zhao M, Wan J, Fang Q, Fang D, Li K. The efficacy of trimetazidine on stable angina pectoris: a meta-analysis of randomized clinical trials. *Int J Cardiol*. 2014;177(3):780-5. doi: 10.1016/j.ijcard.2014.10.149
4. Ferrari R, Camici PG, Crea F, Danchin N, Fox K, Maggioni A, et al. Expert consensus document: A 'diamond' approach to personalized treatment of angina. *Nat Rev Cardiol*. 2018;15(2):120-32. doi: 10.1038/nrcardio.2017.131.
5. DrugBank.5.0 [Internet] [Cited in 2020 Sept 09] Available from: www.drugbank.ca
6. Mannheimer C, Camici P, Chester MR, Collins A, Dejongste M, Eliasson T, et al. The problem of chronic refractory angina; report from the ESC Joint Study Group on the Treatment of Refractory Angina. *Eur Heart J*. 2002;23(5):355-70. doi: 10.1053/euhj.2001.2706.
7. Knuuti J, Wijns W, Saraste A,Capodanno D, Barbato E, Funck-Brentano E, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. *Eur Heart J*. 2020;41(3):407-77. doi: 10.1093/eurheartj/ehz425.
8. Fihn DP, Gardin JM, Abrams J, et al. Berra K, Blankenship JC, Dallas AP,et al. 2012 ACCF/AHA/ACP/AATS/PCNA/SCAI/STS Guideline for the diagnosis and management of patients with stable ischemic heart disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, and the American College of Physicians, American Association for Thoracic Surgery, Preventive Cardiovascular Nurses Association, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *J Am Coll Cardiol*. Dec 2012;60(24):e44-e164. doi: 10.1016/j.jacc.2012.07.013.
9. Sendón JL, Lee S, Cheng ML, Ben-Yehuda O, CARISA study investigators. Effects of ranolazine on exercise tolerance and angina frequency in patients with severe chronic angina receiving maximally-tolerated background therapy: analysis from the Combination Assessment of Ranolazine In Stable Angina (CARISA) randomized trial. *Eur J Prev Cardiol*. 2012;19(5):952-9. doi: 10.1177/2047487312450133.

Influence of Spiritual Well-Being on Blood Pressure, Central Hemodynamics and Endothelial Function

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"Science is not only compatible with spirituality; it is a profound source of spirituality."

Carl Sagan

Introduction

Spirituality[1] and religiosity (S/R) are cultural aspects that have existed since the beginnings of human existence. Having been long considered as opponent to science, only recently, S/R have gained importance in the context of health.[1]

Definitions of S/R have been constantly evolving, according to the need for adaptation to new knowledge. Today, religion has been related to organizational, institutional and dogmatic aspects, that is, the contact with deity occurs through predetermined formats, specific to each religious segment.[2] Spirituality is a wider term, encompassing a quest for personal, psychological and spiritual well-being, and good-quality interpersonal relationships. According to the Department of Studies in Spirituality and Cardiovascular Medicine (DEMCA, *Departamento de Estudos em Espiritualidade e Medicina Cardiovascular*) of the Brazilian Cardiology Society, "spirituality is a set of moral, mental and emotional values that guide thoughts, behaviors and attitudes in life circumstances of intra and interpersonal relationships".[3]

Arterial hypertension (AH) is a highly prevalent disease and the main risk factor for cardiovascular diseases,[4] and hence the main direct and indirect cause of mortality in the world.[5] Since AH is a multifactorial disease, its treatment encompasses both pharmacological and non-pharmacological strategies,[6] focusing on physical and mental well-being.[7,8]

Practices that promote spiritual well-being, allied or not to religiosity, have been associated with the good control of many diseases[9] and reduction of mortality in several situations.[10] There has been evidence of an association between E/R and positive outcomes in heart diseases, such as coronary artery disease (CAD),[11] heart failure (HF),[12] and AH.[13,14]

S/R studies are still incipient, and mostly observational. In general, E/R have been associated with better life habits (less sedentarism, alcohol consumption and smoking),[15] lower blood pressure (BP), lower risk for AH,[16] and better treatment compliance.[17] Spiritual well-being alone may be a cardioprotective factor, as it is correlated with lower levels of BP, fasting glucose, triglycerides and low-density lipoprotein (LDL) cholesterol.[18] On the other hand, an observational study reported an increased likelihood of AH associated with higher frequency of prayer, but a lower likelihood of hypertension associated with variables for meaning and forgiveness.[19]

In light of this, it is important to evaluate the effect of an intervention focusing on spiritual well-being on the control of BP and other hemodynamic parameters. This paper describes the methodology of a clinical trial to evaluate the effects of an intervention in spirituality on peripheral and central BP (cBP) (parameters of arterial stiffness and endothelial function) in hypertensive patients in stages 1 and 2, at low or moderate cardiovascular risk. The parameters of a control group (CG) and an intervention group (IG) will be analyzed before and after 12 months of follow-up in each group and between the groups.

Methods

Type and place of study

This is a randomized non-inferiority trial; data collection will be carried out in the University of Goias Hypertension League (*Liga de Hipertensão Arterial da Universidade Federal de Goiás*) and in the Rio de Janeiro State University (*Universidade do Estado do Rio de Janeiro*). The protocol

Keywords

Spirituality; Religion and Medicine; Blood Pressure; Hemodynamic; Endothelium/physiology; Social Values; Value of Life; Quality of Life

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will be registered on the Brazilian Registry of Clinical Trials (ReCEB, *Registro Brasileiro de Ensaios Clínicos*).

Population, sample, and sampling

The study population will be composed of hypertensive adults (stage 1 or 2) at low or moderate cardiovascular risk, under stable treatment with antihypertensive medications for more than 30 days, which was evaluated by BP measures taken during the last visit.

Sample calculation was performed using the OpenEpi calculator. A systolic blood pressure (SBP) of 130.9 ± 9.2 and 135.81 ± 9.3 mmHg[20] was considered in the IG and the CG, respectively, with a 95% confidence interval and an 80% power of test, with 54 participants in each group.

Stage 3 hypertensive patients (SBP ≥ 180 mmHg and/or diastolic blood pressure [DBP] ≥ 110mmHg) will be excluded.

After patient enrollment, patients who decline to participate in any procedure, and/or show a rise in BP during the follow-up, preventing their participation in the study without changing the drug regimen will be excluded.

Patient recruitment and randomization

The staffs involved in the study will meet for the presentation and discussion about the project, and training of the members for the correct execution of the protocol and uniformization of patient approach, for the sake of methodological rigor.

Patients will be selected based on the last BP recorded in the medical record, and on the classification of AH stage and cardiovascular risk, and will be invited to participate in the study by telephone. Those who accept to participate will be invited for the first visit.

First visit (V0)

Randomization of participants into one of the two groups will be performed by using www. randomizer.org.

All patients will receive information about healthy life habits. Then, the medical history will be taken, and clinical examination and interview will be performed for completion of four questionnaires – Durel,[21,22] willingness to forgive,[23] the gratitude questionnaire,[24] and the spiritual well-being questionnaire.[25] All procedures will be performed by trained investigators, following the same script, to standardize the visits and instructions.

In addition, casual BP will be measured by oscillometry using a Dyna-MAPA AOP device (Cardios, Brazil), that provides measurements of peripheral BP, central BP (cBP), pulse wave velocity (PWV) (using an algorithm and ARC SOLVER equation and expressed as meter/second), and the augmentation index adjusted to 75% of the heart rate (AIx).[26,27] Peripheral BP measurements will be obtained according to the latest Brazilian guidelines on hypertension.[8]

Ambulatory blood pressure monitoring (ABPM) will be performed using a Dyna-MAPA AOP device (Cardios, Brazil), following the latest Brazilian guidelines on ABPM.[28]

Flow-mediated dilation (FMD) will be determined by a high-resolution ultrasound scanner and a robotic arm to obtain a precise positioning and measurement of the brachial artery (UNEX EF 38G), according to the technique proposed by Celermajer et al.[29] and recommendations of the International Brachial Artery Reactivity Task Force.[30,31] FMD is the current gold standard method to evaluate endothelial function; a FMD > 10% indicates a healthy endothelium, and values below that are predictive of increased cardiovascular risk.[32]

Intermediate visit (V1)

An intermediate visit will be held six weeks after V0, by telephone call, to verify patient well-being and encourage adherence to the intervention (IG), highlighting the importance of performing the daily activities proposed and clarifying possible doubts. In-person visits will be scheduled with patients with BP levels above 180/110mmHg and patients with symptoms such as precordial pain or severe headache.

Final visit (V2)

The final visit will be held for both IG and CG after the program proposed, with an acceptable time window of ± three days. All patients will undergo the same procedures of V0.

Intervention group

The intervention will begin in the morning after the V0 and have a duration of 12 weeks. This follow-up period was used in previous non-pharmacological intervention studies with hypertensive patients and shown to be sufficient to detect changes in BP.[33,34]

The intervention will consist of a series of previously recorded videos, messages, short tasks related to the subject of the video and days off (Table 1). Themes related to spirituality, forgiveness, gratitude, optimism, life purpose, and spiritual well-being will be addressed. The content will be available through a smartphone app, which will register the activities performed by each participant daily.

The adherence to the intervention will be considered satisfactory when 75% or more of the proposed tasks are completed.

Control group

The CG will be monitored at the same frequency as the IG. If the results in the IG are significantly better than in the CG, the latter will receive the same treatment at the end of the study period.

Statistical analysis

Data will be analyzed using the Software Stata 14.0. Qualitative variables will be expressed as mean and standard deviation, and quantitative variables as mean and standard deviation or median and interquartile range.

Normality of data distribution will be tested by the Kolmogorov-Smirnov test. Statistical tests will be applied

Table 1 – Sequence of tasks by weekday for the intervention group

Day	Task	Day	Task	Day	Task	Day	Task
1	V 1	22	AT 6	43	R	64	TM 22
2	TM 1	23	TM 8	44	V 9	65	AT 21
3	AT 1	24	R	45	TM 15	66	R
4	R	25	V 6	46	AT 14	67	V 12
5	V 2	26	TM 9	47	TM 16	68	TM 23
6	TM 2	27	AT 7	48	AT 15	69	AT 22
7	AT 2	28	TM 10	49	TM 17	70	TM 24
8	R	29	AT 8	50	AT 16	71	AT 23
9	V 3	30	R	51	R	72	R
10	TM 3	31	V 7	52	V 10	73	V 13
11	AT 3	32	TM 11	53	TM 18	74	TM 25
12	R	33	AT 9	54	AT 17	75	AT 24
13	V 4	34	AT 10	55	TM 19	76	TM 26
14	TM 4	35	TM 12	56	R	77	R
15	AT 4	36	AT 11	57	V 11	78	V 14
16	TM 5	37	AT 12	58	TM 20	79	TM 27
17	R	38	R	59	AT 18	80	TM 28
18	V 5	39	V 8	60	TM 21	81	R
19	TM 6	40	TM 13	61	AT 19	82	V 15
20	AT 5	41	AT 13	62	R	83	TM 29
21	TM 7	42	TM 14	63	AT 20	84	AT 25

V: video; TM: thinking message; AT: activity; R: respite

according to normality of the data for between-group (IG and CG) and within-group comparisons (before and after). If this is the case, the intention-to-treat analysis will be performed, and only with patients who complete the study protocol.

The primary outcome will be peripheral systolic blood pressure and the secondary outcomes will be: cSBP, PWV, mean SBP and FMD.

Ethical aspects

The project was submitted and approved by the Ethics Committee of the UFG General Hospital.

Conclusion

This research will investigate the effects of an intervention based on encouragement and training to achieve spiritual well-being through propensity to forgive, optimism, gratitude, and life purpose on BP behavior.

The study will have a positive impact on clinical practice by presenting the basis for a non-pharmacological approach to the treatment of AH. Also, to our knowledge, this is one of the first clinical trials with this design.

Author Contributions

Conception and design of the research: Teixeira MEF, Vitorino PVO, Brandão AA, Souza ALL, Barbosa TMGA, Esporcatte R, Borba MHE, Avezum A, Barroso WKS; Statistical analysis: Vitorino PVO; Writing of the manuscript: Teixeira MEF; Critical revision of the manuscript for important intellectual content: Vitorino PVO, Brandão AA, Souza ALL, Avezum A, Barroso WKS.

Potential Conflict of Interest

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References

1. Barth WL. A religião cura? Teocomunicação [Internet]. 2014 Aug 12 [cited 2020 Oct 10];44(1):97. doi:org/10.15448/980-6736.2014.1.18224

2. Steinhauser KE, Fitchett G, Handzo GF, Johnson KS, Koenig HG, Pargament KI, et al. State of the Science of Spirituality and Palliative Care Research Part I: Definitions, Measurement, and Outcomes. J Pain Symptom Manage. 2017; 54(3):428–40. doi: 10.1016/j.jpainsymman.2017.07.028.

3. Précoma D, Oliveira G, Simão A, Dutra O, Coelho O, Izar M. Atualização da Diretriz de Prevenção Cardiovascular da Sociedade Brasileira de Cardiologia. Arq Bras Cardiol. 2019;113(4):787–891. doi:http://www.dx.doi.org/10.5935/abc.20190204

4. Olsen MH, Angell SY, Asma S, Boutouyrie P, Burger D, Chirinos JA, et al. A call to action and a lifecourse strategy to address the global burden of raised blood pressure on current and future generations: the Lancet Commission on hypertension. Lancet.2016;388(10060):2665-712. doi: 10.1016/S0140-6736(16)31134-5.

5. World Health Organization. (WHO). A global brief on hypertension | A global brief on Hypertension. Geneva;2013. Document number:WHO/DCO)/WHD/2013.2.

6. Carey RM, Muntner P, Bosworth HB, Wh Document elton PK. Prevention and Control of Hypertension: J Am Coll Cardiol.2018;72(11):1278_93. https://doi.org/10.1016/j.jacc.2018.07.008

7. Cuevas AG, Williams DR, Albert MA. Psychosocial Factors and Hypertension: A Review of the Literature. Cardiol Clin. 2017;35(2):223-30. doi: 10.1016/j.ccl.2016.12.004

8. Barroso WKS, Rodrigues CIS, Bortolotto LA, Mota-Gomes MA, Brandão AA, Feitosa AD de M, et al. Diretrizes Brasileiras de Hipertensão Arterial – 2020. Arq Bras Cardiol.2021;116(1):516-658. doi: https://doi.org/10.36660/abc.20201238

9. Maselko J, Kubzansky L, Kawachi I, Seeman T, Berkman L. Religious service attendance and allostatic load among high-functioning elderly. Psychosom Med. 2007;69(5):464–72. doi: 10.1097/PSY.0b013e31806c7c57.

10. Li S, Stampfer MJ, Williams DR, Vanderweele TJ. Association of religious service attendance with mortality amongWomen. JAMA Intern Med .2016; 176(6):777–85. doi: 10.1001/jamainternmed.2016.1615.

11. Schmidt KE, Quadros AS, Eibel B, Aires AM, Gottschall AM, et al. The influence of cognitive behavioral intervention for anger management on endothelial function in patients with recent mycardial infarction:a randomized clinical trial. Psychosom Med. 2022;84(2):224-30. DOI: 10.1097/PSY.0000000000001039

12. Park CL, Aldwin CM, Choun S, George L, Suresh DP, Bliss D. Spiritual peace predicts 5-year mortality in congestive heart failure patients. Health Psychol.2016;35(3):203-10. doi: 10.1037/hea0000271

13. McIntosh RC, Ironson G, Krause N. Do religious and spiritual identity confer risk for hypertension via psychosocial and lifestyle factors? J Health Psychol. 2020;25(8):1082–97. doi: 10.1177/1359105317748733.

14. Abdala GA, Pinto DR, Moraes OE, Penna D, Moura LVC, Santos DC, et al. Religiosidade e hipertensão: estudo intervencional. Rev Formadores. 2011;4(1):33-42. https://seer-revadventista.com.br/ojs3/index.php/formadores/article/view/102

15. Kobayashi D, Shimbo T, Takahashi O, Davis RB, Wee CC. The relationship between religiosity and cardiovascular risk factors in Japan: A large-scale cohort study. J Am Soc Hypertens.2015;9(7):553-62. doi: 10.1016/j.jash.2015.04.003.

16. Lucchese FA, Koenig HG. Religião, espiritualidade e doença cardiovascular: Pesquisa, implicações clínicas e oportunidades no Brasil. Brazilian J Cardiovasc Surg. 2013;28(1):103–28. doi: 10.5935/1678-9741.20130015

17. Badanta-Romero B, de Diego-Cordero R, Rivilla-García E. Influence of Religious and Spiritual Elements on Adherence to Pharmacological Treatment. J Relig Health. 2018;57(5):1905-17. doi: 10.1007/s10943-018-0606-2.

18. Holt-Lunstad J, Steffen PR, Sandberg J, Jensen B. Understanding the connection between spiritual well-being and physical health: An examination of ambulatory blood pressure, inflammation, blood lipids and fasting glucose. J Behav Med. 2011;34(6):477-88. doi: 10.1007/s10865-011-9343-7

19. Buck AC, Williams DR, Musick MA, Sternthal MJ. An examination of the relationship between multiple dimensions of religiosity, blood pressure, and hypertension. Soc Sci Med. 2009;68 (2):314–22. DOI: 10.1016/j.socscimed.2008.10.010

20. Cramer H, Sellin C, Schumann D, Dobos G. OriginalArbeit: Yoga bei arterieller Hypertonie. Dtsch Arztebl Int. 2018;115(50):833–9. doi: 10.1016/j.socscimed.2008.10.010

21. Koenig HG, Büssing A. The Duke University Religion Index (DUREL): A five-item measure for use in epidemological studies. Religions. 2010;1(1):78–85. https://doi.org/10.3390/rel1010078

22. Taunay TCDE, Gondim F de AA, Macêdo DS, Moreira-Almeida A, Gurgel L de A, Andrade LMS, et al. Validação da versão Brasileira da escala de religiosidade de Duke (DUREL). Rev Psiquiatr Clin. 2012 [cited 2021 Apr 10];39(4):130–5. https://doi.org/10.1590/S0101-60832012000400003

23. DeShea L. A scenario-based scale of Willingness to Forgive. Individ Differ Res. 2003;1(3):201–16. ISSN:1541-745X

24. Emmons RA, Tsang J, McCullough ME. Individual Differences in Gratitude 1 The Gratitude Questionnaire-Six Item Form (GQ-6). J Pers Soc Psychol. 2002;82:112–27. doi: 10.1037//0022-3514.82.1.112

25. Gouveia MJ, Marques M. Versão portuguesa do questionário de bem-estar espiritual (SWBQ): análise confirmatória da sua estrutura factorial. Psicol Saúde Doenças. 2009;10(2):285–93.

26. Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, et al. Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement from the American Heart Association. Hypertension. 2015 Sep 14 [cited 2021 Apr 10];66(3):698–722. doi: 10.1161/HYP.0000000000000033

27. Brandão AA, Amodeo C, Alcântara C, Barbosa E, Nobre F, Pinto F, et al. I posicionamento luso-brasileiro de pressão arterial central. Arq Bras Cardiol. 2017.108(2):100-8. https://doi.org/10.5935/abc.20170011

28. Nobre F, Mion Júnior D, Gomes M, Barbosa E, Rodrigues C, Neves M, et al. 6a Diretrizes de Monitorização Ambulatorial da Pressão Arterial e 4a Diretrizes de Monitorização Residencial da Pressão Arterial. Arq Bras Cardiol. 2018;110(5 supl 1):1-29. doi: 10.5935/abc.20180074

29. Celermajer DS, Sorensen KE, Gooch VM, Miller 0. 1., Sullivan ID, Lloyd JK, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992 Nov 7;340(8828):1111–5. doi: 10.1016/0140-6736(92)93147-f

30. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the international brachial artery reactivity task force. J Am Coll Cardiol. 2002;39(2):257-65. doi: 10.1016/s0735-1097(01)01746-6

31. Thijssen DHJ, Bruno RM, Van Mil ACCM, Holder SM, Faita F, Greyling A, et al. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. Eur Heart J. 2019;40(30):2534–47. doi: 10.1093/eurheartj/ehz350

32. Alexander Y, Osto E, Schmidt-Trucksäss A, Shechter M, Trifunovic D, Duncker DJ, et al. Endothelial function in cardiovascular medicine: A consensus paper of the European Society of Cardiology Working Groups on Atherosclerosis and Vascular Biology, Aorta and Peripheral Vascular Diseases, Coronary Pathophysiology and Microcirculation, and Thr. Cardiovasc Res. 2021;117(1):29-42 doi: 10.1093/cvr/cvaa085

33. Palta P, Page G, Piferi RL, Gill JM, Hayat MJ, Connolly AB, et al. Evaluation of a Mindfulness-Based Intervention Program to Decrease Blood Pressure in Low-Income African-American Older Adults. J Urban Health. 2012; 89(2):308. doi: 10.1007/s11524-011-9654-6

34. Ponte Márquez PH, Feliu-Soler A, Solé-Villa MJ, Matas-Pericas L, Filella-Agullo D, Ruiz-Herrerias M, et al. Benefits of mindfulness meditation in reducing blood pressure and stress in patients with arterial hypertension. J Hum Hypertens. 2018;33(3):237-47. doi: 10.1038/s41371-018-0130-6

New Insights into Medical Therapy for Heart Failure with Preserved Ejection Fraction

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Introduction

Heart failure (HF) phenotypes can be divided into categories according to the left ventricular ejection fraction (EF) – HF with preserved EF (HFpEF; EF ≥ 50%); HF with mildly reduced EF (HFmrEF; EF 41-49%) and HF with reduced EF (HFrEF; EF ≤ 40%).[1] However, HF phenotypes differ beyond just a different EF. While HFpEF develops from an interplay of comorbidities that lead to structural heart disease and HF symptoms, HFrEF usually develops due to a cardiac insult that reduces cardiac output.[1,2] Moreover, while multiple therapies can improve the prognosis of HFrEF, only sodium-glucose cotransporter 2 inhibitors (SGLT2i) improved outcomes in HFpEF in a randomized controlled trial (RCT).[3] In this letter, we explore evidence for medical therapies that could benefit HFpEF patients.

Treatment of HFpEF etiologies and associated conditions

The management of HFpEF etiologies and comorbidities (e.g., hypertension, diabetes, coronary artery disease, obesity, anemia, chronic kidney disease) is essential to avoid disease progression and reduce hospitalization.[1] Patients with transthyretin amyloid cardiomyopathy also benefit from tafamidis, that reduced by 30% and 32% the risk for all-cause mortality and cardiovascular (CV) hospitalizations, respectively, compared with placebo.[4]

Angiotensin-converting-enzyme inhibitors (ACEi), angiotensin receptor blockers (ARBs) and angiotensin receptor-neprilysin Inhibitors

Previous RCTs, such as the PEP-CHF,[5] CHARM-Preserved[6] and I-PRESERVE[7] showed no significant benefit of ACEi or ARBs in HFpEF patients. Khan et al.[8] confirmed these findings but showed, in a pooled analysis of RCTs, a trend towards reduced HF hospitalization risk.[8] Subsequently, sacubitril/valsartan has emerged as a promise to improve outcomes in HFpEF, but failed to meet its primary endpoint of HF hospitalization or CV death in the PARAGON-HF trial.[9] Nonetheless, women with HFpEF may benefit from sacubitril/valsartan, since it reduced by 27% the primary outcome compared with placebo in a prespecified subgroup analysis.[9] Evidence from a meta-analysis of RCTs showed that sacubitril/valsartan led to reductions in NT-proBNP and improvements in quality of life in HFpEF patients.[10] Hence, sacubitril/valsartan could be preferred over ARBs or ACEi in patients with indications for renin-angiotensin system inhibitors due to comorbidities.

Mineralocorticoid receptor antagonists (MRAs)

In the TOPCAT trial, spironolactone did not reduce the primary outcome of CV death, aborted cardiac arrest or HF hospitalization in HFpEF patients compared with placebo, although it was effective among patients with elevated natriuretic peptides.[11,12] Surprisingly, while patients in the Americas experienced an 18% risk reduction of the primary outcome, in Russia and Georgia, spironolactone did not improve prognosis.[11] This can be explained by differences in randomization, patients that did not take the drug, and lower event rates in Russia and Georgia.[11,13] Additional evidence from a meta-analysis showed that spironolactone reduced hospitalizations, improved New York Heart Association (NYHA) class and decreased levels of b-type natriuretic peptide in HFpEF patients.[14]

Diuretics

Due to ethical issues in conducting RCTs for diuretic use, their effects on long-term prognosis in HFpEF are unknown. However, a post-hoc analysis of the CHAMPION trial showed that changes in diuretic and vasodilator therapies according to pulmonary artery pressure reduced by 46% the incidence rate ratio of HF hospitalization in HFpEF with NYHA class III.[15] This reinforces the need of controlling peripheral and pulmonary edema and indicates that diuretics not only control HF symptoms but may also reduce HF hospitalization.

Keywords

Heart Failure; Stroke Volume/drug effects; Angiotensin-Converting Enzyme Inhibitors; Mineralocoirticoids; Receptor Antagonists; Digoxin

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Sodium–glucose cotransporter 2 inhibitors (SGLT2i)

Data from the EMPEROR-Preserved trial showed empagliflozin reduced the risk for the primary endpoint of CV death or HF hospitalization in HFpEF patients compared with placebo.[3] In an exploratory analysis, empagliflozin also reduced HF hospitalizations that required intensive care, hospitalizations that required vasopressors or positive inotropes, and the need for diuretic intensification in outpatients.[16] Moreover, patients assigned to empagliflozin were more likely to have improved NYHA class.[16]

Beta-blockers and other therapies

In a meta-analysis of RCTs, beta-blockers did not reduce the risk for all-cause mortality or CV death in HFpEF patients with sinus rhythm or atrial fibrillation.[17] Digoxin and therapies targeting the nitric oxide-cyclic guanosine monophosphate pathway also failed to improve endpoints in HFpEF.[1,18] Phase III RCTs that investigated pharmacological therapies in HFpEF patients are detailed in Table 1. After reviewing the evidence presented above, we outlined a triple therapy proposal with potential to improve outcomes of HFpEF patients, that is illustrated in Figure 1.

Table 1 – Phase III randomized controlled trials of pharmacological therapies in heart failure with preserved ejection fraction

Study	Drug	Inclusion Criteria	All-Cause Mortality	CV Mortality	CV Death or HF Hospitalization	HF Hospitalization
PEP-CHF[5]	Perindopril	LV wall motion index ≥ 1.4, symptomatic HF treated with diuretic, diastolic dysfunction, age ≥ 70 years	HR: 1.09 (0.75-1.58)	HR: 0.98 (0.63-1.53)	NR	HR: 0.86 (0.61-1.20)
CHARM-Preserved[6]	Candesartan	LVEF > 40%, NYHA II-IV, history of CV hospitalization	NR	HR: 0.99 (0.80-1.22)	HR: 0.89 (0.77-1.03)	HR: 0.85 (0.72-1.01)
I-PRESERVE[7]	Irbesartan	LVEF ≥ 45%, NYHA III-IV or NYHA II with HF hospitalization in the past 6 months, age ≥ 60 years	HR: 1.00 (0.88-1.14)	HR: 1.01 (0.86-1.18)	HR: 0.96 (0.84-1.09)	HR: 0.95 (0.81-1.10)
PARAGON-HF[9]	Sacubitril-Valsartan	HF with LVEF ≥ 45%, NYHA II-IV, left atrial enlargement or LV hypertrophy and BNP ≥ 300 pg/mL or NT-proBNP ≥ 900 pg/mL or HF hospitalization in the last 9 months	HR: 0.97 (0.84-1.13)	HR: 0.95 (0.79-1.16)	RaR: 0.87 (0.75–1.01)	RaR: 0.85 (0.72-1.00)
TOPCAT[11]	Spironolactone	LVEF ≥ 45%, ≥ 1 HF sign and ≥ 1 HF symptom, HF hospitalization within the past 12 months, or BNP ≥ 100 pg/mL or NT-proBNP ≥ 360 pg/mL, age ≥ 50 years	HR: 0.91 (0.77-1.08)	HR: 0.90 (0.73-1.12)	HR: 0.89 (0.77-1.04)	**HR: 0.83 (0.69-0.99)**
EMPEROR-Preserved[3]	Empagliflozin	HF with LVEF ≥ 40%, NYHA II-IV, age ≥ 18 years, NT-proBNP > 300 pg/mL or NT-proBNP > 900 pg/mL for patients with HF and AF	HR: 1.00 (0.87-1.15)	HR: 0.91 (0.76-1.09)	**HR: 0.79 (0.69-0.90)**	**HR: 0.73 (0.61-0.88)**
DIG-PEF[18]	Digoxin	HF with LVEF > 45%, SR	RiR: 0.99 (0.76-1.28)	RiR: 1.00 (0.73-1.36)	RiR: 0.88 (0.70-1.11)	RiR: 0.79 (0.59-1.04)

AF: atrial fibrillation; CV: cardiovascular; HF: heart failure; HR: hazard ratio; LV: left ventricular; LVEF: left ventricular ejection fraction; NT-proBNP: N-terminal-pro hormone b-type natriuretic peptide; NYHA: New York Heart Association; RaR: rate ratio; RiR: risk ratio; SR: sinus rhythm; NR: not reported.

Conclusions

Empagliflozin is the only pharmacological therapy with robust randomized data to support its benefit in HFpEF to this date. However, as discussed above, a combination of diuretics, MRAs and SGLT2i may reduce mortality and hospitalization in HFpEF. Future RCTs investigating novel therapies for HFpEF are needed.

Author Contributions

Conception and design of the research: Correia ETO; Analysis and interpretation of the data, Writing of the manuscript and Critical revision of the manuscript for important intellectual content: Correia ETO Mesquita ET.

