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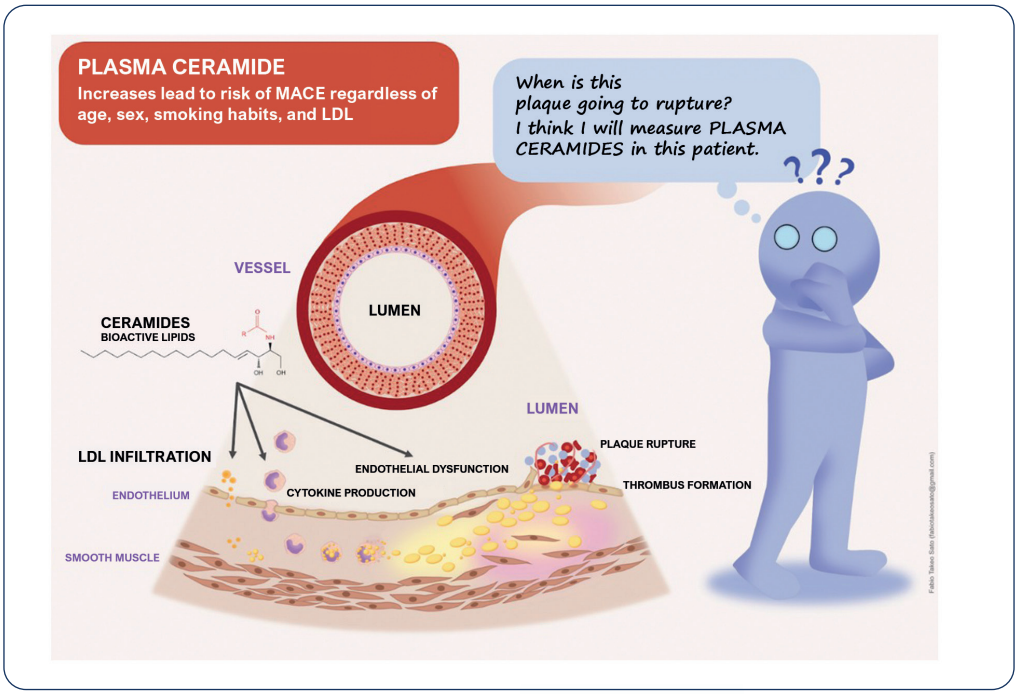

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REVISTA DA SOCIEDADE BRASILEIRA DE CARDIOLOGIA - Publicada desde 1948

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The Year in Basic Research 2021: the Search for Translational Models

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Cardiovascular diseases (CVD) are responsible for approximately 19 million deaths annually in the world.[1] In Brazil, they are involved in one third of deaths.[2] A significant improvement in cardiovascular medicine has been observed in the last decades. However, cardiac failure, which is the final common pathway following heart injury, remains with a high incidence, prevalence, and mortality.[3] A better understanding of CVD may allow the development of new pharmacological and non-pharmacological approaches to their treatment. In 2021, the Arquivos Brasileiros de Cardiologia published articles in the area of basic sciences that were mostly related to experimental models. These studies can provide the basis for a translational approach to expand the understanding of CVD treatment. In this Editorial, we present an overview of recently published articles with emphasis on experimental models for a future translational approach.

The molecular mechanisms involved in the cardiac remodeling development are still widely investigated.[4] MicroRNAs (miRNA) participate in the control of major cellular functions, such as proliferation, differentiation, apoptosis, stress response, and transcriptional regulation. In an elegant study, Xu e Fang[5] observed that miR-34a and miR-125b are downregulated in the heart from patients with diabetic cardiomyopathy at the time of transplant. Additionally, *in vitro* data from rat cardiomyocytes showed that miR-125b and miR-34a overexpression prevents hyperglycemia-induced cardiomyocyte death.

Hypoxemia-mediated apoptosis in cardiomyocytes is a major cause of myocardial injury. Treatment with the vascular endothelial growth factor (VEGF) has been tested to improve tissue perfusion. Despite the interest in VEGF-based gene therapy, its effects are not completely understood. By using transfection of VEGF121 into primary rat cardiomyocytes culture subjected to hypoxia, Zhang et al.[6] showed that VEGF121 positively affects cardiomyocyte proliferation. Ischemic conditioning is a process whereby repeated application of short periods of ischemia alternating with reperfusion protects the myocardium from longer ischemic insults.[7] Despite extensive investigation, no drugs are available to prevent or attenuate ischemia/reperfusion injury. The α2-adrenergic receptor agonist dexmedetomidine, mainly used in analgesia and sedation, attenuated ischemia/reperfusion injury in rats by improving cardiac function and reducing infarcted area.[8] The improvement was associated with decreased myocyte apoptosis and inhibited expression of proteins of the apoptotic pathway PERK/eIF2α/TCF-4/CHOP. A reduction in GRP78 protein, a marker of endoplasmic reticulum stress, was also observed.

Physical exercise is the most important non-pharmacological tool to prevent and treat CVD. Basic and translational research has focused on the mechanisms involved in the benefits of exercise.[9-11] Several studies have shown that exercise improves cardiac remodeling induced by extensive myocardial infarction.[12] Souza et al.[10] observed that, also in a condition of slight cardiac aggression, such as in small size infarction, aerobic exercise on a treadmill for 12 weeks improves functional capacity and preserves left ventricular geometry. Likewise, physical exercise had positive effects in rats with renovascular hypertension.[11] Resistance exercise for 12 weeks increased the activity of antioxidant enzymes and reduced cardiac and renal oxidative damage, characterized by decreased hydrogen peroxide concentration and preserved sulfhydryl groups levels.[11]

The role of natural compounds on the pathophysiology of CVD has attracted the interest of scientists due to its large availability, and low cost and toxicity. L-carnitine is essential to displace fatty acids to mitochondrial oxidation sites. L-carnitine supplementation was shown to reduce the expression of genes involved in inflammation, both in the heart and adipose tissue in diabetic mice.[13] Innovative results were observed with a crude extract of the plant *Sauromatum guttatum* in Sprague-Dawley rats with arterial hypertension induced by excessive salt intake. The administration of the crude extract reduced blood pressure and preserved endothelial function; in aorta isolated from normotensive rats, the extract promoted vascular relaxation.[14] Copaiba oil intake by rats with pulmonary arterial hypertension was accompanied by a systemic antioxidant effect, reduced vascular resistance, and improved right ventricular function.[15] Although the anti-inflammatory and antioxidant effects of orange juice have been known for a long time, there was no study on its effect on infarction-induced cardiac remodeling. Oliveira et al.[16] observed that dietary supplementation with orange juice increases the expression of heme-oxygenase-1, a crucial enzyme in cellular homeostasis with anti-inflammatory, antioxidant and anti-apoptotic effects.

Vitamin D deficiency is associated with increased risk of developing CVD, chronic immune disease, and cancer. However, its supplementation for prevention and control of chronic diseases and CVD has not shown benefits.[17,18] Santos et al.[19] observed that administration of non-hypercalcemic doses of vitamin D to normal rats was followed by metabolic changes and increased cardiac oxidative stress.

Keywords

Cardiovascular Diseases; Experimental Research; Research Design; Ventricular Remodeling; Heart Failure; Exercise; Translacional Medical Research

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Editorial

Doxorubicin is a potent antitumor agent of the anthracycline family, widely used in anticancer therapy. However, its use can result in cardiotoxic effects such as modulation of heme proteins and DNA damage, and cardiomyopathy.[20,21] Currently, there is great interest in agents that can reduce the doxorubicin toxicity. Brito et al.[22] evaluated the effects of resveratrol, a polyphenolic component, on cardiomyocytes from newborn rats treated with doxorubicin. Myocytes from neonates whose mothers had been supplemented during pregnancy with resveratrol had increased viability, antioxidant activity, and protection against gene damage after the addition of doxorubicin.

Basic experimental research allows great advances in the understanding of molecular and cellular mechanisms involved in cardiac performance in physiological and pathological conditions. However, there is still a long way before promising pharmacological and non-pharmacological treatments can be tested in clinical studies and finally incorporated into the therapeutic arsenal currently available for the treatment of cardiovascular diseases.

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Screening for Familial Hypercholesterolemia in Small Towns: Experience from 11 Brazilian Towns in the Hipercolbrasil Program

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Abstract

Background: **Familial hypercholesterolemia (FH) is a genetic disease characterized by elevated serum levels of low-density lipoprotein cholesterol (LDL-C), and it is associated with the occurrence of early cardiovascular disease. In Brazil, HipercolBrasil, which is currently the largest FH cascade screening program, has already identified more than 2000 individuals with causal genetic variants for FH. The standard approach is based on cascade screening of referred index cases, individuals with hypercholesterolemia and clinical suspicion of FH.**

Objectives: **To perform targeted screening of 11 small Brazilian cities with a suspected high prevalence of people with FH.**

Methods: **The selection of cities occurred in 3 ways: 1) cities in which a founder effect was suspected (4 cities); 2) cities in a region with high rates of early myocardial infarction as described by the National Health System database (2 cities); and 3) cities that are geographically close to other cities with a high prevalence of individuals with FH (5 cities). Statistical significance was considered as p value < 0.05.**

Results: **One hundred and five index cases and 409 first-degree relatives were enrolled. The yield of such approach of 4.67 relatives per index case was significantly better (p < 0.0001) than the general HipercolBrasil rate (1.59). We identified 36 IC with a pathogenic or likely pathogenic variant for FH and 240 affected first-degree relatives.**

Conclusion: **Our data suggest that, once detected, specific geographical regions warrant a target approach for identification of clusters of individuals with FH.**

Keywords: **Familial hypercholesterolemia; Genetic Testing; Cardiovascular Disease.**

Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disease that is clinically characterized by elevated blood levels of low density lipoprotein cholesterol (LDL-C), and it is associated with the occurrence of early atherosclerotic cardiovascular disease (ASCVD).[1,2]

The prevalence of FH in the world is estimated to be approximately 1:250 in the heterozygous form and 1:600,000 in the homozygous form. A study conducted by the ELSA-Brasil cohort estimated that the prevalence of individuals with clinical criteria for FH in Brazil is 1:263. Considering these estimates, there would be approximately 760,000 people with FH in Brazil.[4]

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However, although relatively frequent, the heterozygous form is still an underdiagnosed disease.[5] To assist in the identification of individuals with this disease, cascade genetic screening has been used in several countries, such as the Netherlands,[6] the United Kingdom,[7] and Spain.[8] This method has already been recognized as cost-effective for identification as well as prevention of early ASCVD in individuals with FH.[9,10]

In Brazil, HipercolBrasil, which is currently the largest cascade screening program, has existed since 2012,[11] and it has already identified more than 2000 individuals with causal genetic variants for FH. The program currently performs genetic testing on any individual with LDL-C ≥ 230mg/dL (index-case [IC])[12] and in first-degree relatives of those with pathogenic or likely pathogenic variants.

Between July 2017 and July 2019 we tested a new methodology for identifying new individuals with genetic alterations for FH based on the targeting of small municipalities with potentially high FH prevalence.

Here we describe the first results of targeted screening in 11 small Brazilian cities (up to 60,000 inhabitants) with a suspected high prevalence of people with FH.

Methods

The study was conducted at the Genetics and Molecular Cardiology Laboratory of the Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil. The protocol received approval from the Institutional Ethics Committee (CAPPesq protocol l00594212.0.1001.0068).

Study sample

Figure 1 shows inclusion criteria and study design. We enrolled individuals from 11 selected cities with up to 60,000 inhabitants throughout the Brazilian territory. The selection of cities occurred in 3 ways: 1) cities in which a founder effect was suspected, i.e. occurrence of homozygous individuals, but with no history of any degree of relation between parents (Major Vieira, Papanduva, Lagoa do Mato, and Passagem Franca); 2) cities in a region with high rates of dyslipidemia as reported by local physicians (Bom Despacho and Moema);[13] and 3) cities that are geographically close to other cities with a high prevalence of individuals with FH (Bambuí, Pimenta, Luz, Colinas, and Buriti Bravo).

Enrolment of index cases and relatives

In all cities, initial contact was made with the local secretary of health to explain the project and establish an agreement on the partnership. Contact was made via telephone before visiting each city, and an agreement was established by both parties via e-mail. Once in the city, the team was assisted by a health agent appointed by the health secretary. In the cities where there was evidence of a founder effect and in the ones where there were reports of high incidence of dyslipidemia, the sample collection started from family members of previously selected ICs. In these cities, there was also an active search for new ICs from medical records and cholesterol tests carried out in the clinical analysis laboratories of the local healthcare units. Individuals were considered as ICs when they had total cholesterol > 300 mg/dL and/or LDL-C ≥ 210 mg/dL with triglycerides < 300 mg/dL. In these cases, a blood sample was collected to perform a second cholesterol measurement in our laboratory. Those with a confirmed LDL-C ≥ 210 mg/dl in the second measurement were selected for genetic sequencing, while individuals who did not reach this value received a report with the values of total cholesterol and fractions and were excluded from the study.

Genetic sequencing and cascade screening

Blood samples were collected (10 ml of peripheral blood in EDTA tubes) and sent to the Genetics and Molecular Cardiology Laboratory at InCor/HCFMUSP for genetic analysis. Genomic DNA was extracted using QIAamp DNA MiniKit (QIAGEN), following the manufacturer's instructions. IC were sequenced by next generation sequencing in a gene panel comprising the following dyslipidemia-related genes: *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *STAP1*, *LIPA*, *APOE*, *ABCG5*, and *ABCG8*. Bioinformatics analyses were performed in Varstation and CLC Genomic Workbench 9.0 (QIAGEN). Multiplex ligation-dependent probe amplification (MLPA) in *LDLR* was used to screen for copy-number variants in ICs without any missense, nonsense or frameshift variants identified in next generation sequencing. The screening of relatives was performed with Sanger sequencing (for point mutations or small indels) or MLPA (for copy-number variants). Variants were classified following the recommendations of the American College of Medical Genetics and Genomics.[14]

Data analysis

The visual analysis of variable distribution was performed using histograms, and the normality of the data was verified. For continuous variables with normal distribution, the mean and standard deviation were calculated. Categorical variables are shown as frequencies. The differences between frequencies were compared using the chi-square test. The differences between means were compared with unpaired Student's t test or one-way ANOVA, if necessary. The tested variables were normally distributed, and we opted for a parametric test. Statistical significance was considered as p value < 0.05. Statistical analyses were performed with SPSS v19.0 (IBM).

Results

Initially, we collected 230 ICs with at least one cholesterol measure that met the proposed criteria (see Methods). However, 125 of them presented LDL-C values below the threshold after the second measurement and were not further sequenced. In total, 105 ICs and 490 relatives were included in the analysis. Table 1 shows characteristics of the 11 visited cities, Brazilian state, number of inhabitants, and date of each visit. The city with the lowest number of total inhabitants was Moema with 7,028, and the largest was Bom Despacho with 45,624 inhabitants, both in the state of Minas Gerais. The first cities to be visited were Major Vieira and Papanduva (September 2017) and the last were Buriti Bravo and Colinas (February 2019).

Table 2 shows the number of sequenced ICs and relatives per region and their genotype regarding the presence of pathogenic or likely pathogenic variants (positive), no pathogenic variants (negative) or presence of a variant of uncertain significance (VUS), as well as the number of new cases derived from each enrolled IC.

Table 3 shows the three IC groups (negative, positive, or VUS) and their clinical and biochemical data. In total, 105 ICs were sequenced, and pathogenic or likely pathogenic variants were found in 36 (37.8%) individuals, and VUS in 5 (5.25%). Most ICs were female (67.6%), and when the clinical and biochemical characteristics were evaluated among the three groups, there was, as expected, a statistically significant difference regarding baseline (untreated) total cholesterol and LDL-C, with the positive group presenting the highest values of total cholesterol and LDL-C, 382 ± 150 mg/dL and 287 ± 148 mg/dL, respectively. Table 4 shows the clinical and biochemical characteristics of relatives.