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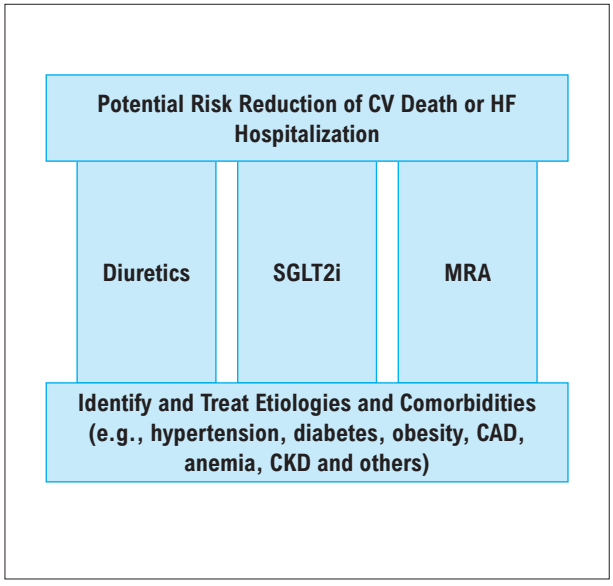

Figure 1 – *A Triple Therapy Proposal for Heart Failure with Preserved Ejection Fraction. CAD: coronary artery disease; CKD: chronic kidney disease; CV: cardiovascular; HF: heart failure; MRA: mineralocorticoid receptor antagonist; SGLT2i: sodium-glucose 2 cotransporter inhibitor. Only empagliflozin has evidence from a robust randomized trial.[3] Post-hoc analyses of the CHAMPION and TOPCAT trials may support the use of diuretics and MRA.[11-13,15]*

References

1. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J. 2021;42(36):3599–726. doi: 10.1093/eurheartj/ehab368
2. Pfeffer MA, Shah AM, Borlaug BA. Heart Failure With Preserved Ejection Fraction In Perspective. Circ Res. 2019;124(11):1598–617. doi: 10.1161/CIRCRESAHA.119.313572
3. Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, et al. Empagliflozin in Heart Failure with a Preserved Ejection Fraction. N Engl J Med. 2021;385(16):1451-61. doi: 10.1056/NEJMoa2107038
4. Maurer MS, Schwartz JH, Gundapaneni B, Elliott PM, Merlini G, Waddington-Cruz M, et al. Tafamidis Treatment for Patients with Transthyretin Amyloid Cardiomyopathy. N Engl J Med. 2018 Sep 13;379(11):1007–16. doi: 10.1056/NEJMoa1805689
5. Cleland JGF, Tendera M, Adamus J, Freemantle N, Polonski L, Taylor J, et al. The perindopril in elderly people with chronic heart failure (PEP-CHF) study. Eur Heart J. 2006 Oct;27(19):2338–45. doi: 10.1093/eurheartj/ehl250
6. Yusuf S, Pfeffer MA, Swedberg K, Granger CB, Held P, McMurray JJV, et al. Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: the CHARM-Preserved Trial. Lancet. 2003 Sep 6;362(9386):777–81. doi: 10.1016/S0140-6736(03)14285-7.
7. Massie BM, Carson PE, McMurray JJ, Komajda M, McKelvie R, Zile MR, et al. Irbesartan in patients with heart failure and preserved ejection fraction. N Engl J Med. 2008 Dec 4;359(23):2456–67. doi: 10.1056/NEJMoa0805450
8. Khan MS, Fonarow GC, Khan H, Greene SJ, Anker SD, Gheorghiade M, et al. Renin-angiotensin blockade in heart failure with preserved ejection fraction: a systematic review and meta-analysis. ESC Heart Fail. 2017 Nov;4(4):402–8. doi: 10.1002/ehf2.12204
9. Solomon SD, McMurray JJV, Anand IS, Ge J, Lam CSP, Maggioni AP, et al. Angiotensin-Neprilysin Inhibition in Heart Failure with Preserved Ejection Fraction. N Engl J Med. 2019 Oct 24;381(17):1609–20. . doi: 10.1056/NEJMoa1908655
10. Salah HM, Fudim M, Al'Aref SJ, Khan MS, Almarzooq ZI, Devabhaktuni SR, et al. Meta-Analysis of Efficacy of Sacubitril/Valsartan in Heart Failure With Preserved Ejection Fraction. Am J Cardiol. 2021 Apr 15;145:165–8. doi: 10.1016/j.amjcard.2021.01.013
11. Pitt B, Pfeffer MA, Assmann SF, Boineau R, Anand IS, Claggett B, et al. Spironolactone for heart failure with preserved ejection fraction. N Engl J Med. 2014 Apr 10;370(15):1383–92. doi: 10.1056/NEJMoa1313731
12. Girerd N, Ferreira JP, Rossignol P, Zannad F. A tentative interpretation of the TOPCAT trial based on randomized evidence from the brain natriuretic peptide stratum analysis. Eur J Heart Fail. 2016 Dec;18(12):1411–4. doi: 10.1002/ejhf.621
13. de Denus S, O'Meara E, Desai AS, Claggett B, Lewis EF, Leclair G, et al. Spironolactone Metabolites in TOPCAT - New Insights into Regional Variation. N Engl J Med. 2017 Apr 27;376(17):1690–2. doi: 10.1056/NEJMc1612601
14. Xiang Y, Shi W, Li Z, Yang Y, Wang SY, Xiang R, et al. Efficacy and safety of spironolactone in the heart failure with mid-range ejection fraction and heart failure with preserved ejection fraction: A meta-analysis of

randomized clinical trials. Medicine. 2019 Mar;98(13):e14967. doi: 10.1097/MD.0000000000014967

15. Adamson PB, Abraham WT, Bourge RC, Costanzo MR, Hasan A, Yadav C, et al. Wireless pulmonary artery pressure monitoring guides management to reduce decompensation in heart failure with preserved ejection fraction. Circ Heart Fail. 2014 Nov;7(6):935–44. doi: 10.1161/CIRCHEARTFAILURE.113.001229

16. Packer M, Butler J, Zannad F, Filippatos G, Ferreira JP, Pocock SJ, et al. Effect of Empagliflozin on Worsening Heart Failure Events in Patients With Heart Failure and Preserved Ejection Fraction: EMPEROR-Preserved Trial. Circulation. 2021 Oct 19;144(16):1284–94. doi: 10.1056/NEJMoa2107038

17. Cleland JGF, Bunting KV, Flather MD, Altman DG, Holmes J, Coats AJS, et al. Beta-blockers for heart failure with reduced, mid-range, and preserved ejection fraction: an individual patient-level analysis of double-blind randomized trials. Eur Heart J. 2018 Jan 1;39(1):26–35. doi: 10.1093/eurheartj/ehx564

18. Ahmed A, Rich MW, Fleg JL, Zile MR, Young JB, Kitzman DW, et al. Effects of Digoxin on Morbidity and Mortality in Diastolic Heart Failure: The Ancillary Digitalis Investigation Group Trial. Circulation. 2006 Aug 1;114(5):397–403. doi: 10.1161/CIRCULATIONAHA.106.628347

Alopecia Universalis after Treatment with Simvastatin and Ezetimibe: Affects on Family

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Abstract

Alopecia areata (AA) is an autoimmune disease that grows in the scalp or in other parts of the body. Alopecia universalis, which is a rare form of alopecia areata, is characterized by a loss of hair that affects the entire body. In the two patients presented in this study, atorvastatin treatment was implemented, with the diagnosis of hypercholesterolemia; however, when the target values were not reached, a combination of simvastatin and ezetimibe was implemented. After a period of simvastatin/ezetimibe treatment, the AA disorder, which began with hair loss on the scalp, spread to the entire body and turned into Alopecia Universalis. Although statins can cause alopecia with autoimmune reactions, they are generally used in the treatment of alopecia due to their immunomodulatory effects.

Case 1

A 69-year-old female patient was followed up in our clinic with the diagnosis of heart failure and coronary artery disease. The patient had a history of hypercholesterolemia. Her Dutch score (clinical score for familial hypercholesterolemia, with a definite diagnosis > 8 points) was calculated as 12 points. The lipid parameters obtained in our clinic were: total cholesterol of 380 mg/dl, low-density lipoprotein (LDL) cholesterol of 299 mg/dl, high-density lipoprotein (HDL) cholesterol of 62 mg/dl, and triglycerides of 93 mg/dl. In the patient's medical history, it was learned that the patient had used 40 mg atorvastatin tablets (Lipitor, Pfizer) for 6 months, 5 years prior, but the treatment was changed to a combination of 40 mg simvastatin and 10 mg ezetimibe (Inegy 10/40, Merck, Sharp & Dohme), since the target values could not be reached. This resulted in the patient losing of hair first from the head and then from the eyebrow, eyelashes, and the hair on the axillar and pubic parts of the body in 2 months. Alopecia universalis increased in approximately 6 months (Figure 1).

Keywords

Alopecia; Autoimmune Diseases; Hypercholesterolemia; Atorvastatin/adverse effects; Ezetimibe, Simvastatin Drug Combination/adverse effects; Genetic

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Case 2

The second case was the 45-year-old son of the female patient, who also showed alopecia universalis (Figure 2). His Dutch score was calculated as 14 points. The patient's lipid parameters were as follows: total cholesterol of 382 mg/dl, LDL cholesterol of 305 mg/dl, HDL cholesterol of 57 mg/dl, and triglycerides of 102 mg/dl. This patient was treated with 40 mg atorvastatin tablets (Lipitor, Pfizer) simultaneously with his mother. When the treatment proved to be ineffective after 5 months, the combination treatment of 40 mg simvastatin and 10 mg ezetimibe (Inegy, Merck/Sharp & Dohme) was implemented. Likewise, after the treatment began, first the hair on the head, then eyebrow, eyelashes, and the hair on the axillar and pubic part of the body began to fall out in 2 months, and alopecia universalis increased in approximately 6 months (Figure 2). Considering that it can be a medicine-related pathology, the application of medicine was stopped; however, no remission was observed in the table. Both cases refused to receive dermatological treatment for the treatment of alopecia.

Discussion

AA is a non-scarring form of alopecia with a patchy loss of hair in the scalp and elsewhere. It can occur at any age, and it is more frequently observed in the second and fourth decades. Generally, it is observed in both sexes with the same frequency. The incidence of this situation that can be accepted as relatively common is 0.15%.[1] Although the pathogenesis of the disease is not completely known, it is an autoimmune disease and apart from genetic factors; the environmental factors such as infection and psychological stress also play an important role in the development of this disease. The family incidence of 10%–20% supports the genetic activation of the disease, which increases to 50% for monozygotic twins.[2] However, alopecia universalis is a rare form of AA, which is defined as the loss of all hair of the head and the body. It constitutes 7%–30% of all AA cases.[3]

The statins are the main therapeutic agents for treating hypercholesterolemia. Effects other than the intended effect during the development of an agent are called pleiotropic effects. Reduction in circulating isoprenoids and

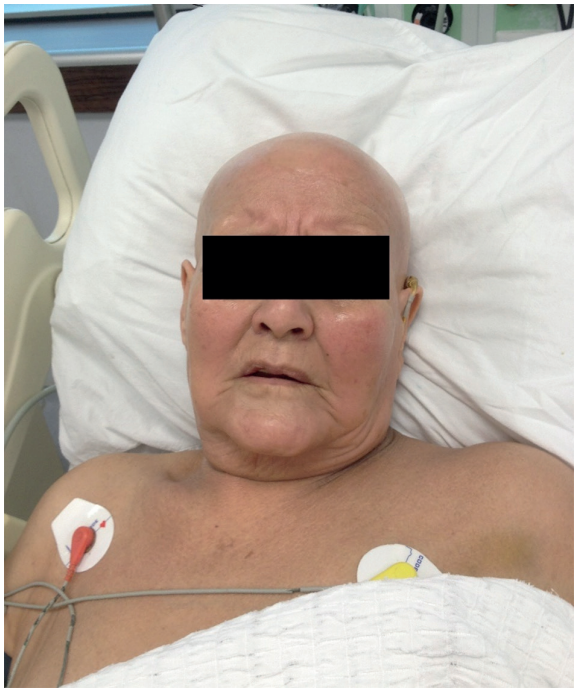

Figure 1 – *Alopecia universalis patient, case 1.*

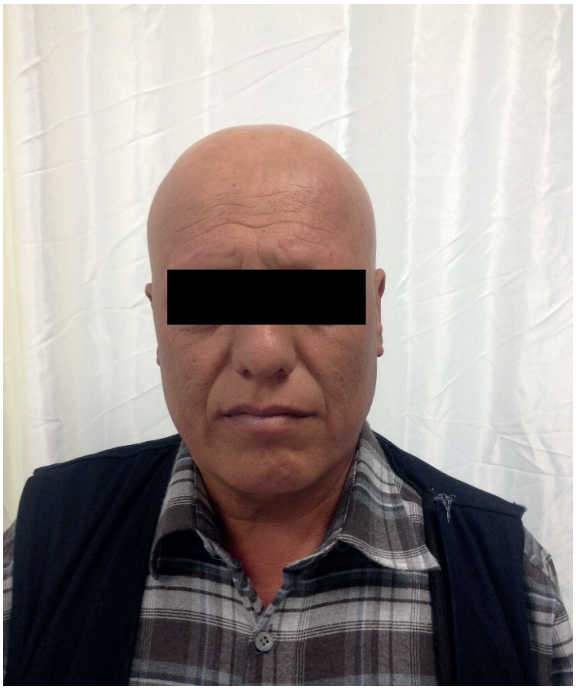

Figure 2 – *Alopecia universalis patient, case 2.*

the inactivation of signaling proteins result in pleiotropic effects of statins, such as anti-inflammatory, antioxidant, antiproliferative, and immunomodulatory effects, plaque stability, and the inhibition of platelet aggregation.[4] Statins execute their immunomodulatory effects, which are pleiotropic, via MHC-II moles, T helper 1 cells, and T helper 2 cells.[5] It is also well-known that in AA, T helper 1 and T helper 2 cells play specific roles.[6] An autoimmune reaction triggering mechanism of statins can cause apoptosis to release autoantigens and hence an autoantibody response. However, this triggers T-lymphocyte activation by causing a change in the cholesterol content of the membrane lipid structure. The result is a T helper 2 reaction that results in autoantibody production by B cells.[7,8] In the literature, there are cases in which simvastatin was used in alopecia treatment because of its immunomodulator effects.[9] Along with this, it is also well-known that statins cause liver damage, such as autoimmune myopathy and autoimmune hepatitis.[10,11] Although hair loss has been reported among the uncommon side effects of statins in the prospectus information, there is one case in which atorvastatin-related hair loss was reported in the literature.[12] Ezetimibe decreases the absorption of cholesterol obtained from the diet. No alopecia case caused by ezetimibe monotherapy has been reported. Moreover, an Ezetimibe/Simvastatine combination related to an autoimmune hepatitis case was reported. However, only a speculation can be done as to whether or not the factor causing this was statin-related or ezetimibe-related.[13] Although statin, ezetimibe, and their combination were used in alopecia treatment because of their immunomodulator effects, ironically, in our cases, it is believed that the combination was temporally associated with the beginning of alopecia in both cases, and may have contributed to the condition. Furthermore, it was observed that genetic factors play a broad role in which the same medicine both causes alopecia and becomes the treatment for alopecia.

Author Contributions

Conception and design of the research, Writing of the manuscript and Critical revision of the manuscript for important intellectual contente: Ozyurtlu F, Cetin N; Acquisition of data: Ozyurtlu F.

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References

1. Delamere FM, Sladden MM, Dobbins HM, Leonardi-Bee J. Interventions for alopecia areata. Cochrane Database Syst Rev.2008;2:CD004413. doi:10.1002/14651858CD004413.pub 2.
2. Goh C, Finkel M, Christos PJ, Sinha AA. Profile of 513 patients with alopecia areata: associations of disease subtypes with atopy, autoimmune disease and positive family history. J Eur Acad Dermatol Venereol.2006;20(9):1055-60. DOI: 10.1111/j.1468-3083.2006.01676.x
3. Hunt N, McHale S. The psychological impact of alopecia. BMJ. 2005;331(7522):951–3. doi: 10.1136/bmj.331.7522.951.
4. Kavalipati N, Shah J, Ramakrishan A, Vasnawala H. Pleiotropic effects of statins. Indian J Endocrinol Metab. 2015;19(5):554-62. doi:10.4103/2230-8210.163106.
5. Chao-Yung Wang, Ping-Yen Liu, James K. Pleiotropic effects of statin therapy: molecular mechanisms and clinical results. Liao Trends Mol Med. 2008;14(1):37-44. DOI: 10.1016/j.molmed.2007.11.004
6. Barahmani N, Lopez A, Babu D, Hernandez M, Donley SE, Duvic M. Serum T helper 1 cytokine levels are greater in patients with alopecia areata regardless of severity or atopy. Clin Exp Dermatol.2010;35(4):409-16. Doi:10.1111/j.1365-2230.2009.03523.
7. Panizzon RG. Lupus-like syndrome associated with statin therapy. Dermatology. 2004; 208(3):276-7. DOI: 10.1159/000077320
8. Noel B. Lupus erythematosus and other autoimmune diseases related to statin therapy: a systematic review. J Eur Acad Dermatol Venereol.2007;21(1):17-24. DOI: 10.1111/j.1468-3083.2006.01838.x
9. Morillo-Hernandez C, Lee JJ, English JC 3rd. Retrospective outcome analysis of 25 alopecia areata patients treated with simvastatin/ezetimibe.. J Am Acad Dermatol. 2019 Sep;81(3):854-7. DOI: 10.1016/j.jaad.2019.04.047
10. Nazir S, Lohani S, Tachamo N, Poudel D, Donato A. Statin-Associated Autoimmune Myopathy: A Systematic Review of 100 Cases. J Clin Rheumatol. 2017Apr;23(3):149-54.DOI: 10.1097/RHU.0000000000000497
11. Russo MW, Scobey M, Bonkovsky HL. Drug-induced liver injury associated with statins. Semin Liver Dis. 2009 Nov;29(4):412-22. DOI: 10.1055/s-0029-1240010
12. Segal AS. Alopecia associated with atorvastatin. Am J Med. 2002 Aug 1;113(2):171. doi: 10.1016/s0002-9343(02)01135-x.
13. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012. PMID: 31643176 Bookshelf ID:NBK547852

Septal Ablation with Radiofrequency and the Use of New Technologies in Patients with Hypertrophic Cardiomyopathy in an Electrophysiology Laboratory

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Dear Editor,

We have read, with great interest, the article "Septal ablation with catheters and radiofrequency guided by echocardiography for treatment in patients with obstructive hypertrophic cardiomyopathy (OHC): First experience", published recently by Valdigem et al.[1] in the journal *Arquivos Brasileiros de Cardiologia*.

In this study, the authors evaluated the effects of endocardial ablation by radiofrequency (RF) of the interventricular septum with reduction of the ventricular-arterial gradient and improvement in functional class in 12 patients with OHC. Catheters with solid 8-mm tips were used to apply the thermo-controlled RF. The energy intensity was of 80 Watts, with a maximum temperature of 60ºC. The target for ablation was the region with the highest gradient in the left ventricle outflow tract and was identified by the transesophageal echocardiogram. The authors observed an average reduction of the gradients obtained from 96.8±34 mmHg to 36.1±23 mmHg (p=0.0001) during a 1-year follow-up, with a clinical improvement in all patients of the series. They concluded that the septal ablation with RF is an effective, safe strategy and represents a new option to treat OHC patients with high and symptomatic gradients. We would like to congratulate the authors for their fine results in using a technological device that is of easy access, as well as for bringing new and relevant information about the procedure, which is still under development.

From August 2020 to January 2021, our team conducted an ablation with RF of the interventricular septum in two patients (a 44-year-old man and a 38-year-old woman) with symptomatic OHC, who were refractory to clinical treatment, both undergoing follow-up for more than 12 months. However, in contrast to the technique described by Valdigem et al.,[1] we used new imaging techniques, including electroanatomic mapping (EAM) and intracardiac echocardiography (ICE) (Figure 1). The EAM allowed us to define the localization of the intraventricular conduction system and conferred greater safety in the application of RF (avoiding the left bundle branch block or complete atrioventricular block). The geometric construction produced by the EAM of the left and right ventricles also provided important information on the definition of the area to be treated. The ICE allowed us to follow the production of the lesions of RF in the interventricular septum and the evolution of the edema near the left ventricle outflow track during the procedure, without the need for an echocardiographer. Additionally, the ablation with radiofrequency was optimized with the use of irrigated-tipped catheters, and the lesions were controlled by the VISITAG SURPOINT (J&J)[2] software in order to standardize its depth.

The procedure's interruption criteria used by Valdigem et al.[1] was an acute 25% drop in the ventricular-arterial gradient. However, some authors suggest that the excessive septal ablation to reach these indexes can acutely provoke a paradoxical and acute increase of the gradient with the risk of significant pulmonary congestion after the ablation.[3] Our impression is that the use of a purely anatomic strategy, with septal applications directly above the left branch, with an Ablation Index target of between 600 and 700, using an 3.5-mm irrigated catheter (50 Watts and 43ºC) and a continuous evaluation of the edema of the left ventricle outflow tract with the ICE, may well make the procedure safer.

The wide range of series published to date do not give value to the immediate gradient, suggesting that the greatest benefit in the reduction of the gradient occurs between 9 and 12 months of ablation.[4,5] Our patients witnessed a significant reduction of the interventricular gradient, with an average drop from 91±22 mmHg to 27±14 mmHg, approximately 12 months after the initial procedure, and a further reduction in the first post-operative day of 22±6 mmHg, both with a significant improvement in the symptoms. The patient is currently at the functional class II level. The use of an irrigated catheter can cause more predictable lesions, but it can also contribute in treating pulmonary congestion, as described by the authors. The simultaneous use of the ICE to follow up on the RF applications can also prevent the occurrence of *"Stem Pops"*, a common fact in prolonged applications and with high energy. Additionally, the intracardiac echocardiogram aids in monitoring the risk of excessive applications by accompanying the formation of a septal edema. Nevertheless, one of our patients presented a medical condition of immediate pulmonary congestion after the ablation, which was resolved with the use of diuretics and non-invasive ventilation. Both the use of the irrigated catheter as well as the significant

Keywords

Radiofrequency Ablation; Hypertrophic Cardiomyopathy; Electrophysiology.

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edema in the outflow tract may have contributed to the medical condition presented by the patient. Further studies are warranted in order to compare different techniques, as well as to standardize what would be the ideal means through which to create the lesions, which minimize the risk of acute increases in the ventricular-arterial gradient after the ablation.

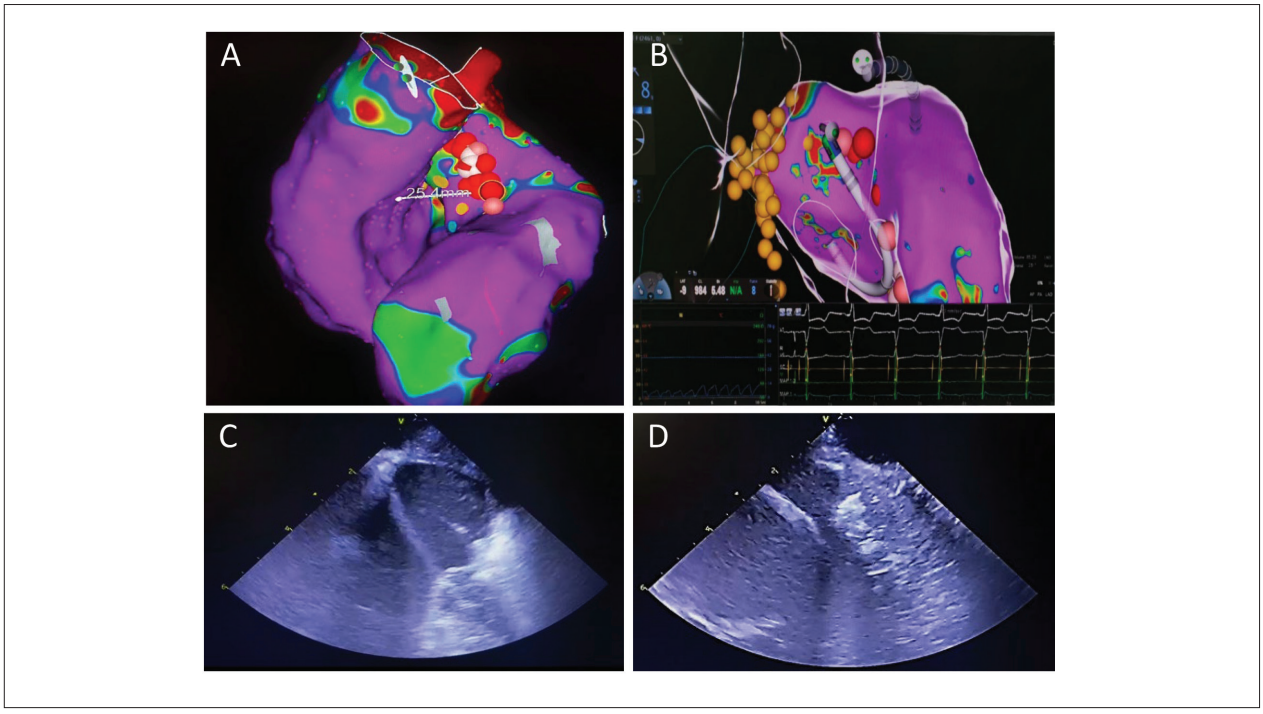

Figure 1 – *Carto 3-guided electroanatomic maps of the right and left ventricle. This picture shows the point with the largest septal thickness (25 mm). The points in red represent the region where the radiofrequency was applied. B) Beginning of the application of radiofrequency. The yellow points represent the areas to be avoided in which the conduction system was identified. C) Hyperechogenicity of the septal region, evaluated continually using the intracardiac echocardiogram during the application of radiofrequency. It is possible to identify the catheter in the strap resting on the septal region. D) At the end of the procedure, an intense edema is observed in the septal region, associated with hyperechogenicity near the left ventricle outflow tract.*

References

1. Valdigem BP, Correia EB, Moreira DAR, Bihan DL, Pinto IMF, Abizaid AAC, et al. Septal Ablation with Radiofrequency Catheters Guided by Echocardiography for Treatment of Patients with Obstructive Hypertrophic Cardiomyopathy: Initial Experience. Arq Bras Cardiol. 2022;118(5):861-872. doi: 10.36660/abc.20200732.
2. Okumura Y, Watanabe I, Iso K, Nagashima K, Sonoda K, Sasaki N, et al. Clinical Utility of Automated Ablation Lesion Tagging Based on Catheter Stability Information (VisiTag Module of the CARTO 3 System) with Contact Force-Time Integral During Pulmonary Vein Isolation for Atrial Fibrillation. J Interv Card Electrophysiol. 2016;47(2):245-252. doi: 10.1007/s10840-016-0156-z.
3. Lawrenz T, Lawin D, Radke K, Stellbrink C. Acute and Chronic Effects of Endocardial Radiofrequency Ablation of Septal Hypertrophy in HOCM. J Cardiovasc Electrophysiol. 2021;32(10):2617-2624. doi: 10.1111/jce.15203.
4. Cooper RM, Shahzad A, Hasleton J, Digiovanni J, Hall MC, Todd DM, et al. Radiofrequency Ablation of the Interventricular Septum to Treat Outflow Tract Gradients in Hypertrophic Obstructive Cardiomyopathy: A Novel Use of CARTOSound® Technology to Guide Ablation. Europace. 2016;18(1):113-20. doi: 10.1093/europace/euv302.
5. Crossen K, Jones M, Erikson C. Radiofrequency Septal Reduction in Symptomatic Hypertrophic Obstructive Cardiomyopathy. Heart Rhythm. 2016;13(9):1885-90. doi: 10.1016/j.hrthm.2016.04.018.

Reply

Congratulations for your interest in the procedure,[1] we believe that new protocols for ablation and especially for post-operative care should be encouraged, given that even the way of releasing the energy and the localization of the point where the radiofrequency is applied are not a consensus among authors (note that Lawrenz, in a publication in August 2021, presented data with a bilateral application and only in the right septum).[2]

After a careful review of the first 40 cases carried out by our research group (publication pending), we observed that differences related to the morphology of the interventricular septum can be significant in the choice of the location to

Letter to the Editor

begin the ablation. More homogeneous hypertrophic septa tend to present a migration from the gradient in the apical direction. Thus, the ablation guided only by the anatomy can result in more extensive lesions unnecessarily. Moreover, residual medioventricular gradients can also occur, which would require a second approach.

Another benefit for the use of the transesophageal echocardiogram (TEE) would be the localization of the real point where the gradient began. More often, the gradient begins in the apical region than in the thicker region of the septum, marked by the point of greater aliasing, illustrated by the color flow mapping echocardiogram (Figure 1). The TEE presents images with a better visualization of the left ventricle outflow tract than does the intracardiac echocardiogram, information that is of utmost importance for the adequate alignment of the catheter.

The echocardiographic evaluation differentiates the shapes of valvular obstructions (in aortic stenosis) from the subvalvar obstructions, through the spectral image of the Continuous Doppler (Figure 2). This information is essential in cases of sequential stenosis, as occurs in the cases of septal ablation by pre-TAVI radiofrequency (in which this method has shown a promising role).

Furthermore, the TEE provides information about the adjacent structures, including the application of radiofrequency, enabling the ready identification of possible complications, such as lesions in tendinous cords, or the anterior cusp of the mitral valve.

One of the main parameters of success in the intra-procedure is the reduction in mitral regurgitation, resulting from the reduction in the systolic anterior motion (SAM) of the mitral valve due to the reduction in the obstruction of the left ventricle outflow tract.

Once again, we would like to congratulate the authors for their considerations and for their efforts to simplify the procedure and increase its diffusion. If it should be of your interest, we are willing to participate in joint analyses and sharing of experiences.