Figure 2 shows the geographic distribution of the 11 cities located in 3 Brazilian states, the number of registered cases, the number of individuals genotyped, and the number of individuals with a pathogenic variant.

Brazilian states, from top to bottom: Maranhão, Minas Gerais, and Santa Catarina

Table 5 shows all the encountered variants and the location where they were identified. In total, 21 different variants were identified with 3 variants appearing more frequently. Observed frequencies for these 3 variants suggest that they have founder effects in these localities. Six homozygous patients and one compound heterozygous in trans were found.

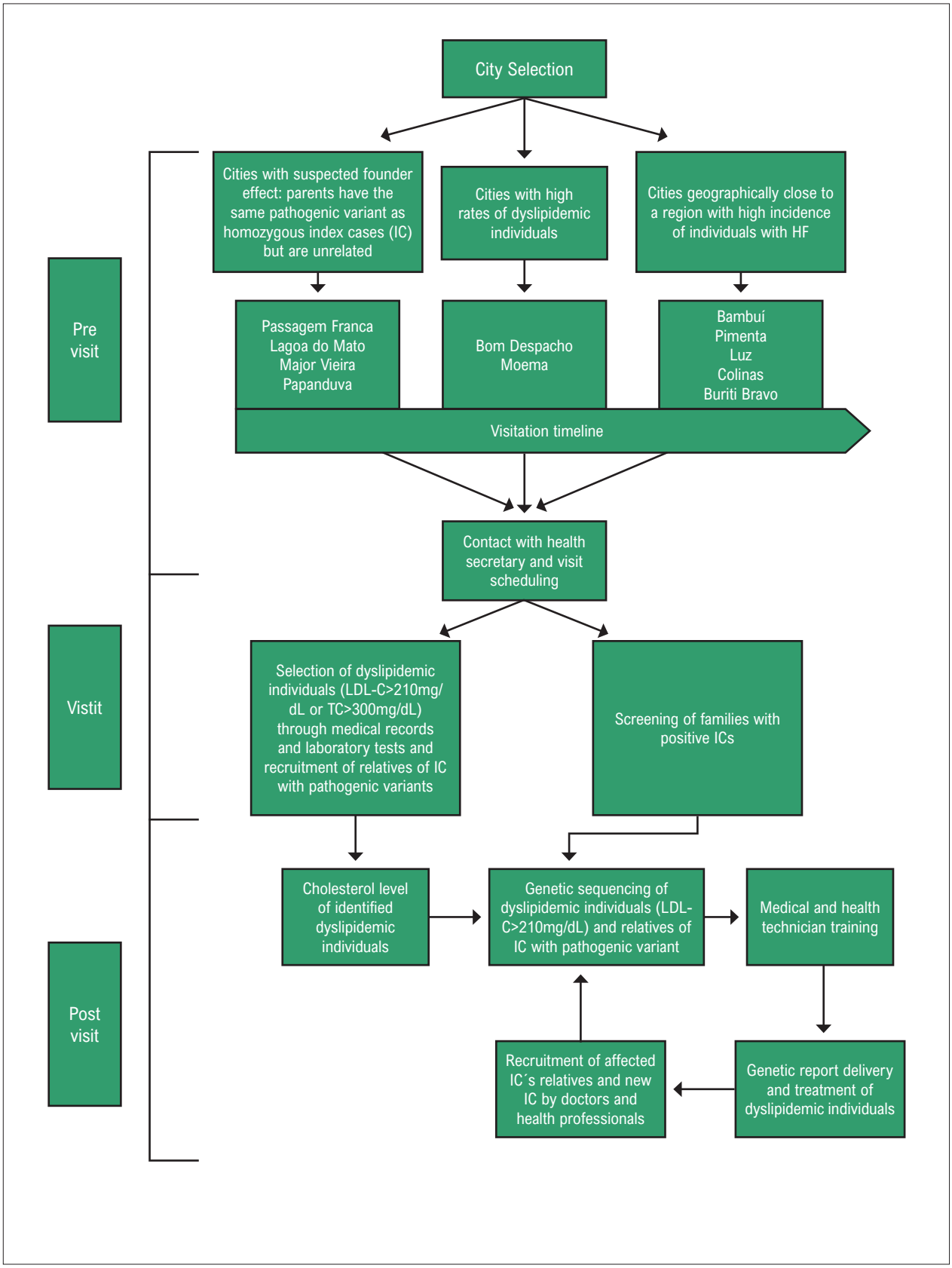

Figure 1 – *Methodology for selecting cities, capturing ICs and relatives and training health care professionals to continue cascade genetic screening.*

Table 1 – Overall characteristics of sampled municipalities

City	Brazilian state	Total inhabitants (IBGE Census)	Visit date	N of expected cases (1:263)[4]	N of positive cases identified
Bambuí	Minas Gerais	22,709	Dec 2018	86	2
Bom Despacho	Minas Gerais	45,624	Aug 2018	173	45
Buriti Bravo	Maranhão	23,827	Feb 2019	91	0
Colinas	Maranhão	42,196	Feb 2019	160	4
Lagoa do Mato	Maranhão	10,955	Apr 2018	42	32
Luz	Minas Gerais	17,492	Dec 2018	67	6
Major Vieira	Santa Catarina	8,103	Sep 2017	31	47
Moema	Minas Gerais	7,028	Aug 2018	27	36
Papanduva	Santa Catarina	18,013	Sep 2017	68	48
Passagem Franca	Maranhão	17,296	Apr 2018	66	50
Pimenta	Minas Gerais	8,236	Dec 2018	31	6

IBGE: Brazilian Institute of Geography and Statistics.

Table 2 – ICs and relatives collected per region and their genotypes for the presence of FH genetic variants

Origin	ICs			Relatives			Number of relatives per identified ICs	Number of genotyped individuals per city
	Negative	Positive	VUS	Negative	Positive	VUS		
Bambuí	0	1	0	0	1	0	1	2
Bom Despacho	15	11	2	34	31	3	2.4	96
Buriti Bravo	4	0	0	0	0	0	0	4
Colinas	6	1	1	1	3	0	0.5	12
Lagoa do Mato	3	2	0	25	30	0	11	60
Luz	21	4	1	0	2	0	0.08	28
Major Vieira	1	3	0	48	44	0	23	96
Moema	1	4	0	36	32	0	13.6	73
Papanduva	4	2	1	50	46	0	13.7	103
Passagem Franca	3	5	0	55	45	0	12.5	108
Pimenta	6	2	1	0	4	0	0.4	13
Total	64	35	6	249	238	3	4.7	595

IC: index case; VUS: variant of uncertain significance; FH: Familial hypercholesterolemia.

Discussion

This study describes the results of the implementation of a cascade screening system for FH in 11 small Brazilian cities.

Despite the known cost benefits of cascade screening for FH, worldwide implementation has been suboptimal. Different local barriers and implementation hurdles have to be identified and overcome. How to implement cascade screening in small localities, for example, has been mainly overlooked. This challenge is greater in a continent-sized country like Brazil, where, in addition to the enormous geographic distances, there is inequality in access to health services. We have described the experience of HipercolBrasil in conducting comprehensive cascade screening in small towns in Brazil. In this new model, cascade genetic screening was carried out in cities that showed evidence of a higher prevalence of FH due to previous finding of individuals with the homozygous phenotype from the same city, or because those regions had reported elevated frequency of myocardial infarction.

Cities that had evidence of a founder effect were the ones that presented a higher identification of individuals affected per each IC analyzed (in descending order Major Vieira, Papanduva, Lagoa do Mato, and Passagem Franca). In these cities, we started from homozygous individuals whose parents were non-related and were born in different geographic regions. Clearly, whenever this situation is flagged by a cascade screening program, it

Table 3 – Clinical and biochemical characteristics of negative, positive, and VUS-altered ICs

	Negative IC	(64)	Positive IC	(36)	IC VUS	(5)	p value
Females %	45 (70.3)	64	21 (58.3)	36	5 (100)	5	0.134
Males %	19 (29.7)	64	15 (41.7)	36	-	5	
Age (years)	54±15	64	44±19	36	56±16	5	0.015
Use of lipid lowering drugs	32 (50.0)	64	24 (66.7)	36	3 (60.0)	5	0.261
Early CAD	2 (3.1)	64	4 (11.1)	36	-	5	0.297
Xanthomas	3 (4.7)	64	3 (8.3)	36	1 (20.0)	5	0.365
Xanthelasmas	4 (6.3)	64	1 (2.8)	36	-	5	0.696
Corneal arcus	2 (3.1)	64	3 (8.3)	36	-	5	0.345
Current TC	279±65	62	316±107	36	302±28	5	0.102
Current LDL-C	195±56	64	234±104	36	207±35	5	0.051
Baseline TC	322±33	60	382±150	32	305±43	5	0.008
Baseline LDL-C	233±24	59	287±148	34	229±20	4	0.022

CAD: coronary artery disease; IC: index case; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; VUS: variant of uncertain significance. Early CAD defined as atherosclerotic cardiovascular disease event < 55 and 60 years of age in males and females, respectively; lipids in mg/dL; baseline lipids = untreated.

Table 4 – Clinical and biochemical characteristics of negative and positive relatives

	Negative relatives	N (249)	Positive relatives	N (240)	p value
Females %	136 (54.6)	249	135 (56.3)	240	0.504
Males %	113 (45.4)	249	105 (43.8)	240	
Age (years)	40±21	249	38±21	240	0.710
In use of lipid lowering drugs	31 (12.4)	249	93 (38.8)	240	0.001
Early CAD	2 (0.8)	249	9 (3.8)	240	0.034
Xanthomas	6 (2.4)	249	17 (7.1)	240	0.013
Xanthelasmas	11 (4.4)	249	34 (14.2)	240	0.001
Corneal arcus	1 (0.4)	249	9 (3.8)	240	0.009
Current TC	198±51	114	309±86	127	0.001
Current LDL-C	124±42	192	233±75	198	0.001
Baseline TC	220±191	97	318±97	130	0.001
Baseline LDL-C	126±41	169	243±82	178	0.001

CAD: coronary artery disease; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol. Early CAD defined as atherosclerotic cardiovascular disease event < 55 and 60 years of age in males and females, respectively; lipids in mg/dL; baseline lipids = untreated.

deserves the deployment of a city-wide approach, because the costs-benefits of this scenario are the most advantageous. Implementing the genetic cascade in small towns proved to be more efficient when compared to the genetic cascade performed by Hipercol Brasil[11] considering that the rates of family members per IC were 4.7 and 1.6, respectively ($p < 0.0001$).

It is important that the rate of tested family members per IC was also higher in cities with suspected founder effects. This probably occurred because these cities had a small number of inhabitants, and most relatives had some degree of familial relation. This did not occur in Bom Despacho, a city considerably larger than the others (45,624 inhabitants), and, although the number of family members collected was similar to that of other cities, there was a higher number of ICs collected (28) decreasing the rate of relatives/IC to 2,4. This situation exemplifies the tenuous equilibrium between city size and the success of the described approach.

Visited cities that were geographically close to cities with suspected founder effects (Bambuí, Buriti Bravo, Colinas, Pimenta, and Luz) had a low uptake of ICs and, consequently, a low number of identified relatives. This suggests that

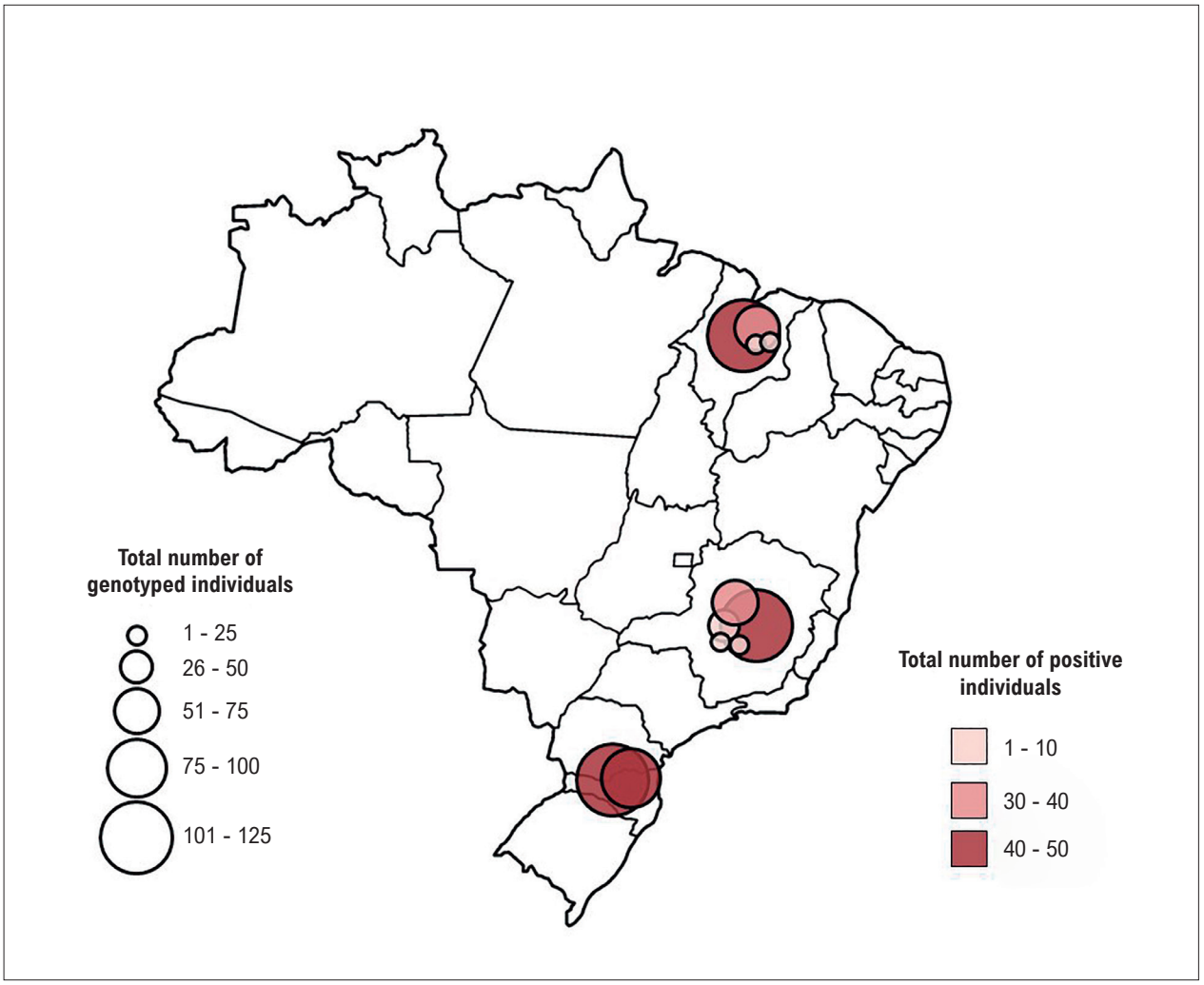

Figure 2 – *Geographical distribution of cases, number of genotyped individuals, and number of individuals with an identified pathogenic variant (positive).*

concentrating efforts in the selected municipality, as opposed to extending the approach to nearby towns, should be prioritized, and the capture of nearby potential cases should be left to the usual cascade screening mechanism.