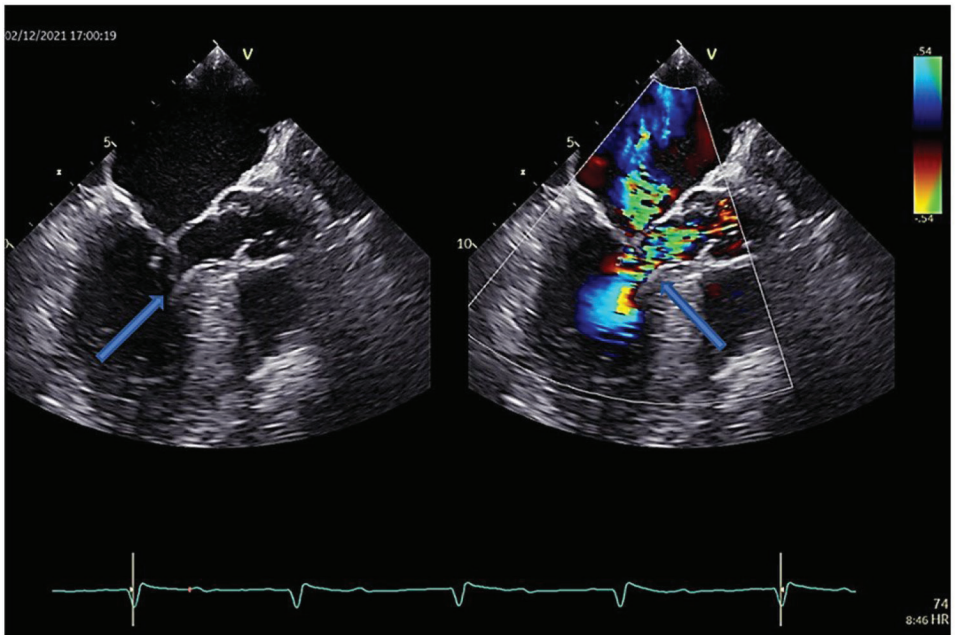

Figure 1 – *Intra-procedure TEE with and without color (at 150°): evaluation of the mitral valve, identification of the SAM and its localization. From this angle, we were able to more easily identify the location of greater acceleration of the flow and the degree of mitral valve regurgitation.*

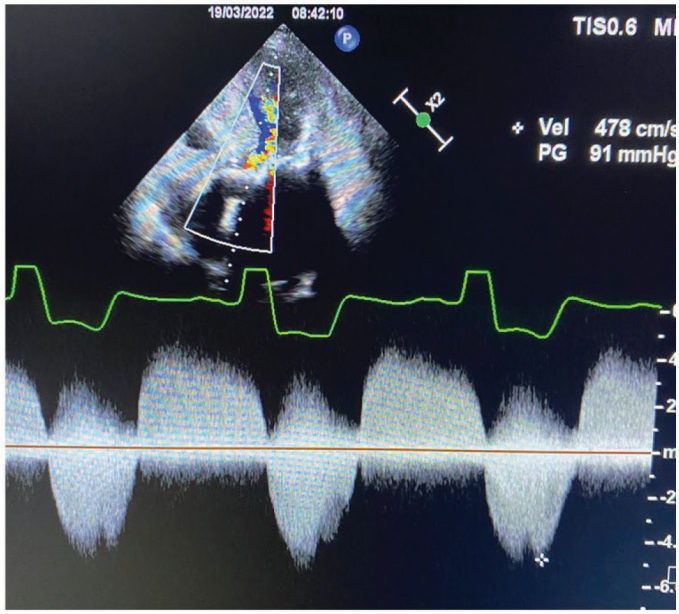

Figure 2 – *Continuous Doppler image with the shapes related to aortic stenosis and subvalvar obstruction*

References

1. Chokr M, Mayrink M, Vandoni PMP, Linhares PV, Sousa IBS, Castello Júnior HJ, et al. Septal Ablation with Radiofrequency and the Use of New Technologies in Patients with Hypertrophic Cardiomyopathy in an Electrophysiology Laboratory. Arq Bras Cardiol. 2022; 119(4):634-637.
2. Lawrenz T, Lawin D, Radke K, Stellbrink C. Acute and Chronic Effects of Endocardial Radiofrequency Ablation of Septal Hypertrophy in HOCM. J Cardiovasc Electrophysiol. 2021;32(10):2617-24. doi: 10.1111/jce.15203.

Brazilian Society of Cardiology Guidelines on the Analysis and Issuance of Electrocardiographic Reports – 2022

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Note: These guidelines are for information purposes and should not replace the clinical judgment of a physician, who must ultimately determine the appropriate treatment for each patient.

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Adail Paixao Almeida	Nothing to be declared
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Introduction

This review of electrocardiography guidelines is a result of advances in the understanding of various diseases, with important repercussions to the electrocardiographic tracing. Some professionals may imagine that electrocardiogram (ECG) interpretation has not gone through changes with time; they certainly ignore recently described diseases and other health problems whose electrophysiological mechanisms are better understood now than in the past. Some electrocardiographic parameters are important prognostic markers in Chagas disease, and changes considered as predictors of mortality can be identified in the general population (ECG-age). A crucial question is: when to recommend an ECG?

The ECG is a simple, low cost, non-invasive exam that provides an idea of an individual's cardiac condition and can eventually identify situations with risk of sudden death. Therefore, ECG findings within normal limits may anticipate that the ventricular function should be normal or close to normal, which is an important factor at first contact with the patient.

We believe that all persons should undergo an ECG at some point in their lives, and this examination should only be repeated in case of clinical need. Some guidelines state a class IIb indication for an ECG in asymptomatic individuals of the general population and a class IIa indication in case of hypertension and/or diabetes.[1]

The possibility of transmitting ECG tracings by the internet allowed the diffusion of this technology in various underprivileged regions of our country and the achievement of better standards of health assistance. In recent years, a significant increase (with millions of analyzed ECGs) has been seen in studies of artificial intelligence and automatic interpretation systems as additional tools for electrocardiography. Some results were able to demonstrate the ability of these new systems to identify some arrhythmias, as well as predict their appearance, in addition to anticipating outcomes such as ischemic stroke. Therefore, we expect that this update helps standardize the issuing of electrocardiographic reports by medical doctors allowing easier electrocardiographic understanding.

1. Standards for the Analysis and Issuance of Electrocardiographic Reports

1.1. Standards for Electrocardiographic Analysis

Three characteristics should be considered for an accurate electrocardiographic interpretation:

- Age: ECG characteristics depend on age and are clearly seen in age groups of newborns, infants, children, and adolescents up to 16 years of age. In the first 2 groups, these changes are faster (Section 13). Adults may also show negative T waves exclusively in V1.[2]
- Body type: slender individuals sometimes have their hearts in the upright (vertical) position, and the resulting axes, especially the P wave and QRS complex, are normally shifted to the right with a clockwise rotation in the frontal plane leads. On the other hand, in short and broad individuals with hearts in horizontal position, deviations are usually to the left (frontal plane).
- Sex: In female adults, negative T waves are commonly observed in the right precordial leads, with a larger QTc than male individuals and children.

1.2. The Electrocardiographic Report[1,3-5]

1.2.1. Descriptive Report

a) Analysis of the rhythm and quantification of the heart rate (HR);
b) Analysis of the duration, amplitude, and morphology of the P wave, and duration of the PR interval;
c) Electrical axis determination: P wave, QRS complex, and T wave;
d) Analysis of the duration, amplitude, and morphology of the QRS complex;
e) Analysis of ventricular repolarization and description of ST-segment, T wave, QT, and U changes, when present.

1.2.2. Final Report

It should contain the synthesis of diagnoses listed in these guidelines. Abbreviations can be used in reports, scientific texts, and protocols, among other documents, between parentheses and after the standard diagnostic definition.

1.2.3. Automated Report

In recent years, technological development has brought significant improvements to the accuracy of automatic measurements made by the currently available equipment, making automated interpretation an important auxiliary tool for the medical report. However, the verification of automatic measurements by a medical doctor is of paramount importance. The simple use of automatic metric and vector measurements, as well as reports issued by these systems, if not revised, are not recommended.

1.2.4. Reports Via the Internet

Tele-ECG systems[6-8] send ECGs performed remotely to referral centers for report issuance. The technique for performing ECGs (by the performing units), as well as the interpretation and reports (by the referral centers), should follow the most recent national and international guidelines. These are a part of telecardiology, which also comprehends other examinations in this specialty that are performed, recorded, and transmitted from one site to another for remote interpretation. Some examples include the monitoring of pacemakers, Holter monitors, and the cardiac event recorder. Among the numerous benefits of telecardiology, we cite:

a) Pre-hospital care at the patient's own location;
b) Reductions in time and costs for the patient;
c) Faster triage by specialists;
d) Access to specialists in case of accidents and emergencies;
e) Facilitated management of health care resources;
f) Increased safety of post-surgical patients during rehabilitation;
g) Cooperation and integration between researchers for sharing clinical records;
h) Access to educational programs of training and qualification.

According to various authors, telecardiology is a socially and economically advantageous activity for service providers, sponsors, and patients. It is recognized as a useful tool in locations that are distant from large city centers.

2. Tracing Technical Quality Analysis

2.1. Tracing Technical Evaluation Criteria

2.1.1. Calibration of the Electrocardiograph

In analogic equipment, calibration should always be verified. The normal pattern must have 1 mV (10 mm). On the other hand, in modern computerized equipment with digitized tracing, the calibrator pattern is verified automatically. Digital filters should follow internationally accepted recommendations, especially those from the AHA. For adults and adolescents, high-frequency filter cutoffs of at least 150 Hz should be used. For children, filter cutoffs of up to 250 Hz. Filters with lower frequencies can interfere when capturing pacemaker spikes. Low-frequency filters should use a 0.05 Hz cutoff. Some equipment use bidirectional filters.[9]

2.1.2. Lead Reversal

Figure 2.1 shows the correct position of peripheral electrodes — right arm (RA), left arm (LA), right leg (RL), and left leg (LL) — with their respective colors (red, yellow, black, and green).

2.1.2.1. Lead Switches

2.1.2.1.1. Transposition of Upper Limb Electrodes

DI leads with negative waves and aVR with positive waves.

2.1.2.2. Lower Limb Electrode Swapped for Upper Limb Electrodes

Isoelectric line or very small wave amplitudes in D2 (right arm) or D3 (left arm). Swapping upper limb electrodes with lower limb electrodes shows this pattern in D1 due to the negligible potential difference in the upper limbs.

2.1.2.3. Left Arm Electrode Swapped for Left Leg Electrode

This is the most difficult lead reversal to detect. The QRS axis tends to shift to the left. It may look like a normal ECG, but it produces the following alterations:

a) Inverted P wave in D3;
b) Positions of D1 and D2 are changed; QRS voltage is higher in D1 and lower in D2;
c) In D3, P, QRS, and T are inverted. Positions of aVL and aVF are also changed. The aVR lead does not change.

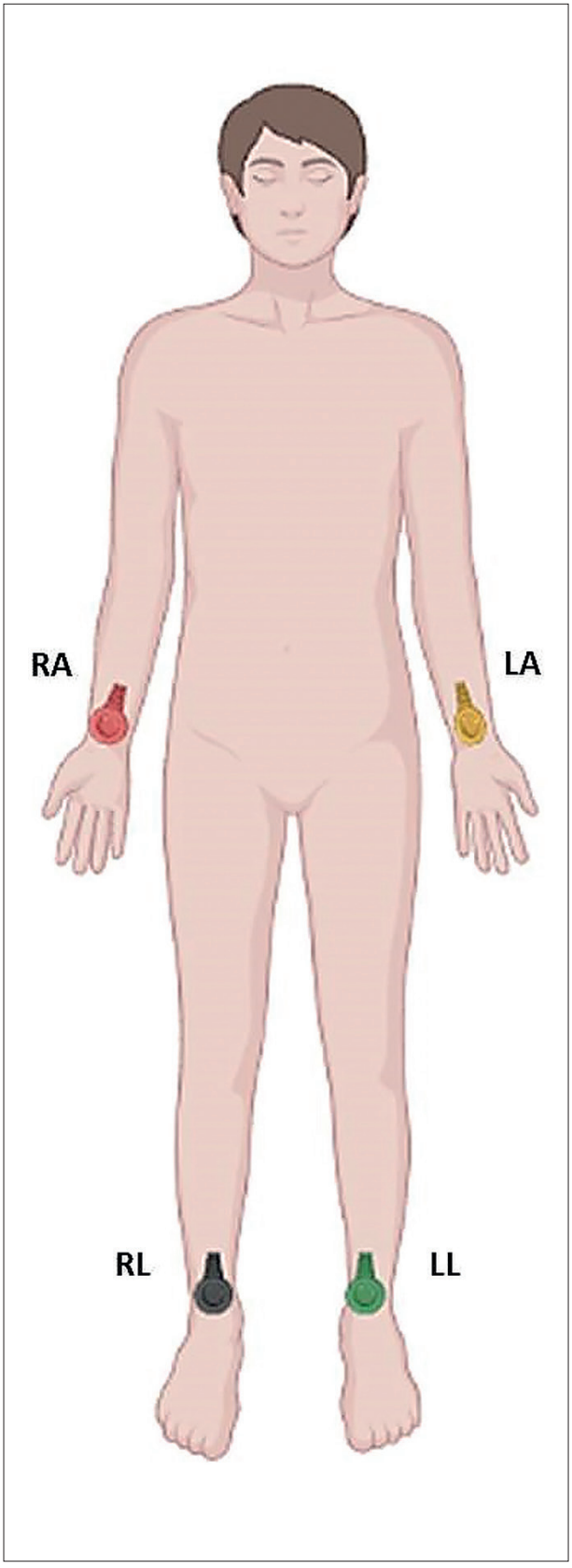

Figure 2.1 – *Positioning of peripheral electrodes.*
RA: right arm; LA: left arm; RL: right leg; LL: left leg.

2.1.2.4. Precordial Electrode Reversals

The normal progression of the R wave from V1 to V6 is changed.

2.1.2.5. Misplacement of the V1 and V2 Electrodes

V1 and V2 electrodes incorrectly positioned above the second intercostal space may produce an rSr' pattern simulating end conduction delay (or an IRBBB pattern), or an rS morphology from V1 to V3 and a negative P wave in V1, simulating left atrial hypertrophy.

2.1.3. Other Interferences

2.1.3.1. Muscle Tremors

Muscle tremors may interfere with the baseline, mimicking electrocardiographic changes such as atrial flutter and ventricular fibrillation[10] in patients with Parkinson's disease.

2.1.3.2. Neurostimulation

Patients with central nervous system disorders who require electrical stimulation devices can present artifacts that mimic cardiac pacemaker spikes.

2.1.3.3. Cold, Fever, Hiccups, and Psychomotor Agitation

These conditions produce baseline artifacts and can mimic arrhythmias such as atrial fibrillation and atrial flutter.

2.1.3.4. "Large Precordial Electrode"

The use of conductive gel as a continuous strip on the precordium results in similar tracings in V1–V6, corresponding to the mean electric potential in these leads.[3]

2.1.3.5. Baseline Wander

It may be caused by loose electrodes, movement of the limbs, breathing, or when the patient is in a wheelchair. When the patient is in a wheelchair, other artifacts can also be recorded.

2.1.3.6. Other Electrical and Electromagnetic Interferences

These result from interferences from electrical lines or equipment and cell phones. Prior to performing the ECG, the patient should be requested to put away all metallic objects and cell phones. Transcutaneous pacemakers can produce spikes, which could be mistaken for a false capture. The filter used in the ECG is also important since it sometimes creates a false capture failure, which generates a pause represented by an isoelectric line between 2 beats.[11-12]

2.1.3.7. Alterations Due to the Malfunction of Software and Computerized Electrocardiographic Signal Acquisition Systems

Data acquisition by computerized systems, in some older electrocardiographs, can seldom present specific

problems that are not yet completely understood. In the absence of an electrocardiographic signal in one of the electrodes, for example, the system may counterbalance the other acquired signals and create bizarre QRS complexes. Twelve-lead simultaneous equipment, which automatically measure P wave and QRS durations, can overestimate these measurements because the software uses the first and the last wave (among the 12 leads) for generating these values.

3. Heart Rhythm Analysis

3.1. Analysis of the P Wave, HR, and Rhythm

Population-based studies on normal electrocardiographic ranges have been used for many years as reference for our population, even though it is known that ethnic differences influence what is considered normal. In 2017, among all the information obtained by the ELSA-Brasil study, a research paper on the normal values for the Brazilian population without heart disease was published.[13]

The parameters addressed on Section 3 refer to adult ECGs. Pediatric ECGs will be addressed on Section 13.

3.1.1. Definition of Sinus Rhythm

This is the physiological rhythm of the heart, originating from the upper right atrium and observed on the surface ECG as positive P waves in the D1, D2, and aVF leads regardless of the presence of a QRS complex. The P axis may vary from 0º to +90º. The maximum amplitude and duration of the normal P wave are 2.5 mm and 110 ms, respectively. Changes in its morphology may happen depending on the HR and orientation (P-wave axis) in the observed leads.[14]

3.1.2. Frequency of the Sinus P Wave

The normal HR during waking hours ranges from 50 bpm to 99 bpm.[14-16]

3.2. Analysis of Supraventricular Rhythm Alterations

3.2.1. Definition of Cardiac Arrhythmia

Cardiac arrhythmia is due to abnormality of formation and/or conduction of the electrical impulse across the myocardium.[17] After the definition (or not) of the presence of sinus rhythm, cardiac arrhythmia is investigated.

3.2.2. Supraventricular Arrhythmia

This rhythm originates above the bundle of His. The site of origin of this arrhythmia should be identified whenever possible. When this is not possible, the generic term "supraventricular" will be used.

3.2.3. Presence of Sinus P Wave

3.2.3.1. Sinus Arrhythmia

It is usually physiologic and depends on the autonomic nervous system, being characterized by a variation in PP intervals between 160 ms and 220 ms during sinus rhythm. Phasic variations are related to breathing (frequently seen in children), as opposed to nonphasic variations.

3.2.3.2. Sinus Bradycardia

Sinus bradycardia is a sinus rhythm with HR below 50 bpm.

3.2.3.3. Second-degree Sinoatrial Block

The second-degree exit block of sinus depolarization results in a lack of P wave in a cycle. Type I sinoatrial block is characterized by progressively shorter PP cycles before the block. Type II sinoatrial block shows no differences between PP cycles, and the pause corresponds to 2 previous PP cycles. Sinoatrial block I is not visible on a standard ECG. Third-degree blocks are seen as atrial or junctional escape rhythm.

3.2.3.4. Interatrial Blocks (IAB)

These are conduction delays between the right and left atria, which can be classified as first-degree (P wave duration of 120 ms or longer), second-degree (these patterns are transitory), and third-degree or advanced (P wave duration of 120 ms or longer, biphasic or plus-minus in the inferior wall, related with supraventricular arrhythmias and Bayés syndrome).[18,19]

3.2.3.5. Sinus Tachycardia

Sinus Tachycardia is a sinus rhythm with a HR of 100 bpm or higher.

3.2.4. Absence of P Wave before the QRS

3.2.4.1. Atrial Fibrillation (AFib)

Disorganized atrial electrical activity, with rates ranging from 450 to 700 beats/min and a variable ventricular response. The baseline can be isoelectric, with fine or coarse irregularities, or have a combination of these changes (f-waves). Regular RR intervals indicate atrioventricular (AV) dissociation. The ventricular response of an AFib can be calculated from a 6-s tracing (number of QRS complexes in this period and multiplied by 10). We then have the following possibilities of ventricular response (during resting ECG):

(1) AFib rhythm with slow ventricular response, when HR is ≤ 50 bpm;

(2) AFib rhythm with adequate HR control, when ventricular response is between 60 bpm and 80 bpm;

(3) AFib rhythm with inadequate HR control, when ventricular response is between 90 bpm and 110 bpm;

(4) AFib rhythm with rapid ventricular response, when HR is > 110 bpm.

3.2.4.2. Atrial Flutter

Atrial flutter is an oganized atrial electrical activity (macroreentrant mechanism) across a large area of the right atrium. It is named typical atrial flutter when it runs through (and is dependent on) the cavotricuspid isthmus (CTI). Macroreentry can occur both in the counterclockwise (90% of the cases) or clockwise (10%) directions. In the former, it is named typical atrial flutter and in the latter, it is named REVERSE typical atrial flutter. In a typical atrial flutter, the known F waves have rates of 240 bpm to 340 bpm and a characteristic pattern: a sawtooth aspect, being negative in the inferior leads and generally positive in V1. Varying degrees of AV conduction may occur, and when higher than 2:1, the detection of negative F waves is easier. On the other hand, F waves have higher rates in the reverse atrial flutter (between 340 bpm and 430 bpm). F waves are positive in the inferior leads, and also widened. When it comes to the ECG, it is not possible to differentiate between a REVERSE typical atrial flutter and left atrial tachycardia (originating from the right superior pulmonary vein). The so-called atypical atrial flutter does not go through the CTI. Therefore, this classification includes scar-related atrial tachycardias, atrial tachycardias arising from the inferior vena cava, and reentrant tachycardias originating from the mitral valve annulus, which are all very difficult to diagnose on the ECG (receiving the generic name of atrial tachycardia).

3.2.4.3. Junctional Rhythm

Junctional rhythm is an escape rhythm originating from the AV junction, with QRS which are similar or slightly different from sinus rhythm. This aberrancy is due to a different origin of the stimulus and not to phasic aberrant conduction, which depends on the stimulus being altered by phase 3 (early) or 4 (late) of the action potential. It can happen with no visible P wave on ECG. These "positions" of the P wave are due to the conduction velocities of electrical impulses to the atria and ventricles. By reaching the ventricles first and the atria second, the P wave is located within or after the QRS complex. Junctional escape rhythm is defined when HR is < 50 bpm. Active junctional rhythm is determined when HR > 50 bpm. Finally, tachycardia is defined if HR > 100 bpm.

3.2.4.4. Junctional Extrasystole

Junctional extrasystole is an early ectopic beat originating from the AV junction. There are 3 possible electrocardiographic presentations for this phenomenon:

a) Negative P wave in the inferior leads with a short PR interval;
b) Lack of atrial activity preceding the QRS complex (P wave buried within the QRS);
c) Negative P wave in the inferior leads after the QRS complex;

The QRS complex has similar morphology and duration to the baseline rhythm, although aberrant conduction may occur (see Items 3.2.8.1 and 3.2.8.2).

3.2.4.5. Common atrioventricular nodal reentrant tachycardia (AVNRT)[20]

This type of tachycardia happens within the AV node, and nodal reentry is its electrophysiological mechanism. In ninety percent, one circuit uses a fast pathway (retrograde) and the other uses a slow pathway (anterograde) and it is called common AVNRT. When the QRS is narrow during tachycardia, pseudo S waves can be seen in the inferior leads and an rSr' (pseudo r') morphology can be seen in V1, reflecting atrial activation from AV node to sinus node direction. This retrograde atrial activation, in most cases, occurs within 80 ms of QRS onset (RP < 80 ms). Sometimes, the atrial activation wave is buried within the QRS and is thus not seen on the ECG. There are some similarities between common AVNRT and orthodromic AV reciprocating tachycardia (AVRT). RP interval is used to distinguish them and it will be described next. In cases of common AVNRT with a wide QRS, differential diagnoses must consider monomorphic ventricular tachycardia.

3.2.4.6. Orthodromic AV Reciprocating Tachycardia (AVRT)

This type of reentrant tachycardia uses the normal conduction system in the anterograde direction and an accessory pathway in the retrograde direction. In general, the QRS is narrow and the P wave is retrograde, being more commonly located in the ST-segment. The P wave can present diverse morphologies, according to the location of the accessory pathway. The RP interval is > 80 ms.

3.2.5. Occurrence of a Non-sinus P Wave Before the QRS Complex

3.2.5.1. Ectopic Atrial Rhythm

Ectopic atrial rhythm corresponds to atrial activity occurring in a different location from the anatomic region of the sinus node. Thus, the P wave has a different morphology (polarity) from that characterizing sinus rhythm.

3.2.5.2. Multifocal Atrial Rhythm

It is originated from multiple atrial foci, with an HR < 100 bpm, recognized on the ECG by the presence of at least 3 different P wave morphologies and 3 different PR intervals. PP and PR intervals are frequently variable, and one P wave is seen for each QRS complex; blocked P waves may occur.

3.2.5.3. Junctional Rhythm

As mentioned on Item 3.2.4.3, it is characterized by negative P waves in the D2, D3, and aVF leads, in addition to a short PR interval. Junctional escape rhythm is defined when HR < 50 bpm. Active junctional rhythm is determined when HR is > 50 bpm; junctional tachycardia is defined when HR > 100 bpm.

3.2.5.4. Delayed Atrial Beat

A delayed atrial beat can be considered a "replacement" atrial beat. It is frequently seen when a temporary interruption of normal sinus automaticity occurs as a consequence of sinus node inhibition / failure. It can be from right or left atrium, usually late, and it has a P wave of non-sinus morphology.

3.2.5.5. Premature Atrial Complex (PAC)

Premature atrial complex is an early atrial ectopic beat. It may recycle the baseline PP interval.

3.2.5.6. Blocked or Non-conducted PAC

Ectopic beat that originates from the atrium sometimes cannot be conducted to the ventricles, thus a premature P wave without a QRS complex can be seen. There are two main causes for the lack of conduction: a very early premature atrial complex that reaches the AV node within its absolute refractory period, or a previous His-Purkinje conduction system disease. PACs not conducted to ventricles may lead to bradycardia.

3.2.5.7. Atrial Tachycardia

Atrial tachycardia is an atrial rhythm that originates from a region other than the sinus node (characterized by a P wave of a different morphology) with an atrial rate > 100 bpm. Variable AV conduction is common.

3.2.5.8. Multifocal Atrial Tachycardia

It has the same characteristics of multifocal atrial rhythm with an atrial rate > 100 bpm.

3.2.5.9. Uncommon AV Nodal Reentrant Tachycardia (AVNRT)

Its location is exactly the same to common AVNRT (3.2.4.5), but the circuit activation happens in the reverse direction (10%). Ventricular activation occurs through the fast pathway (anterograde) and the atrial activation occurs through the slow pathway (retrograde) and it is called uncommon AVNRT; this is why retrograde atrial activation happens later, with a characteristic longer RP interval than PR. Therefore, uncommon AVNRT is not a differential diagnosis for common AVNRT or orthodromic AVRT.

3.2.5.10. Permanent Junctional Reciprocating Tachycardia (Coumel Tachycardia)

Permanent junctional reciprocating tachycardia is a supraventricular tachycardia that uses a particular accessory pathway (with an exclusive and decremental retrograde conduction). It is characterized by tachycardia with a long RP interval and its differential diagnoses include those described on Items 3.2.5.7 and 3.2.5.9.

3.2.6. Pauses

Pauses are defined by a lack of P wave and QRS complex with an interval > 1.5 s. Clinical significance is considered when longer than 2 s. The occurrence of pauses may be related to sinus arrest, non-conducted PAC, sinoatrial block, and AV block.

3.2.6.1. Sinus Arrest

Sinus arrest corresponds to a pause in sinus activity > 1.5 times the basic PP cycle.

3.2.6.2. Sinus Node Dysfunction

The inability of the sinus node to maintain HR above the physiological need for the present situation is named sinus node dysfunction. On the ECG, this abnormality (or dysfunction) encompasses sinus pause, sinoatrial block, sinus bradycardia, replacement rhythms, AFib, atrial flutter, and tachy-brady syndrome, among other disorders.[21]

3.2.7. Classification of Supraventricular Tachycardias Based on the RP Interval

The RP interval is a commonly used measure for characterizing supraventricular tachycardia. Its measurement is done from the beginning of the QRS complex to the following P wave (RP). Depending on the position of this P wave, the RP interval can be short (P wave before the midpoint of 2 consecutive QRS) or long (P wave located after the midpoint of 2 QRS). Therefore, paroxysmal supraventricular tachycardias can be divided into:

(a) Short RP (normally up to 120–140 ms), as observed in common AVNRT and orthodromic AVRT;

(b) Long RP, as observed in atrial tachycardia, uncommon AVNRT, and Coumel tachycardia (permanent junctional reciprocating tachycardia).[22]

3.2.8. Supraventricular Arrhythmias with a Wide QRS Complex

3.2.8.1. Aberrant Conduction

Supraventricular stimulus with hampered propagation in the conduction system, generating a QRS complex of different morphology when compared to the baseline QRS complex; it may resemble a bundle-branch block pattern, a fascicular block pattern, or both.

3.2.8.2. PAC with Aberrant Conduction

PAC with aberrant conduction is an early P wave followed by a QRS complex with a bundle-branch block pattern or fascicular block pattern, or both.

3.2.8.3. Supraventricular Tachycardia (SVT) with Aberrant Conduction

It is a generic denomination for the aforementioned tachycardias presenting with aberrant conduction.

3.2.8.4. Antidromic AV Reentrant Tachycardia

Reentrant tachycardia uses an accessory pathway in an anterograde direction and the conduction system in a retrograde direction. The aberrant QRS is characterized by the presence of ventricular pre-excitation. Differential diagnoses must consider ventricular tachycardia. The presence of 1:1 retrograde atrial depolarization favors accessory pathway conduction involvement and AV dissociation is diagnostic of ventricular tachycardia.

4. AV Conduction

4.1. Defining a Normal AV Conduction

The period from the beginning of the P wave until the beginning of the QRS complex determines the PR interval, when there is atrial activation and physiological delay in the AV junction and/or the His-Purkinje system. Its duration is 120–200 ms, considering a maximum HR of 90 bpm. The PR interval varies according to HR and age.

4.1.1. Delayed AV Conduction[23-26]

Before studying them it is essential to remember the normal decremental conduction related to the AV node, which is an important electrophysiological characteristic of the AV node. This property refers to a reduction in the conduction velocity of the electrical impulse in the AV node, and it can be estimated through the PR interval on conventional ECG. This interval is considered normal in adults when between 120 ms and 200 ms, depending on age and HR.

Delayed AV conduction occurs when atrial impulses have a delay or fail to reach the ventricles.

Anatomically, abnormal AV node delayed conduction can be located in the AV node itself (nodal block), in the His-Purkinje bundle (intra-His block), or below this structure (infra-His block). Nodal conduction delays normally present narrow QRS complexes (< 120 ms) and have a good prognosis; these are expressed by an increased PR interval. On the other hand, intra- and infra-His delays usually have wide QRS complexes and worse disease progression. In these cases, a normal PR interval is uncommon.

We highlight that the AV node is greatly influenced by the autonomic nervous system; therefore, in situations where parasympathetic tone prevails (during sleep, in athletes), first-degree AV block and/or type I second-degree AV block may be seen, even without AV node lesion.

4.1.1.1. First-degree AV Block

In this case, the PR interval is > 200 ms in adults, for an HR between 50 bpm and 90 bpm.

4.1.1.2. Type I Second-degree AV Block (Mobitz I)

In this case, AV conduction gradually slows down (Wenckebach phenomenon). Typically, there is a progressive increase in the PR interval; these increases are gradually shorter until AV conduction is blocked and a sinus beat cannot be conducted. Therefore, there is a gradual increase in the PR interval with simultaneous shortening of RR intervals until a P wave is blocked. This cycle may be repeated for variable periods, where the PR interval immediately after the blocked beat should be the smallest, and the next interval should have the largest proportional increase when compared to the ones that follow. The ratios for this block may vary such as 5:4; 4:3; 3:2 conduction.

4.1.1.3. Type II Second-degree AV Block (Mobitz II)

An abrupt failure of AV conduction happens in this situation. There is 1:1 AV conduction with a fixed PR interval, then a sudden P wave is blocked followed by new 1:1 AV conduction with a similar PR interval to the previous ones. The block is located at an intra/infra-His Purkinje site.

4.1.1.4. 2:1 Second-degree AV Block

It is characterized by alternating conducted and blocked sinus P waves. Most of 2:1 AV blocks are located at an intra/infra His-Purkinje site. The diagnosis of non-conducted PAC should be excluded.