Conclusion

Cascade screening in small cities (fewer than 60,000 inhabitants) with a founder effect proved to be effective. However, some points might be of great importance in order for the cascade screening to be effective, and the following might be considered before deciding which cities to track: establishment of a formal partnership and explicit interest on the part of the local health department in receiving the program and performing the cascade screening; availability of clinical analysis laboratory datasets to carry out a retrospective survey of cholesterol tests; and dissemination via radio stations and social media regarding the disease and the program for greater adherence by the inhabitants.

This study is limited by the relative number of cities evaluated considering the continental size of Brazil. However, it suggests that the designed approach may be useful for detecting individuals with FH. In conclusion, our data suggest that, once detected, specific geographical regions warrant a targeted approach for the identification of clusters of FH individuals.

Author Contributions

Conception and design of the research: Jannes CE, Pereira AC; Acquisition of data: Jannes CE, Silvino JPP, Lima IR, Tada MT; Analysis and interpretation of the data: Jannes CE, Silvino JPP, Pereira AC; Statistical analysis: Silva PRS, Pereira AC; Obtaining financing: Jannes CE, Krieger JE, Pereira AC; Writing of the manuscript: Jannes CE, Oliveira TGM, Santos RD, Pereira AC; Critical revision of the manuscript for intellectual content: Silvino JPP, Santos RD, Krieger JE, Pereira AC.

Potential Conflict of Interest

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

Table 5 – FH pathogenic variants, likely pathogenic variants and VUS found per city

Gene	Variant	Variant Classification	Bambuí	Bom Despacho	Luz	Pimenta	Moema	Buriti Bravo	Colinas	Lagoa do Mato	Passagem Franca	Major Vieira	Papanduva	Total
LDLR	Duplication from exon 4 to 8 (b)	Pathogenic	0	0	0	0	0	0	0	0	0	45[b]	41	86
LDLR	Duplication from promoter to exon 6	Pathogenic	0	0	0	0	0	1	4	29	49[a]	0	0	83
LDLR	p.Asp224Asn	Pathogenic	0	39	4	0	34	0	0	0	0	0	0	77
LDLR	p.Cys222*	Pathogenic	0	0	0	0	0	0	0	0	0	0	5	5
LDLR	c.1359-1G >C	Pathogenic	0	0	0	5	0	0	0	0	0	0	0	5
LDLR	p.Gly592Glu	Pathogenic	0	0	0	0	0	0	0	0	0	2	0	2
LDLR	p.Ala771Val	Pathogenic	0	0	1	0	0	0	0	0	0	0	0	1
LDLR	p.Pro699Leu	Pathogenic	0	0	1	0	0	0	0	0	0	0	0	1
LDLR	p.Asp601His	Likely Pathogenic	2	0	0	0	2	0	0	0	0	0	0	4
LDLR	p.Cys34Arg	Likely Pathogenic	0	1	0	0	0	0	0	0	0	0	0	1
LDLR	p.Arg257Trp	Likely Pathogenic	0	0	0	0	0	0	0	0	0	0	1	1
LDLR	p.Ser854Gly	Likely Pathogenic	0	2	0	0	0	0	0	0	0	0	0	2
LDLR	c.-228G>C	VUS	0	0	0	0	0	0	1	0	0	0	0	1
LDLR	p.Ala30Gly	VUS	0	0	0	1	0	0	0	0	0	0	0	1
APOB	p.Ala2790Thr	VUS	0	0	0	0	0	0	0	0	0	0	1	1
APOB	p.Met499Val	VUS	0	1	0	0	0	0	0	0	0	0	0	1
PCSK9	p.Arg237Trp	VUS	0	4	0	0	0	0	0	0	0	0	0	4
PCSK9	p.Arg357Cys	VUS	0	0	1	0	0	0	0	0	0	0	0	1
STAP1	p.Pro176Ser	VUS	0	0	0	1	0	0	0	0	0	0	0	1
LDLR	p.Cys222*	Pathogenic	0	0	0	0	0	0	0	0	0	0	1[c]	1[c]
LDLR	Duplication from exon 4 to 8	Pathogenic												
PCSK9	p.Arg215Cys	Likely Pathogenic	0	0	0	0	0	0	0	1	0	0	0	1[c]
APOB	p.Asp2213Asn	VUS												
APOB	p.Val3290Ile	VUS												
PCSK9	p.Arg215Cys	Likely Pathogenic	0	0	0	0	0	0	0	1	0	0	0	1[c]
APOB	p.Val3293Ile	VUS												
PCSK9	p.Arg215Cys	Likely Pathogenic	0	0	0	0	0	0	0	1	0	0	0	1[c]
APOB	p.Asp2213Asn	VUS												

2 homozygotes (b) 4 homozygotes (c) compound heterozygous in trans. VUS: variant of uncertain significance ; FH: Familial hypercholesterolemia.

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Study Association

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

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Targeted Screening of Familial Hypercholesterolemia in 11 Small Brazilian Cities: An Effective Approach to Detect Clusters of Affected Individuals

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Short Editorial related to the article: Screening for Familial Hypercholesterolemia in Small Towns: Experience from 11 Brazilian Towns in the Hipercolbrasil Program

Familial hypercholesterolemia (FH) is an autosomal codominant disease associated with high levels of LDL-cholesterol and premature atherosclerotic cardiovascular disease.[1] The condition is underrecognized, and screening tools must be implemented to improve diagnosis and promote early treatment.[2] The screening methods include universal, selective, cascade, reverse cascade, and oportunistic screening;[3,4] however, in small cities in certain regions, where founder effects can be present, target search for affected individuals can be an interesting option. The HipercolBrasil is a genetic cascade screening program carried out in the Heart Institute, with more than 2,000 patients identified with pathogenic variants,[5] and from these results, index cases (IC) from small cities with pathogenic variants were selected to amplify the cascade. The program performs genetic tests for FH in individuals with confirmed LDL-C ≥ 210 mg/dL in two separate analyses: (IC)[6] and in first-degree relatives of those in whom pathogenic or likely pathogenic variants were identified.

In our country, the recommendations for genetic testing follow the First Brazilian Guideline for Familial Hypercholesterolemia,[7] endorsed by the Update of the Brazilian Guideline for Familial Hypercholesterolemia – 2021.[8]

In the article by Jannes et al.,[9] the authors used a targeted screening method applied to candidates from 11 small Brazilian cities (with less than 60,000 inhabitants) with a suspected high prevalence of people with FH. They selected four cities with suspected founder effect (Major Vieira, Papanduva, Lagoa do Mato, and Passagem Franca); two cities in regions with high rates of dyslipidemia and early myocardial infarction, as described by the National Health System database (Bom Despacho and Moema); and five cities that were geographically close to other cities with a high prevalence of individuals with FH (Bambuí, Pimentas, Luz, Colinas, and Buriti Bravo). One-hundred and five index cases and 409 first-degree relatives were enrolled from those cities. Using such approach, the authors found 4.67 relatives per index case, which was significantly higher ($p < 0.0001$) compared with the general HipercolBrasil rate (1.59). The methods used to confirm the diagnosis of FH were next-generation sequencing (NGS) with a panel including *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *STAP1*, *LIPA*, *APOE*, *ABCG5* and *ABCG8* genes. The genetic screening was complemented by MLPA (multiplex ligation-dependent probe amplification) in the *LDLR* gene to detect gene copy number variations (CNVs) associated with FH when no mutation was identified. This study has shown that the rates of FH detection were higher than the HipercolBrasil program and were also higher in cities with founder effects. On the other hand, in cities close to those in which founder effects were present, the detection rate of index cases was lower, consequently, the number of affected relatives.

In a previous publication, the authors reported that their FH cascade screening program could predict family enrollment based on IC features, useful information for devising better and more effective screening approaches for at-risk individuals.[10] However, there is an important gap in risk perception, cholesterol management, and other aspects related to FH.[11] The strategy of target screening in small cities can be effective, but some issues must be taken into account before choosing the city to screen. The participation of the local health system, availability of previous laboratory results, and advertisement of this campaign in different social media may improve adherence of the population and better results.

Keywords

Hyperlipoproteinemia Type II/genetics; Genetic Testing/methods; Gain of Function Mutation; Early Detection; Genetic Diseases, Inborn

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Relationship of Lung Function and Inspiratory Strength with Exercise Capacity and Prognosis in Heart Failure

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Abstract

Background: Spirometry is underused in heart failure (HF) and the extent to which each defect associates with exercise capacity and prognosis is unclear. Objective: To determine the distinct relationship of continuous %predicted FVC (ppFVC) and FEV_1/FVC with: 1) maximal inspiratory pressure (MIP), left ventricular ejection fraction (LVEF), exercise performance; and 2) prognosis for the composite of cardiovascular death, heart transplantation or left ventricular assist device implant.

Methods: A cohort of 111 HF participants (AHA stages C/D) without diagnosed pneumopathy, spirometry, manovacuometry and maximum cardiopulmonary test. The association magnitudes were verified by linear and Cox (HR; 95% CI) regressions, age/sex adjusted. A $p<0.05$ was considered significant.

Results: Age was 57±12 years, 60% men, 64% in NYHA III. Every 10%-point increase in FEV_1/FVC [β 7% (95% CI: 3–10)] and ppFVC [4% (2-6)] associated with ventilatory reserve (VRes), however only ppFVC associated with MIP [3.8 cmH_2O (0.3-7.3)], LVEF [2.1% (0.5-3.8)] and VO_2peak [0.5 mL/kg/min (0.1–1.0)], accounting for age/sex. In 2.2 years (mean), 22 events occurred, and neither FEV_1/FVC (HR 1.44; 95% CI: 0.97–2.13) nor ppFVC (HR 1.13; 0.89–1.43) was significantly associated with the outcome. Only in the LVEF ≤50% subgroup (n=87, 20 events), FEV_1/FVC (HR 1.50; 1.01–2.23), but not ppFVC, was associated with greater risk.

Conclusions: In chronic HF, reduced ppFVC associated with lower MIP, LVEF, VRes and VO_2peak, but no distinct poorer prognosis over 2.2 years of follow-up. Distinctively, FEV_1/FVC was associated only with VRes, and, in participants with LVEF ≤50%, FEV_1/FVC reduction proportionally worsened prognosis. Therefore, FEV_1/FVC and ppFVC add supplementary information regarding HF phenotyping.

Keywords: Respiratory Insufficiency; Respiratory Muscles; Ventricular Function; Exercise Tolerance; Risk Assessment.

Introduction

Heart failure (HF) and poor lung function frequently coexist, emerging from several mechanisms: septal thickening and parenchymal congestion; impaired pulmonary vascular function and microvascular hypoperfusion; airway dysregulation and remodeling; inspiratory and peripheral skeletal muscle weakness; imbalanced chemo-, ergo- and metaboreflex for ventilatory control; heart enlargement; and decreased bronchial conductance.[1-3] However, spirometry is largely underused in HF. Even in co-prevalent HF and in chronic obstructive pulmonary disease (COPD), 80% of the individuals performed echocardiography, but <50% undergo spirometry.[4-6]

Subclinical ventilatory alterations are present in early HF stages, contributing to dyspnea and exercise intolerance.[3] Airway obstruction can be found mostly in non-compensated states and restrictive defects are described, particularly in chronic and stable subjects.[7,8] It must be acknowledged that baseline spirometric defects improve cardiopulmonary exercise test (CPX) interpretation for differential diagnosis of effort limitation,[8,9] and identify mortality risk in HF with preserved ejection fraction (HFpEF) or HF with reduced ejection fraction (HFrEF).[10,11]

However, the association of spirometry parameters with exercise limitation and prognosis in HF is still controversial,[8] given its use in variable HF severity status and phenotypes, the possible differential contribution of each obstructive and restrictive defects, and the poorly explored potential of non-linear relationships between dynamic lung and heart dysfunctions.[12] We hypothesized that in chronic stable HF,

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impaired forced vital capacity (FVC) and forced expired volume in 1 second (FEV_1)/FVC ratio is differently associated with other functional parameters at rest and in exercise, and consequently with poorer prognosis. Therefore, we aimed to (1) define the extent to which FEV_1/FVC and FVC are associated with left ventricular ejection fraction, respiratory strength and exercise responses; and (2) determine their associations with major incidental cardiovascular events (cardiovascular death, heart transplant and left ventricular assist device-LVAD).

Methods

Study Population and Clinical Characteristics

This cohort enrolled 158 consecutive HF subjects referred to the Laboratory of Physiology (Universidade de Brasília, Brasília, Brazil) for CPX from June 2015 to July 2016, followed up to at least over 24 pre-planned months. Subjects with HF, regardless of etiology or LVEF, were enrolled. They were required to be clinically stable in the previous three months (no decompensation or hospitalizations), free from diagnosed pulmonary disease (COPD, emphysema or use of bronchodilators), without medical conditions which precluded a maximal cycle-ergometer CPX. Participants had echocardiography (HD 11XE, Phillips, Amsterdam, Netherlands) done within one month from enrollment. For this analysis, we included 111 HF subjects, as spirometry data were unavailable for 43 participants, and 4 of them were unable to perform the spirometry maneuvers adequately, therefore without interpretable quality.[13]

On the first day, subjects underwent clinical evaluation, followed by respiratory strength assessment, and spirometry after a 30-minute rest. CPX was performed on the following day. Echocardiography was performed according to standard recommendations;[14] pulmonary artery systolic pressure (PASP) was estimated from Doppler-echocardiography tricuspid regurgitation jet peak velocity, when available. Hypertension and diabetes were defined based on self-report, use of medication, or measurements at the medical appointment (blood pressure above 140/90 mmHg and fasting glucose ≥126 or random glucose ≥200 mg/dL, respectively). Dyslipidemia was defined as LDL ≥160 mg/dL or use of lipid-lowering agents. Smoking status was self-reported. The referring cardiologist informed the primary HF etiology and pharmacological prescription.

All participants signed a written informed consent and institutional review board approval was obtained from the Ethics Review Board of Universidade de Brasilia (CAAE 50414115.4.0000.0030).

Assessment of Pulmonary Function and Respiratory Strength

Spirometry was performed according to recommendations.[13] FEV_1 was obtained from the volume of exhaled gas on the first second of expiration. FVC was obtained from the volume of gas vigorously exhaled after maximal inspiratory effort (Microlab, Carefusion, Yorba Linda, USA). The best of 5 forced expirations was used. Predicted reference values were derived from Brazilian equations.[15] The continuous FEV_1/FVC and percent predicted FVC (ppFVC) were considered the main primary exposures. As a sensitivity approach, we also analyzed terciles of each metric, and dichotomic obstructive and non-obstructive patterns (FEV_1/FVC ≤70 and >70 respectively), and restrictive and non-restrictive patterns (ppFVC <80% and ≥80%, respectively).

Maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) were measured according to standard recommendations,[16] and were obtained with a digital transducer (MVD300®, Globalmed. Porto Alegre. Brazil). Subjects were sitting. and used a nose clip and a mouthpiece. MIP was determined at the maximum inspiration effort from near the residual volume, against an occluded airway with a minor air leak (2 mm). MEP was determined at the maximum expiration effort from near the vital capacity, against an occluded airway. Three to 5 reproducible (≤10% of variation between values) maneuvers were performed, sustained for at least 1 second each. They were separated by 1-minute rest and the highest value was used for analysis.[16] Low MIP was considered when MIP was ≤ 80 cmH_2O in men and ≤60 cmH_2O in women.[17]

Cardiopulmonary Exercise Test

Subjects underwent a maximum symptom-limited CPX,[18] using cycle-ergometer ramp protocol (Corival, Lode, Netherlands) and ventilatory expired gas analysis cart (Quark CPET, Cosmed, Italy). Volume and gas calibration was performed before each test. Minute ventilation (VE), oxygen uptake (VO_2), and carbon dioxide output (VCO_2) were acquired breath-by-breath and averaged over 10-second intervals. The ventilatory anaerobic threshold (VAT) was determined by the V-slope method. Peak VO_2 was expressed as the highest 10-second averaged sample obtained during the final plateau, if the patient reached it, or the highest 20-seconds average sample from the final minute of a symptom-limited test, if not. The VE/VCO_2 slope was calculated from a linear regression equation, from the start of the test to the exercise peak. Given the fatigue reported in preliminary MVV tests (not shown) underestimating subsequent forced maneuvers or the inability to perform a sustained and reproductible measurement, particularly in patients at a more advanced stage of the disease, we were unable to use the gold-standard measured MVV uniformly to ensure comparability. Therefore, the ventilatory reserve was estimated from FEV_1 (calculated as 100-[VE/(FEV_1x40)100]).[18] Circulatory power was calculated from the VO_2 and systolic blood pressure product at peak, and ventilatory power from the quotient of peak systolic blood pressure and VE/VCO_2 slope.[19]

Incidental Events

The incidental endpoint was composite and included cardiovascular mortality, heart transplantation or LVAD implantation after enrollment in the study. Surveillance occurred every three months by making telephone calls, reviewing medical charts or by confirmation from local death certificate services.

Statistical Approach

Characteristics were described using mean and standard deviation for continuous variables, and absolute numbers and percentages for categorical variables. Kolmogorov-Smirnov test was applied and continuous variables showed normal distribution. For the cross-sectional analysis, linear regression assessed the associations between FEV_1/FVC and ppFVC exposures, and cardiac structure, respiratory strength and CPX variables as outcomes, in unadjusted and age- and sex-adjusted models, shown as ß-coefficient and 95% confidence interval (95% CI), per 10 percentage points increase in each spirometry variable. As the continuous variables were normally distributed and observations within each model were independent from each other (Pearson's bivariate correlation coefficients <0.35 between each exposure and outcome), linear regression assumptions were verified. To address potential non-linear cardiopulmonary associations, we also tested restricted cubic spline models using 3 to 7 knots, unadjusted and age- and sex-adjusted.

For sensitivity analysis, we determined the following categories: a) sex-specific terciles of FEV_1/FVC and ppFVC, with the first tercile representing the worst and the third tercile, the best lung function; and b) dichotomic obstructive (FEV_1/FVC ≤70) and non-obstructive (FEV_1/FVC>70) spirometry patterns or restrictive (ppFVC <80%) and non-restrictive (ppFVC ≥80%) patterns. Linear and logistic regressions and chi-square tests for trend were used to assess associations. To address potential asymmetries between included and excluded subjects, these groups were compared using the chi-square test for categorical variables, and the independent samples t-test for continuous variables.

For the prospective analysis, Cox regression was used to determine the magnitude of association of 10-percentage points decrease in spirometry variable with incidental composite endpoint, shown as hazard ratio (HR) and 95% CI. Non-linear associations were investigated using restricted cubic spline regression with the number of knots selected to minimize the AIC model (3 to 7 knots tested). The proportional hazards assumption was tested for all models using Schoenfeld residuals, and no violations were detected. As a sensitivity approach, four Cox regression sub-analyses were performed for each spirometric exposure, restricting them exclusively to subjects with: LVEF ≤50%; LVEF >50%; low MIP; and normal MIP.

A two-sided p-value <0.05 was considered significant for all analyses. Statistical analysis was performed using Stata software version 14.2 (Stata Corp LP, College Station, Texas, USA).

Results

Among the 111 HF subjects, ischemic etiology was predominant, AHA stages C or D, treated according to guidelines, including 24 subjects with LVEF >50% (Table 1). Approximately half of the subjects had restrictive (ppFVC <80%) pattern, one quarter had obstructive (FEV_1/FVC ≤70) pattern and 14 subjects (13%) had combined dysfunctions, while 40 of them (36%) had normal spirometry. From the 26 (23%) patients with body mass index (BMI) greater than 30 kg/m², 15 of them (65%) showed a ppFVC <80%. Among 57 patients with ppFVC <80% (51%), 15 had BMI>30 kg/m². Low MIP was a frequent finding. The average peak VO_2 was low, assuring a maximal effort criterion. General or leg muscle fatigue were the overall limiting symptoms. No wheezing or cyanosis was observed. Five patients had ventilatory reserve lower than 20%, including 4 with 10% to 15%; they had baseline restrictive (3) or combined (2) spirometric defects and LVEF <34%. Among them, RQ range was 1.09 and 1.22. Subjects not included due to missing or poor-quality spirometry had similar characteristics as the included subjects, except for younger mean age (51.6±14.2 years). (Supplemental Table S1).

Relationship of FEV_1/FVC with Functional Variables and Prognosis

Modeled continuously, FEV_1/FVC was proportionally associated with FEV_1 and with the ventilatory reserve from CPX, such that every 10-percentage points increase in FEV_1/FVC, was associated with 200 mL (95% CI 100–310 mL, p<0.001) FEV_1 increase, and with 7% points (95% CI 3–10%; p<0.001) increase in estimated ventilatory reserve, after adjusting for age and gender (Table 2). Although low MIP was a common finding in subjects with an obstructive pattern (n=15, 54%), the frequency was similar compared to the non-obstructive pattern (n=36, 47%; p=0.54), and MIP was not associated with continuous FEV_1/FVC (p=0.90). Additionally, a non-linear association was observed between FEV_1/FVC and FEV_1, such that this relationship is more robust if FEV_1/FVC is below 75% (Figure 1A). No other cardiopulmonary structure or function metric was associated with FEV_1/FVC. These findings were also consistent across FEV_1/FVC terciles (Supplemental Table S2).

At a mean follow-up of 2.2±0.7 years, 15 subjects had cardiovascular death outcome, 3 had heart transplant and 4 had LVAD implant. Lower FEV_1/FVC tended to increase the risk for the composite endpoint, however linearly not significant when accounting for age and sex (Table 3). Conversely, a non-linear association between FEV_1/FVC and the composite endpoint was observed, such that the risk decreases in association with FEV_1/FVC above 75. (Figure 2).

Two sensitivity analyses were performed. First, excluding subjects with LVEF>50% (n=24) from the 87 remaining subjects, 20 events occurred. In this scenario, every 10-percentage points decrease in FEV_1/FVC was associated with a 50% increase in the likelihood of the incident composite outcome per year of observation, accounting for age and sex (p=0.04) (Supplemental Figure S1). Among those with LVEF>50%, only two events occurred. Second, amongst subjects with a low MIP (n=51, 13 events), low FEV_1/FVC was associated with heightened risk for the primary outcome (HR 1.72; 1.14-2.61; p=0.009), while in the subgroup with normal MIP (n=57, six events), it was not associated with the outcome (HR 0.98; 0.36–2.69) (Supplemental Figure S1)

Relationship of Percent predicted FVC with functional variables and prognosis

Accounting for age and sex, every 10-percentage points increase in adjusted ppFVC was proportionally associated with a linear increase in FEV_1, by 230 mL (95% CI 190–270 mL, p<0.001). (Table 2). MIP also increased by 3.8 cmH2O (95% CI 0.3–7.3, p=0.03), but non-linear analysis showed

Table 1 – Characteristics of heart failure population at baseline (n=111). Values expressed as mean±SD or n (%)

Subjects, n	111
Demographics and clinical characteristics	
Age, years	57.4 ± 11.8
Male, n (%)	67 (60%)
Etiology, n (%)	
Chagas	32 (29%)
Ischemic	43 (39%)
Idiopathic	23 (21%)
Other	13 (12%)
BMI, kg/m^2	26.6 ± 4.8
BMI >30 kg/m^2; n (%)	26 (23%)
Medical history	
Hypertension, n (%)	63 (57%)
Diabetes, n (%)	20 (18%)
Current smokers, n (%)	29 (26%)
Dyslipidemia, n (%)	44 (40%)
NYHA, n (%)	
I	15 (13%)
II	25 (22%)
III	71 (64%)
Medications and devices	
Beta-blockers, n (%)	100 (90%)
ACEi/ARB, n (%)	94 (84%)
Spironolactone, n (%)	73 (66%)
Digoxin, n (%)	22 (20%)
Statin, n (%)	70 (63%)
Furosemide, n (%)	67 (60%)
Pacemaker/ICD, n (%)	25 (22%)
Pulmonary function	
FEV_1, L	2.3 ± 0.7
FVC, L	3.0 ± 0.9
Percent predicted FVC, %	80 ± 17
Percent predicted FVC <80%	57 (51%)
FEV1/FVC	75 ± 9
FEV_1/FVC ≤70	28 (25%)
MEP, cmH_2O	84.7 ± 40.1
MIP, cmH_2O	75.4 ± 35.4
Low MIP, n (%)	51 (49%)
Echocardiographic characteristics	
LVEF, %	38.4 ± 15.0
LVEF >50%, n (%)	24 (23%)
LA volume index, mL/m^2	44.7 ± 16.7
Estimated PASP, mmHg	38.9 ± 12.0
Cardiopulmonary test	
Peak power, W	80.3 ± 30.6
Peak heart rate, bpm	118 ± 26
Peak systolic pressure, mmHg	151 ± 25
Absolute peak VO_2, mL/min	966 ±401
Relative peak VO_2, mL/kg/min	13.4 ± 4.6
RER	1.23 ± 0.18
Absolute VO_2 at VAT, mL/min	618 ± 281
Relative VO_2 at VAT, mL/kg/min	8.6 ± 3.5
O_2 pulse, mL/beat	8.4 ± 3.1
OUES	1145 ± 465
VE max, (L/min)	45.0 ± 16.6
Ventilatory reserve, %	48 ± 19
VE/VCO2 slope	37.3 ± 8.1
Circulatory power, mmHg.mL/kg/min	2165 ± 1024
Ventilatory power, mmHg	4.3 ± 1.4

BMI: body mass index; FEV_1: forced expired volume in 1 second; FVC: forced vital capacity; NYHA: New York Heart Association functional class; ACEi: angiotensin converting enzyme inhibitors; ARB: angiotensin II receptor blockers; ICD: implantable cardioverter defibrillator; MEP: maximal expiratory pressure; MIP: maximal inspiratory pressure; LVEF: left ventricular ejection fraction; LA: left atrium; PASP: pulmonary artery systolic pressure; VO_2: oxygen consumption; OUES: oxygen uptake efficiency slope; VE: minute ventilation; VE/VCO_2 slope: VE/carbon dioxide production; RER: respiratory exchange ratio; VAT: ventilatory anaerobic threshold.

Table 2 – Continuous relationship of spirometric patterns (per 10-percentage points increase in each FEV_1/FVC and ppFVC) with cardiopulmonary function in heart failure subjects at baseline

Cardiac and pulmonary function		FEV_1/FVC		% Predicted FVC	
		Coefficient (95% CI)	p	Coefficient (95% CI)	p
FEV^1, L	Model 1	0.24 (0.10; 0.38)	0.001	0.25 (0.18; 0.31)	<0.001
	Model 2	0.20 (0.10; 0.31)	<0.001	0.23 (0.19; 0.27)	<0.001
MEP cmH_2O	Model 1	2.7 (-6.6; 11.9)	0.57	-0.9 (-5.8; 4.1)	0.73
	Model 2	0.6 (-7.5;8.8	0.88	-1.5 (-5.8; 2.9)	0.50
MIP, cmH_2O	Model 1	0.9 (-0.6; 8.2)	0.81	4.1 (0.2; 8.1)	0.04
	Model 2	0.4 (-6.1; 6.9)	0.90	3.8 (0.3; 7.3)	0.031
LV ejection fraction, %	Model 1	1.9 (-1.0; 4.9)	0.20	2.2 (0.6; 3.9)	0.007
	Model 2	1.6 (-1.4; 4.7)	0.28	2.1 (0.5; 3.8)	0.013
Peak power, W	Model 1	1.5 (-4.6; 7.6)	0.63	4.2 (0.9; 7.5)	0.012
	Model 2	0.3 (-4.3; 4.9)	0.89	3.5 (1.1; 6.0)	0.005
Absolute peak VO_2, mL/min	Model 1	30 (-50; 111)	0.45	35 (-9; 78)	0.11
	Model 2	18 (-49; 86)	0.59	27 (-9; 63)	0.14
Relative peak VO_2, mL/kg/min	Model 1	-0.3 (-1.3; 0.6)	0.47	0.6 (0.1; 1.1)	0.02
	Model 2	-0.4 (-1.3; 0.4)	0.30	0.5 (0.1; 1.0)	0.028
Respiratory exchange ratio	Model 1	-0.01 (-0.05; 0.02)	0.48	0.02 (-0.003; 0.04)	0.09
	Model 2	-0.01 (-0.05; 0.02)	0.40	0.01 (-0.003; 0.03)	0.10
Absolute VO_2 at VAT, mL/min	Model 1	-8 (-66; 49)	0.77	12 (-20; 44)	0.45
	Model 2	-12 (-67; 44)	0.68	13 (-18; 44)	0.42
Relative VO_2 at VAT, mL/kg/min	Model 1	-0.7 (-1.4; -0.004)	0.05	0.2 (-0.2; 0.6)	0.31
	Model 2	-0.7 (-1.4; 0.007)	0.05	0.2 (-0.2; 0.6)	0.25
O_2 pulse, mL/beat	Model 1	0.4 (-0.2; 1.0)	0.21	0.1 (-0.2; 0.5)	0.45
	Model 2	0.4 (-0.2; 0.9)	0.18	0.1 (0.2; 0.4)	0.47
OUES	Model 1	62 (-31; 155)	0.19	19 (-32; 70)	0.47
	Model 2	48 (-34; 130)	0.25	9 (-36; 54)	0.70
VE max, L/min	Model 1	-0.4 (-3.7; 2.9)	0.82	1.6 (-0.2; 3.4)	0.08
	Model 2	-0.6 (-3.3; 2.1)	0.68	1.5 (0.02; 2.9)	0.05
Ventilatory reserve, %	Model 1	7.4 (3.9; 10.9)	<0.001	4.6 (2.8; 6.5)	<0.001
	Model 2	6.8 (3.3; 10.3)	<0.001	4.3 (2.5; 6.2)	<0.001
VE/VCO_2 slope	Model 1	-0.2 (-2.0; 1.7)	0.87	-0.6 (-1.6; 0.3)	0.19
	Model 2	0.2 (-1.6; 2.0)	0.85	-0.5 (-1.5; 0.5)	0.35
Circulatory power, mmHg.mL/kg/min	Model 1	-20 (-202; 163)	0.83	85 (-14; 183)	0.09
	Model 2	-43 (-212; 125)	0.61	72 (-19; 163)	0.12
Ventilatory power, mmHg	Model 1	0.08 (-0.20; 0.35)	0.59	0.12 (-0.03; 0.27)	0.11
	Model 2	0.04 (-0.22; 0.31)	0.76	0.10 (-0.04; 0.24)	0.17

FEV_1: forced expired volume in 1 second; FVC: forced vital capacity; MEP: maximum expiratory pressure; MIP: maximum inspiratory pressure; LV: left ventricular; VO_2: oxygen consumption; VAT: ventilatory anaerobic threshold; OUES: oxygen uptake efficiency slope; VE max: maximum minute ventilation; VCO_2: carbon dioxide production. Model 1: unadjusted; Model 2: age, gender. Note: p-values refer to the respective linear regression analysis.