4.1.1.5. Advanced or High-grade Second-degree AV Block

AV conduction happens in less than 50% of all sinus beats in a 3:1 ratio, 4:1 ratio, or higher. In general, AV conduction is noticed by a constant PR interval for each beat conducted. Most of them are located at an intra/infra His-Purkinje site. Junctional escapes may occur.

4.1.1.6. Third-degree or Complete AV Block

Stimuli of sinus origin are unable to reach and depolarize the ventricles; a focus below the blocked region, therefore takes ventricular rhythm control. As a result, there is no relationship between atrial and ventricular electrical activities (AV dissociation), which is translated on the ECG as P waves unrelated with QRS complexes. The rate of the sinus rhythm is higher than that of the ventricular escape rhythm. Third-degree AV block may be intermittent or permanent. AV Blocks originating from the supra-Hisian region may present escapes that resemble the baseline ECG. On the other hand, an infra-Hisian origin shows large QRS complexes.

4.1.1.7. Paroxysmal AV Block

Paroxysmal AV block is a series of a sudden, consecutive blocked P waves.

4.1.2. Ventricular Pre-excitation[27-30]

In patients with ventricular pre-excitation, muscle fibers remain within the fibrous tissue and act as accessory pathways for conducting the electrical impulse between the atria and ventricles. These additional pathways may be located in any part of the AV annulus (Figure 4.1). The

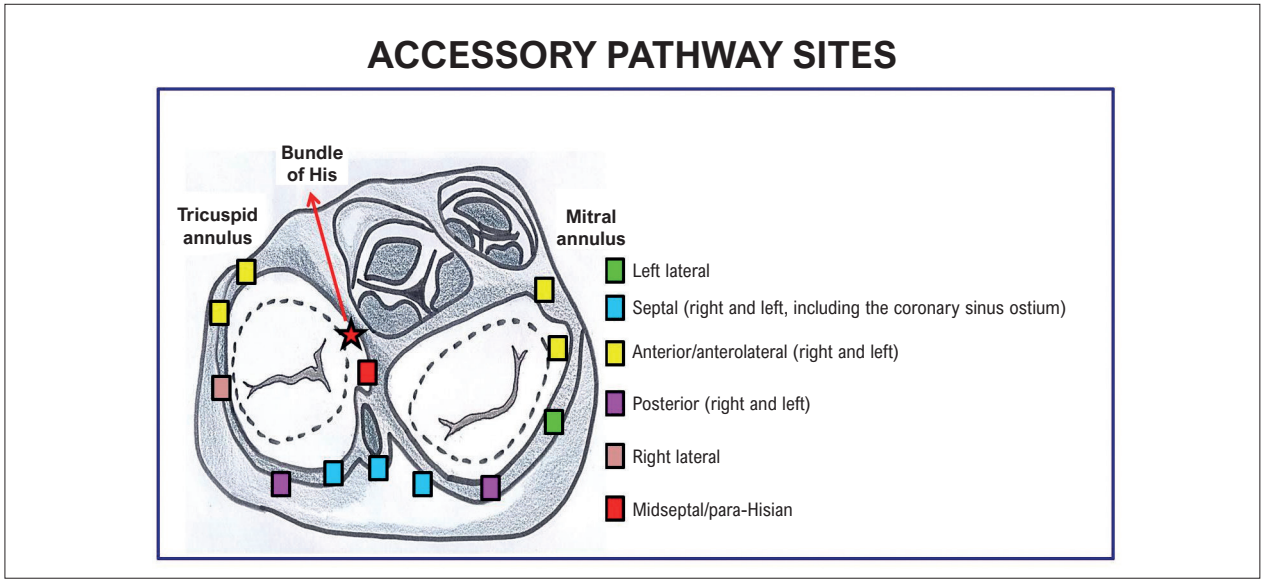

Figure 4.1 – *Accessory pathway sites on the tricuspid and mitral valve annuli*

classical pattern is characterized by a PR interval < 120 ms during sinus rhythm in adults and < 90 ms in children (varying with age and HR); a slurring (delta wave) on the initial portion of the QRS complex close to the P wave; QRS duration > 120 ms in adults and > 90 in children; and secondary changes in ST-segment and T wave. These ECG findings with the diagnosis of symptomatic paroxysmal SVT characterizes Wolff-Parkinson-White (WPW) syndrome. The accessory pathway can be anatomically located using 12-lead ECG. Left-sided pathways are the most common (50% of the cases), followed by posteroseptal pathways (25%), right-sided pathways (15%), and anteroseptal pathways (10%). Anterior regions of the AV annulus are superior; this way, local accessory pathways determine activation in the superoinferior direction, with a positive delta wave in the inferior leads. On the other hand, the basal posterior region is inferior, with a consequently negative delta wave in the inferior leads. Differential diagnosis should occasionally be performed with a short PR interval and no delta wave, which are present in Lown-Ganong-Levine syndrome,[31] and a normal PR interval with ventricular pre-excitation, which is present in fasciculoventricular pathways such as the Mahaim variant.[32]

Accessory pathways can be divided when the QRS complex is predominantly positive (R) in V1 and V2, which indicates a left accessory pathway. When the QRS complex is negative (QS or rS), the accessory pathway is located to the right. Left-sided accessory pathways appear on the ECG as a negative delta wave in the D1 and/or aVL leads, a positive delta wave in the D2, D3, and aVF leads, and in V1 and V2. Right-sided accessory pathways present a positive delta wave in D1, D2, and aVL leads, and a normally negative one in D3 and aVR leads, as well as V1. The frontal plane QRS axis is shifted leftwards. On the other hand, posteroseptal pathways show a negative delta wave in D2, D3, and aVF. The importance of recognizing the locations of anteroseptal and midseptal pathways concerns their proximity to the bundle of His, which is associated with a higher risk during catheter ablation. In both locations, the delta wave is positive in the D1, D2, and aVL leads, while it is negative in D3 and aVR and positive/isoelectric in aVF, with a normal QRS axis. In 80% of the cases, the R/S transition occurs in V2.[32]

The analysis of QRS complexes in V1 and V2 will define whether they are located to the right or to the left.[33] Several algorithms can be used to localize the accessory pathway based either on the polarity of the QRS complex or the accessory pathway.[34-36] It is important to note that diseases such as hypertrophic cardiomyopathy and familial forms of glycogen storage disease (Fabry disease) may mimic the presence of pre-excitation.

4.1.3. Other Mechanisms of Changes in the AV Relationship (normal AV node conduction)

4.1.3.1. AV Dissociation

AV dissociation is caused by the following mechanisms: replacement, interference, AV block, and arrhythmia.[37] Two dissociated rhythms take place, one is of atrial origin (usually a sinus rhythm with a regular PP interval), and the other is of junctional or ventricular origin, also with a regular RR interval. Both foci rates are similar (isorhythmic dissociation). Ventricular rhythm may be hyperautomatic.

4.1.3.2. Retrograde Atrial Activation

Retrograde atrial activation can be observed when the activation is originated from junctional or ventricle sites stimulation. There is a retrograde conduction, usually through the AV node or an accessory pathway. It is mandatory that a negative P wave after a QRS complex is found in the inferior leads.

5. Analysis of Ventricular Activation

5.1. Normal Ventricular Activation

5.1.1. Definition of a Normal QRS

The QRS complex is considered normal when its duration is < 120 ms in all leads and its amplitude is 5–20 mm in the frontal plane leads and 10–30 mm in the precordial leads, with a normal orientation of the electrical axis.[38,39]

5.1.2. Normal Electrical Axis in the Frontal Plane

The normal limits of the frontal plane QRS axis are normally -30º and +90º.

5.1.3. Normal Ventricular Activation in the Transversal Plane

It is expected a smooth transition of the typical rS morphology in V1 to the qR pattern in V6. So, from V1 to V6 there is a progressive increase of the r wave and decrease of the S wave amplitudes. In general, intermediate RS pattern (transition zone) occurs in V3 or V4.[16]

5.1.4. Analysis of Ventricular Rhythm Alterations

5.1.4.1. Definition of Cardiac Arrhythmia

Cardiac arrhythmia can be defined as a change in frequency, formation, and/or conduction of the electrical impulse across the myocardium.[17]

5.1.4.2. Ventricular Arrhythmia

Ventricular arrhythmia is an arrhythmia that originates below the bundle of His, usually seen with a wide QRS.

5.1.4.3. Analysis of Ventricular Arrhythmias

5.1.4.3.1. Premature Ventricular Complex (PVC)[40]

PVC is a beat that originates in the ventricle before it is expected, in most cases with a postextrasystolic pause and recycling the RR interval. In the absence of a pause, it is named interpolated PVC. PVCs usually have a QRS > 120 ms. Exceptionally they can be < 120 ms (PVCs originating from the ventricular septum or close to the conduction system). Regarding their morphology, they can be monomorphic (the same morphology at the same lead) or polymorphic (two or more morphologies at the same lead). According to their recurrence, they can be classified as isolated, paired, bigeminal, trigeminal, quadrigeminal, or concealed.

5.1.4.3.2. Ventricular Escape Beat

Ventricular escape beat is characterized when ventricular depolarization occurs late. It appears due to the temporary inhibition of anatomically higher rhythms.

5.1.4.3.3. Ventricular Escape Rhythm – Idioventricular Rhythm

An idioventricular rhythm originates in the ventricles, with a HR < 40 bpm, and replaces anatomically higher rhythms that were inhibited or blocked.

5.1.4.3.4. Accelerated Idioventricular Rhythm

An accelerated idioventricular rhythm originates in the ventricle (wide QRS), with HR > 40 bpm (50–130 bpm, more frequently 70–85 bpm) as a result of increased automaticity. It is not a subsidiary rhythm and competes with the baseline rhythm of the heart. It is usually self-limited and associated with ischemic myocardial disease (reperfusion/ischemia).[41]

5.1.4.3.5. Ventricular Tachycardia (VT)

VT is a cardiac rhythm that presents three or more successive beats at a rate > 100 bpm.

5.1.4.3.5.1. Monomorphic VT

Monomorphic VT is characterized by uniform QRS morphology in the same lead.

5.1.4.3.5.2. Polymorphic VT

Polymorphic VT is a fast ventricular rhythm with 3 or more different wide QRS morphologies.[42]

5.1.4.3.5.3. Torsades des Pointes (TdP)

TdP is a wide QRS polymorphic tachycardia with a QRS that "rotates" around the baseline (twisting motion). It is normally preceded by long-short cycles (sinus beat-PVC) and is due to a long QT interval in sinus rhythm, which can be congenital or secondary to medications, electrolyte imbalance, or certain heart diseases.[43]

5.1.4.3.5.4. Bidirectional VT[44]

Tachycardia of ventricular origin where the right bundle-branch (or rarely the left bundle-branch) is constantly blocked, while the anterosuperior and posteroinferior divisions of the left bundle-branch are blocked in an alternating mode, beat by beat. In the frontal plane, a beat with positive QRS is alternated with a beat with negative QRS, generating the bidirectional aspect. This type of arrhythmia is related with digitalis toxicity, severe myocardial disease due to advanced cardiomyopathy, and cases with no structural heart disease such as catecholaminergic polymorphic ventricular tachycardia; it usually precedes polymorphic VT.

5.1.4.3.5.5. VT Length

Sustained: a tachycardia that lasts > 30 s or is associated to symptoms of hemodynamic instability. Non-sustained: a tachycardia that lasts < 30 s and there is no symptoms of hemodynamic instability.

5.1.4.3.6. Fusion Beat

Fusion beat corresponds to a beat that is generated from two sites: an activation from the ventricle and another from

the atria. Electrocardiographically, it presents a P wave followed by a wide QRS (a hybrid morphology between a supraventricular beat and a beat of ventricular origin). Fusion beats are seen in the following situations: ventricular pre-excitation, VT, parasystole, and some PVCs.

5.1.4.3.7. Supraventricular Capture Beat During Idioventricular Rhythm

This is a beat originating from the atrium that can overcome the anatomical or functional conduction block in the AV junction and completely or partially depolarize the ventricle; in case of partial depolarization, a fusion beat occurs.

5.1.4.3.8. Ventricular Parasystole

Ventricular parasystole corresponds to the beat that originates from a ventricular site and competes with the sinus rhythm (a parallel pacemaker with a permanent entry block and occasional exit block). It is electrocardiographically visible for its own rate, fusion beats, periods of interectopic intervals with a multiple denominator, and variable coupling intervals.[45]

5.1.4.3.9. Ventricular Fibrillation (VF)

VF is characterized by bizarre and chaotic waves, with variable amplitude and ventricular rate. It corresponds to one of the clinical presentations of cardiac arrest. It can be preceded by VT or TdP that degenerated into VF.

5.1.4.4. Criteria for Differentiating Wide QRS Complex Tachycardias[46-57]

Most wide QRS complex tachycardias (80%) are of ventricular origin, and the presence of structural heart disease reinforces this possibility. AV dissociation, fusion beats, and/or capture beats (with different QRS) strongly suggest a VT diagnosis. Some algorithms, such as those by Brugada and by Vereckei[48] (widely known) and others, help differentiate those wide QRS tachycardias (Table 5.1).[49-54] Figures 5.1 and 5.2 show ECG features of Brugada and Steuer criteria for diagnosing VT.

6. Cardiac Chambers hypertrophy

6.1. Atrial hypertrophy

6.1.1. Left Atrial Hypertrophy

- P wave duration: ≥ 120 ms (D2 lead), sometimes a P wave with two peaks (right and left atrial components ≥ 40 ms);
- Morris Index: P wave with an increased negative component in V1 lead (negative component of ≥ 1 mm²).

Table 5.1 – Electrocardiographic criteria for differentiating supraventricular tachycardia with aberrant conduction from ventricular tachycardia

Autor	Wellens[49] (1978)	Brugada[46] (1991)	Steuer[51] (1994)	Vereckei [54] (2008)	Pava[55] (2010)	Jastrzebski[56] or VT Escore (2016)	Santos Neto[57] (2021)
Findings and steps of analysis for each algorithm	AV dissociation	Absence of RS in the precordial leads	Predominantly negative QRS complexes from V4 to V6	Initial R wave in the aVR lead	Interval from the onset of the QRS complex to the apex of the R wave ≥ 50 ms in the D2 lead	Dominant R wave in the V1 lead	Predominantly negative polarity in 4 leads: DI, D2, V1, and V6
	QRS complex > 140 ms (RBBB)	RS ≥ 100 ms	QS complex in one or more leads from V2 to V6	Initial r or q > 40 ms		Initial r wave > 40 ms in V1 or V2	Predominantly negative polarity in 3 out of 4 leads
	QRS complex > 160 ms (LBBB)	AV dissociation	AV dissociation	Notch on the descending limb of a predominantly negative QRS		Notch on the S wave in the V1 lead	Predominantly negative polarity in 2 out of 4 leads
	QRS axis beyond -30º	Morphological criteria		Relação Vi/Vt ≤ 1		Initial R wave in the aVR lead	
	Mono or biphasic QRS in V1 (RBBB)					Interval from the onset of the QRS complex to the apex of the R wave ≥ 50 ms in the DII lead	
	QR or QS in V6 (LBBB)					Absence of RS in the precordial leads	
						AV dissociation	

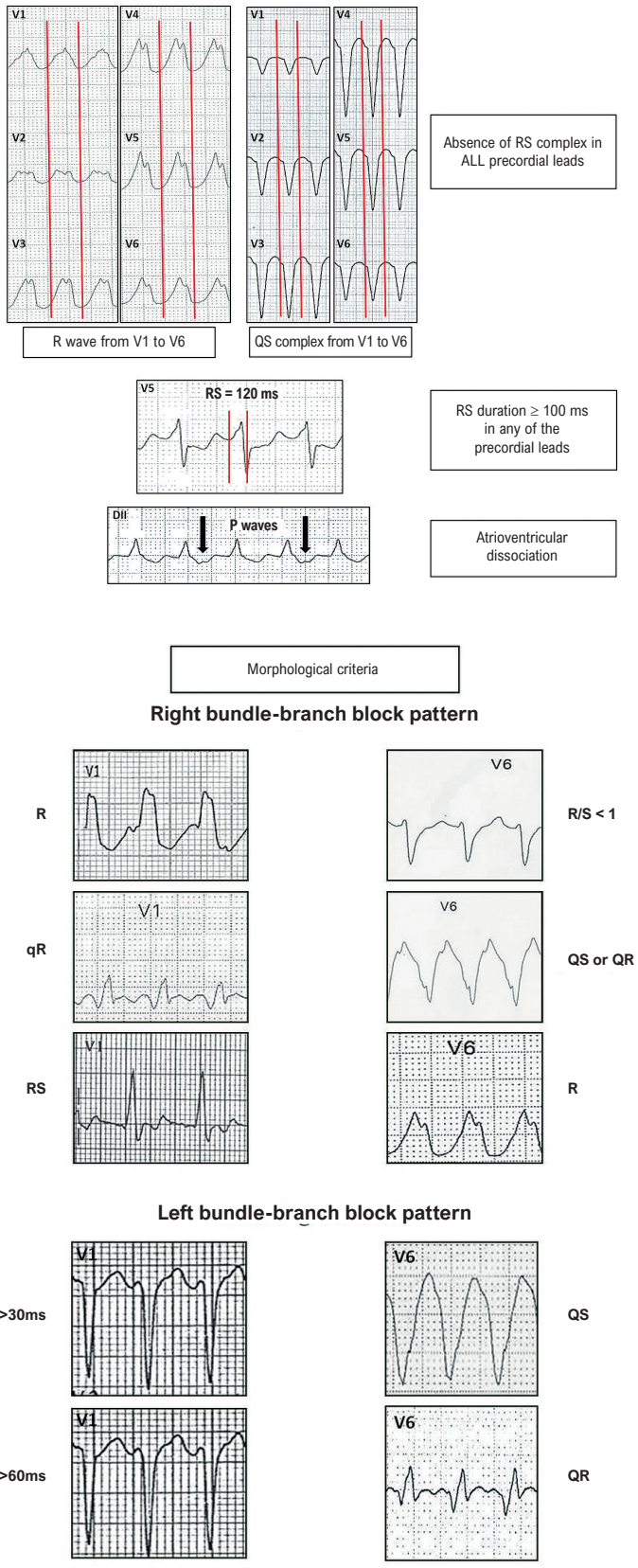

Figure 5.1 – *Examples of Brugada criteria for diagnosing ventricular tachycardia.*

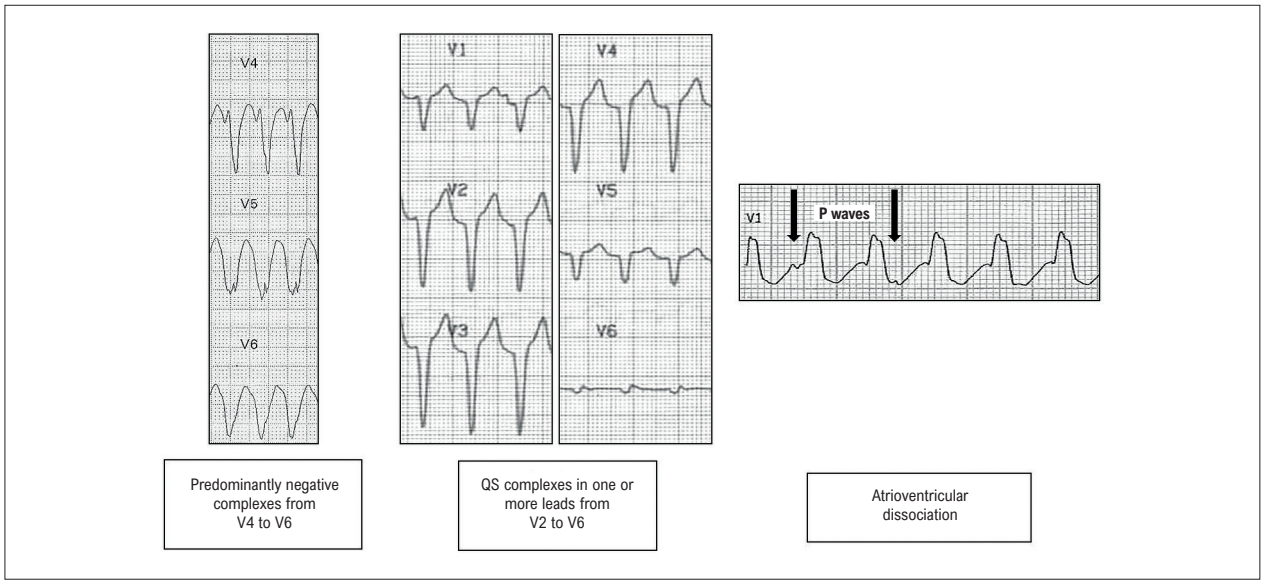

Figure 5.2 – *Steuer criteria for diagnosing ventricular tachycardia.*

6.1.2. Right Atrial Hypertrophy

Isolated right atrial hypertrophy is rare but is frequently associated with right ventricular hypertrophy. ECG findings:

- P waves amplitude: > 0.25 mV or 2.5 mm (D2 lead);
- V1 lead: positive initial portion > 0.15 mV or 1.5 mm;
- Some indirect signs: Peñaloza-Tranchesi (low-voltage QRS complex in V1 with an abruptly QRS amplitude increase in V2) and Sodi-Pallares (QR, Qr, qR, or qRS complexes in V1).

6.1.3. Biatrial hypertrophy

There are both characteristics of right and left atrial hypertrophy.

6.1.4. Left Ventricular Hypertrophy[58-68]

Although the echocardiogram has a high accuracy for identifying LVH, the ECG has important prognostic significance when abnormal. The currently used criteria include:

6.1.4.1. Romhilt-Estes Criteria[66]

According to these criteria, LVH is present when 5-point score is achieved. Limitations: LBBB, RBBB, atrial flutter, atrial tachycardia, atrial fibrillation.

a) *3 points*: increased QRS amplitude (≥ 20 mm in the frontal plane and/or ≥ 30 mm in the transversal plane); strain pattern (free of digitalis influence); Morris index;

b) *2 points*: left QRS axis deviation beyond -30º;

c) *1 point*: increased ventricular activation time (VAT) or intrinsicoid deflection > 40 ms; increased QRS duration (> 90 ms) in V5 and V6; and a strain pattern under digitalis influence.

6.1.4.2. Sokolow-Lyon Index[60]

LVH is considered when the sum of S wave amplitude (V1 lead) + R wave amplitude (V5/V6 lead) is ≥ 35 mm. In young people, this threshold can be 40 mm. This index should not be used in athletes.

6.1.4.3. Cornell Index[58]

LVH is considered when the sum of R wave amplitude (aVL lead) + S wave amplitude (V3 lead) is ≥ 28 mm in men and ≥ 20 mm in women.

6.1.4.4. Peguero-Lo Presti[67,68]

LVH is considered when the sum of the deepest S wave in all 12 leads + S wave in V4 is ≥ 28 mm in men and ≥ 23 mm in women.

6.1.4.5. Changes in Ventricular Repolarization

A flat T wave in the left leads (D1, aVL, V5, and V6), or a strain pattern (ST depression ≥ 0.5 mm with a negative and asymmetrical T wave).

6.1.5. Right Ventricular Hypertrophy[69-72]

6.1.5.1. QRS Axis

A QRS axis shift located to the right beyond +110° - frontal plane.

6.1.5.2. Tall R Wave

A tall R wave in V1 and V2 (R/S ratio ≥ 1), and deep S waves in the opposite leads (V5 and V6).

6.1.5.3. qR or qRs Morphology

A qR or qRs morphology in V1 (or V1 and V2) is one of the most specific signs of RVH. It indicates an increased intraventricular pressure (systolic).

6.1.5.4. rsR' Morphology

A triphasic pattern (rsR'), with prominent R wave in the right precordial leads V1 and V2. It indicates an increased intraventricular pressure (diastolic) with an enlarged chamber.

6.1.5.5. Ventricular Repolarization

ECG can present a strain pattern (ST-segment depression with a negative T wave) in the right precordial leads (V1, V2, and, sometimes, V3).

6.1.5.6. Seattle Criteria for RVH

RVH is considered when the sum of R wave in V1 + S wave in V5–V6 > 10.5 mm (and right axis deviation > 120º).

6.1.6. Biventricular Hypertrophy

a) Frontal plane QRS axis deviated to the right, associated with voltage criteria for LVH;
b) An ECG typical of RVH, associated with one or more of the following features:
   b.1) deep Q waves in V5 and V6 and in inferior leads;
   b.2) Increased R wave voltage in V5 and V6;
   b.3) Sokolow-Lion criterion for LVH (S in V1-V2 + R in V5-V6);
   b.4) Intrinsicoid deflection in V6 ≥ 40 ms.
c) Large biphasic QRS complexes, with R-S > 50 mm, in mid-precordial leads (V2 to V4 - Katz-Wachtel phenomenon).

6.1.7. Differential Diagnoses for Increased QRS Amplitude[73]

Increased QRS amplitude is most commonly seen with ventricular hypertrophy. However, QRS may be increased in normal individuals in the following situations:

a) Children, adolescents, and young adults;
b) Slender individuals;
c) Athletes;
d) Women who underwent mastectomy surgery;
e) Vagotonia.

7. Analysis of Intraventricular Blocks (Conduction Delay)

7.1. Intraventricular Blocks[74,75]

Although the concept of "bundle-branch block" is well established in the literature, various degrees of delays in the intraventricular propagation of electrical impulses can occur, leading to changes in the morphology and duration of the QRS complex. These changes in intraventricular conduction may be fixed or intermittent, and also rate-dependent. These blocks may be caused by structural changes in the His-Purkinje conduction system or in the ventricular myocardium (necrosis, fibrosis, calcification, infiltrative diseases, or vascular insufficiency), or functional changes (due to the relative refractory period of part of the conduction system), generating aberrant intraventricular conduction.

7.1.1. Left Bundle-Branch Block (LBBB)[76,77]

a) Wide QRS ≥ 120 ms (as an essential condition); classical manifestations of LBBB, however, width ≥ 130 ms in women and ≥ 140 ms in men);
b) Absence of a q wave in D1, aVL, V5, and V6; variants may have a q wave in aVL only.
c) Wide R waves, with notches and/or mid-terminal slurring in D1, aVL, V5, and V6;
d) Delayed r wave progression from V1 to V3 (sometimes with QS complexes);
e) Intrinsicoid deflection in V5 and V6 ≥ 50 ms.
f) QRS axis between -30º and +60º;
g) ST-segment depression and asymmetrical T wave opposed to the mid-terminal delay.

7.1.1.1. LBBB in Association with LVH[78-79]

The electrocardiographic diagnosis of LVH in association with LBBB is not trivial due to changes in the QRS complex inherent to the LBBB. Studies show variable results regarding the accuracy of electrocardiographic criteria for LVH:

a) Left atrial hypertrophy;
b) QRS duration > 150 ms;
c) R wave in aVL > 11 mm;
d) S waves in V2 > 30 mm and in V3 > 25 mm;
e) QRS axis beyond -40º;
f) Sokolow-Lyon Index ≥ 35 mm.

7.1.1.2. LBBB in Association with RVH[80] (at Least 2 out of 3 Criteria)

a) Low voltage in the precordial leads;
b) Prominent R wave in aVR;
c) R/S ratio < 1 in V5.

7.1.2. Right Bundle-Branch Block (RBBB)[81,82]

a) Wide QRS ≥ 120 ms as an essential condition;
b) Slurred S waves in D1, aVL, V5, and V6;
c) qR waves with slurred R wave in aVR;
d) rSR' or rsR' with thickened R' in V1;
e) Variable QRS axis, usually shifted to the right in the frontal plane;
f) Asymmetrical T wave opposed to the delay of the end of the QRS complex.

7.1.2.1. End Conduction Delay

This expression may be used when there is a subtle conduction disturbance in the right bundle-branch. It can be a normal variant and sometimes is also called incomplete right bundle-branch block.

7.1.3. Left Fascicular Blocks[83-92]

A conduction delay that affects one of the left bundle-branch divisions may generate an upward/leftward shift (LAFB) or a downward/rightward shift (LPFB) or an anterior shift (left anteromedial fascicular block) of the QRS axis.

7.1.3.1 Left Anterosuperior Fascicular Block (LAFB)[83-87]

a) QRS axis ≥ -45º;
b) rS complex in D2, D3 and aVF with an S3 greater than S2; QRS duration < 120 ms;
c) S wave amplitude ≥ 15 mm in D3 (or equivalent area);
d) qR complex in D1 and aVL with an intrinsicoid deflection time ≥ 50 ms or qRs complex with a minimal "s" wave in D1;
e) qR complex in aVL with slurred R wave;
f) Slow r wave progression from V1 to V3;
g) Presence of S wave from V4 to V6.

7.1.3.2. Left Anteromedial Fascicular Block[88-90]

a) qR complex from V1 to V4.
b) ncreasing R wave from V1 to V3 (≥ 15 mm) and decreasing QRS complex amplitude from V4 to V6;
c) QRS duration < 120 ms;
d) No deviation of the frontal plane QRS axis;
e) T waves generally negative in the right precordial leads.

All the mentioned criteria are valid in the absence of RVH, septal hypertrophy, or old lateral myocardial infarction.

7.1.3.3 Left Posteroinferior Fascicular Block (LPFB)[83-85,91,92]

a) Frontal plane QRS axis shifted to the right > +90º;
b) qR complex in D2, D3 and aVF with R3 > R2 and an intrinsicoid deflection > 50 ms;
c) R wave in D3 > 15 mm (or equivalent area);
d) Intrinsicoid deflection duration increased in aVF, V5–V6 ≥ 50 ms;
e) rS complex duration < 120 ms in D1; slower R wave progression may occur from V1–V3;
f) S wave from V2 to V6.

All these criteria are valid in the absence of a slender body type, RVH, and old lateral myocardial infarction.[80,91]

7.1.4. Right Fascicular Blocks[82]

7.1.4.1. Right Superior Fascicular Block

a) rS complex in D2, D3, and aVF with S2 > S3 (differentiating it from the LAFB);
b) Rs complex with an S wave > 2 mm in D1, rS complex duration < 120 ms, rS complex in D1 or D1, D2, and D3 (S1, S2, S3);
c) Slurred S waves in V1–V2/V5–V6 or, eventually, rSr' complex in V1 and V2;
d) qR complex with slurred R wave in aVR.

7.1.4.2. Right Inferior Fascicular Block

a) R wave in D2 > R wave in D3;
b) rS complex duration < 120 ms in D1;
c) Frontal plane QRS axis shifted to the right > +90º;
d) Slurred S waves in V1–V2/V5–V6 or, eventually, rSr' complex in V1 and V2;
e) qR complex with slurred R wave in aVR.

Given the difficulty in recognizing right fascicle blocks, the term "intraventricular end conduction delay" may be used.

7.1.5. Bundle-Branch and Fascicular Blocks Association[93]

7.1.5.1. LBBB in Association with LAFB

An LBBB with a frontal plane QRS axis shifted to the left, beyond -30º, suggests the presence of LAFB.

7.1.5.2. LBBB in Association with LPFB

An LBBB with a frontal plane QRS axis shifted downwards and to the right, beyond +60º, suggests an association with LPFB, RVH, or congenital heart disease.

7.1.5.3. RBBB in Association with LAFB

An RBBB with a frontal plane QRS axis shifted to the left, beyond -30º, suggests the presence of LAFB.[94,95]

7.1.5.4. RBBB in Association with LPFB

An RBBB with a frontal plane QRS axis shifted downwards and to the right, beyond +120º, suggests an association with LPFB.

7.1.5.5. RBBB in Association with LAFB and Left Anteromedial Fascicular Block

RBBB in association with LAFB and left anteromedial fascicular block follows the same bundle-branch and fascicular block criteria described above.

7.1.5.6. LAFB in Association with Left Anteromedial Fascicular Block

LAFB and left anteromedial fascicular block follows the same fascicular block criteria described above.

7.1.5.7. Masquerading Bundle-Branch Block[96,97]

RBBB pattern in V1 (R or rR' complex) and an LBBB pattern in the frontal plane leads with LAFB. The S wave in D1 is normally absent or below 1 mm. In the presence of these associations, axis deviations are more prominent.

7.1.6. Special Situations Involving Intraventricular Conduction

7.1.6.1. Peri-infarction Conduction Block[98]

QRS complex duration is increased in the presence of an abnormal Q wave due to myocardial infarction (inferior or lateral leads). QRS complex final portion is increased (QR complex).

7.1.6.2. Peri-ischemia Block[98,99]

Peri-ischemia block occurs when there is a transient increase in QRS complex duration with ST-segment elevation (acute phase).

7.1.6.3. QRS Complex Fragmentation (fQRS)[99,100]

Presence of notches in the R or S waves in 2 contiguous leads in the absence of bundle-branch block. With a narrow QRS, notches are more clearly seen in the inferior leads. With bundle-branch block more than 2 notches are needed. This diagnosis should be differentiated from end conduction delays when the notch appears in the S wave in V1 and V2. The more leads with fragmentation are observed, the worse the prognosis.

7.1.6.4. Atypical LBBB[101]

In a patient with previous LBBB with a new infarction, there are deep and wide Q waves, a QS complex pattern in V1–V4, and QR complex in V5–V6, with QRS fragmentation.

7.1.6.5 Parietal or Purkinje/Muscle or Focal Intraventricular Block[102]

This dromotropic disturbance is located between the Purkinje fibers and the muscle. It is seen in severe hypertrophies and cardiomyopathies. May be associated with LAFB or LVH, and the duration of the QRS complex is ≥ 120 ms, without LBBB morphology or LBBB with LAFB morphology.

8. Analysis of the ECG In Coronary Heart Disease

It is important to highlight that a normal ECG does not exclude the presence of a coronary event. Specific clinical guidelines for acute coronary syndromes should be followed.[103,104]

8.1. Diagnostic Criteria for Myocardial Ischemia[105]

8.1.1. Presence of Ischemia

a) *Hyperacute phase* – peaked and symmetrical T wave as the initial presentation;

b) *Subendocardial ischemia* – positive, symmetrical, and peaked T wave;

c) *Subepicardial ischemia* – negative, symmetrical, and peaked T wave. This alteration is currently attributed to a pattern of reperfusion or edema instead of real ischemia of the subepicardial region.[106]

8.1.2. Circumferential or Global Ischemia[107,108]

A peculiar situation during an angina episode, with ST-segment depression in 6 or more leads, particularly in V4–V6, along with negative T waves associated with an ST-segment elevation > 0.5 mm in aVR.

8.1.3. Secondary Changes

Secondary changes in the T wave are those not fitting within the definition of ischemic waves, especially due to asymmetry and the presence of other diagnostic characteristics such as chamber hypertrophy or intraventricular blocks.

8.2. Subendocardial and Subepicardial Injury: Diagnostic Criteria

a) *Subepicardial injury* – J-point and ST-segment elevation, with upper concavity or convexity (more specific) of the segment in 2 contiguous leads, of at least 1 mm in the frontal plane and left precordial leads. In precordial leads (V1 to V3), ST-segment elevation should be ≥ 1.5 mm in women, ≥ 2 mm in men aged 40 years or older, and ≥ 2.5 mm in men aged less than 40 years;[109]

b) *Subendocardial injury*[109] – J-point and ST-segment depression, horizontal or downsloping ≥ 0.5 mm, in 2 contiguous leads, at 60 ms after the J-point.

Note: to diagnose injury one should consider the concomitant presence of changes in the T wave and ST-segment, recognized in at least 2 concordant leads.

8.3. Definition of Myocardial Fibrosis

An area with fibrosis (old myocardial infarction) is considered when ventricular activation does not occur as expected and does not suggest intraventricular conduction disturbance. Myocardial fibrosis (old myocardial infarction) is characterized by pathological Q waves in 2 contiguous leads, with duration ≥ 40 ms, associated or not with amplitude > 25% of the QRS amplitude, or a reduced R wave in an area where it is expected and should be present.

8.4. Topographic Analysis of Ischemia, Injury, and Necrosis

8.4.1. ECG Topographic Analysis of Ischemic Manifestations (Meyers)

a) Anteroseptal wall – V1, V2, and V3 leads;

b) Anterior wall – V1, V2, V3, and V4 leads;

c) Anterolateral wall – V4 to V5, V6, D1, and aVL leads;

d) Extensive anterior wall – V1 to V6, D1, and aVL leads;

e) Lateral wall – D1 and aVL leads and/or V5 and V6 leads;

f) Inferior wall – D2, D3, and aVF leads.

Note: The term "posterior wall" should no longer be used due to current evidence indicating that the recording of leads V7–V9 refers to the lateral wall.[110]

8.4.2. ECG Topographic Analysis of Ischemic Manifestations in Association with Magnetic Resonance Imaging[111]

a) Septal wall – Q wave in V1 and V2 leads;
b) Anteroapical wall – Q wave in V1, V2 to V3–V6 leads;
c) Anteromedial wall – Q wave (qS complex or r wave) in D1, aVL, occasionally in V2 and V3 leads;
d) Lateral wall – Q wave (qR1 complex or r wave) in D1, aVL, V5–V6 and/or RS complex in V1 lead;
e) Inferior wall – Q wave in D2, D3, and aVF leads.

The sites mentioned above present the best anatomic correlations in acute coronary syndromes with ST-segment elevation and in necrosis (when present). Topographic sites may vary in the case of cardiomegaly or major structural alterations.

8.4.3. Electrocardiographic Correlation with the Culprit Artery (Table 8.1)[112]

In Figure 8.1 we find the correlation between the culprit artery and the wall/ventricular segment involved.

8.5. Particular Areas of Infarction

8.5.1. Right Ventricle Myocardial Infarction

ST-segment elevation in the right precordial leads (V1, V3R, V4R, V5R, and V6R), particularly with ST-segment elevation > 1 mm in V4R. ST-segment elevation in right ventricle infarctions appears for a short period of time due to the low oxygen consumption of the right ventricular muscle. In general, this infarction is associated with low inferior wall and/or lateral wall infarctions of the left ventricle.[113]

8.5.2. Atrial Infarction

Atrial infarction can be recognized by the presence of PR segment elevation > 0.5 mm. It can be associated with atrial arrhythmias.[114]

8.6. Differential Diagnoses[115]

8.6.1. Subepicardial Ischemia

It should be differentiated from secondary changes in ventricular repolarization in LVH or bundle-branch blocks (asymmetrical T wave).

8.6.2. Acute Myocardial Infarction (AMI) with ST-segment Elevation

It should be differentiated from:

a) Early repolarization;
b) Pericarditis and myocarditis;
c) Former AMI with dyskinetic area and persistent ST-segment elevation (left ventricular aneurysm);
d) Acute pancreatitis;
e) Hyperkalemia;
f) Catecholaminergic syndromes;
g) Brugada syndrome.

8.7. Association of Myocardial Infarction with Bundle-Branch Blocks

8.7.1. Myocardial Infarction in the Presence of RBBB

The electrocardiographic diagnosis of myocardial infarction is not hindered by the presence of an RBBB.

8.7.2. Myocardial Infarction in the Presence of LBBB

The presence of an LBBB hinders the recognition of an associated myocardial infarction. In LBBB, the conduction delay begins with the disappearance of the first vector and mid to terminal ventricular activation impairment. In septal infarctions, a larger and wider R wave is seen (as opposed to

Table 8.1 – Correlation between electrocardiographic leads and culprit artery

		ST-segment elevation			ST-segment depression
Left coronary branch		aVR			V2-V6; I,L
Anterior descending coronary artery	Before the first septal branch	V1 - V4		I, L	II, III, F
Anterior descending coronary artery	Between the septal and diagonal branches	V1 - V6		I, L	
Long left anterior descending coronary artery (post-crux cordis)	After the septal and diagonal branches	V2 - V6		I, L	V2-V6; I,L
Proximal right coronary artery		V4 - V6	II < III, F		I, L, V1 - V3
Mid-distal right coronary artery			II < III, F	I, L	I, L, V1 - V3
Distal right coronary artery			II < III, F		I, L
Right coronary artery (right ventricle)		V1, V3R, V4R	II < III, F		
Circumflex coronary artery		V4 - V6	II > III, F	I, L	V1 - V3
Circumflex coronary artery (right ventricle)		V1, V3R, V4R; V4 - V6	II > III, F	I, L	

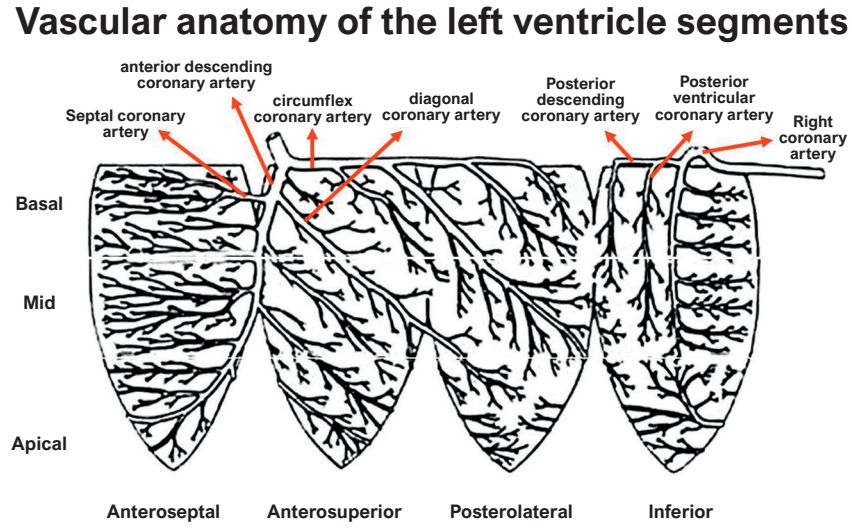

Figure 8.1 – *Correlation between culprit artery and wall/ventricular segment (adapted from Selvester RH et al.)*[112]

the usually small or absent r wave in LBBB) in V1 and/or V2, associated with a q wave in V5 and V6. In lateral infarctions, we notice slurred or notched S waves in the ascending phase. Inferior infarctions result in slurred or notched S waves in D2, D3, and aVF.[116]

ST-segment deviations may allow the identification of recent myocardial infarction, according to criteria defined by Sgarbossa et al. Five points or more indicate a high accuracy in the identification of myocardial infarction with ST-segment elevation.[117]

a) 5 points: ST-segment elevation ≥ 1mm concordant with the QRS/T;
b) 3 points: ST-segment depression ≥ 1 mm in V1, V2, and V3;
c) 2 points: ST-segment elevation ≥ 5 mm discordant with the QRS/T.

9. Analysis of Ventricular Repolarization

9.1. Ventricular Repolarization

The analysis of ventricular repolarization is extremely complex, as it represents the interaction of various systems that can be expressed through segments and electrical waves. The repolarization phenomenon received greater attention after the recognition of its contribution to the risk stratification of major arrhythmic events and sudden cardiac death.

9.1.1. Normal Ventricular Repolarization

Ventricular repolarization comprehends the period between the end of the QRS and the end of the T wave (or U wave, when present). In this context, the following elements should be analyzed:

9.1.1.1. J-point

J-point is located at the end of the QRS complex. Its position is used to identify ST-segment deviations.

9.1.1.2. ST-Segment

ST-segment is located between the J-point and the T wave. It should be at the same level as the PR segment. Variation up to 0.5 mm (up or down) is considered within the normal range.

9.1.1.3. T Wave

A normal T wave is asymmetrical, with a slower onset and a faster ending. It is also positive in almost all leads, and its amplitude corresponds to 10%-30% of the QRS amplitude. It is always negative in aVR and may be negative only in V1 and/or D3.

9.1.1.4. U Wave

U wave is the last and smallest deflection in the ECG. When present, it occurs soon after the T wave and before the P wave of the next cycle. Its polarity is the same as that of the preceding T wave, and in most cases its amplitude corresponds to 5%-25% of the preceding T wave. In general, it is visible only in low heart rates and its genesis is attributed to the following:

a) Late repolarization of the Purkinje fibers;
b) Slow repolarization of papillary muscles;
c) Late residual potentials in the septum;
d) Electromechanical coupling;
e) M cell activity;
f) Delayed afterdepolarization (triggered activity).

9.1.1.5. QT interval and Corrected QT Interval (QTc)

a) *QT* – Measurement from the beginning of the QRS to the end of the T wave. It represents the total duration of ventricular electrical activity;

b) *QTc* – QT is modified by the heart rate. In general, its correction (QTc) uses the Bazett formula:

$$QTc = \frac{QT^{*}}{\sqrt{RR}}$$

* QT measured in milliseconds and RR distance measured in seconds.

The Bazett formula,[118] although widely used for calculating QTc, presents limitations for HRs below 60 bpm or above 90 bpm. In these cases, linear formulas such as those by Framingham[119] and Hodges[120] should be used.

QT and QTc values do not need to be reported, but they should always be checked for normality. QTc values vary with gender and are accepted as normal when up to 450 ms for men and 470 ms for women. For children, the upper limit of normality is 460 ms,[121] and QT is considered short when below 340 ms.[122]

The measurement of the QT interval in bundle-branch blocks is controversial, and a simplified correction was recently proposed by Bogossian: $QTm = QT_{LBBB} - 0.5\ QRS_{LBBB}$.[123]

9.1.2. Variants of Ventricular Repolarization

9.1.2.1. Early Repolarization Pattern

Historically, the ECG with "early repolarization" has always been considered normal. Some publications have associated the slurring or notching of the final portion of the QRS (also named early repolarization) with a higher death rate; thus caused scientific turmoil regarding the benignity of this condition. Early repolarization is characterized by the mandatory presence of a notch or slurring of the final portion of the QRS complex; J-point elevation may or may not be found.[124]

J wave with a straightened aspect of the ST in the inferior leads (or associated with lateral leads) may be an electrical marker of ventricular tachyarrhythmias risk.[125-129]

In the last decades, great advances have linked the ventricular repolarization features to risk stratification of major arrhythmic events and sudden cardiac death. These include the dispersion of ventricular repolarization as a marker of the non-uniform recovery of myocardial excitability and the recognition of cyclic (macrovolt or microvolt) T-wave alternans. It is important to consider significant changes in polarity, duration, and morphology of the electric phenomena described above as ventricular repolarization alterations.

10. Channelopathies and Other Genetic Alterations

10.1. Genetics and the ECG

In recent years, the improvement of genetic mapping techniques allowed a deeper understanding and differentiation of potentially fatal clinical conditions with characteristic electrocardiographic patterns. Within this group, we highlight conditions that affect structurally normal hearts, such as channelopathies and others with myocardium involvement such as hypertrophic cardiomyopathy and arrhythmogenic right ventricular cardiomyopathy.

10.1.1. Channelopathies

Cardiac channelopathies are a result of genetic mutations or malfunctioning of ion channels, resulting in important modifications of the action potential. Some specific electrocardiographic findings associated with syncope (at rest or triggered by exercise), as well as ventricular arrhythmias in structurally normal hearts, should raise the hypothesis of channelopathies.

10.1.1.1. Congenital Long QT Syndrome[129,130]

Congenital long QT syndrome was the first described and most studied channelopathy. It allowed the understanding of the relationship between molecular biology and genetics, and the association with clinical manifestations, risk stratification, and treatment. Congenital long QT syndrome represents the main cause of a negative autopsy in cases of sudden death among young people.

Its main characteristic is the prolongation of the QTc on the ECG, with values > 460 ms. Clinically the presence of syncope or cardiac and respiratory arrest triggered by emotional and physical stress should raise the hypothesis of long QT syndrome. People with this condition are at a high risk of polymorphic VT, syncope, and sudden death (when polymorphic VT degenerates into VF). *Torsades des pointes* (TdP) is a polymorphic VT in a person with long QT. Although 16 genes have been linked to mutations associated with long QT syndrome (LQT), 3 of them correspond to 75% of all diagnosed cases: KCNQ1 (LQT1), KCNH2 (LQT2), and SCN5A (LQT3). Some triggers are gene-specific: exercise is more strongly associated with LQT1, emotional stress is associated with LQT2, and bradycardia is associated with LQT3. Characteristics on the ECG include:

a) LQT1: T wave with a wide base and delayed beginning;
b) LQT2: T wave with low amplitude, usually with notch;
c) LQT3: delayed T wave, with a huge ST-segment.

10.1.1.2. Short QT Syndrome[131-133]

Described in 2000, short QT syndrome is characterized by a short QT interval (< 340 ms) associated with AFib and sudden cardiac death. There is increased activity of potassium channels (phase 3 of the action potential) resulting in shortening of the QT interval. Genes related with this syndrome are KCNH2, KCNQ1, and KCNJ2. When the ECG highlights short QTc (< 370 ms) and a distance < 120 ms between the J point and the peak of the T wave, a diagnosis of short QT is suspected.

10.1.1.3. Brugada Syndrome[134-137]

Brugada syndrome is a channelopathy caused by sodium channels defect. In most cases it happens in the right ventricular epicardium, affecting men more than women (8:1). Some individuals present syncope and or/cardiac arrest due to VF, besides family history of sudden death. In many cases, these events happen during rest and sleep, and may also be triggered by hyperthermia and some medications, culminating in sudden death.

Brugada syndrome has a dominant autosomal inheritance and is responsible for 20% of all sudden deaths with a normal heart at autopsy. It is a genetically heterogeneous condition, involving at least 13 genes. More than 200 mutations have been described, most of them occur in genes that affect the sodium channels (SCN5A) and it can be identified in only 20% to 25% of all cases.

A J-point elevation ≥ 2 mm in V1 and V2 leads, followed by a slow ST-segment depression with upward convexity and a T-wave inversion, characterizes the type 1 pattern. The diagnosis of Brugada syndrome is made by the electrocardiographic finding of the type 1 pattern in association with symptoms.

Type 2 pattern is characterized by a J-point elevation in V1 and V2 < 2 mm and a saddle-shaped ST-segment. This pattern is highly suspect but does not confirm the diagnosis. Its transient pattern hinders diagnosis, and in doubtful cases, ECG should be recorded with upper precordial leads. The electrodes are positioned in the second and third right and left intercostal spaces, which allow better assessment of the right ventricle outflow tract. This electrode position increases ECG sensitivity for type 1 pattern diagnoses.[138]

Brugada phenotype is characterized by the presence of Brugada type 1 ECG pattern in a person with no history of aborted sudden death or syncope, or sudden death in first-degree relatives.

Brugada phenocopy is not the same as Brugada phenotype, and is characterized by an electrocardiographic pattern that is presumably identical to the syndrome, but caused by many other conditions. It presents a transmural gradient (epicardium to endocardium) resulting from a sharp notch on the epicardial action potential, mediated by I_{to} channels and by the loss of the action potential dome. Among the described situations, there are metabolic alterations, mechanical compression by extra-cardiac structures, ischemia, myocardial/pericardial disease, and some medications.[139] The ECG with Brugada pattern disappears after the resolution of these conditions.

10.1.1.4. Catecholaminergic Tachycardia[140,141]

Catecholaminergic tachycardia affects individuals during childhood and adolescence. Some of them report syncopal episodes and a family history of sudden death. Hereditary or sporadic mutations in ryanodine channels, responsible for regulation of the intracellular calcium, are responsible for 50% to 60% of all cases of catecholaminergic polymorphic VT. The resting ECG may be normal or with sinus bradycardia and/or U waves. Bidirectional ventricular arrhythmia triggered by exercise testing or isoproterenol infusion is typical.

Other frequent findings include PVC, which are usually isolated, intermittent, bigeminal, and paired, increasing in density with exercise.

10.1.2. Genetic Diseases with Primary Cardiac Involvement

10.1.2.1. Arrhythmogenic Right Ventricular Cardiomyopathy (Dysplasia)[142-144]

Arrhythmogenic right ventricular cardiomyopathy is a genetic disease with primary involvement of the right ventricle. There is a replacement of myocytes with fibrofatty tissue and it is associated with arrhythmias, heart failure, and sudden death. ECG is characterized by a low voltage and longer duration QRS complex in V1/V2 (epsilon wave, present in 30% of the cases), associated with negative, rounded, and asymmetrical T waves from V1 to V4. It is associated with PVC originating from the right ventricle (LBBB morphology) and may have a superior or inferior orientation. The finding of negative T waves up to V6 suggests left ventricle involvement.

10.1.2.2. Hypertrophic Cardiomyopathy[145,146]

Hypertrophic cardiomyopathy is a primary heart disease with genetic basis. It has mostly autosomal dominant inheritance, affects 1:500 live births. There are several described mutations and with severe segmental or diffuse ventricular hypertrophy. In almost 75% the ECG is altered, and in the pediatric population it has a good sensitivity.[147] It is characterized by rapid and deep Q waves in the inferior and/or precordial (lateral) leads, generally associated with classical ECG signs of LVH and accompanied by characteristic ST-T changes (deep and negative T waves).

10.1.3. Genetic Diseases with Secondary Cardiac Involvement

10.1.3.1. Muscular Dystrophy[148]

Muscular dystrophy is a group of diseases that predominantly affect voluntary muscles. In some of them, there is respiratory and heart muscle involvement. The most common ECG findings are tall R wave in V1 and V2 (R/S ratio > 1), deep Q wave in V6, D1, and aVL, right bundle-branch conduction delay, QS complexes in D1, aVL, D2, and D3, and abnormal ventricular repolarization.

11. Electrocardiographic Pattern in Specific Clinical Situations

11.1. Clinical Conditions that Can Modify the ECG

Besides heart diseases, some peculiar ECG pattern can be recognized due to systemic diseases, metabolic disorders, and use of medications. In some of them, such as long QT, WPW, and Brugada syndrome, the ECG is the most sensitive and specific diagnostic test.[149] On the other hand, its sensitivity

decreases in conditions such as myocardial infarction, pericarditis, and digitalis toxicity, although it is still one of the main diagnostic methods in clinical practice. Myocardial infarction and WPW syndrome, due to their prevalence and importance, are analyzed in separate chapters of this guideline. Other situations were grouped in this section.

We will now analyze the highly specific diagnostic features for the following conditions, in alphabetical order. However, we recommend that the terms "ECG suggestive of" or "ECG consistent with" should be used in the final ECG reports.

11.1.1. Digitalis Action

The use of digitalis can be recognized by ST-T depression with upper concavity (reverse tick or Salvador Dali sagging) and shortened QTc interval. Several arrhythmias may occur in case of digitalis toxicity, especially PVC. Bidirectional ventricular tachycardia and atrial tachycardia (variable AV conduction) is highly suggestive of digitalis toxicity, as well as bradiarrhythmias (first-degree AV block and type I second-degree AV block).

11.1.2. Drug-induced ST-T Changes

Drugs that interfere with the ST-T (increasing QTc interval) can be found on the following electronic address: http://www.azcert.org/medical-pros/drug-lists/drug-lists.cfm.[150]

11.1.3. Electrical Alternans

Electrical alternans is characterized by alternately higher and lower QRS amplitudes. It is cyclic and not related with the respiratory cycle, in successive QRS complexes.

11.1.4. T-wave Alternans (TWA)

The clinical applicability of T-wave alternans has been increasingly investigated. TWA is characterized by beat by beat modification of amplitude, shape, and orientation of the T wave (J-point and/or ST-segment). It may be intermittent or permanent. On the 12-lead ECG, these variations may be macroscopic (macrovolt alternans) or so small that computerized algorithms are required for its analysis (microvolt alternans).

11.1.5. Acute Injury of the Central Nervous System

Hemorrhagic injury of the central nervous system (CNS) can produce giant negative T waves (more rarely positive) simulating subepicardial ischemia that is called cerebral T wave. Increased QTc interval can also be observed. All ECG alterations are reversible after CNS treatment.

11.1.6. Interatrial Communication

An individual with an interatrial communication can present a right end conduction delay and, sometimes, RVH. If an upward and leftward deviation of the QRS axis is also present it is related with the *ostium primum* defect. Supraventricular arrhythmias, such as atrial fibrillation/flutter, is not uncommon.

11.1.7. COVID-19

Cardiac impairment due to COVID-19 may reach 44% of severe cases; electrocardiographic alterations were observed in up to 93% of hospitalized patients with critical illness. The reasons for myocardial alterations with changes in the ECG include cytokine storm, hypoxic injury, electrolyte imbalance, plaque rupture, coronary artery spasm, microthrombi, or direct endothelial or myocardial injury. The ECG may present supraventricular tachycardia (sinus tachycardia, atrial fibrillation, atrial flutter, AVNRT), malignant ventricular arrhythmia (monomorphic and polymorphic VT and VF), bradycardia, and AV blocks (second- and third-degree), increased QT interval, RBBB and LBBB, QRS axis rightward deviation, ST-segment elevation or depression, T-wave inversion, pathological Q waves, and signs of pulmonary thromboembolism (sinus tachycardia/atrial fibrillation, RVH, RBBB, T-wave inversion from V1 to V3, S1Q3T3 pattern). Moreover, COVID-19 can uncover the Brugada pattern in individuals with this disease.[151]

11.1.8. Pericardial Effusion

In pericardial effusion, ECG can present dielectric effect (see Item 11.14), sinus tachycardia, and electrical alternans.

11.1.9. Dextrocardia

Individuals with dextrocardia present negative P, QRS and T waves in D1 and V6. Positive QRS complexes in V1 and negative QRS in V6 are also observed (main differential diagnosis with reversal of the upper limb electrodes).

11.1.10. Dextroposition

Individuals with dextroposition may manifest a negative or minus-plus P wave in D1, a deep Q wave in D1 and aVL, and qRS complexes in the right precordial leads.

11.1.11. Electrolyte Imbalance

11.1.11.1. Hyperkalemia

ECG changes depend on serum potassium concentrations, and they occur sequentially: symmetrical T wave with increased amplitude and a narrow base, decreased QTc interval, intraventricular conduction delay (wide QRS), and decreased P wave amplitude until its disappearance, with sinoventricular rhythm.

11.1.11.2. Hypokalemia

ECG can present increased U wave amplitude; ST-segment and T-wave depression; increased QTU interval. The QT interval should preferably be measured in the aVL lead because the U wave tends to be more isoelectric.

11.1.11.3. Hypocalcemia

In patients with hypocalcemia, ECG can show flattening and increased duration of the ST-segment with an increase in the QTc interval.

11.1.11.4. Hypercalcemia

In patients with hypercalcemia, ECG can show shortening and eventual disappearance of the ST-segment, with a consequent shortening of the QT interval.

11.1.12. Chronic Obstructive Pulmonary Disease (COPD)

COPD frequently presents ECG with dielectric effect (see Item 11.1.14); rightward QRS axis shift; rS complex from V1 to V6. Right chambers hypertrophy is associated to heart disease and a rightward shift of the P axis, near +90º (P *pulmonale*).

11.1.13. Antiarrhythmic Drugs

Antiarrhythmic drugs can be related with proarrhythmia. In the following sections, we will present the drugs that can change the ECG.

11.1.13.1. Amiodarone

Amiodarone is a Class III antiarrhythmic drug; it may cause PR interval prolongation, sinus bradycardia, increased QTc (with no increase in the dispersion of repolarization), and T wave alterations. All of them are more clearly seen in the precordial leads with bifid or flattened T waves. These changes can also be observed in the frontal plane leads (it differentiates them from bifid T waves in children, which are limited to the precordial leads).

11.1.13.2. Propafenone

Propafenone is a Class IC antiarrhythmic drug that may cause AV blocks and complex ventricular arrhythmias (paired PVC, nonsustained VT, and polymorphic VT), especially in patients with coronary artery disease.

11.1.13.3. Sotalol

Sotalol is a Class III antiarrhythmic drug and may cause sinus bradycardia, AV blocks, and increased QTc interval (with polymorphic VT).

11.1.14. Dielectric Effect

Dielectric effect can present an ECG with low QRS voltage (< 0.5 mV in the frontal plane and < 1.0 mV in the precordial leads). It may result from a large pericardial effusion, pleural effusion, emphysema, COPD, morbid obesity, or anasarca. Hypothyroidism and infiltrative heart diseases can also present a low voltage pattern.

11.1.15. Pulmonary Embolism

Patients with pulmonary embolism can present sinus tachycardia, right end conduction delay, acute QRS axis rightward deviation, ST-segment depression (V1 to V3), and negative T waves from V1 to V3. The classical S1Q3T3 pattern may occur.

11.1.16. Ashman or Gounaux-Ashman Phenomenon[17]

Ashman phenomenon is an aberrant conduction of a beat from supraventricular origin. It can happen after a long-short cycle. Due to its characteristic long refractory period (compared to the left bundle-branch) the aberrancy occurs more frequently at the right bundle-branch. Irregular rhythms such as atrial fibrillation, atrial flutter and atrial tachycardia favor this phenomenon.[152,153] Concealed transeptal retrograde conduction is responsible for the following consecutive beats with aberrancy.

11.1.17. Hypothermia

Patients with hypothermia can present bradycardia, prolonged QT interval and J wave or Osborn wave (notch with large amplitude and short duration at the final portion of a QRS.

11.1.18. Hypothyroidism

In severe cases (myxedema), it may present bradycardia, low voltage, and diffuse alterations of ventricular repolarization.