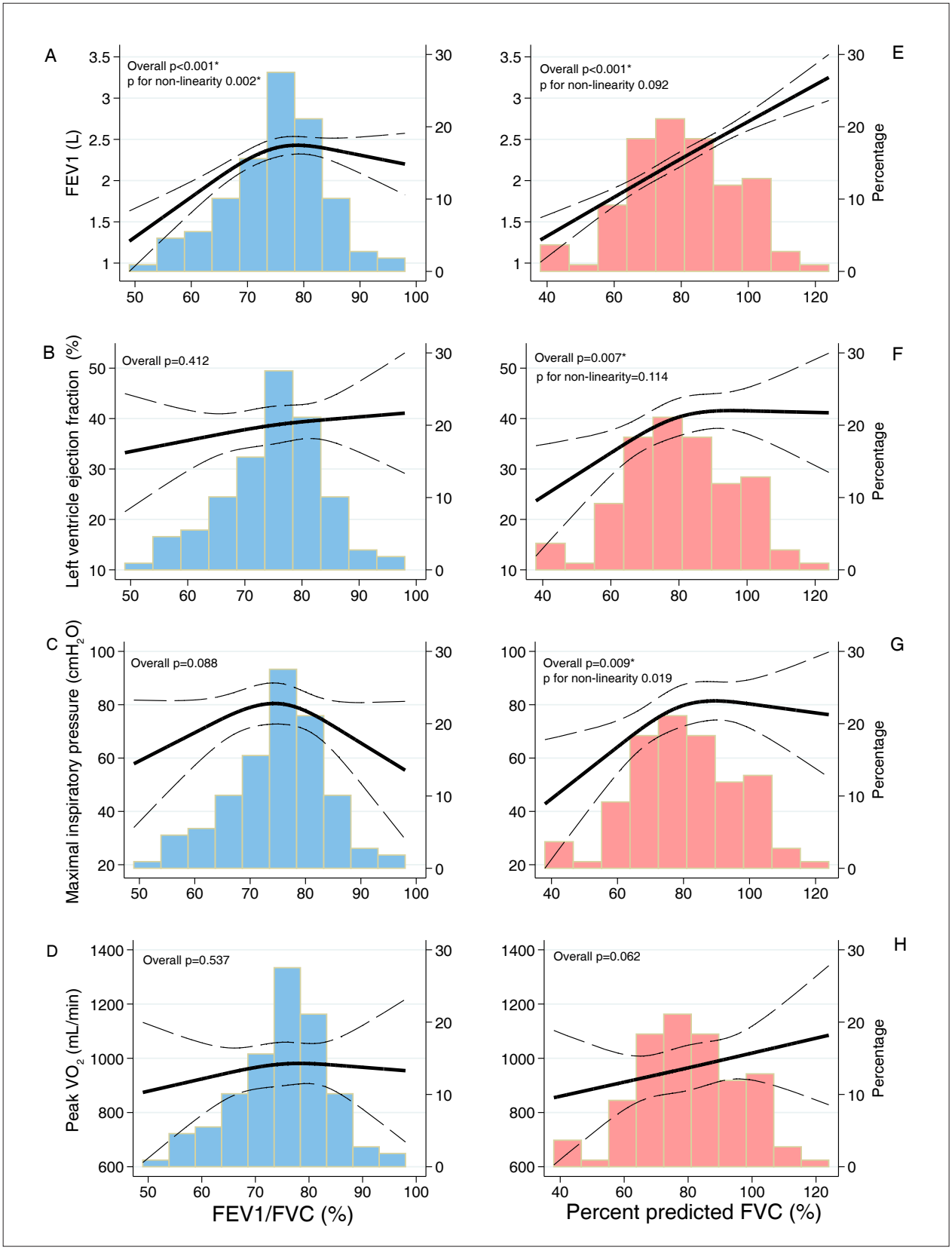

Figure 1 – *Continuous association of FEV_1/FVC (blue) and percent predicted FVC (light red) with FEV_1, LVEF, MIP and VO_2peak at baseline 5 using restricted cubic splines. Models were constructed using restricted cubic splines with 3 knots. *p <0.05 in models further adjusted for age and sex.*

that such association was more robust for ppFVC <80% (Table 2 and Figure 1G). Low MIP was more frequent in HF subjects with restrictive pattern (n=34, 65%) when compared to those without restrictive pattern (n=17, 32%; p<0.001). LVEF increased by approximately 2% points for every 10-percentage points increase in ppFVC, which, also, was more prominent for ppFVC<80% (Figure 1F). Regarding CPX, the greater ppFVC, the greater peak power, relative peak VO_2 and ventilatory power in adjusted models. No other cardiopulmonary structure or function metric was associated with ppFVC, either continuously (Table 2) or across terciles (Supplemental Table S3).

Lower ppFVC was not able to distinguish HF subjects under higher risk for the composite endpoint on the primary (Table 3 and Figure 2) or on the sensitivity analysis (Supplemental Figure S2).

Discussion

In a real-world cohort with 111 chronic HF subjects in classes C or D, within a broad ejection fraction range, we investigated how normal-to-severe spirometric dysfunction spectrum related to resting and exercise functional metrics and to major incidental cardiovascular events. Across airway

Table 3 – Association of spirometry variables at baseline with incidental composite outcome (cardiovascular mortality, heart transplant or left ventricular assist device implant; 22 events) among heart failure subjects (n=111), with mean follow-up of 2.2±0.7 years

	n	Events	Unadjusted HR (95% CI)*	p	Age/sex adjusted HR (95% CI)*	p
FEV_1/FVC						
Obstructive	28	9	2.45 (1.05-5.77)	p=0.039	2.28 (0.95-5.44)	p=0.064
Non-obstructive	83	13				
Continuous	111	22	1.48 (1.00-2.18)	p=0.050	1.44 (0.97-2.13)	p=0.069
ppFVC						
Restrictive	57	14	1.83 (0.77-4.37)	p=0.172	1.86 (0.78-4.44)	p=0.163
Non-restrictive	54	8				
Continuous	111	22	1.16 (0.92-1.46)	p=0.207	1.13 (0.89-1.43)	p=0.306

** Per 10-unit decrease. Note: p-values refer to the respective Cox regression analysis.*

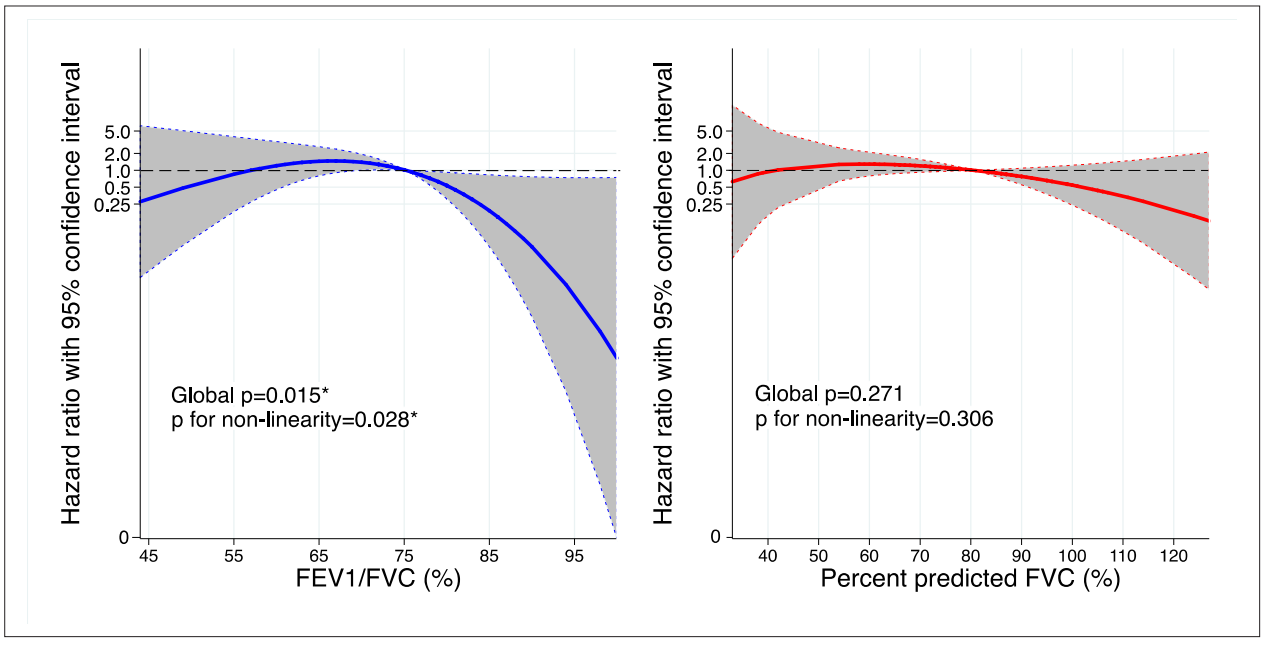

Figure 2 – *Continuous associations of FEV1/FVC (blue) and percent predicted FVC (red) at baseline with the composite outcome (cardiovascular death, heart transplant, and left ventricular assist device implant), in a mean follow-up of 2.2±0.7 years (22 events). Models were constructed for the primary exposure variables (FEV_1/FVC and percent predicted FVC) using restricted cubic splines with 3 knots. Linear corresponds to Cox regression analysis. *p <0.05 in models further adjusted for age and sex.*

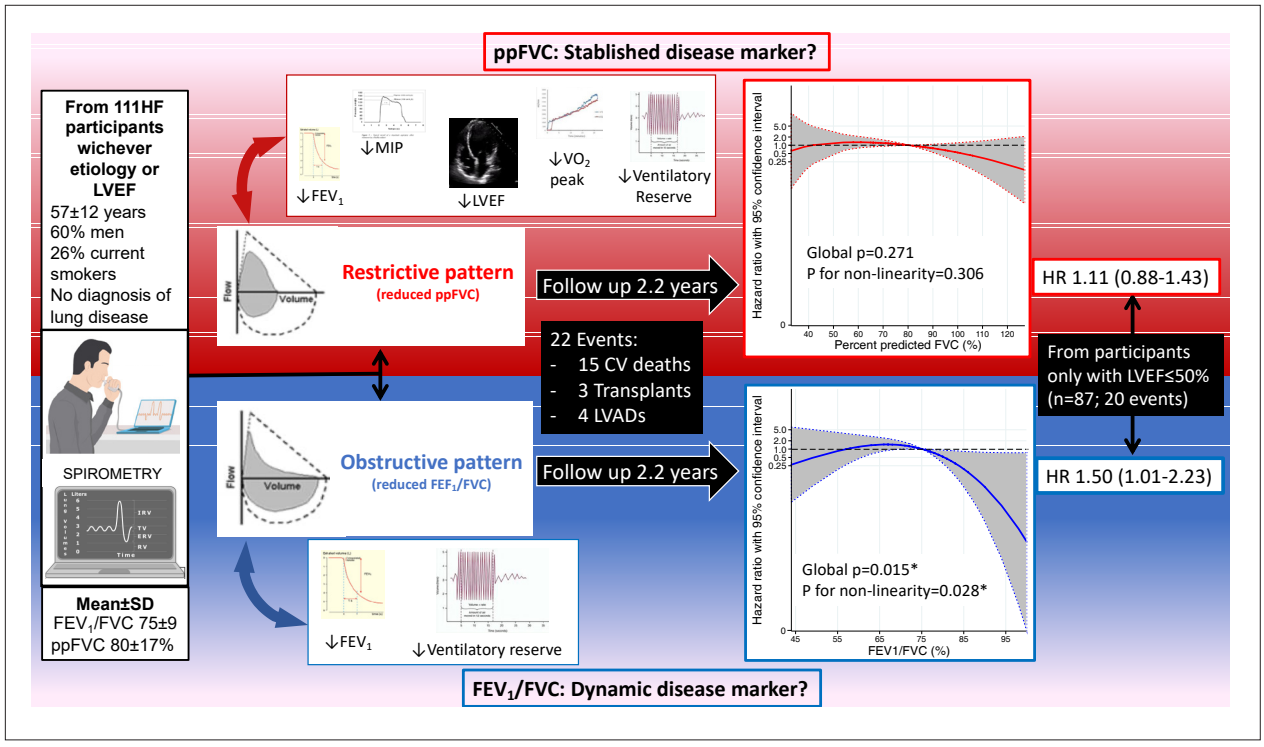

Figure 3 – *Visual abstract of the main findings: FEV_1/FVC and ppFVC differently characterize HF patients. HF: heart failure; LVEF: left ventricular ejection fraction; FEV1: forced expiratory volume in 1 second; ppFVC: percent predicted of forced vital capacity; MIP: maximal inspiratory pressure; CV: cardiovascular; LVAD: left ventricular assist device; HR: hazard ratio. HF: Although ppFVC was associated with other functional variables than the ventilatory reserve, only FEV_1/FVC was associated with a relatively short-term prognosis, particularly for low ejection fraction, suggesting that each marker may add different information regarding HF phenotyping.*

obstruction and vital capacity impairment ranges, accounting for age and sex, both low FEV_1/FVC and ppFVC were associated with reduced exercise ventilatory reserve, but only low ppFVC was associated with low ejection fraction, inspiratory weakness, and reduced exercise capacity; and more prominent when ppFVC was lower than 80%. Although such lung dysfunctions were common, the risk for the composite endpoint of cardiovascular death, heart transplant or LVAD implant was non-linearly associated with FEV_1/FVC only, not with ppFVC, suggesting a better prognosis in the non-obstructive pattern (FEV_1/FVC >75%). Additionally, among the low LVEF and low MIP subgroups, only reduced FEV_1/FVC distinguished greater risk. Therefore, FEV_1/FVC and ppFVC differently phenotype clinical aspects of HF patients (Figure 3).

Resting Lung Function and Exercise Performance Relationship

Direct HF effects on airways, such as vascular congestion, parenchyma and alveolar edema and interstitial fibrosis, are related to acute/subacute airway diameter constriction and to subacute/chronic lung volume reductions.[1-3] As a result, FEV_1 and FVC decrease independently or concurrently, suggesting that even subclinical dysfunction could contribute differently to exercise intolerance. Accordingly, FEV_1 was more robustly associated with aerobic capacity (peakVO_2) than LVEF.[8,20] Therefore, the maximum voluntary ventilation (derived from FEV_1), is expected to be impaired in HF, proportionally to underlying severity of lung dysfunction. However, exercise intolerance in HF subjects is multifactorial and reduced ventilatory reserve at peak only partially expresses lung contribution.[7] Indeed, we observed that ventilatory reserve increased 7% and 4% points for every 10% points increase in FEV_1/FVC and ppFVC, respectively. However, ventilatory reserve was as low as 38% in FEV_1/FVC and 39% in the lowest terciles of ppFVC on average, which is greater than the 20% expected to unequivocally assume a ventilatory constraint in peak exercise, supporting the cardiocirculatory limitation as to the primary – but not unique – effort limitation origin in our subjects.

Parameters such as OUES (Oxygen Uptake Efficiency Slope) and VE/VCO_2 slope are less dependent on peak effort and are more sensitive to distinguishing ventilatory from cardiocirculatory constraints.[20,21] The average values of low OUES, high VE/VCO_2 slope and low O_2 pulse in our study actually suggest a cardiocirculatory limitation predominantly, but the lack of association between resting FEV_1/FVC and ppFVC with other exercise ventilatory variables, including ventilatory efficiency, was contrary to our initial hypothesis. Therefore, the ability of resting spirometry to precisely measure ventilatory contribution to exercise impairment, other than ventilatory reserve, may be limited, overwhelmed by the cardiocirculatory component in more advanced HF stages, as in our cohort, with patients at stages C/D, predominantly in NYHA III (64%) and mean peakVO2 of 13 mL/kg/min.[22]

Regarding other functional variables, only ppFVC, not FEV_1/FVC, was additionally associated with resting MIP, but not MEP, with LVEF and with peak power and peak relative VO_2, accounting for age and sex.