11.1.19. Chronic Renal Failure

Patients with chronic renal failure can present ECG abnormalities related to hyperkalemia and hypocalcemia (discussed elsewhere), and these findings can be associated with decreased renal function.

11.1.20. Pericarditis[154]

The following electrocardiographic alterations may be seen in the acute phase of the inflammatory process, usually in this order:

a) *PR-segment depression* in D1, D2, aVF, and from V2 to V6. PR segment elevation in aVR; it may also occur in V1;

b) *ST segment* – diffuse elevation with upper concavity, except for V1 and aVR. There are no associated Q waves;

c) *T wave* – slightly increased and symmetrical in the initial phase. Characteristically, it is not inverted in the presence of ST elevations. It may be inverted in the chronic phase of the disease, after normalization of the ST segment, but rarely with enough depth to resemble the ischemic T-wave pattern.

11.1.21. Chemotherapy Drugs[155-158]

The development of new chemotherapy drugs has increased patient's survival. ECG abnormalities may occur in patients with chemotherapy-induced myocardial injury. These alterations are characterized by a complex pathogenesis and may depend both on the direct toxicity of chemotherapy drugs (with electrophysiological substrate) and on direct injure to the myocardium, endocardium, and pericardium (due to ischemia, inflammation, or radiation); this is why alterations accompanying cardiac dysfunction are

unspecific and can precede symptoms, even before ECG alterations appear. Electrocardiographic findings are better identified through with serial ECGs. These include sinus tachycardia, T-wave flattening or inversion, increased QT interval, and low QRS voltage.

Some arrhythmias, such as TdP, and VT/VF may occur during treatment. The most well-known drugs are anthracyclines; however, alkylating agents (cyclophosphamide), antimetabolites (5-fluorouracil), antimicrotubule agents (paclitaxel), immunomodulating drugs (thalidomide), and targeted therapy can cause cardiac damage.

12. The ECG in Athletes

12.1. The Importance of Understanding the Athlete's ECG[159-164]

Many physiological adaptations occur during sport training and some of them can lead to specific electrocardiographic findings, even without anatomical/structural changes. That's why the interpretation of athletes' ECGs becomes a challenging task. With the inclusion of the resting ECG in pre-participation evaluations, we should be aware of specific recommendations for this population. At the present time, athlete's ECG findings can be divided into 3 categories:

12.1.1. Normal ECG Findings (Group 1)

- Increased QRS voltage for LVH or RVH;
- Right bundle-branch conduction delay;
- Early repolarization/ST-segment elevation;
- ST-segment elevation followed by T-wave inversion (V1 to V4) in black athletes;
- T-wave inversion (V1 to V3) in athletes aged less than 16 years;
- Sinus bradycardia/sinus arrhythmia;
- Ectopic atrial rhythm or junctional rhythm;
- First-degree AV block;
- Type I second-degree AV block.

12.1.2. Abnormal ECG Findings (Group 2)

- T wave inversion in other situations;
- ST-segment depression;
- Pathological Q waves;
- LBBB;
- QRS duration ≥ 160 ms;
- Epsilon wave;
- Ventricular pre-excitation;
- Prolonged QT interval;
- Brugada type 1 pattern;
- Severe sinus bradycardia (< 30 bpm);
- Pr interval ≥ 400 ms;
- Type II second-degree AV block;
- Third-degree AV block;
- Two or more PVC;
- Atrial tachyarrhythmias;
- Ventricular arrhythmias.

12.1.3. Borderline ECG Findings (Group 3)

- Upward and leftward QRS axis deviation;
- Left atrial hypertrophy;
- Upward and rightward QRS axis deviation;
- Right atrial hypertrophy;
- RBBB.

Athletes with a normal ECG do not need further investigation if they are asymptomatic and have no family history of hereditary heart diseases and/or sudden death. On the other hand, athletes with findings of group 2 should undergo investigation of pathological cardiovascular conditions associated with sudden death. All findings mentioned in this group (2) could be manifestations of structural alterations. Finally, athletes with a borderline ECG (and asymptomatic and no family history of hereditary heart diseases and/or sudden death) are exempted from further investigation if they present only one of the findings in group 3. If these athletes present 2 or more findings from group 3, pathological cardiovascular conditions associated with sudden death in athletes should be investigated.

13. The ECG in Children

13.1. Introduction

Although the general principles for the interpretation of children's and adults' ECGs are similar, the analysis of pediatric ECGs constitutes a challenge for clinical practice. This is mainly due to electrocardiographic patterns that are specific in children (Table 13.1). Such patterns are related with age and anatomical/ physiological changes intrinsic to human development.[165]

Table 13.1 – Normal electrocardiographic findings in children

Shorter PR interval and narrower QRS complexes
A right QRS axis deviation in the first year of life
Prominent Q waves in the inferior and lateral leads
The analysis of ventricular repolarization is more important than QRS amplitude in the diagnosis of ventricular hypertrophy
Early repolarization
Negative T wave from V1 to V4 until 12 years of age
Bifid or notched T wave in the right precordial leads
Prominent U wave
Common and physiological rhythm findings: • Prominent U wave • Sinus arrhythmia, ectopic atrial rhythm • First-degree and type I second-degree AV blocks • Sporadic atrial and ventricular extrasystoles

The ECG of newborns reflects the hemodynamic effects of the fetal circulation on the right ventricle, as well as anatomical and physiological changes resulting from the transition to neonatal circulation. Up to 32 weeks of pregnancy, the left ventricle is larger than the right ventricle. From this phase and until the end of pregnancy, the right ventricle prevails due to the progressive increase in pulmonary vascular resistance.[166] During birth, lung aeration leads to a sharp drop in pulmonary arterial pressure, while the removal of the placenta and closure of the arterial duct increase systemic vascular resistance.[167] In general, at the end of the first month of life, the left ventricle size equals the right ventricle, and then anatomically prevails over it.[166,167] Most of these adaptive changes happen after birth and before the first year of life. Maturation of the autonomic nervous system, physical growth, and changes in the position of the heart occur progressively up to adult age.[168] As a result, the normal ECG changes rapidly during the first weeks of life, and the child starts to gradually present electrocardiographic patterns that are similar to those of an adult only by the age of 2 to 3 years.[167]

13.2. Technical Aspects

The ECG of children should include the classical 12 leads, which may be complemented by V3R and V4R leads in case of suspected right chamber hypertrophy.[169] Artifacts are common and usually caused by inadequate electrode placement, chest wall deformities, and movements (voluntary or not) that are intrinsic to each age group.[170]

Reference tables and graphs of age-related variations of electrocardiographic parameters are frequently consulted (necessary task).[170] Most of these values, particularly considering the first year of life, derive from Canadian data by Davignon et al.[171] Despite the publication of more recent studies[172] this continue to be the main reference in clinical practice (Table 13.2). It is still debatable whether these data may be extrapolated to the general public. To date, 2 studies have been published based on the Brazilian population. One of them, including almost 100 term newborns with a normal ECG in the first week of life, showed electrocardiographic parameters that were different from those published by Davignon.[173] The second study, with a population over 1 year of age, included more than a million children.[174] Another controversial situation is the computerized and automatic ECG reports. It has questionable accuracy in pediatrics and its routine use is not yet recommended.[170]

13.3. Electrocardiographic Parameters and their Variations

The pediatric ECG should be systematically evaluated according to the patient's age group (Table 13.2), and its analysis should consider the same criteria as that in adults: rhythm, heart rate, P wave (axis, amplitude, and duration), AV conduction, QRS complex (axis, amplitude, duration, and morphology), ST-segment, T wave, and U wave. Measurement of the QT interval and QTc calculation should be routinely performed.[175]

13.3.1. Heart Rate and Sinus Rhythm

Contractile mass and ventricular compliance are relatively lower in children, particularly during the first year of life. As a result, their cardiac output depends basically on the HR, which is much higher in children than in adults. A healthy newborn can present HR between 150–230 bpm. Normal HR increases from the date of birth until the first and second months of life; on the sixth month, it returns to values similar to those of the first day. From this point on, HR progressively decreases and, around 12 years-old, it reaches values considered normal for adults.[168]

13.3.1.1. Possible Alterations

13.3.1.1.1. Sinus Arrhythmia

Very frequent in children, it is generally phasic and associated with breathing.[165] It is less noticeable in higher HR and in newborns, especially in the first week of life.

13.3.1.1.2. Sinus Tachycardia

Sinus tachycardia is considered when sinus rhythm presents HR above the 98th percentile for the age (in general lower than 220 bpm).[170,171] Sinus tachycardia may have various causes, of which the most frequent are: physical activity, fever (10-bpm increase in HR for each 1ºC increase in body temperature), anemia, and dehydration.[170]

13.3.1.1.3. Sinus Bradycardia

Sinus bradycardia is considered when sinus rhythm presents HR below the second percentile for the age [170,171] (Table 13.2). It may have various etiologies, such as infections, respiratory failure, hypothermia, hypothyroidism, and increased intracranial pressure. In newborns, the occurrence of transient sinus bradycardia can be associated with the transplacental passage of anti-Ro/SSA antibodies, especially in mothers with systemic lupus erythematosus or other connective tissue diseases. Patients with cardiac channelopathies such as LQT3 and Brugada syndromes may also manifest sinus bradycardia.

13.3.1.1.4. Other Bradycardias

A sudden prolongation of the PP interval is common and occurs in almost half of all normal newborns and one-sixth of adolescents. These pauses are frequently related with an increased vagal tone[165] and some of them can be followed by supraventricular or ventricular escape beats.[168]

13.3.2. P wave and Atrial Electrical Activity

The characteristics of atrial activation remain relatively constant in all ages. P wave axis is very important to establish the rhythm site of origin, visceroatrial situs, and cardiac position.[170] The sinus P-wave axis is between 0 and +90 degrees. The normal P wave should not exceed 0.12 s and 2.5 mm (duration and amplitude); these parameters present few variations in different pediatric age groups (Table 13.2).

Table 13.2 – Normal electrocardiographic parameters by age

	0–1 day	1–3 days	3–7 days	7–30 days	1–3 months	3–6 months	6–12 months	1–3 years	3–5 years	5–8 years	8–12 years	12–16 years
HR	94 - 155	91 - 158	90 - 166	106 - 182	120 - 179	105 - 185	108 - 169	89 - 152	73 - 137	65 - 133	62 - 160	60 - 120
P (mV)	0.01 - 0.28	0.03 - 0.28	0.07 - 0.29	0.07 - 0.30	0.07 - 0.26	0.04 - 0.27	0.06 - 0.25	0.07 - 0.25	0.03 - 0.25	0.04 - 0.25	0.03 - 0.25	0.03 - 0.25
PR D2 (seconds)	0.08 - 0.20	0.08 - 0.14	0.07 - 0.15	0.07 - 0.14	0.07 - 0.13	0.07 - 0.15	0.07 - 0.16	0.08 - 0.15	0.08 - 0.16	0.09 - 0.16	0.09 - 0.17	0.09 - 0.18
QRS (seconds)	0.02 - 0.10	0.02 - 0.07	0.02 - 0.07	0.02 - 0.08	0.02 - 0.08	0.02 - 0.08	0.03 - 0.08	0.03 - 0.08	0.03 - 0.07	0.03 - 0.08	0.04 - 0.09	0.04 - 0.09
QRS axis	59 - 189	64 - 197	76 - 191	70 - 160	30 - 115	7 - 105	6 - 98	7 - 102	6 - 104	10 - 139	6 - 116	9 - 128

HR: heart rate; mV: millivolts.

13.3.2.1. Possible Alterations

13.3.2.1.1. Atrial Hypertrophy

P-wave amplitude is increased in right atrial hypertrophy, which is more clearly seen in D2 lead. Left atrial hypertrophy is characterized by an increase in P-wave duration (according to the age percentile) and/or its negative deflection in V1 (> 40 ms in duration and > 0.1 mV in amplitude).[168]

13.3.2.1.2. Junctional Rhythm

Junctional rhythm is characterized by a negative P wave in inferior leads (preceding, concomitant, or after the QRS complex). It can occur in up to one-third of normal children. It is more common during sleep, but it can happen during waking hours; in general, it does not have any pathological significance.

13.3.3. PR Interval and AV Conduction

The PR interval increases with age, and presents an inverse relationship with HR. It varies according to autonomic tone (Table 13.2).

13.3.3.1. Possible Alterations

13.3.3.1.1. AV Blocks

First-degree and type I second-degree AV block episodes occur in around 10% of normal children and up to 20% of normal adolescents. There are occasional periods of 2:1 AV block. They are more common during sleep, but can happen during waking hours, especially in individuals with vagotonia and in athletes.[165]

Type II and advanced second-degree AV block, as well as third-degree AV block, are usually pathological and can occur isolated or in association with complex cardiac malformations. The isolated form of congenital third-degree AV block affects 1:20 000 live births and is usually related with the transplacental passage of anti-Ro/SSA and anti-La/SSB antibodies.[168]

13.3.3.1.2. Short PR Interval and Ventricular Pre-excitation

A short PR interval can be detected in cases of junctional or other ectopic atrial rhythm. Pompe and Fabry diseases can also present it.[168,170]

Ventricular pre-excitation is characterized by a shortened PR interval with a delta wave.[170] Intermittent ventricular pre-excitation is not uncommon among newborns and children. Even when ventricular pre-excitation is persistent, the typical ECG pattern can be subtle. In these cases, the mid-precordial leads (V3-V4) can show them better. Wolf-Parkinson-White syndrome has an incidence of 0.15% to 0.3% in the pediatric population. An increased prevalence of ventricular pre-excitation is observed in individuals with hypertrophic cardiomyopathy, Ebstein anomaly, L-transposition of the great arteries, and cardiac tumors.

13.3.4. Ventricular Electrical Activity

The most prominent changes in ventricular electrical activity occur during a child's first year of life. In the first days of life, the frontal plane QRS axis is directed downward and to the right, varying between 55º and 200º and reflects the predominance of the right ventricle over the left ventricle. It is less evident in tracings of preterm newborns, since fetuses with less than 32 weeks of gestational age have a larger left ventricle in comparison with the right ventricle. As the infant grows, the QRS axis deviates to the left, and when the baby reaches 6 months, it is at 65º.[166] On the transversal plane, QRS axis is directed rightwards and anterior at birth. Still in the first week of life, the QRS axis deviates to the left and maintains an anterior orientation, resulting in an increased R wave in V6 with persistent pure R waves in V1. On the transversal plane, the posterior QRS axis deviation is progressive. Thus, the R wave decreases slowly in V1 throughout the first year

of life, even when exhibiting normal patterns in V5 and V6.[166] The morphology of QRS complexes in the precordial leads changes with the child development. This is explained by the modification of the ventricular electrical activation axis. The following aspects are present:

- The amplitude of the R wave in V1 increases during the first month of life and slowly decreases over the following several years. The amplitude, in this lead, should be < 18 mm in the first year of life and < 10 mm after this period;
- From birth to the sixth month of life, the R wave should be larger in V1 than in V6. R wave amplitude in V1 becomes virtually equal to that in V6 between 6 and 12 months of age. From this point on, it increases in V6 and progressively decreases in V1;
- Q waves are normal and can be very pronounced in the inferior and left precordial leads, and represent the septum activation, although they are absent in D1 and aVL. The Q waves amplitude varies with the child's age and with the ECG lead. Its duration should not exceed 0.03 s (Table 13.2).

In newborns, the QRS complex can be very narrow (in general, below 0.08 s). Its duration increases progressively with age, especially from the third year of life on (Table 13.2).

13.3.4.1. Possible Alterations

13.3.4.1.1. Changes in the QRS Axis and Amplitude

A leftward axis deviation is present in many conditions, such as ventricular septal defects, tricuspid atresia, and WPW syndrome, but it can also be a normal variant. A rightward axis deviation can happen in Noonan syndrome, even in the absence of major pulmonary hypertension and in the presence of RVH.[170]

- RVH: should be suspected in the presence of a positive T wave in V1 after the first week of life and increased amplitudes of the R wave in V1 and S wave in V6. The QR pattern in V1 is usually seen in cases of pressure overload, and the rSR' is seen in cases of right ventricular volume overload;[168]
- LVH: the ECG has limited accuracy for detecting LVH in children. The signs that help the most when diagnosing LVH are an increased S wave in V1, increased R wave amplitude in V6, and T wave abnormalities in V5 and V6;[168]
- Biventricular hypertrophy: wide and isodiphasic complexes in the mid-precordial leads — the Katz-Wachtel phenomenon. The R + S waves sum > 60 mm in V4 is very specific and can occur, for example, in cases of large ventricular septal defects.[170]

13.3.4.1.2. Q-wave Alterations

Pathological Q waves can be seen in children with anomalous coronary artery, ventricular pre-excitation, myocarditis, cardiomyopathy, and muscular dystrophies;[175] they are frequent in patients with hypertrophic cardiomyopathy, especially in the anterolateral leads (V4 to V6, D1 and aVL). In general, these are associated with signs of ventricular hypertrophy and ST-segment and T wave alterations.[176] It should be noted that the presence of a Q wave in V1 is always pathological.

13.3.4.1.3. Intraventricular Conduction Disturbances

Bundle-branch blocks diagnosis is determined by QRS duration and the patient's age (Table 13.2). RBBB can occur in some heart diseases such as Ebstein anomaly and after corrective surgery for congenital malformations such as Fallot tetralogy and interventricular communication. Isolated congenital forms of RBBB or LBBB are rare. Tricuspid atresia, ostium primum interatrial communication, anomalous coronary artery, and AV septal defects may be associated with left anterior fascicular block. LBBB is less frequent in children. The presence of LBBB in patients with severe cardiomyopathy is due to significant left ventricle/conduction system impairment. LBBB is generally associated with a poor prognosis.[168,170]

13.3.4.1.4. Epsilon Wave and Arrhythmogenic Right Ventricular Cardiomyopathy (Dysplasia)

See item 10.1.2.1.

13.3.5. Ventricular Repolarization

Ventricular repolarization is evaluated by measurement of the QT interval and the analysis of ST-segment morphology, T wave, and U wave in different leads.[168]

13.3.5.1. QT Interval

The QT interval presents an inverse relationship with the heart rate — the higher the HR, the shorter the QT interval, and vice-versa. In children, some peculiarities should be analyzed:[168]

- The QT interval should be measured in D2, V5, and V6 – use the longest one for calculating QTc;
- In higher heart rates, the P wave may overlap with the T wave and make QT measurement more difficult, especially in case of prolonged P wave;
- The U wave can be very prominent in children and should not be included in the QT interval if well separated from the T wave. In case of fusion between T and U waves, or when the U wave is too wide (> 50% of the T wave), the tangent method should be used;
- In cases of sinus arrhythmia, QTc should be calculated through the mean value of measurements obtained in various cardiac cycles;
- At 4 days old, children of both sexes have a mean QTc of 400 ± 20 ms. Around 2 months of life, there is a physiological prolongation of the QTc (mean value 410 ms); it then decreases progressively until 6 months of life, when it returns to the values recorded in the first week;
- In children, a normal QTc is up to 440 ms (97.5th percentile).[168]

- Although its routine use in pediatric cardiovascular screening is still under debate, the ECG has a crucial role in the early diagnosis of life-threatening arrhythmic heart diseases manifested during childhood and adolescence, mainly long QT syndrome (see below).

13.3.5.1.1. Possible Alterations

13.3.5.1.1.1. Long QT Syndrome

Long QT syndrome manifests predominantly during childhood and adolescence — few patients have symptoms during the first year of life.[177] Sudden death is the initial presentation of long QT syndrome in up to 12% of the cases.[177] Although this disease is relatively rare, the effort employed in its screening is justified by the efficacy of early treatment and prevention of sudden death.[168] Differential diagnoses should include secondary causes of QTc prolongation — see Section 11 for more details. During the first months of life, the infants of mothers with autoimmune diseases that express anti-Ro/SSA antibodies can present a very prolonged QTc, which is normally a transient finding that normalizes around the sixth month of life.[168]

13.3.5.1.1.2. Short QT Syndrome

See item 10.1.1.2.

13.3.5.2. ST-Segment

ST-segment should always be evaluated and the J-point position is fundamental to identify ST-segment elevation or depression. Thus, J-point position should be compared to the isoelectric line, which is usually at the level of the PQ segment. In newborns and infants, the TP segment (isoelectric line between the T wave and the following P wave) is more commonly used as reference for the baseline.[164]

13.3.5.2.1. Possible Alterations

13.3.5.2.1.1. ST-Segment Deviations

Slight ST-segment deviations are common during the first month of life (usually < 2 mm). ST-segment elevation up to 3 mm can be seen in the right precordial leads and is considered a normal finding (specially from 1 year of age).[168] Ventricular hypertrophy, cardiomyopathies, pericarditis, ventricular pre-excitation, anomalous coronary artery origin, and medications, among other factors, may alter ventricular repolarization and lead to ST-segment elevation or depression. Despite its low sensitivity, ST-segment depression presents good specificity for diagnosing ventricular hypertrophy. Cases of anomalous origin of the left coronary branch (arising from the pulmonary artery) are manifested as an extensive anterior infarction (usually after the first month of life).[170]

13.3.5.2.1.2. Early Repolarization

See item 9.1.2.1.

13.3.5.2.1.3. Brugada Electrocardiographic Pattern

Brugada pattern is rare in children and it has a much lower frequency than among adults.[177] For more details, see item 10.1.1.3.

13.3.5.3. T Wave

During birth, positive T waves in the right precordial leads are normal and are probably due to the physiological adaptation of the right ventricle to new hemodynamic characteristics and lower myocardial elasticity. In normal children, after the second or third day of life, the T wave vector is deviated posteriorly and to the left. Thus, the T wave becomes negative in V1 at the end of the first week. From 7 days to 7 years of life, positive T waves in V1 are usually associated with RVH.[170] The T wave can remain negative from V1 to V4 (juvenile T-wave pattern) until age 12–14 years, when it becomes positive from V2 to V6. The presence of negative T waves in these leads after this period can be considered a normal variant in 1–3% of the cases, but they must be investigated.[170,178,179] Pericarditis, myocarditis, cardiomyopathies, myocardial ischemia, ventricular hypertrophy, and hydroelectrolytic imbalance may also lead to changes in the T wave. Symmetrical, negative, and wide T waves are not uncommon in the precordial leads in patients with hypertrophic cardiomyopathy. In cases of children with severe acute brain injury large negative T waves in various leads can be seen. This is known as "cerebral T waves" (Item 11.1.5).

13.3.5.4. U Wave

U Wave is not always visible on the ECG, but it can be prominent in children, in cases of hypokalemia, with antiarrhythmic drugs, and long QT syndrome.

13.4. Heart Rhythm Disorders

The electrocardiographic criteria used for assessing cardiac arrhythmias in children follow those used for adults (see Section 3).

13.5. Identification of Situs, Cardiac Position, and Ventricular Inversion

The identification of *situs* through the ECG is fundamentally based on the orientation of the P wave, which is positive in D1 and V6 in *situs solitus* and negative in *situs inversus*.[166] In this case, electrode reversal and left atrial rhythm are the main differential diagnoses.

In patients with *situs solitus* and levocardia, the P wave axis and the QRS axis are in the left lower quadrant (frontal plane). In *situs inversus* with dextrocardia, the P wave axis and the QRS axis are in the right lower quadrant. The P wave axis and the QRS axis are located in different quadrants when there is a disagreement between *situs* and cardiac position, such as in dextrocardia with *situs solitus* (which is usually associated with complex congenital heart diseases).[170]

The orientation of the first (5–20 ms) QRS vectors is important for determining the position of the ventricles. In case of ventricular inversion, the first vectors are directed towards the left and Q waves are not seen in D1 and V6.[166]

14. The ECG during Cardiac Pacing

14.1. Cardiac Pacing

Basically, the ECG of an individual with a cardiac implantable electronic device (CIED) is characterized by the presence or absence of spikes (artifacts resulting from pulse energy emission by pacing).

Except for implantable monitors (loop recorders), all other CIED (pacemakers, cardiac resynchronization devices, and implantable cardioverter-defibrillators – Table 14.1) are capable of emitting spikes, especially for treating bradycardias. So, the identification of the type of CIED based on ECG interpretation frequently is not possible. On the other hand, proper device functioning, as well as dysfunctions, can be recognized on the ECG. Pacing configuration, unipolar or bipolar, determines the size of the spike. In unipolar pacing, potential difference is established between the pulse generator and the lead tip, which potential difference results in a large amplitude vector. Consequently, the spikes should have large amplitudes too. In bipolar pacing, potential difference is established between the anode and cathode on the lead tip; the vector generated by the potential difference should thus be small and the spikes recorded in this mode should also be small (sometimes almost imperceptible).

The terms and codes (5-letter code – Table 14.2) used for describing CIED properties follow an international standard by the North American Society of Pacing and Electrophysiology (Naspe) and the British Pacing and Electrophysiology Group (BPEG).[180] Figure 14.1 illustrates the algorithm for identifying the mode of operation of CIED.

14.1.1. Basic Terms

a) Spike — corresponds to the electrical impulse emitted by the CIED;

b) Capture — artificial tissue depolarization after the spike;

c) Basic rate — atrial/ventricular pacing rate without interference of spontaneous beats;

d) AV interval (AVI) — interval between spontaneous (sensed) or paced (spike) atrial activity and ventricular pacing;

e) Interventricular interval (IVI) — interval between 2 ventricular spikes, programmable by telemetry, available in cardiac resynchronization devices, and that can be sometimes identified on a resting ECG;

f) Maximum rate limit (MRL) — maximum pacing rate. In single-chamber devices, the maximum rate is reached with the activation of an adaptive rate pacing sensor. In dual-chamber devices, the maximum rate is reached in response to atrial sensitivity (frequency of P waves) or also by rate sensor activation;

g) Sensitivity — ability to recognize atrial (P waves) or ventricular (QRS complex) spontaneous electrical events;

Table 14.1 – Types of CIED and their classical indications.

CIED	Basic properties	Main indication
Conventional pacemaker	Atrial and/or ventricular pacing	Bradiarrhythmias
Cardiac resynchronization device	Atriobiventricular pacing	Refractory heart failure with LBBB
Implantable cardioverter-defibrillator	Atrial and/or ventricular pacing and therapies for ventricular tachyarrhythmia	Prevention of sudden cardiac death
Implantable cardioverter-defibrillator and cardiac resynchronization device	Atriobiventricular pacing Anti-ventricular tachyarrhythmia therapies	Refractory heart failure with LBBB Prevention of sudden cardiac death

CIED: cardiac implantable electronic devices.

Table 14.2 – 5-letter code for electrocardiographic identification of the mode of operation of CIED

I Chambers paced	II Chambers sensed	III Response to sensing	IV Rate modulation	IV Multisite pacing
O: none	O: none	O: none	O: none	O: none
V: ventricle	V: ventricle	T: trigger		A: atrium
A: atrium	A: atrium	I: Inhibited		V: ventricle
D: dual (A+V)	D: dual (A+V)	D: dual (A+V)		D: dual (A+V)
S: Single-chamber (A or V)	S: Single-chamber (A or V)		R: rate modulation	

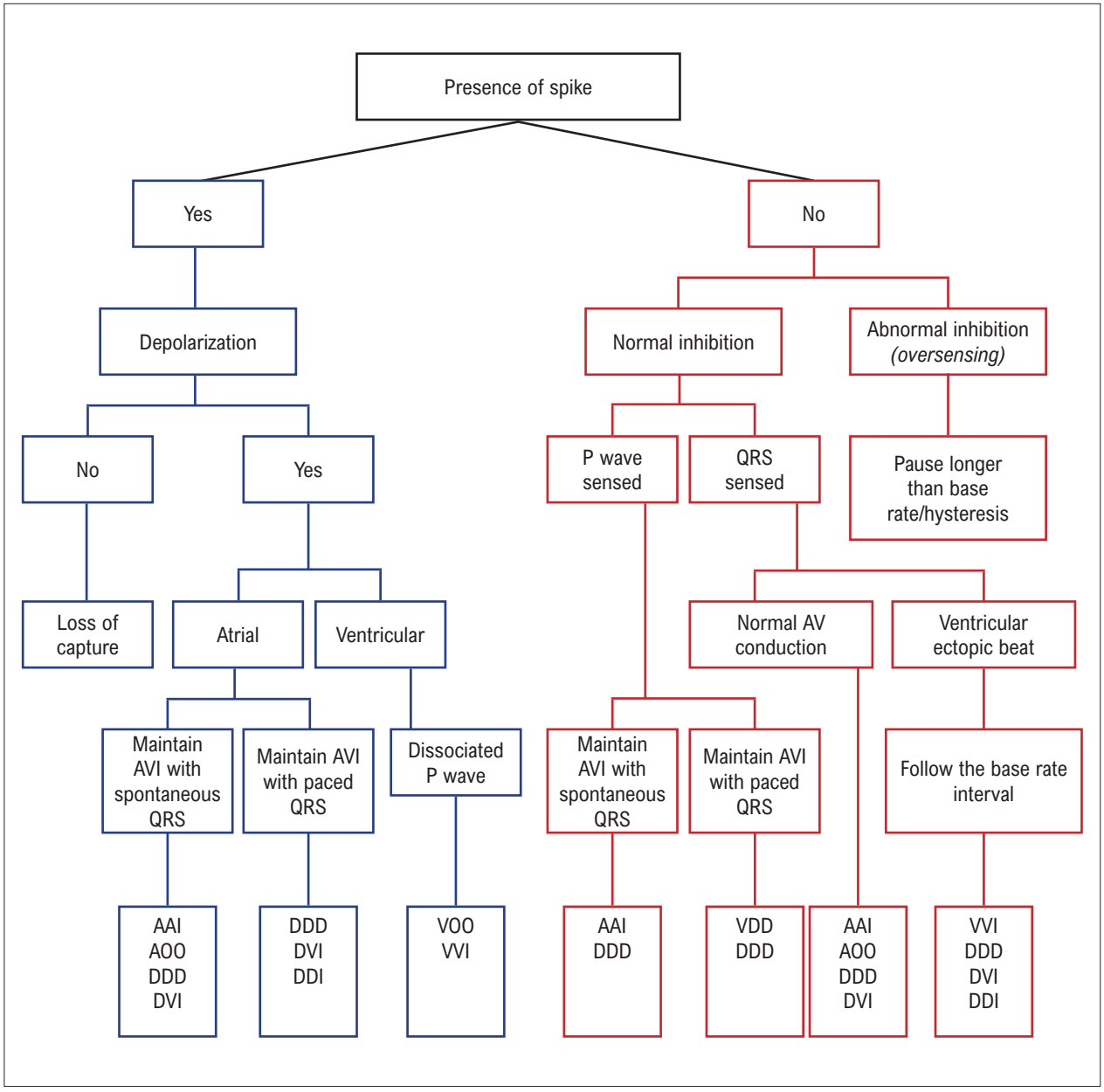

Figure 14.1 – *Algorithm for interpretation of ECG of individuals with CIED.*[181]

h) Normal inhibition — the pacing activity is inhibited by the intrinsic rhythm (no spikes).