Given the severe yet stable characteristics of HF subjects in this study, the discrepant correlations for each exposure could possibly result from the primary influence of HF in reducing total lung capacity, disproportionally decreasing FVC relative to FEV_1, therefore attenuating the FEV_1/FVC effect to predict exercise responses.[1] Accordingly, a restrictive pattern is usual in the HF syndrome,[1,7,8] particularly in HFrEF.[23] Additionally, the direct relationship of ppFVC with LVEF supports the hypothesis that a potential enlarged and dysfunctional heart relates to the aforementioned reduced lung volume due to space-occupying and congestive vascular and parenchymal effects. Such alterations, aggravated by inspiratory weakness, can compromise breathing mechanics in response to increasing demands, further reducing lung compliance, and all may contribute to exercise limitation, represented by the associated low-peak VO_2.[1,7,8]

Interestingly, only ppFVC was associated with the MIP. In HF, reduced vital capacity is associated with low MIP and diaphragm dysfunction,[24,25] which plays a significant role in exercise limitation in HFrEF[26,27] and HFpEF,[28] and demonstrates independent prognostic relevance.[29] Consistent with our findings, generalized skeletal muscle dysfunction and structural abnormalities, particularly the diaphragm, largely contributes to exercise intolerance in both HFrEF and HFrEF.[30] Automaticity and constant work overload, even at rest, uniquely characterize the diaphragm predisposition to early dysfunction in HF syndrome more prominently than expiratory and other peripheral muscles.[31]

Lung Function and Cardiovascular Prognosis

Lung function decline, beginning subclinically, have shown an association with incidental heart failure in unselected general populations.[32,33] Additionally, in subjects with stable HFrEF, spirometry significantly predicted all-cause mortality.[34] Olson et al.[34] studied 134 HFrEF subjects with peak VO_2 of 19 mL/kg/min and 66% NYHA classes I and II, and showed that lower FEV_1 and FVC had lower survival rates. In contrast, in advanced pre-transplant HFrEF, Georgiopoulou et al. demonstrated that spirometry provided no prognostic information.[35] In our cohort, positioned between the previous studies regarding severity, continuous FEV_1/FVC and ppFVC were not able to distinguish HF subjects with increased risk for major cardiovascular events in linear models.

Probably, given the chronic HF stages and the predominant cardiocirculatory limitation from the CPX, as demonstrated, ventilatory impairment contribution provided minor additional prognostic information for the whole LVEF range HF. Attempting to address a potential dynamic relationship of such complex biological interaction, we investigated non-linear associations for possible ranges or thresholds under differential risk across the spectrum of both exposures. We found a non-linear association between FEV_1/FVC and the composite endpoint, in which HF subjects with FEV_1/FVC greater than 75% decreased the likelihood of having major cardiovascular events in a mean follow-up of 2.2 years. We can hypothesize that: 1) COPD, the leading cause of airway obstruction which frequently coexists with HF, could have been undetected, increasing the risk burden;[4] or 2) from primary HF effects on respiratory system, the FEV_1 changes may be more sensitive than FVC in shorter time periods, influenced by dynamic changes in small and mid-bronchi calipers.[23] Reduced vital capacity, a hallmark of advanced HF,[9] was less sensitive in distinguishing the risk of incidental events throughout this follow-up.

Potential contributions from lung function to incidental cardiovascular events also appear to differ between HFpEF and HFrEF phenotypes. Restricting the analysis to a subset of subjects with LVEF ≤50%, a decrease in FEV_1/FVC, but not across the ppFVC spectrum, identified a higher risk for the composite endpoint, while no conclusion could be made for those with LVEF >50% with only two events. Similarly, in the inspiratory weakness subset, decreasing FEV_1/FVC distinguished greater risk for major cardiovascular events, which was not observed among those without weakness or across the ppFVC spectrum. We could speculate that the obstructive pathophysiology pattern adversely impacted mostly those with reduced LVEF and with inspiratory weakness.

Limitations

Several limitations should be noted. As an observational study, causality could not be addressed, and residual and unmeasured confounders may exist for the observations described. Only a subset of subjects was included, with complete spirometry, necessary for this study, and, given the younger age of excluded subjects, involuntary selection bias could be present. Spirometry measures were performed without bronchodilators, so reversible obstruction remained undetected; additionally, restrictive patterns were based only on FVC, because more precise and direct measures of volumes and capacities were unavailable, which could have limited the ability to detect true lung volume restriction, but could increase the external validity of findings. Also, an important mechanism of exercise limitation could be due to air trapping, which cannot be detected by spirometry.

Unfortunately, measured MVV was unfeasible to all participants, which could have influenced ventilatory reserve metrics, most likely underestimated. Even so, only 5 participants had <20% ventilatory reserve. However, we traded off potential unreliable results for uniform and comparable values throughout the cohort using an estimated MVV measure. Despite a reasonable correlation between FEV_1 and MVV (r^2=0.82),[36] we acknowledge that MVV must be performed prior to CPET at the individual level whenever possible.

We also acknowledge that low exercise oxygen saturation may represent ventilation-perfusion mismatch or ventilatory restraint, which is not exclusively, but most frequently associated with respiratory system impairment, particularly advanced COPD, interstitial lung diseases and pulmonary hypertension,[18] conditions excluded at study enrollment. However, an oximeter compatible to our CPX system was

unavailable during data acquisition and the existing fingertip probe produced unreliable peak values. Therefore, we resorted to the unremarkable physical exam (no peak exercise wheezing or cyanosis) to assume that hypoxia was unlikely.

Lastly, the relatively short follow-up time and, consequently, event rate, may have limited the ability to detect prognostic associations with ppFVC rather than FEV_1/FVC, given the more chronic behavior of the former.

Implications for Clinical Practice

Spirometry and manovacuometry are extensively available pulmonary function tools, although underused in HF.[4,26] Interpretation of ventilatory defects can be challenging in these subjects, particularly in those with HFpEF, in whom phenotype variation can potentially overlap heart and lung symptoms and fewer data are available.[9] However, they can provide valuable information on HF impact on the respiratory system, differentiating from undiagnosed (and undertreated) lung disease, better interpreting CPX, identifying potential therapeutic targets (rehabilitation, ventilatory training) and defining prognostic risk factors, emphasizing that spirometry is an available and feasible tool, which must be performed prior to CPX and to support risk stratification in HF. Subclinical and early stages alterations in lung function may predict future cardiovascular events. Adding to that knowledge, our study suggests that also in more chronic and stable HF, the presence and type of lung dysfunction help to better interpret exercise responses and to identify subjects at higher risk.

Conclusion

In a real-world cohort with chronic HF subjects, irrespective of ejection fraction range, continuous FEV_1/FVC and ppFVC at baseline were directly associated with ventilatory reserve at exercise, accounting for age and sex. However, only low ppFVC was additionally associated with low ejection fraction, inspiratory weakness, and reduced exercise capacity. Within a 2.2-year mean follow-up, only FEV_1/FVC, but not ppFVC, distinguished HF subjects at higher risk for major cardiovascular events, which were more prominent among those with reduced ejection fraction and low inspiratory pressure. Therefore, FEV_1/FVC and ppFVC add different information regarding HF phenotyping.

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Author Contributions

Conception and design of the research: Ramalho SHR, Lima ACGB, Cipriano GFB, Cipriano Junior G; Acquisition of data: Ramalho SHR, Lima ACGB, Silva FMF, Souza FSJ, Cipriano GFB, Cipriano Junior G; Analysis and interpretation of the data: Ramalho SHR, Cahalin LP, Cipriano GFB, Cipriano Junior G; Statistical analysis: Ramalho SHR; Obtaining financing: Lima ACGB, Cipriano GFB, Cipriano Junior G; Writing of the manuscript: Ramalho SHR; Critical revision of the manuscript for intellectual content: Ramalho SHR, Lima ACGB, Silva FMF, Souza FSJ, Cahalin LP, Cipriano GFB, Cipriano Junior G.

Potential Conflict of Interest

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

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*Supplemental Materials

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Lung Function and Inspiratory Muscle Strength in Heart Failure: Can They be Considered Potential Prognostic Markers?

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Short Editorial related to the article: Relationship of Lung Function and Inspiratory Strength with Exercise Capacity and Prognosis in Heart Failure

Heart failure (HF) is a complex syndrome considered a major public health problem. Different HF subtypes are classically defined based on left ventricular ejection fraction (LVEF).[1] Although its prognosis has improved in recent decades – explained, in part, by major therapeutic advances[2] – HF persists with a high burden of mortality and negatively influences the quality of life.[3,4] In this sense, common symptoms experienced in this disease, such as shortness of breath and exercise intolerance, contribute greatly to this sharp decline in the quality of life of individuals.[5] Another condition, considered an important risk factor, which usually accompanies HF, is pulmonary dysfunction.[6] The respiratory impairments observed in HF may be related to several reasons, such as an impairment of lung mechanics and gas diffusion,[7] as well as respiratory muscle weakness – aggravating increased dyspnea, being a major limitation on physical exercise.[8]

Spirometry is a widely used test that allows the analysis of lung function – by measuring the amount of air inhaled and exhaled to the maximum. Because chronic obstructive pulmonary disease shares similar signs and symptoms with HF, its identification in individuals with HF can be challenging; in this sense, spirometry can help confirm the diagnosis.[9] About evaluating the potential severity of some lung diseases, the exercise test can also be useful, observing a series of parameters, such as the ratio of forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC).[10] The severity of the disease can still be classified based on FEV_1 when it is below the lower limit of normal (ranging from mild when ≥70% of predicted to very severe when <35% of predicted). Although HF itself can lead to a decrease in FEV_1 and FVC of about 20% of predicted,[10] in addition to the fact that a worse FEV_1 may predict higher mortality,[11] convincing evidence examining the prognostic role of FEV_1 in the HF setting still needs further investigation.

Keywords

Heart Failure; Respiratory Insufficiency; Respiratory Muscles; Ventricular Function; Exercise Tolerance; Risk Assessment

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In this issue of the ABC, Ramalho et al.[12] shared data from a cohort study of 111 Brazilian adults (mean age: 57 years; 40% women) with chronic HF, no diagnosed lung disease, and who underwent a test of respiratory muscle strength and spirometry; participants were subsequently followed for a mean of 2.2 years. Some of the aims of the study were to analyze the FEV1/FVC ratio with (a) maximal inspiratory pressure, (b) LVEF, and (c) patients' prognosis – the latter being defined as a composite of cardiovascular (CV) death, emergency heart transplantation or left ventricular assist device implantation. Overall, the initial mean LVEF was 38%, but 24 of the patients had an LVEF >50%; the vast majority of the sample (64%) was in class III using the NYHA classification. Ischemic heart disease and Chagas' disease were the main etiologies observed (39% and 29%, respectively). The patients were relatively well treated, receiving optimal medical therapy (beta-blockers in 90%, renin-angiotensin-aldosterone system inhibitors in 84%, and mineralocorticoid receptor antagonists in 66%).

This article has several interesting findings worth highlighting. Both FVC and FEV_1/FVC did not correlate with a better or worse prognosis during the mean follow-up. On the other hand, after a sensitivity analysis, a low FEV_1/FVC was indicated to be a potential risk marker for increased major adverse CV events in the theoretically more severe individuals, i.e., with LVEF <50%. Furthermore, a higher risk of CV events was observed in those with both reduced maximal inspiratory pressure and FEV_1/FVC (hazard ratio 1.72; 95% confidence interval, 1.14 to 2.61).

Decades ago, Tockman et al.[13] reported FEV_1 as an independent predictor for CV mortality after following a cohort of apparently healthy men. In other observational studies that evaluated the prognosis of maximal inspiratory pressure in patients with HF, Hamazaki et al.[14] reported a lower incidence of clinical events in patients with a wide variety of LVEF (majority in NYHA functional class II) when a higher maximal inspiratory pressure was present, after cardiac rehabilitation sessions and at a mean follow-up of 1.8 years, even after adjustment for confounding factors. Meyer et al. suggested that inspiratory muscle strength could be useful in risk stratification of patients.[15]

Despite interesting findings, which to some extent corroborate previous studies, the study by Ramalho et al.[12] does not allow us to make causal inferences safely due to its observational design. It should be interpreted in light of this and other possible limitations. Although it is well established that HF is commonly characterized by abnormality of

respiratory muscles, the consequent decline in quality of life, and possibly worse prognosis, it would be premature to definitively conclude a direct association between maximal inspiratory pressure or FEV_1/FVC with increased risk of CV events in this population, regardless of LVEF. Despite these comments, this study provides important information to the literature, and it rekindles the possibility that FEV_1/FVC can be used as a prognostic tool, offering incremental information in the HF scenario, especially in the group of patients considered at higher risk. Still, it would be prudent to state that the relationship between these markers and the prognosis of these individuals remains uncertain.

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Mortality from Heart Failure with Mid-Range Ejection Fraction

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Abstract

Background: **The prognostic importance of the classification 'heart failure (HF) with mid-range ejection fraction (EF)' remains uncertain.**

Objective: **To analyze the clinical characteristics, comorbidities, complications, and in-hospital and late mortality of patients classified as having HF with mid-range EF (HFmrEF – EF: 40%-49%), and to compare them to those of patients with HF with preserved EF (HFpEF – EF > 50%) and with HF with reduced EF (HFrEF – EF < 40%) on admission for decompensated HF.**

Methods: **Ambispective cohort of patients admitted to the cardiac intensive care unit due to decompensated HF. Clinical characteristics, comorbidities, complications, and in-hospital and late mortality were assessed. The software R was used, with a 5% significance, for the tests chi-square, analysis of variance, Cox multivariate, and Kaplan-Meier survival curve, in addition to machine-learning techniques (Elastic Net and survival tree).**

Results: **519 individuals were included between September 2011 and June 2019 (mean age, 74.87 ± 13.56 years; 57.6% were men). The frequencies of HFpEF, HFmrEF and HFrEF were 25.4%, 27% and 47.6%, respectively. Previous infarction was more frequent in HFmrEF. The mean follow-up time was 2.94 ± 2.55 years, with no statistical difference in mortality between the groups (53.8%, 52.1%, 57.9%). In the survival curve, there was difference between neither the HFpEF and HFmrEF groups, nor the HFpEF and HFrEF groups, but between the HFmrEF and HFrEF groups. Age over 77 years, previous HF, history of readmission, dementia and need for vasopressors were associated with higher late mortality in the survival tree.**

Conclusion: **The EF was not selected as a variable associated with mortality in patients with decompensated HF.**

Keywords: **Heart Failure; Mortality; Mid-Range Ejection Fraction.**

Introduction

Heart failure (HF) is a complex systemic clinical syndrome, defined as cardiac dysfunction that causes inadequate blood supply to meet tissue metabolic needs.[1] It is the third cause of cardiovascular death in developed countries and an important cause of morbidity and hospitalization.[2] In Brazil, the mortality rate from HF in absolute numbers had a non significant decrease from 2008 to 2015.[3] In the BREATHE registry, the first Brazilian multicenter registry of acute HF, patients with decompensated HF had a high in-hospital mortality rate.[4] Heart failure was the main cardiovascular cause of hospitalizations in Brazil between 2008 and 2017, with 2.380.133 paid authorizations for hospitalization, accounting for 21% of the total number.[3]

Mortality related to HF, as well as the need for admission due to that syndrome, is closely associated with the assessment of left ventricular ejection fraction (EF), which is used for HF diagnosis, treatment, and prognosis. In 2016, The European Society of Cardiology issued a HF guideline with a new EF classification, introducing the concept of HF with mid-range EF (HFmrEF) for patients with EF ranging from 40% to 49%.[5] According to that classification, HF with EF equal to or greater than 50% was named HF with preserved EF (HFpEF), while HF with EF below 40% was named HF with reduced EF (HFrEF).[5]

The relevance of the HFmrEF classification for clinical practice remains uncertain regarding the change in the individualized diagnostic and therapeutic approach for that category. The CHART-2 Study, published in 2017, with 3480 patients from the 'Registry in the Tohoku District' followed up for 1 year, has shown that the clinical characteristics of patients with HFmrEF were different, suggesting that HFmrEF represented a transitional status or an overlap zone between HFpEF and HFrEF.[6]

Because of the remaining doubts in the literature, this study aimed to analyze the clinical characteristics, comorbidities, complications, and in-hospital and late mortality of patients

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classified as having HFmrEF, in addition to compare them to those of patients with HFpEF and HFrEF on admission due to decompensated HF. The analysis of those data can provide better understanding about the importance of HFmrEF for the therapeutic approach and prognosis of Brazilian patients admitted due to HF.

Methods

Ambispective cohort of patients admitted to the cardiac intensive care unit due to decompensated HF, from September 2011 to June 2019. Patients aged > 18 years and meeting the Framingham and Boston criteria were included, while 203 multiple admissions were excluded, only the last admission being considered. Information on late all-cause mortality was extracted from the site of the General Internal Affairs of Justice from Rio de Janeiro (http://www4.tjrj.jus.br/SEIDEWEB/default.aspx). Patients were assessed for 3 years regarding the outcome 'death from all causes'.

The following variables were assessed: age, sex, heart rate on admission, family history of coronary artery disease and myocardial revascularization, and presence of comorbidities, such as diabetes, hypertension, atrial fibrillation, chronic kidney disease (glomerular filtration rate < 60mL/min/1.73m^2), infarction, HF, stroke, and dementia. The previous use of beta-blockers, angiotensin-converting-enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB), and nitrates was evaluated. In addition, the following were assessed: creatinine and BNP levels on admission; need for coronary angiography; use of indwelling urinary catheter and of vasopressors; and dialysis treatment.

The variables were collected by using a standardized questionnaire. Echocardiogram on admission and the Teichholz's formula or Simpson's rule were used to measured and classify EF. The patients were separated into three groups according to their EF, considering HFpEF, HFmrEF and HFrEF in accordance with the classification of the last guideline.[5,7]

This study's project was submitted to and approved by the Committee on Ethics and Research on 09/18/2019 (certification of presentation for ethical appreciation number 18502319.3.0000.5249; appraisal: 3.582.453). Because this is an ambispective analysis of data collected in a partially prospective way, written informed consent was waived.

Statistical analysis

The normal distribution of the continuous variables was assessed by use of the Kolmogorov-Smirnov test. The results were presented as mean ± standard deviation (continuous variables) or number of occurrence and percentage (categorical variables). The means were compared by use of the chi-square test for categorical variables and analysis of variance (1-way ANOVA). The Kaplan-Meier curve was used to analyze survival over time, and the Tarone-Ware test for comparisons between the groups.[8,9]

The semi-parametric Cox model, sequentially estimated by use of Elastic Net, a machine-learning regularization technique, was used for the initial selection of variables, and then re-estimated by use of maximum likelihood and the significant variables put aside. Survival tree (machine learning) was used to identify the explanatory variables of mortality over time. The software R was used for statistical analyses at 5% significance level.[10]

The widths of the confidence intervals were not adjusted to multiplicity, thus, they should noy be used to infer the definitive treatment. The Cox models were used to calculate the measures of association (relative risks) and their respective 95% confidence intervals.

Results

This study included 519 individuals with a mean age of 74.87 ± 13.56 years, and 57.6% were men. The frequency distributions of HFpEF, HFmrEF and HFrEF were 25.4%, 27%, and 47.6%, respectively. All continuous variables were normally distributed. The male sex was more frequent in the HFmrEF and HFrEF groups as compared to the HFpEF group. The occurrence of previous HF and permanent atrial fibrillation was significantly higher in the HFpEF group, while that of previous myocardial infarction was higher in the HFmrEF group. The previous use of beta-blockers was similar in the groups, while that of ACEI and ARB was higher in the HFmrEF and HFrEF groups. There was an increasing gradient between the need for vasopressor use and the EF reduction (Table 1).

The mean follow-up duration was 2.94 ± 2.55 years. During follow-up, 287 (52.3%) patients died and, during hospitalization, 75 (14.5%) died, with no statistical difference between groups (Figure 1). When analyzing the specific causes of in-hospital death, there was a higher frequency of infectious causes, represented by septicemia and pneumonia, accounting for 7.3% and 4.2%, respectively. They were followed by diseases of the circulatory system, represented by HF and acute and chronic ischemic heart disease, accounting for 5.6%, 3.7% and 3.4%, respectively.

In the Kaplan-Meier survival curve[8] (Figure 2), the Tarone Ware test[10] shows no significant difference when comparing survival between the HFpEF and HFmrEF groups (p=0.27) and between the HFpEF and HFrEF groups (p=0.21). However, there was a significant statistical difference between the HFmrEF and HFrEF groups (p=0.02).

The multivariate analysis of the Cox model (Table 2) identified 13 variables associated with the risk of death during follow-up. Of those variables, the following stand out because of their clinical importance and higher relative risk: need for monitoring of urinary output with indwelling urinary catheter, report of readmission, previous coronary artery bypass grafting surgery, previous dementia and HF, need for dialysis treatment, and use of vasopressors.

The survival tree helps identify the patterns of shorter survival, considering the set of all variables (Figure 3). Age over 77 years and need for vasopressors were associated with higher mortality. The second pattern of higher mortality was patients older than 77 years with previous HF or dementia. The use of vasopressors and readmission were the third pattern associated with higher mortality regardless of age. Creatinine on admission over 1.48 mg/dL was the subsequent pattern of higher mortality.

Table 1 – Clinical characteristics of patients with heart failure with preserved, mid-range and reduced ejection fraction

Variables	HFpEF	HFmrEF	HFrEF	Total	p
n (%)	132(25.4%)	140(27%)	247(47.6%)	519	-
Age (mean)	77.8±15.8	74.2±11.9	73.6±12.8	74.8±13.5	0.13#
Men	45(34.1%)	87(62.1%)	167(67.6%)	299(57.6%)	**<0.001**
EF (mean)	66.9±8.9	45.1±3.3	30.3±7.6	43.6±16.6	**<0.001#**
BNP (mean)	3807	4969	6301	5307	0.17#
DM	43(32.6%)	52(37.1%)	93(37.8%)	188(36.2%)	0.59
SAH	109(82.6%)	109(77.9%)	91(77.3%)	409(78.8%)	0.46
Permanent AF	40(30.3%)	20(14.3%)	41(16.6%)	101(19.5%)	**0.001**
CKD* (GFR <60ml/min/1.73m2)	21(15.9%)	26(18.6%)	30(12.1%)	77(14.8%)	0.21
MI *	22(16.7%)	48(34.3%)	65(26.3%)	135(26.3%)	**0.004**
HF *	56(42.4%)	35(25%)	96(38.9%)	187(36%)	**0.005**
Stroke*	12(9.1%)	9(6.4%)	37(6.5%)	37(7.1%)	0.59
Previous dementia	14(10.6%)	15(10.7%)	17(6.9%)	46(8.9%)	0.32
Previous beta-blocker	55(41.7%)	60(42.9%)	94(38.1%)	209(40.3%)	0.60
Previous ACEI/ARB	13(9.8%)	48(34.3%)	73(29.3%)	134(25.8%)	**<0.001**
Use of vasopressors	10(7.6%)	21(15%)	59(23.9%)	90(17.3%)	**<0.001**

Values shown as mean and standard deviation. HF: heart failure; EF: ejection fraction; HFpEF: HF with preserved EF; HFmrEF: HF with mid-range EF; HFrEF: HF with reduced EF; ACEI: angiotensin-converting-enzyme inhibitor; AF: atrial fibrillation; ARB: angiotensin receptor blocker; BNP: brain natriuretic peptide; CKD: chronic kidney disease; DM: diabetes mellitus; GFR; glomerular filtration rate; MI: myocardial infarction; SAH: systemic arterial hypertension. (*) on admission; # ANOVA, other variables, chi-square.

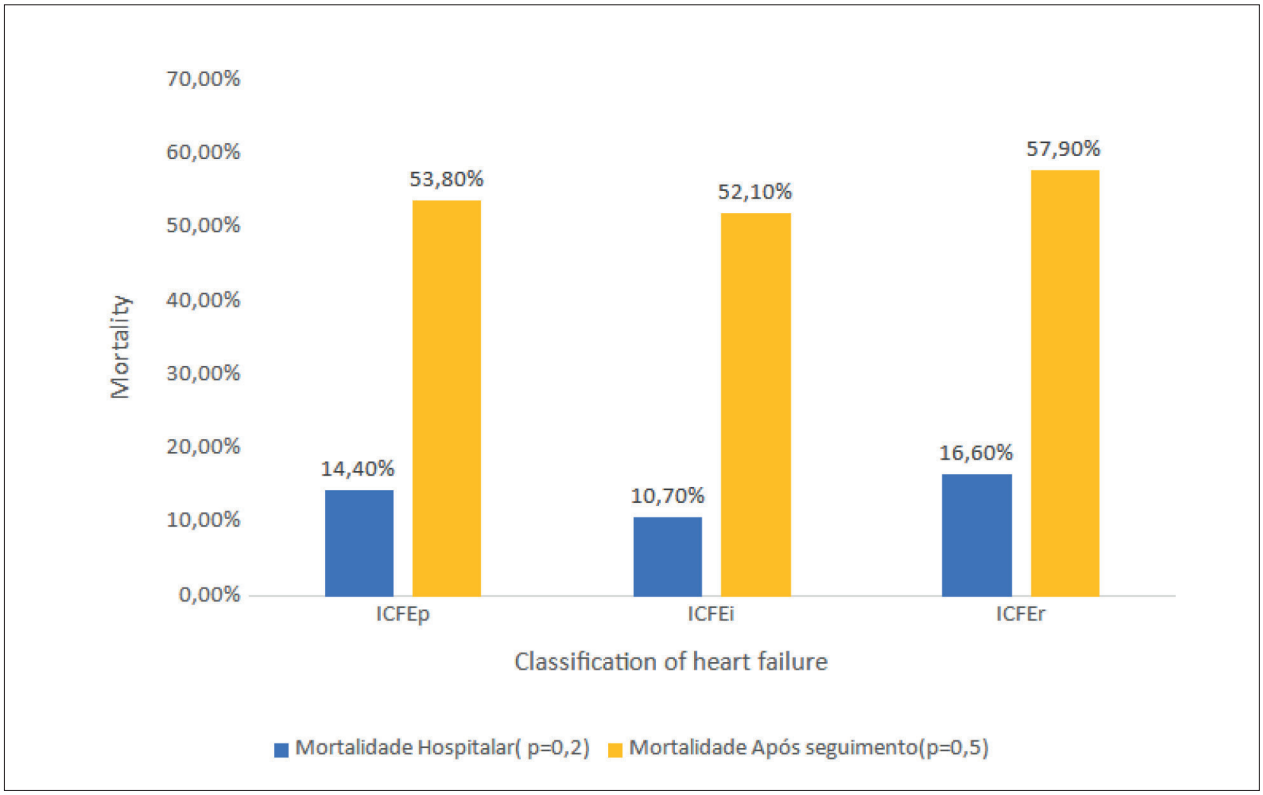

Figure 1 – *In-hospital mortality e após o follow-up (2,94 years) em patients hospitalizados por HFpEF, HFmrEF e HFrEF.*

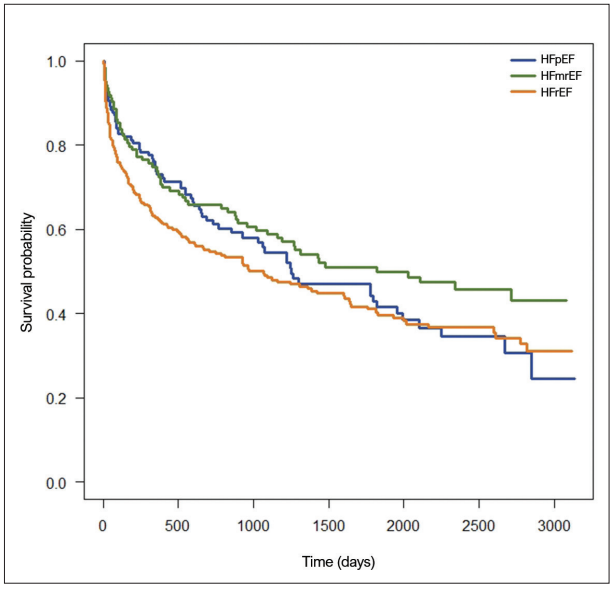

Figure 2 – *Kaplan-Meier survival curve[8] of patients with heart failure with preserved ejection fraction (HFpEF), heart failure with mid-range ejection fraction (HFmrEF) and heart failure with reduced ejection fraction (HFrEF) during the study period.*

Discussion

This study assessed a prospective cohort of patients admitted due to decompensated HF and used artificial intelligence to identify characteristics of HFmrEF regarding in-hospital and late mortality, relating it to the other groups categorized according to EF. Previous infarction was more frequent in HFmrEF and there was no statistical difference in mortality in the groups during the follow-up of 2.94 ± 2.55 years. In addition, in the survival curve, patients with HFpEF did not differ from those with HFmrEF, and patients with HFpEF did not differ from those with HFrEF; however, statistical difference was evidenced between patients with HFmrEF and HFrEF. Age over 77 years, presence of previous HF, history of readmission, presence of dementia and need for vasopressors were associated with higher late mortality in the survival tree. It is worth noting that EF was not selected as a variable associated with mortality in patients with decompensated HF.

Meta-analysis published in 2018, with 606 762 adult patients, compared the hospitalization rate and mortality from HFmrEF to those from HFpEF and HFrEF. The results suggested significant differences in all-cause mortality and noncardiac mortality between the HFrEF and HFmrEF group. In addition, the HFpEF group differed significantly from the HFmrEF group regarding cardiac death. Hospitalization associated with HF showed no difference between the groups.[11] This finding was similar to that from the present study, in which all-cause mortality differed between the HFmrEF and HFrEF groups. The authors from the meta-analysis emphasized the importance of concomitant comorbidities for the findings related to mortality.[11] In addition, higher prevalence of myocardial infarction was observed in the HFmrEF group, as well as of permanent atrial fibrillation in the HFpEF group.

Another meta-analysis from 2018 with 109 257 patients from 12 studies analyzed the clinical characteristics, hospitalization, and all-cause mortality in the three groups categorized according to EF. The authors reported significant differences in the baseline characteristics, in cardiovascular and all-cause mortality, and on admission due to HF in the three categories. In that meta-analysis, the patients with HFmrEF were older, mostly men and had less ischemic heart disease as compared to the patients with HFrEF.[12] A gradient of frequency was observed in age, sex, presence of ischemic heart disease,

Table 2 – Cox model for the outcome mortality with mean follow-up of 2.94 ± 2.55 years

Variables	Coefficient (RR)	95% confidence interval	p value
FHCAD	0.56	0.33 -0.96	0.037
Coronary angiography	0.61	0.38 - 0.99	0.004
Previous nitrate	0.68	0.51 - 0.91	0.009
Creatinine on admission	0.88	0.79 - 0.98	0.002
HR on admission	0.98	0.98 - 0.99	0.001
Age	1.03	1.02 – 1.04	<0.001
Use of IUC	1.48	1.14 - 1.94	<0.001
Readmission	1.52	1.18 – 1.96	0.001
Previous CABG	1.63	1.13 - 2.35	0.008
Dementia	1.72	1.21 - 2.44	0.002
Previous HF	2.24	1.73 - 2.90	<0.001
Dialysis treatment	2.56	1.62 - 4.04	<0.001
Vasopressor	2.91	2.06 - 4.11	<0.001

RR: relative risk; FHCAD: family history of coronary artery disease; HR: heart rate; IUC: indwelling urinary catheter; CABG: coronary artery bypass grafting surgery; HF: heart failure.