14.1.2. Analysis of the Electrocardiographic Characteristics of CIED

a) Normal device operation — when normal capture and sensitivity are observed;

b) Loss of atrial and/or ventricular capture (intermittent or persistent) — absence of depolarization in the paced chamber (presence of a spike, but without triggering a P wave or QRS complex);

c) Sensitivity failure:

c.1) Excessive sensitivity (oversensing) — inappropriate sensitivity, resulting in the misidentification of spurious electrical signal that does not correspond to depolarization inhibiting spikes emission (electromagnetic interference, myopotentials, T wave, etc.);

c.2) Reduced sensitivity (undersensing) — inability to recognize spontaneous depolarization. It may occur due to inadequate programming or due to changes in the intrinsic signal (the system does not "see" the P wave or QRS complex).

d) Fusion beats — correspond to the artificial activation of cardiac tissue simultaneously to spontaneous depolarization, resulting in hybrid complexes. The pacemaker spike is followed by a P wave (atrial fusion) or a QRS complex (ventricular fusion), which morphology is in between capture and spontaneous beats;

e) Pseudofusion beats — spontaneous activation of cardiac tissue, concurrent with the device spike, which has no effect on the P wave or QRS complex (ventricular and atrial pseudofusion, respectively). The morphology of the wave following the spike is similar to that of the spontaneous beat;

f) Pacemaker-mediated tachycardia — restricted to AV CIED and characterized by ventricular triggering from a retrograde P wave. Therefore, this arrhythmia is caused by a circular movement in which the artificial pacing is the anterograde component of the circuit and the retrograde component is anatomic (normal or anomalous pathway);

g) Pacemaker-conducted tachycardia — tachyarrhythmia also involving AV CIED, characterized by the presence of supraventricular arrhythmia that, when perceived by the atrial channel, triggers ventricular capture at high rate, maintaining some characteristics of spontaneous arrhythmia;

h) Pacemaker-induced tachycardia — changes in sensitivity or electromagnetic interference that cause atrial or ventricular arrhythmias caused by inadequate pacing.

15. Tele-electrocardiography

Telemedicine is the remote delivery of health care services with the use of information and communication technologies in situations where one (or two) health care professionals and the patient are not physically present.[182] Tele-ECG systems record the electrocardiographic tracing obtained at a distance through different means and data transfer technologies. A remote physician analyzes and interprets the electrocardiographic tracing, and the report is made and sent electronically. Tele-ECG is related to ECG development. In 1905, Einthoven described the transtelephonic transmission of the ECG from the university hospital to the physiology laboratory at the University of Leiden, located 1.5 km away.[183]

With the advent of the computerized ECG[184] and systems capable of transmitting electrocardiographic tracings via the internet, the ECG with the specialist report became available in locations far from large counties centers. Tele-ECG units began to be implemented in Brazil in the 2000s, improving access to the electrocardiographic diagnosis and early recognition of relevant electrocardiographic abnormalities that could be fatal.[185]

Specific infrastructure is required to implement and run a tele-ECG department (Table 15.1). The central ECG interpretation unit should have a team of cardiologists, information technology (IT) specialists, and administrative assistants. Complete IT infrastructure with computers, hardware, software, and data protection and storage systems are vital for proper functioning.

Table 15.1 – Technical requirements for implementing tele-ECG

TECHNICAL STANDARDS
ANVISA registration
ABNT NBR IEC 60601-1 (general safety standard)
ABNT NBR IEC 60601-1-1 (safety of electromedical systems)
ABNT NBR IEC 60601-1-2 (electromagnetic compatibility)
ABNT NBR IEC 60601-1-4 (programmable electromedical systems)
ABNT NBR IEC 60601-2-25 (safety of electrocardiographs)
ABNT NBR IEC 60601-2-251 (safety standard, including the essential performance of electrocardiographs, single- and multi-channel recorders and analyzers)
MINIMUM EQUIPMENT REQUIREMENTS
Desktop or laptop computer
At least 1 USB port (2.0 or 3.0)
CD/DVD drive
4 GB memory
Intel Pentium® processor
Windows 7, 8, or 10
250GB hard drive or more
RECOMMENDATIONS
12 leads
High-quality tracing (1200 samples/second/channel)

The remote health care units that perform the ECG should have a digital electrocardiograph (approved by the corresponding federal agencies), internet connection, equipment, and services that allow audio or video communication, in addition to training all professionals involved.[182,186] We recommend that the original electrocardiographic signal be transmitted from images generated by the electrocardiograph itself or by professional scanners, avoiding low-quality digitization or image distortions that could hinder or prevent the ECG analysis.[182]

Tele-ECG has been shown to be an effective strategy in the rationalization of access to complementary propaedeutics, early diagnostics, prioritization of referrals, and organization of waitlists in health care systems, improving cost-effectiveness and health care assistance (Table 15.2).[187] Especially in rural areas, the prehospital tele-ECG performed in patients with acute coronary syndrome reduced door-to-balloon time and long-term mortality.[188,189] The detection of AF [190]and some channelopathies such as Brugada syndrome was also improved.[191] Moreover, the use of tele-ECG databases is essential for nationwide epidemiological studies.[192]

The constant growth of healthcare-related technology extended new perspectives in tele-ECG. The use of artificial intelligence (AI) techniques in ECG is increasing exponentially, with good results in the automatic diagnosis

Table 15.2 – Benefits of the tele-ECG[187]

Quick electrocardiographic diagnosis, enabling the identification of normal and abnormal cases
Prehospital care at the patient's location
Access to specialists in case of accidents and emergencies
Reductions in time and costs for the patient
Faster triage by specialists
Help and guidance to non-specialists
Easy management of health care resources
Increased safety of postoperative patients during rehabilitation
Cooperation and integration between researchers for sharing clinical records
Access to educational programs of training and qualification
A second opinion

of electrocardiographic abnormalities,[193,194] and in the development of new cardiovascular risk markers.[195] The wearable equipment such as the chest strap HR monitor, adhesive ECG patches, smartphones, and smartwatches allowed the early identification of possible cardiac arrhythmias, especially AF.[196] These portable and easy-to-use devices allowed fast HR recording in the patient's daily life, in any environment and at any time, followed by automatic and real-time interpretation via AI. A significant limitation of this technology still is the cost. As a consequence of modern techniques, we cannot ignore a possible increase in workload (recording and sending information from patients to their physicians), false-positive cases resulting from artifacts, and the increased emotional burden to some patients when "discovering" a cardiac arrhythmia. The following years should elucidate the role of new methodologies and technologies in clinical practice. However, with these advancements, we believe the ECG can reach new uses and applications.

References

1. Greenland P, Alpert JS, Beller GA, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, et al. American College of Cardiology Foundation; American Heart Association. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2010 Dec 14;56(25):e50-103. doi: 10.1016/j.jacc.2010.09.001.
2. Said SA, Bloo R, de Nooijer R, Slootweg A. Cardiac and non-cardiac causes of T-wave inversion in the precordial leads in adult subjects: A Dutch case series and review of the literature. World J Cardiol. 2015 Feb 26;7(2):86-100. doi: 10.4330/wjc.v7.i2.86. PMID: 25717356; PMCID: PMC4325305.
3. Moffa PJ, Sanches PC. Eletrocardiograma normal e patológico. São Paulo: Editora Roca, 2001.
4. Grindler J, Silveira MAP, Oliveira CAR, Friedmann AA. Artefatos Técnicos. In: Friedmann AA, Grindler JO,Rodrigues CA. (eds). Diagnóstico diferencial no eletrocardiograma. Barueri (SP): Editora Manole; 2007. Cap.20; p. 187-194.
5. Fisch C. Electrocardiography and vectorcardiography. In: Braunwald E (ed). Heart disease: a textbook of cardiovascular medicine. Philadelphia: W.B.Saunders; 1984. p. 200.
6. Bashshur RL, Shannon GW. History of telemedicine: evolution, context, and transformation. USA: Mary Ann Liebert, Inc. Publisher; 2009.
7. Lin SH, Liu JH, Wei J, Yin WH, Chen HH, Chiu WT. A business model analysis of telecardiology service. Telemed J E Health. 2010 Dec;16(10):1067-73. doi: 10.1089/tmj.2010.0059. PMID: 21186990.
8. Oliveira Junior MT, Canesin MF, Marcolino MS, Ribeiro AL, Carvalho AC, et al., Sociedade Brasileira de Cardiologia. Diretriz de telecardiologia no cuidado de pacientes com síndrome coronariana aguda e outras doenças cardíacas.. Arq Bras Cardiol. 2015 Jun;104(5 Suppl 1):1-26. doi: 10.5935/abc.20150057. Epub 2015 Jun 1. PMID: 26039716.
9. García-Niebla J, Llontop-García P, Valle-Racero JI, Serra-Autonell G, Batchvarov VN, de Luna AB. Technical mistakes during the acquisition of the electrocardiogram. Ann Noninvasive Electrocardiol. 2009;14(4):389-403. doi: 10.1111/j.1542-474X.2009.00328.x.
10. Llinas R, Henderson GV. Images in clinical medicine. Tremor as a cause of pseudo-ventricular tachycardia. N Engl J Med. 1999 Oct 21;341(17):1275. doi: 10.1056/NEJM199910213411705.
11. Aslanger E, Bjerregaard P. Mystery of "bizarre electrocardiogram" solved. J Electrocardiol.2011;44(6):810-1 doi:10.1016/j.jelectrocard.2011.04.001
12. Aslanger E, Yalin K. Electromechanical association: a subtle electrocardiogram artifact. J Electrocardiol. 2012;45(1):15-7. doi: 10.1016/j.jelectrocard.2010.12.162.
13. Pinto Filho MM,Ribeiro ALP.Electrocardiographic findings in Brazilian adults without heart disease:GLSA-Brasil. Arq Bras Cardiol.2017;109(5):416-24. doi:10.5935/abc.20170146,
14. Yang XS, Beck G J, Wilkoff BL. Redefining normal sinus heart rate J Am Coll Cardiol.1995;25(2 Suppl 1):193 A.
15. Palatini P. Need for a revision of the normal limits of resting heart rate. Hypertension.1999;33(2):622-5. Doi:10.1161/o1.hyp.33.2.622
16. Mason JW, Ramseth DJ, Chanter DO, Moon TE, Goodman DB, Mendzelevski B. Electrocardiographic reference ranges derived from 79,743 ambulatory subjects. J Electrocardiol.2007;40(3):228-34. Doi: 10.1016/j.jelectrocard.2006.09.003.
17. Andries E, Brugada P, Brugada J, Steurer G, Podrid PJ. A practical approach to the diagnosis of a tachycardia with a wide QRS complex. In: Podrid P, Kowey P. Cardiac arrhythmia: mechanisms, diagnosis and management. Baltimore: Williams & Wilkins;1995.p:1022-1050. ISBN:0683069055
18. Bayés de Luna A, Platonov P, Cosio FG, Cygankiewicz I, Pastore C, Baranowski R, et al. Interatrial blocks:a separate entity from left atrial enlargement: a consensus report. J Electrocardiol. 2012 Sep;45(5):445-51. doi: 10.1016/j.jelectrocard.2012.06.029.
19. Elosua R, Escobar-Robledo LA, Massó-van Roessel A, Martínez-Sellés M, Baranchuk A, Bayés-de-Luna A. ECG patterns of typical and atypical advanced interatrial block: prevalence and clinical relevance. Rev Esp Cardiol (Engl Ed). 2021;74(9):807-10. doi: 10.1016/j.rec.2021.02.008.
20. Gomes JA, Mehta D, Langan MN. Sinus node reentrant tachycardia. Pacing Clin Electrophysiol. 1995 May;18(5 Pt 1):1045-57. doi: 10.1111/j.1540-8159.1995.tb04747.x.
21. Benditt D, Sakaguchi S, Goldstein M. Sinus node dysfunction: Pathophysiology, clinical features, evaluation, and treatment. In: Zipes DP,Jalife J,(eds). Cardiac electrophysiology: from cell to bedside. Phyladelphia:Saunders;1995. p.1215-1247.

22. Page RL, Joglar JA, Caldwell MA, Calkins H, Conti JB, Deal BJ, et al. Evidence Review Committee Chair‡. 2015 ACC/AHA/HRS Guideline for the Management of Adult Patients With Supraventricular Tachycardia: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society.Circulation.2016;133(14):e471-505. doi:10.1161/CIR0000000000000310.

23. Erikssen J, Otterstad JE. Natural course of a prolonged PR interval and the relation between PR and incidence of coronary heart disease. A 7-year follow-up study of 1832 apparently healthy men aged 40-59 years. Clin Cardiol. 1984 Jan;7(1):6-13. doi: 10.1002/clc.4960070104.

24. Crisel RK, Farzaneh-Far R, Na B, Whooley MA. First-degree atrioventricular block is associated with heart failure and death in persons with stable coronary artery disease: data from the Heart and Soul Study. Eur Heart J. 2011 Aug;32(15):1875-80. doi: 10.1093/eurheartj/ehr139.

25. Barold SS, Ilercil A, Leonelli F, Herweg B. First-degree atrioventricular block. Clinical manifestations, indications for pacing, pacemaker management & consequences during cardiac resynchronization. J Interv Card Electrophysiol. 2006 Nov;17(2):139-52. doi: 10.1007/s10840-006-9065-x.

26. Holmqvist F, Daubert JP. First-degree AV block-an entirely benign finding or a potentially curable cause of cardiac disease? Ann Noninvasive Electrocardiol. 2013 May;18(3):215-24. doi: 10.1111/anec.12062.

27. Wolff L, Parkinson J, White PD. Bundle-branch block with short P-R interval in healthy young people prone to paroxysmal tachycardia. Ann Noninvasive Electrocardiol.2006;11(4):340-53. https://doi.org/10.1111/j.1542-474X.2006.00127.x

28. Boineau JP, Moore EN. Evidence for propagation of activation across an accessory atrioventricular connection in types A and B pre-excitation. Circulation.1970;41(3):375-97. doi: 10.1161/01.cir.41.3.375.

29. Burchell HB, Frye RL, Anderson MW, McGoon DC. Atrioventricular and ventriculoatrial excitation in Wolff-Parkinson-White syndrome (type B). Temporary ablation at surgery. Circulation.1967;36(5):663-72. doi: 10.1161/01.cir.36.5.663.

30. Hara WN. Localização vectorcardiográfica das vias acessórias atrioventriculares em pacientes com síndrome de Wolff-Parkinson-White submetidos à ablação por radiofrequência. Tese. São Paulo: Escola Paulista de Medicina da Universidade Federal de São Paulo;1974.

31. Lown B, Ganong WF, Levine SA. The syndrome of short P-R interval, normal QRS complex and paroxysmal rapid heart action. Circulation. 1952;5(5):693-706. doi: 10.1161/01.cir.5.5.693.

32. Mahaim I. Kent's fibers and the A-V paraspecific conduction through the upper connections of the bundle of His-Tawara. Am Heart J. 1947;33(5):651-3. doi: 10.1016/0002-8703(47)90080-x.

33. Lorga Filho A, Sosa E, Scanavacca M, D'Avila A, Kuniyoshi R, de Horta J, et al. Electrocardiographic identification of mid-septal accessory pathways in close proximity to the atrioventricular conduction system. Pacing Clin Electrophysiol.1996;19(11 Pt 2):1984-7. Doi:10.1111/j.1540-8159.1996.tb03266.x.

34. Moss JD, Gerstenfeld EP, Deo R, Hutchinson MD, Callans DJ, Marchlinski FE, Dixit S. ECG criteria for accurate localization of left anterolateral and posterolateral accessory pathways. Pacing Clin Electrophysiol. 2012 Dec;35(12):1444-50. doi: 10.1111/pace.12011.

35. Arruda MS, McClelland JH, Wang X, Beckman KJ, Widman LE, Gonzalez MD, et al. Development and validation of an ECG algorithm for identifying accessory pathway ablation site in Wolff-Parkinson-White syndrome. J Cardiovasc Electrophysiol.1988;9(1):2-12. doi:10.1111/j.1540-8167.1998.tb00861.x.

36. Crinion D, Baranchuk A. Algorithms to identify accessory pathways' location on the 12-lead electrocardiogram. Card Electrophysiol Clin. 2020;12(4):465-74. doi: 10.1016/j.ccep.2020.08.007.

37. Spodick DH. Truly isorhythmic A-V dissociation. Am J Geriatr Cardiol. 2007;16(1):53 Jan-Feb;16(1):53. doi: 10.1111/j.1076-7460.2007.5966.x.

38. Bayés de Luna A, Gaugi Gené C, Soler Soler J, Fort de Ribot R. Eletrocardiologia clínica. Barcelona: Editora Científico-Médica;1977.

39. Durrer D. The human heart: some aspects of its excitation. Trans Stud Coll Physicians Phila. 1966 Jan;33(3):159-70. PMID: 5906405.

40. Bisteni A,Sodi-Pallares D, Medrano GA, Pileggi F. A new approach for the recognition of ventricular premature beats. Am J Cardiol. 1960 Mar;5:358-69. doi: 10.1016/0002-9149(60)90083-7. PMID: 13801031.

41. Terkelsen CJ, Sørensen JT, Kaltoft AK, Nielsen SS, Thuesen L, Bøtker HE, Lassen JF. Prevalence and significance of accelerated idioventricular rhythm in patients with ST-elevation myoc ardial infarction treated with primary percutaneous coronary intervention. Am J Cardiol. 2009 Dec 15;104(12):1641-6. doi: 10.1016/j.amjcard.2009.07.037.

42. Brady WJ, De Behnke DJ, Laundrie D. Prevalence, therapeutic response, and outcome of ventricular tachycardia in the out-of-hospital setting: a comparison of monomorphic ventricular tachycardia, polymorphic ventricular tachycardia, and torsades de pointes. Acad Emerg Med. 1999 Jun;6(6):609-17. doi: 10.1111/j.1553-2712.1999.tb00414.x.

43. Trinkley KE, Page RL 2nd, Lien H, Yamanouye K, Tisdale JE. QT interval prolongation and the risk of torsades de pointes: essentials for clinicians. Curr Med Res Opin. 2013 Dec;29(12):1719-26. doi: 10.1185/03007995.2013.840568.

44. Leenhardt A, Extramiana F, Milliez P, Denjoy I, Thomas O, Meddane M,et al. Tachycardies ventriculaires bidirectionnelles [Bidirectional ventricular tachycardias]. Arch Mal Coeur Vaiss. 2003 Dec;96(Spec 7):27-31. PMID: 15272518.

45. Chung KY, Walsh TJ, Massie E. Double ventricular parasystole. Am Heart J 1964;67:162-5. doi: 10.1016/0002-8703(64)90364-3.

46. Brugada P, Brugada J, Mont L, Smeets J, Andries EW. A new approach to the differential diagnosis of a regular tachycardia with a wide QRS complex. Circulation. 1991 May;83(5):1649-59. doi: 10.1161/01.cir.83.5.1649.

47. Antunes E, Brugada J, Steurer G, Andries E, Brugada P. The differential diagnosis of a regular tachycardia with a wide QRS complex on the 12-lead ECG: ventricular tachycardia, supraventricular tachycardia with aberrant intraventricular conduction, and supraventricular tachycardia with anterograde conduction over an accessory pathway. Pacing Clin Electrophysiol. 1994 Sep;17(9):1515-24. doi: 10.1111/j.1540-8159.1994.tb01517.x.

48. Vereckei A, Duray G, Szénási G, Altemose GT, Miller JM. Application of a new algorithm in the differential diagnosis of wide QRS complex tachycardia. Eur Heart J. 2007 Mar;28(5):589-600. doi: 10.1093/eurheartj/ehl473.

49. Wellens HJ, Bär FW, Lie KI. The value of the electrocardiogram in the differential diagnosis of a tachycardia with a widened QRS complex. Am J Med. 1978 Jan;64(1):27-33. doi: 10.1016/0002-9343(78)90176-6.

50. Wellens HJ. Electrophysiology: ventricular tachycardia: diagnosis of broad QRS complex tachycardia. Heart. 2001:86(5):579-85. doi: 10.1136/heart.86.5.579.

51. Steurer G, Gürsoy S, Frey B, Simonis F, Andries E, Kuck K, Brugada P. The differential diagnosis on the electrocardiogram between ventricular tachycardia and preexcited tachycardia. Clin Cardiol. 1994 Jun;17(6):306-8. doi: 10.1002/clc.4960170606.

52. Marriot HJ. Differential diagnosis of supraventricular and ventricular tachycardia. Cardiology 1990;77(3):209-20. doi: 10.1159/000174602.

53. Drew BJ, Scheinman MM. Value of electrocardiographic leads MCL1, MCL6 and other selected leads in the diagnosis of wide QRS complex tachycardia. J Am Coll Cardiol. 1991 Oct;18(4):1025-33. doi: 10.1016/0735-1097(91)90762-x.

54. Vereckei A, Duray G, Szénási G, Altemose GT, Miller JM. New algorithm using only lead aVR for differential diagnosis of wide QRS complex tachycardia. Heart Rhythm. 2008 Jan;5(1):89-98. doi: 10.1016/j.hrthm.2007.09.020.

55. Pava LF, Perafán P, Badiel M, Arango JJ, Mont L, Morillo CA, Brugada J. R-wave peak time at DII: a new criterion for differentiating between wide complex QRS tachycardias. Heart Rhythm. 2010 Jul;7(7):922-6. doi: 10.1016/j.hrthm.2010.03.001.

56. Jastrzebski M, Sasaki K, Kukla P, Fijorek K, Stec S, Czarnecka D. The ventricular tachycardia score: a novel approach to electrocardiographic diagnosis of ventricular tachycardia. Europace. 2016 Apr;18(4):578-84. doi: 10.1093/europace/euv118.

57. Santos Neto F, Pisani CF, Darrieux FCDC, Cirino CMF, Hachul DT, Santos AM, et al. Validation of a Simple Electrocardiographic Algorithm for Detection of Ventricular Tachycardia. Arq Bras Cardiol. 2021 Mar;116(3):454-463. doi: 10.36660/abc.20190501.

58. Casale PN, Devereux RB, Kligfield P, Eisenberg RR, Miller DH, Chaudhary BS, Phillips MC. Electrocardiographic detection of left ventricular hypertrophy: development and prospective validation of improved criteria. J Am Coll Cardiol. 1985 Sep;6(3):572-80. doi: 10.1016/s0735-1097(85)80115-7.

59. Devereux RB, Phillips MC, Casale PN, Eisenberg RR, Klingfield P. Geometric determinants of electrocardiographic left ventricular hypertrophy. Circulation.1983;67(4):907-11. DOI: 10.1161/01.cir.67.4.907

60. Sokolow M, Lyon TP. The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. Am Heart J 1949;37(2):161-86. DOI: 10.1016/0002-8703(49)90562-1

61. Pringle SD, MacFarlane PW, McKillop JH,Lorimer AR,Dunn FG. Pathophysiologic assessment of left ventricular hypertrophy and strain in asymptomatic patients with essential hypertension. J Am Coll Cardiol. 1989;13(6):1377-81. doi: 10.1016/0735-1097(89)90314-8

62. Reichek N, Devereux RB. Left ventricular hypertrophy: Relationship of anatomic echocardiographic and electrocardiographic findings. Circulation 1981;63(6):1391-8. doi: 10.1161/01.cir.63.6.1391.

63. Romhilt DW, Bove KE, Norris RJ, Conyers E, Conradi S, Rowlands DT, et al. A critical appraisal of the electrocardiographic criteria for the diagnosis of left ventricular hypertrophy. Circulation. 1969;40(2):185-95. DOI: 10.1161/01.cir.40.2.185

64. Fragola PV, Autore C, Ruscitti GC, Picelli A, Cannata D. Electrocardiographic diagnosis of left ventricular hypertrophy in the presence of left bundle branch block: a wasted effort. Int J Cardiol. 1990;28(2):215-21. DOI: 10.1016/0167-5273(90)90063-b

65. Scott RC. The correlation between ECG patterns of ventricular hypertrophy and the, anatomic findings. Circulation. 1960;21:256-91. doi: 10.1161/01.cir.21.2.256.

66. Romhilt D, Estes E. A point score system for the ECG diagnosis of left ventricular hypertrophy. Am Heart J 1968;75(6):752-8. doi: 10.1016/0002-8703(68)90035-5.

67. Peguero JG, Lo Presti S, Perez J, Issa O, Brenes JC, Tolentino A. Electrocardiographic Criteria for the Diagnosis of Left Ventricular Hypertrophy. J Am Coll Cardiol. 2017;69(13):1694-703 . doi: 10.1016/0002-8703(68)90035-5.

68. Tavares CAM, Samesima N, Hajjar LA, Godoy LC, Padrão EMH, Lazar Neto F, et al. Clinical applicability and diagnostic performance of electrocardiographic criteria for left ventricular hypertrophy diagnosis in older adults. Sci Rep. 2021 Jun 1;11(1):11516. doi: 10.1038/s41598-021-91083-9.

69. McCaughan D, Koroxenidis GT, Hopff LG, William C. et al. New vectorcardiographic criteria for the diagnosis of acquired right ventricular hypertrophy: Comparison with standard electrocardiographic criteria (P). [Abstract] Circulation. 1963;28(4):766.

70. Myers GB, Klein HA, Stofer BE. The electrocardiographic diagnosis of right ventricular hypertrophy. Am Heart J. 1948;35(1):1-40. doi: 10.1016/0002-8703(48)90182-3.

71. Gamboa R, Hugenholtz PG, Nadas AS. Corrected (Frank), uncorrected (cube), and standard electrocardiographic lead systems in recording augmented right ventricular forces in right ventricular hypertension. Brit Heart J. 1966;28(1):62-74. doi: 10.1136/hrt.28.1.62.

72. Flowers NC, Horan LG. Hypertrophy and infarction: Subtle signs of right ventricular enlargement and their relative importance. In: Schlant RC, Hurst JW. Advances in Electrocardiography. New York: Grune & Straton, 1972.

73. Okin PM, Roman MJ, Devereux RB, Kligfield P. Time-voltage area of the QRS for the identification of left ventricular hypertrophy. Hypertension. 1996;27(2):251-8. doi: 10.1161/01.hyp.27.2.251.

74. Castellanos Jr A, Lemberg L. Diagnosis of isolated and combined block in the bundle branches and the divisions of the left branch. Circulation. 1971;43(6):971-6. doi: 10.1161/01.cir.43.6.971.

75. Scheinman M, Brenman BA. Clinical and anatomic implications of intraventricular conduction blocks in acute myocardial infarction. Circulation. 1972;46(4):753-60. doi: 10.1161/01.cir.46.4.753.

76. Tan NY, Witt CM, Oh JK, Cha YM. Left Bundle Branch Block: Current and Future Perspectives. Circ Arrhythm Electrophysiol. 2020 Apr;13(4):e008239. doi: 10.1161/CIRCEP.119.008239.

77. Kusumoto FM, Schoenfeld MH, Barrett C, Edgerton JR, Ellenbogen KA, Gold MR,et al. 2018 ACC/AHA/HRS Guideline on the Evaluation and Management of Patients With Bradycardia and Cardiac Conduction Delay: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines, and the Heart Rhythm Society. Circulation. 2019 Aug 20;140(8):e333-e381. doi: 10.1161/CIR.0000000000000627. Erratum in: Circulation. 2019 Aug 20;140(8):e504-e505.

78. Auricchio A, Fantoni C, Regoli F, Carbucicchio C, Goette A, Geller C,et al. Characterization of left ventricular activation in patients with heart failure and left bundle-branch block. Circulation. 2004 Mar 9;109(9):1133-9. doi: 10.1161/01.CIR.0000118502.91105.F6.

79. Tavares CAM, Samesima N, Lazar Neto F, Hajjar LA, Godoy LC, Padrão EMH, et al. BMC Cardiovasc Disord. 2021 Nov 17;21(1):547. doi: 10.1186/s12872-021-02332-8.

80. Denis B, Arnaud P. [Right ventricular diastolic overloading in left bundle-branch block]. Ann Cardiol Angeiol (Paris). 1990 Apr;39(4):213-7. PMID: 2142390

81. Castellanos A Jr, Maytin 0, Arcebal AG, Lemberg L. Significance of complete right bundle branch block with right axis deviation in absence of right ventricular hypertrophy. Brit Heart J. 1970;32(1):85-92. doi: 10.1136/hrt.32.1.85.

82. Rosenbaum MB. Types of right bundle branch block and their clinical significance. J Eletrocardiol 1968;1(2):221-32. doi: 10.1016/s0022-0736(69)81017-4.

83. Rosenbaum MB, Elizari MV, Lazzari JO. Los Hemibloqueos. Buenos Aires: Paidos;1967.

84. Tranchesi J, Grinberg M, Moffa PJ,Spiritus MO, Serro-Azul LG. Os bloqueios das divisões do ramo esquerdo (hemibloqueios). Conceitos atuais. Arq Bras Cardiol 1971;24(3):77-90. PMID: 5162306

85. Demoulin JC, Kulbertus HE. Histopathological examination of concept of left hemiblock. Br Heart J. 1972;34(8):807-14. doi: 10.1136/hrt.34.8.807.

86. Rosenbaum MB, Elizari MV, Levi RJ, Nau GJ, Pisani N, Lazzari JO, et al. Five cases of intermittent left anterior hemiblock. Am J Cardiol. 1969;24(1):1-. doi: 10.1016/0002-9149(69)90044-7.

87. Benchimol A, Desser KB, Massey BJ. Coexisting left anterior hemiblock and inferior wall myocardial infarction. Vectorcardiographic features. Am J Cardiol 1972;29(1):7-14. DOI: 10.1016/0002-9149(72)90409-2

88. Hoffman I, Mehte J, Hilssenrath J, Hamby RI. Anterior conduction delay: A possible cause for prominent anterior QRS forces. J Eletrocardiology 1976;9(1):15. doi: 10.1016/s0022-0736(76)80004-0.

89. Iwamura N, Kodama I, Shimizu T,Yamada K, Irata Y,Toyama J. Functional properties of the left septal Purkinje network in premature activation of the ventricular conduction system. Am Heart J. 1978;95(1):60-9. doi: 10.1016/0002-8703(78)90397-6.

90. Tranchesi J, Moffa PJ, Pastore CA, de Carvalho Filho E, Tobias NM. Bloqueio da divisão ântero-medial do ramo esquerdo do feixe de His nas coronariopatias. Caracterização vetorcardiográfica. Arq Bras Cardiol .1979;32(6):355-60. PMID: 508112

91. Brenes PC, Medrano GA, Sodi-Pallares D. El bloqueo de Ia subdivision posterior de Ia rama izquierda del haz de His. Estudio clínico, eletro y vetorcardiográfico. Arch Inst Cardiol Méx. 1970;40(5):621-34. PMID: 5532175

92. Demoulin JC, Kulbertus HE. Histopathologic correlates of left posterior fascicular block. Am J Cardiol. 1979;44(6):1083-8. doi: 10.1016/0002-9149(79)90173-5.

93. Dhingra RC, Wyndham C, Amat-y-Leon F Denes P, Wu D, Srindhar, et al. Incidence and site of atrioventricular block in patients with chronic bifascicular block. Circulation. 1979;59(2):238-46. doi: 10.1161/01.cir.59.2.238.

94. Kulbertus H, Collignon P, Humblet L. Vectorcardiographic study of QRS loop in patients with left superior axis deviation and right bundle-branch block. Br Heart J. 1970;32(3):386-92. doi: 10.1016/0002-8703(70)90417-5.

95. Fragola PV, Autore C, Magni G,Albertini M, Pierangeli L, Ruscitti G, et al. et al. Limitations of the electrocardiographic diagnosis of left ventricular hypertrophy: the influence of left anterior hemiblock and right bundle branch block. Int J Cardiol. 1992;34(1):41-8. doi: 10.1016/0167-5273(92)90080-m.

96. Elizari MV, Baranchuck A, Chiale PA. Masquerading bundle branch block : a variety of right bundle branch block with left anterior fascicular block. Expert Rev Cardiov Ther. 2013;11(1); 69-75. doi: 10.1586/erc.12.142.

97. Dhanse S, Kareen H, DevasiT, Sudhakar RM. Masquerading bundle branch block : a poor prognosis sign revisited . J Clin Diagn Res. 2016; 10(9):OD01-OD02 doi: 10.7860/JCDR/2016/20572.8472.

98. Flowers NC, Horan LG, Wylds AC, Crawford W, Sridharan MR, Horan CP, et al. Relation of peri-infarction block to ventricular late potentials in patients with inferior wall myocardial infarction. Am J Cardiol. 1990 Sep 1;66(5):568-74. DOI: 10.1016/0002-9149(90)90483-h

99. Lemmert ME, de Jong JS, van Stipdonk AM, Crifins HJ, Sridharan MR, Horan CP, et al. Electrocardiographic factors playing a role in ischemic ventricular fibrillation in ST elevation myocardial infarction are related to the culprit artery. Heart Rhythm. 2008 Jan;5(1):71-8. doi: 10.1016/j.hrthm.2007.09.011.

100. Brenyo A, Pietrasik G, Barsheshet A, Huang DT, Polonsky B, McNitt S, et al. QRS fragmentation and the risk of sudden cardiac death in MADIT II. J Cardiovasc Electrophysiol. 2012 Dec;23(12):1343-8. doi: 10.1111/j.1540-8167.2012.02390.x.

101. Benezet-Mazuecos J, Ibanez B, Farre J. Atypical left bundle branch block in dilative "burned-out" phase of hypertrophic cardiomyopathy. Pacing Clin Electrophysiol. 2005 Dec;28(12):1357-9. doi: 10.1111/j.1540-8159.2005.00279.x.

102. Nogami A. Purkinje-related arrhythmias part I: monomorphic ventricular tachycardias. Pacing Clin Electrophysiol. 2011 May;34(5):624-50. doi: 10.1111/j.1540-8159.2011.03044.x.

103. Amsterdam EA, Wenger NK, Brindis RG, Casey DE, Ganiats TG, Holmes DG, et al. American College of Cardiology; American Heart Association Task Force on Practice Guidelines; Society for Cardiovascular Angiography and Interventions; Society of Thoracic Surgeons; American Association for Clinical Chemistry. 2014 AHA/ACC Guideline for the Management of Patients with Non-ST-Elevation Acute Coronary Syndromes: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2014 Dec 23;64(24):e139-228. doi: 10.1016/j.jacc.2014.09.017.

104. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J. 2016;37(3):267-315. doi: 10.1093/eurheartj/ehv320

105. Krone RJ, Greenberg H, Dwyer EM Jr, Kleiger RE, Boden WE. Long-term prognostic significance of ST segment depression during acute myocardial infarction. The Multicenter Diltiazem Postinfarction Trial Research Group. J Am Coll Cardiol. 1993;22(2):361-7. doi: 10.1016/0735-1097(93)90038-3.

106. Bayes de Luna A, Zareba W, Fiol M, Nikus K, Birnbaum Y, et al. Negative T wave in isquemic heart disease: A consensus article. Ann Noninvasive Electrocardiol. 2014;19(5):426–41.

107. Knotts RJ, Wilson JM, Kim E HD, Birnbaum Y, Huang HD, Birnbaum Y. Diffuse ST depression with ST elevation in aVR: Is this pattern specific for global ischemia due to left main coronary artery disease? J Electrocardiol.2013;46(3):240-8. doi: 10.1016/j.jelectrocard.2012.12.016.

108. Yamaji H, Iwasaki K, Kusachi S, Murakami T, Hirami R, Hamamoto H, et al. Prediction of acute left mais coronary artery obstruction by 12-lead electrocardiography. ST segment elevation in lead aVR with less ST elevation in lead V1. J Am Coll Cardiol. 2001;38(5):1348-54. doi: 10.1016/s0735-1097(01)01563-7.

109. Thygesen K(1), Alpert JS, Jaffe AS, Simooms ML, Chaitman BR, White HD, et al. Writing Group on the Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction. ESC Committee for Practice Guidelines (CPG). Third universal definition of myocardial infarction. Eur Heart J. 2012;33(20):2551-67. doi: 10.1093/eurheartj/ehs184.

110. Bayés de Luna A, Rovai D, Pons Llado G,Gorgels A, Chaitman BR, White HD, et al. The end of an electrocardiographic dogma: a prominent R wave in V1 is caused by a lateral not posterior myocardial infarction-new evidence based on contrast-enhanced cardiac magnetic resonance-electrocardiogram correlations. Eur Heart J. 2015 Apr 21;36(16):959-64. doi: 10.1093/eurheartj/ehv035.

111. Nucifora G, Miani D, Di Chiara A, Piceoli G, Artico J, Puppato M, et al. Infarct-like acute myocarditis: relation between electrocardiographic findings and myocardial damage as assessed by cardiac magnetic resonance imaging. Clin Cardiol. 2013 Mar;36(3):146-52. doi: 10.1002/clc.22088.

112. Selvester RH, Sanmarco ME, Solomon JC, Wagner GS. The ECG: QRS change. In: Wagner GS (ed) Measurement and intervention. The Hague: Martinus Nijhoff Publishers; 1982. p.23-50.

113. Lopez-Sendon J, Coma-Canella I, Alcasena S, et al. Electrocardiographic findings in acute right ventricular infarction: sensitivity and specificity of electrocardiographic alterations in right precordial leads V4R, V3R, V1, V2, and V3. J Am Coll Cardiol. 1985;6(6):1273-9. doi: 10.1016/s0735-1097(85)80213-8.

114. Lazar, EJ, Goldberger J, Peled H, Sherman M, Frisfman WH. Atrial infarction: diagnosis and management. Am Heart J. 1988;116(4):1058-63. doi: 10.1016/0002-8703(88)90160-3.

115. Bairey CN, Shah PK, Lew AS, Hulse S. Electrocardiographic differentiation of occlusion of the left circumflex versus the right coronary artery as a cause of inferior acute myocardial infarction. Am J Cardiol 1987;60(7):456-9. doi: 10.1016/0002-9149(87)90285-2.

116. Nikus K, Birnbaum Y, Fiol-Sala M, Rankinen J, de Luna AB. Conduction Disorders in the Setting of Acute STEMI. Curr Cardiol Rev. 2021;17(1):41-9. doi: 10.2174/1573403X16666200702121937.

117. Sgarbossa EB, Pinski SL, Barbagelata A, et al. Electrocardiographic diagnosis of evolving acute myocardial infarction in the presence of left bundle-branch block. GUSTO-1 (Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries) Investigators. N Engl J Med 1966;334(7):481-7. doi: 10.1016/0002-9149(87)90285-2.

118. Bazett HC. An analysis of the time-relations of electrocardiograms. Heart.1920(7):353-70.

119. Sagie A, Larson MG, Goldberg RJ, Bengtson JR, Levy D. et al. An improved method for adjusting the QT interval for heart rate (the Framingham Heart Study). Am J Cardiol 1992;79(7):797–801. DOI: 10.1016/0002-9149(92)90562-d

120. Hodges M, Salerno D, Erlien D. Bazett's QT correction reviewed - Evidence that a linear QT correction for heart is better, (Abstract). J Am Coll Cardiol.1983;1(2):694.

121. Camm JA, Marik M, Yap GY. Acquired long-QT syndrome. Philadelphia:Blackwell Publishing; 2004. pp. 47-48.

122. Gollob MH, Redpath CJ, Roberts JD. The Short QT Syndrome - Proposed Diagnostic Criteria. J Am Coll Cardiol. 2011;57(7):802-12. doi: 10.1016/j.jacc.2010.09.048.

123. Bogossian H, Linz D, Heijman H,Heijman J, Bimpong-Buta NY, Bandorski D, et al. QT evaluation in patients with bundle branch block . Int J Cardiol Heart Vasc.2020;30:100636. doi: 10.1016/j.ijcha.2020.100636.

124. Macfarlane PW, Antzelevitch C, Haissaguerre M, Huikuri HV, Potse M, et al.The early repolarization pattern: aa consensus paper. J Am Coll Cardiol. 2015 Jul 28;66(4):470-7. doi: 10.1016/j.jacc.2015.05.033.

125. Haissaguerre M, Derval N, Sacher F, Jesel l, Deisenholer I, Roy L, et al. Sudden cardiac arrest associated with early repolarization. N Engl J Med 2008;358(19):2016–23. doi: 10.1056/NEJMoa071968.

126. Kukla P, Jastrz□bski M, Pérez-Riera AR. Some controversies about early repolarization: the Haïssaguerre syndrome. Ann Noninvasive Electrocardiol. 2015;20(5):409-18. doi: 10.1111/anec.12263.

127. Tikkanen JT, Anttonen O, Junttila MJ, Aro AA, Kerola T, Rissanen HA et al. Long-term outcome associated with early repolarization and electrocardiography. Engl J Med, 2009;361(26):2529–37. doi: 10.1056/NEJMoa0907589

128. Macfarlane PW, Antzelevitch C, Haisaguerre M, Huikuri HV, Potse M, Rosso R, et al. The Early Repolarization Pattern. A consensus paper. J Am Coll Cardiol.2015;68(4):470-7. doi: 10.1056/NEJMoa0907589

129. Laksman ZW, Gula LJ, Saklani P, Cassagneau R, Steinberg C, Conacher S, et al. Early repolarization is associated with symptoms in patients with type 1 and type 2 long QT syndrome. Heart Rhythm. 2014;11(9):1632-8. doi: 10.1016/j.hrthm.2014.05.027.

130. Moss AJ. New insights into the arrhythmogenic substrate of the long QT syndrome. Circulation.2014;130(22):1929-30. doi: 10.1161/CIRCULATIONAHA.114.013232.

131. Cross B, Homoud M, Link M, Foote C, Garlitski AC, Weinstock J, Estes NA 3rd. The short QT syndrome. J Interv Card Electrophysiol. 2011 Jun;31(1):25-31. doi: 10.1007/s10840-011-9566-0.

132. Gaita F, Giustetto C, Bianchi F,Schimpf R, Haissguerre M, Calo L, et al. Short QT syndrome: pharmacological treatment. J Am Coll Cardiol. 2004 Apr 21;43(8):1494-9. doi: 10.1016/j.jacc.2004.02.034.

133. Wolpert C, Schimpf R, Veltmann C, Giustetto C, Gaita F, Borggrefe M. Clinical characteristics and treatment of short QT syndrome. Expert Rev Cardiovasc Ther. 2005 Jul;3(4):611-7. doi: 10.1016/j.jacc.2004.02.034.

134. Bayés de Luna A, Brugada J, Baranchuk A, Borggrefe M, Breithardt G, Goldwasser D, et al. Current electrocardiographic criteria for diagnosis of Brugada pattern: a consensus report. J Electrocardiol. 2012 Sep;45(5):433-42. doi: 10.1016/j.jelectrocard.2012.06.004.

135. Sarquella-Brugada G, Campuzano O, Arbelo E, Brugada J, Brugada R. Brugada syndrome: clinical and genetic findings. Genet Med. 2015;2016;18(1):3-12. PMID: 25905440 Review.

136. Hong K, Brugada J, Oliva A, Berruezo-Sanchez A, Potenza D, Pollevick GD, et al. Value of electrocardiographic parameters and ajmaline test in the diagnosis of Brugada syndrome caused by SCN5A mutations. Circulation. 2004 Nov 9;110(19):3023-7. doi: 10.1161/01.CIR.0000144299.17008.07.

137. Postema PG, Wolpert C, Amin AS,Probst V, Borggrefe M, Poden DH, et al. Drugs and Brugada syndrome patients: review of the literature, recommendations, and an up-to-date website (www.brugadadrugs.org). Heart Rhythm. 2009 Sep;6(9):1335-41. doi: 10.1016/j.hrthm.2009.07.002.

138. Sangwatanaroj S, Prechawat S, Sunsaneewitayakul B, Tungsanga K, Tosukhooowong P. et al. New electrocardiographic leads and the procainamide test for the detection of the Brugada sign in sudden unexplained death syndrome survivors and their relatives. Eur Heart J. 2001 Dec;22(24):2290-6. DOI: 10.1053/euhj.2001.2691.

139. Baranchuk A, Nguyen T, Hyung Ryu M, Femenía F, Zareba W, Wilde AM. et al. Brugada phenocopy:new terminolçogy and proposed classification. Ann Noninvasive Electrocardiol 2012;17(4):299–314. DOI: 10.1111/j.1542-474X.2012.00525.x

140. Hayashi M, Denjoy I, Extramiana F, Maltret A,Buisson NR, Lupoglazo JM, et al. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. Circulation,2009;119(18):2426-34. doi: 10.1161/CIRCULATIONAHA.108.829267.

141. Roston TM, Vinocur JM, Maginot KR, Mohammed S, Salerno JC, Etheridge SP, et al. Catecholaminergic polymorphic ventricular tachycardia in children: analysis of therapeutic strategies and outcomes from an international multicenter registry. Circ Arrhythm Electrophysiol. 2015 Jun;8(3):633-42. doi: 10.1161/CIRCEP.114.002217.

142. Te Riele AS, James CA, Philips B, Rastegar N, Bhonsale A, Groenweg J, et al. Mutation-positive arrhythmogenic right ventricular dysplasia/cardiomyopathy: the triangle of dysplasia displaced. J Cardiovasc Electrophysiol. 2013;24(12):1311-20. doi: 10.1111/jce.12222.

143. Zhang L, Liu L, Kowey PR, Fontaine GW. The electrocardiographic manifestations of arrhythmogenic right ventricular dysplasia. Curr Cardiol Rev. 2014 Aug;10(3):237-45. doi: 10.2174/1573403x10666140514102928.

144. Hoffmayer KS, Bhave PD, Marcus GM, James CA, Tichnell C, Chopra N, et al. An electrocardiographic scoring system for distinguishing right ventricular outflow tract arrhythmias in patients with arrhythmogenic right ventricular cardiomyopathy from idiopathic ventricular tachycardia. Heart Rhythm. 2013;10(4):477-82. doi: 10.1016/j.hrthm.2012.12.009.

145. El-Saiedi SA, Seliem ZS, Esmail RI. Hypertrophic cardiomyopathy: prognostic factors and survival analysis in 128 Egyptian patients. Cardiol Young.2014;24(4):702-8. doi: 10.1017/S1047951113001030.

146. Semsarian C, Ingles J, Maron MS, Maron BI. New perspectives on the prevalence of hypertrophic cardiomyopathy. J Am Coll Cardiol. 2015;65(12):1249-54. doi: 10.1016/j.jacc.2015.01.019.

147. Gilbreath HR, Castro D, Iannaccone ST. Congenital myopathies and muscular dystrophies. Neurol Clin. 2014 Aug;32(3):689-703, viii. doi: 10.1016/j.ncl.2014.04.006.

148. Bönnemann CG, Wang CH, Quijano-Roy S,Deconinck N, Bertini E, Ferreiro A, et al. Members of International Standard of Care Committee for Congenital Muscular Dystrophies. Diagnostic approach to the congenital muscular dystrophies. Neuromuscul Disord. 2014;24(4):289-311. doi: 10.1016/j.nmd.2013.12.011.

149. Santos MA, Costa F de A, Travessa AF, Bombig MT, Fonseca FH, Luna Filho B. et al. [Duchenne muscular dystrophy: electrocardiographic analysis of 131 patients]. Arq Bras Cardiol. 2010;94(5):620-4. doi: 10.1590/s0066-782x2010005000024.

150. Arizona Cert – Center for Education and Research on Therapeutics [Cited 2009 April 28].Available from: http://www.azcert.org/medical-pros/drug-lists/drug-lists.cfm

151. Long B, Brady WJ, Bridwell RE, Ramzy M, Montrief T, Singh M, et al. Electrocardiographic manifestations of COVID- 19. Am J Emerg Med.2021;41:93-103. doi: 10.1016/j.ajem.2020.12.060.

152. Gouaux JL, Ashman R. Auricular fibrillation with aberration stimulating ventricular paroxysmal tachycardia. Am Heart J. 1947;34(3):388-73. doi: 10.1016/0002-8703(47)90487-0.

153. Kennedy LB, Leefe W, Leslie BR. The Ashman phenomenon. J La State Med Soc. 2004 May-Jun;156(3):159-62. PMID: 15233390

154. Spodick DH. Electrocardiogram in acute pericarditis. Distributions of morphologic and axial changes by stages. Am J Cardiol. 1974;33(4):470-4. doi: 10.1016/0002-9149(74)90603-1.

155. Liang X, Wang Y, Yin X, Gong X, Pan S, Chen Z, et al. Electrocardiographic Characteristics of Breast Cancer Patients Treated with Chemotherapy. Cardiol Res Pract. 2020 Dec 9;2020:6678503. doi: 10.1155/2020/6678503.

156. Liu B, An T, Li M, Yi Z, Li C, Sun X, et al. The association between early-onset cardiac events caused by neoadjuvant or adjuvant chemotherapy in triple-negative breast cancer patients and some novel autophagy-related polymorphisms in their genomic DNA: a real-world study. Cancer Commun (Lond). 2018 Dec 4;38(1):71. doi: 10.1186/s40880-018-0343-7.

157. Buza V, Rajagopalan B, Curtis AB. Cancer Treatment–Induced Arrhythmias Focus on Chemotherapy and Targeted Therapies. Circ Arrhythm Electrophysiol.2017;10(8):e005443. doi: 10.1161/CIRCEP.117.005443.

158. Hajjar LA ,Costa IBS,Queiroga M, Hoff P, Maria Del Pilar Estevez MP, Fonseca S, et al. Brazilian Cardio-oncology Guideline – 2020. Arq Bras Cardiol. 2020 Nov;115(5):1006-43. doi: 10.36660/abc.20201006.

159. Lawless CE, Best TM. Electrocardiograms in athletes: interpretation and diagnostic accuracy. Med Sci Sports Exerc. 2008;40(5):787-98. doi: 10.1249/MSS.0b013e318164dd18.

160. Zorzi A, El Maghawry M, Corrado D. Evolving interpretation of the athlete's electrocardiogram: From European Society of Cardiology and Stanford criteria, to Seattle criteria and beyond. J Electrocardiol. 2015;48(3):283-91. doi: 10.1016/j.jelectrocard.2015.01.007.

161. Corrado D, Pelliccia A, Heidbuchel H, Sharma S, Link M, Basso C, et al.. Section of Sports Cardiology European Association of Cardiovascular Prevention and Rehabilitation. Recommendations for interpretation of 12-lead electrocardiogram in the athlete Eur Heart J. 2010;31(2):243-59. doi: 10.1093/eurheartj/ehp473

162. Riding NR, Sheikh N Adamuz C, Watt V, Farooq A, Whyte GP, et al. Comparison of three current sets of electrocardiographic interpretation criteria for use in screening athletes. Heart. 2015 Mar;101(5):384-90. doi: 10.1136/heartjnl-2014-306437.

163. Drezner JA, Sharma S, Baggish A, Papadakis M, Wilson MG, Prutkin JM, et al. International Criteria for Electrocardiographic Interpretation in Athletes: Consensus Statement. Br J Sports Med. 2017 May;51(9):704-731. doi: 10.1136/bjsports-2016-097331.

164. Drezner JA, Ackerman MJ, Anderson J, Ashley E, Asplund CA, Baggish AL, et al.Electrocardiographic interpretation in athletes: the 'Seattle Criteria'..Br J Sports Med. 2013 Feb;47(3):122-4. doi: 10.1136/bjsports-2012-092067.PMID: 23303758

165. Dickinson D. The normal ECG in childhood and adolescence. Heart. 2005; 91: 1626-16302Dickinson D. The normal ECG in childhood and adolescence. Heart. 2005; 91(12):1626-30. DOI: 10.1136/hrt.2004.057307

166. Tobias N. O eletrocardiograma normal da criança. In:Tranchesi J. Eletrocardiograma normal e patológico. 7.ed. São Paulo:Editora Roca;2001. p:141-50. ISBN: 9788572413237

167. Tipple M. Interpretation of electrocardiograms in infants and children. Images Paediatr Cardiol. 1999;1(1):3-13.

168. Schwartz P, Garson A, Paul T, Stramba-Badiale M, Vetter V, Villain E, Wren C. Guidelines for the interpretation of the neonatal electrocardiogram. Eur Heart J. 2002; 23(17):1329-44.

169. Kligfield P, Gettes LS, Bailey JJ, Childers R, Deal BJ, William Hancock WE, et al. Recommendations for the Standardization and Interpretation of the Electrocardiogram: Part I: The Electrocardiogram and Its Technology: A Scientific Statement From the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society Endorsed by the International Society for Computerized Electrocardiology. Circulation .2007;115(10):1306-24. doi: 10.1161/CIRCULATIONAHA.106.180200.

170. Bronzetti G, Mariucci E, Bonvicini M, Picchio F. L'ECG in età pediátrica: cosa deve sapere Il cardiologo dell'adulto. G Ital Cardiol. 2011;12(6):408-18. DOI: 10.1714/835.9306

171. Davignon A, Rautaharju P, Boiselle E, Soumis E, Megelas M, Choquette A. Normal ECG Standards for infants and children. Pediatr Cardiol. 1980; 1:123-31. https://doi.org/10.1007/BF02083144

172. Rijnbeek P, Witsenburg M, Schrama E, Hess J, Kors J, New normal limits for the paediatric electrocardiogram. Eur Heart J. 2001;22(8):702-11. DOI: 10.1053/euhj.2000.2399

173. Marina de Souza Pimenta. Avaliação eletrocardiográfica de RN normais na primeira semana de vida. [Tese de Doutorado, Programa de Cardiologia]. Orientador: Prof. Dr. Carlos Alberto Pastore. Coorientadora: Profa. Dra. Vera Lúcia Jornada Krebs. Faculdade de Medicina da Universidade de São Paulo: São Paulo.

174. Palhares DMF, Marcolino MS, Santos TMM, da Silva JLP, Gomes PR, Ribeiro LB, et l. Normal limits of the electrocardiogram derived from a large database of Brazilian primary care patients. BMC Cardiovasc Disord. 2017 Jun 13;17(1):152. doi: 10.1186/s12872-017-0572-8.

175. Pastore C, Pinho C, Germiniani H,Samesima N, Pereira Filh HG, Kruse JCI, et al. et al. Diretrizes da Sociedade Brasileira de Cardiologia sobre Análise e Emissão de Laudos Eletrocardiográficos. Arq Bras Cardiol. 2009; 93(3 supl.2): 1-19. doi: 10.5935/abc.20160054.

176. Sanches P, Moffa P. O eletrocardiograma nas pericardites e miocardites e cardiomiopatias. In: Tranchesi J. – Eletrocardiograma normal e patológico. 7.ed. São Paulo:Editora Roca;2001.p: 623-50.

177. Priori S, Wilde A, Horie M, Cho Y, Beher ER,Blom N, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES and AEPC in June 2013. Heart Rhythm. 2013 Dec;10(12):1932-63. doi: 10.1016/j.hrthm.2013.05.014.

178. Aro A, Anttonen O, Tikkanen J, Juntilla MJ, Kerola T, Rissanen H, et al. Prevalence and Prognostic Significance of T-Wave Inversions in Right Precordial Leads of a 12-Lead Electrocardiogram in the Middle-Aged Subjects. Circulation.2012;125(21):2572-7. doi: 10.1161/CIRCULATIONAHA.112.098681.

179. Marcus F, McKenna W, Sherrill D, Basso C, Bauce B, Bluemke DA, et al. Diagnosis of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia Proposed Modification of the Task Force Criteria. Circulation. 2010;121:1533–1541. https://doi.org/10.1161/CIRCULATIONAHA.108.840827

180. Bernstein AD, Daubert JC, Fletcher RD, Hayes DL, Luderitz B, Reynolds WR, et al. The revised NASPE/BPEG generic code for antibradycardia, adaptive-rate, and multisite pacing. North American Society of Pacing and Electrophysiology/British Pacing and Electrophysiology Group. Pacing Clin Electrophysiol. 2002 Feb;25(2):260-4. doi: 10.1046/j.1460-9592.2002.00260.x.

181. Martinelli Filho M, Siqueira SF, Nishioka SAD. Atlas de Marcapasso: a função através do eletrocardiograma. 2. ed. São Paulo: Editora Atheneu; 2012. ISBN: 978-8538802907

182. Oliveira Jr MT, Canesin MF, Marcolino MS, Ribeiro ALP, de Carvalho ACC, Reddy S, et al. Diretriz de telecardiologia no cuidado de pacientes com síndrome coronariana aguda e outras doenças cardíacas. Arq Bras Cardiol. 2015;104(Supl 1):1-26. DOI: 10.5935/abc.20150057

183. Barold SS. Willem Einthoven and the birth of clinical electrocardiography a hundred years ago. Card Electrophysiol Rev. 2003;7(1):99-104. doi: 10.1023/a:1023667812925.

184. Savage DD, Rautaharju PM, Bailey JJ, Horton MR, Hadden W, Lacroix AZ, et al. The emerging prominence of computer electrocardiography in large population-based surveys. J Electrocardiol. 1987;20(Suppl):48-52. PMID: 2961839.

185. Alkmim MB, Figueira RM, Marcolino MS, Silva C, Abreu MP, et al. Improving patient access to specialized health care: the Telehealth Network of Minas Gerais, Brazil. Bull World Health Organ. 2012;90(5):373–8. doi: 10.2471/BLT.11.099408.

186. Lopes MA, Oliveira GM, Ribeiro AL, Pinto FJ, Rey HCV, Zimerman LI, et al. Arq Bras Cardiol. 2019 Nov;113(5):1006-56. doi: 10.5935/abc.20190205.

187. Andrade MV, Maia AC, Cardoso CS, Alkmim MB, Ribeiro ALP. Cost-benefit of the Telecardiology service in the state of Minas Gerais: Minas Telecardio project. Arq Bras Cardiol. 2011;97(4):307-16. doi: 10.1590/s0066-782x2011005000080

188. Lazarus G, Kirchner HL, Siswanto BB. Prehospital tele-electrocardiographic triage improves the management of acute coronary syndrome in rural populations: A systematic review and meta-analysis. J Telemed Telecare. 2020; Sep 30;1357633X20960627.

189. Nascimento BR, Brant LCC, Marino BCA, Passaglia LG, Ribeiro ALP. Implementing myocardial infarction systems of care in low/middle-income countries.Heart.2019;105(1):20-6. doi: 10.1136/heartjnl-2018-313398.

190. Busch MC, Gross S, Alte D, Kors JA, Völzke H, Ittermann T, et al. Impact of atrial fibrillation detected by extended monitoring—A population-based cohort study. Ann Noninvasive Electrocardiol. 2017;22(6):e12453. doi: 10.1111/anec.12453.

191. Amir M, Munizu M, Mappangara I, Adam ATS. Telemedicine for detecting Brugada Syndrome in eastern Indonesia: A multi-center prospective observational study. Ann Med Surg. 2021;65:102334. doi: 10.1016/j.amsu.2021.102334.

192. Ribeiro ALP, Paixão GMM, Gomes PR, Ribeiro MH, Ribeiro AH, Canazart JA, et al. Tele-electrocardiography and bigdata: The CODE (Clinical Outcomes in Digital Electrocardiography) study. J Electrocardiol. 2019; 575:S575-S78. doi: 10.1016/j.jelectrocard.2019.09.008.

193. Ribeiro AH, Ribeiro MH, Paixão GMM, Oliveira DM, Gomes PR, Canazart JA, et al. Automatic diagnosis of the 12-lead ECG using a deep neural network. Nat Commun. 2020;11(1):1760. doi: 10.1038/s41467-020-15432-4.

194. Smith SW, Walsh B, Grauer K, Wang K, Rapin J, Li J, et al. A deep neural network learning algorithm outperforms a conventional algorithm for emergency department electrocardiogram interpretation. J Electrocardiol. 2019; 52:88-95. doi: 10.1016/j.jelectrocard.2018.11.013.

195. Attia ZI, Noseworthy PA, Lopez-Jimenez F, Asirvatham SJ, Deshmukh AJ, Gersh BJ, et al. An artificial intelligence-enabled ECG algorithm for the identification of patients with atrial fibrillation during sinus rhythm: a retrospective analysis of outcome prediction. Lancet. 2019; 394(10201):861-7. doi: 10.1016/S0140-6736(19)31721-0

196. Varma N, Cygankiewicz I, Turakhia MP, Heidbuchel H, Hu YF, Chen LY, et al. 2021 ISHNE/HRS/EHRA/APHRS Expert Collaborative Statement on mHealth in Arrhythmia Management: Digital Medical Tools for Heart Rhythm Professionals: From the International Society for Holter and Noninvasive Electrocardiology/Heart Rhythm Society/European Heart Rhythm Association/Asia-Pacific Heart Rhythm Society. Circ Arrhythm Electrophysiol. 2021 Feb;14(2):e009204. doi: 10.1161/CIRCEP.120.009204.