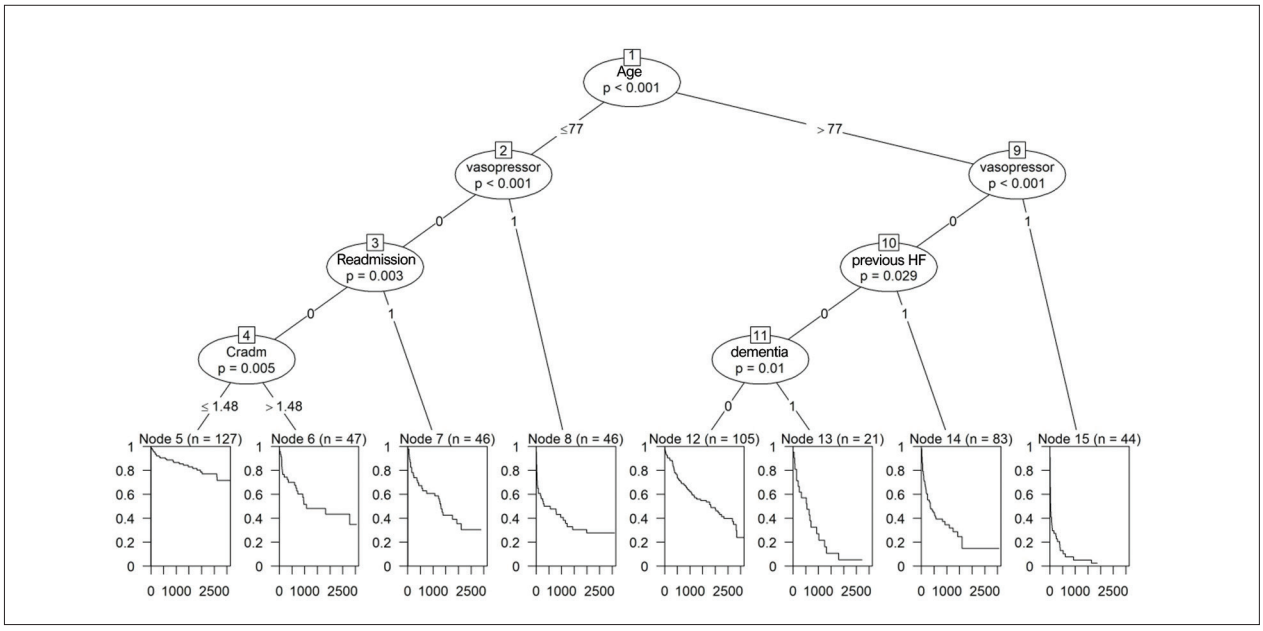

Figure 3 – *Survival tree of patients admitted due to heart failure. Cradm: creatinine on admission; HF: heart failure.*

hypertension, atrial fibrillation, chronic obstructive pulmonary disease, and glomerular filtration rate reduction according to the EF categorization. The same occurred regarding the use of beta-blockers and ACEI. Over approximately 3 years, the number of deaths from all causes was lower in HFmrEF than in HFrEF, but higher than that in HFpEF. Similarly, in HFmrEF, cardiovascular mortality and hospitalizations were lower as compared to HFrEF and slightly higher as compared to HFpEF. These findings suggest that HFmrEF, regarding data and outcomes, occupies a mid-position between HFrEF and HFpEF, more associated with worse prognosis outcomes as compared to HFpEF, but less associated with worse prognosis outcomes as compared to HFrEF. It is worth noting that the studies included were observational with heterogeneous populations and samples of different sizes. Only five studies reported data on hospitalization due to HF and cardiovascular death, indicating that the result should be interpreted carefuly.[12]

It is worth noting that the studies cited considered neither the relationship of the variables and their associations with the outcome over time, nor the interactions between all variables. In our study, the mean age was approximately 75 years, higher than that in the literature, in the cited meta-analysis (62 years) or in the BREATHE Registry (64 years).[4] This might explain the cut-off point of 77 years in the survival tree. In addition, there was a predominance of the male sex among patients with HFmrEF and HFrEF.[13]

The infectious causes, septicemia and pneumonia, were listed as having the highest in-hospital specific mortality in the sample. A study[14] has shown that the cardiovascular prognosis of recent-onset HF improved substantially from 2002 to 2014 (hazard ratio: 0.73; 95% CI: 0.68-0.80) for patients younger and older than 80 years. However, among those older than 80 years of age, the drop in cardiovascular mortality was totally compensated by non-cardiovascular mortality, in which case, the treatment changed the way elderly patients died, as observed in our study.

The presence of dementia syndrome, especially not related to the use of vasopressors on admission, was a factor of worse prognosis evidenced on the survival analysis. A recent study has reported functional decline in 15% of the patients, and, in 80%, that decline occurred prior to admission from decompensated HF and associated with a higher long-term risk for outcome composed by hospitalization and all-cause or HF death, similarly to our findings.[15]

The presence of previous HF in this sample was related to higher mortality, as well as to readmissions due to HF and need for inotropic agents, identified by use of the machine-learning technique. These three variables indicate worse prognosis of patients admitted with decompensated HF and are markers of severity that do not depend on EF. Patients admitted due to HF have a high rate of re-hospitalization in up to 6 months (30% to 40%),[16] and the risk of death after hospitalization due to HF remains increased from 12 to 18 months from the index event,[17] being one of the variables used to indicate heart transplantation.[18] The rates of readmission due to HF in young adults are similar to those of the elderly, suggesting that the re-hospitalization risk is present regardless of age.[19]

Chronic kidney dysfunction and HF often coexist and share several risk factors, such as diabetes, hypertension and hyperlipidemia, which compound the prognosis of decompensated chronic HF.[20] In addition, the cardiorenal syndrome, characterized by kidney function worsening during hospitalization due to HF or right after discharge, contributes to worsen the prognosis of decompensated HF.[21] Creatinine level on admission greater than 1.48 mg/dL has been associated with worse prognosis in individuals under the age of 77 years, representing a higher risk for kidney dysfunction, cardiorenal syndrome and need for dialysis.

There are several models to predict mortality from HF, such as the *Get With the Guidelines-Heart Failure* (GWTG-HF)[22] and the *Meta-Analysis Global Group in Chronic Heart Failure* (MAGGIC),[23] with unsatisfactory accuracy and without validation for the Brazilian population. Algorithms using deep learning, such as DAHF, improved the ability to predict mortality from HF during hospitalization and after 12 and 36 months from admission; however, they have not been developed for the Brazilian population.[24] This study's strength resides in the selection, through Elastic Net and survival trees (machine learning), of patterns of clinical presentation associated with worse in-hospital and late mortality in patients admitted with decompensated HF to a Brazilian cardiac intensive care unit.

One limitation of this study is its single-center nature, in addition to the lack of information on all the medications used prior to admission, such as diuretics. Thus, there is a potential bias of selection inherent in observational studies. There is, in the multiple analyses of the independent variables and mortality, exploratory nature. These characteristics might hinder the external validity of the findings. Regarding internal validity of data, some statistics, such as means and relative risks, are more important. The hypothesis that the EF categorization would be a predictor of in-hospital and late death in this sample was not corroborated by the analysis using machine learning. In this context, death related to decompensated HF seems to represent the sum of aging and progressing organ failures.

Conclusion

There was no statistical difference in mortality between the groups in the follow-up of 2.94 ± 2.55 years. The survival curve showed difference neither between the HFpEF and HFmrEF groups, nor between the HFpEF and HFrEF groups, but between the HFmrEF and HFrEF groups. Age older than 77 years, HF prior to admission, history of readmission, dementia, creatinine level on admission greater than 1.48 mg/dL, and need for vasopressors were associated with higher late mortality on the survival tree.

Author Contributions

Conception and design of the research: Dutra GP,Gomes BFO,Junior PRC,Petriz JLF,Nascimento EM, Pereira BB, Oliveira GMM.

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.

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Prognosis of Heart Failure with Mid-Range Ejection Fraction: A Story or a Version?

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Short Editorial related to the article: Mortality from Heart Failure with Mid-Range Ejection Fraction

The category "heart failure (HF) with mid-range ejection fraction" (HFmrEF), i.e., with left ventricular ejection fraction (LVEF) between 40-49%, was first described in 2016 in the European Society of Cardiology Guidelines on the syndrome.[1] After that, much of the worldwide cardiology community has adopted HF's classification into three categories of LVEF (reduced, mid-range and preserved), including the Brazilian Society of Cardiology (2018),[2] despite existing uncertainties about the real meaning of the new classification and, more importantly, what the identification of HFmrEF subgroup would impact on clinical practice. Unlike most, the American Heart Association and the American College of Cardiology (2013) has used 'borderline' HF with preserved LVEF (HFpEF) to define patients with LVEF between 41 and 49%, which was not updated in the document of 2017.[3,4]

In this context, in 2021, different international cardiological societies published a report proposing a universal definition and classification of HF. Regarding the classification by LVEF, although attractive from a clinical and epidemiological point of view, authors reviewed the limitations of its use from different aspects and proposed HF categories in which therapeutic strategy would be different. HFmrEF became synonymous with "lightly reduced" LVEF HF, now including LVEF between 41-49%, which was also adopted by Brazilian HF Guidelines update, 2021.[5,6]

In recent years, a large volume of clinical research has been published to understand better the HFmrEF population concerning its morbidity and prognosis. Patients classified as 'intermediate' seem to exhibit an overlap in clinical features, biomarkers, cardiac imaging findings and clinical outcomes compared to those with reduced LVEF HF (HFrEF) and HFpEF. However, there is a tendency towards greater similarity with patients with HFrEF. Patients with HFmrEF, such as HFrEF, are younger than in HFpEF and exhibit a higher prevalence of ischemic heart disease and male gender, while, in general, they have a lower proportion of atrial fibrillation.[1,7] However, this description may vary depending on the cohort studied or the clinical scenarios evaluated (e.g., outpatients or inpatients).[7]

Regarding clinical outcomes, studies have observed higher total mortality in HFrEF, and patients with HFmrEF, in general, were in the intermediate situation or closer to the cases of HFpEF.[8,9] On the other hand, a recent meta-analysis (2021) of 27 prospective studies found that total annual mortality was significantly lower in HFmrEF (37.5%) than in HFrEF (43.7%) and HFpEF (47.3%). Cardiovascular mortality, in turn, was lower in HFpEF, higher in HFrEF and intermediate in HFmrEF, the group that had the lowest incidence of hospitalization for HF.[10]

HF prognosis, on the other hand, is not necessarily related to the LVEF.[5] HFmrEF accounts, on average, for 10-20% of HF cases, and in many patients, intermediate LVEF represents a transitional and dynamic state, in which one can be facing recovery from trough HFrEF or a worsening towards trough HFrEF.[6,11] The topic is still quite controversial, making new studies necessary, involving populations from different geographic regions and varied clinical scenarios.

In this issue, Dutra et al.[12] evaluated the prognosis of an ambispective cohort of 519 patients with decompensated HF admitted to the intensive care unit of a single Brazilian center during a mean follow-up of almost three years.[12] Of the total sample, 27.0%, 25.4% and 47.6% had HFmrEF, HFpEF and HFrEF, respectively. The mean age was high, with patients with HFmrEF and HFrEF slightly younger than those with HFpEF. Like other articles, male gender was more frequent in HFmrEF and HFrEF, and atrial fibrillation was significantly more prevalent in HFpEF. In-hospital mortality was high (14.5%), predominantly for noncardiovascular causes, as was mortality at long-term (52.3%). The authors observed lower mortality in HFmrEF compared to HFrEF, which was statistically significant. Furthermore, finally, they identified 'patterns' (groups of variables) associated with worse survival, with the combination of age at admission > 77 years and the need for vasopressor therapy being the one with the worst prognosis. Dementia, prior HF, hospital readmission and baseline serum creatinine >1.48 mg/dL were also associated, alone or in groups, with higher mortality at late follow-up.

Keywords

Cardiovascular Diseases/physiopathology; Heart failure/physiopathology;Prognosis; Heart Failure/epidemiology; Prognosis; Stroke; Atrial Fibrillation; Mortality

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The study by Dutra et al.[12] is useful and pertinent to investigating such a current and controversial content in representatives of the Brazilian population. Some limitations preclude definitive conclusions, most of which have already been discussed by the authors in the publication, but the study adds information that joins previous data, also exploratory for the most part, in advancing the understanding of HFmrEF. In 2021, Petersen et al. published the follow-up results of a prospective cohort (n=380) of decompensated HF admitted to a tertiary hospital in Rio Grande do Sul, Brazil, in which 31.8%, 16.6% and 51.6% had HFrEF, HFmrEF and HFpEF, respectively.[13] Patients were younger and had lower in-hospital mortality (7.6%) than in the study of Dutra et al.[12] For total long-term mortality (primary outcome), the rates were also high, without detecting differences between the categories of HF. The cardiovascular cause was the main responsible for these observed deaths, and in exploratory multivariate models, HFmrEF and HFrEF were associated with a higher risk of cardiovascular mortality.

Although the study by Dutra et al.[12] does not definitively conclude on the clinical, etiological or prognostic characteristics of HFmrEF, their data feed the knowledge gap about this subgroup of patients with HF. Soon, we hope that new and consistent scientific evidence will inform us whether HFmrEF patients are intermediaries of two extremes or, indeed, a specific subgroup.

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Microalbuminuria and its Prognostic Significance in Patients with Acute Heart Failure with Preserved, Mid-Range, and Reduced Ejection Fraction

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Abstract

Background: **The prevalence and significance of microalbuminuria have not been well studied in patients with different heart failure subtypes.**

Objective: **The prevalence and significance of microalbuminuria have not been well studied in patients with different heart failure subtypes. Therefore, we aimed to investigate the frequency and prognostic value of microalbuminuria in patients hospitalized for acute heart failure (AHF) with preserved ejection fraction (HFpEF), mid-range ejection fraction (HFmrEF), and reduced ejection fraction (HFrEF).**

Methods: **All consecutive adult patients referred to the hospital due to AHF between June 2016 and June 2019 were enrolled. Microalbuminuria is defined as urinary albumin to creatinine ratio (UACR) level in the range of 30–300 mg/g. Hospital mortality was the endpoint of this study**

Results: **Of the 426 AHF patients (mean age 70.64 ± 10.03 years, 53.3 % female), 50% had HFrEF, 38.3% had HFpEF, and 11.7% had HFmrEF at presentation.The prevalence of microalbuminuria was 35.2%, 28.8%, and 28.0% in HFrEF, HFpEF, and HFmrEF, respectively. A total of 19 (4.5%) patients died during the in-hospital course, and in-hospital mortality was higher in HFrEF patients (6.6%) compared to patients with HFpEF (2.5%) and HFmrEF (2.0%). Multivariate analysis showed that the presence of microalbuminuria predicted in-hospital mortality in patients with HFrEF and HFmrEF but not in HFpEF.**

Conclusion: **Although microalbuminuria was common in all subgroups of AHF patients, it has been found to predict prognosis only in patients with HFrEF and HFmrEF.**

Keywords: **Albuminuria/physiopathology; Prognosis; Heart Failure; Stroke Volume; Hospitalization; Adults; Mortality.**

Introduction

Heart failure (HF) has been classified into three groups based on left ventricular ejection fraction (LVEF) in current guidelines; HF with reduced EF (HFrEF), HF with mid-range EF (HFmrEF), and HFwith preserved EF (HFpEF).[1] Acute heart failure (AHF), which can be developed in all types of HF, is a significant cause of mortality and healthcare costs in industrialized and developing countries.[2,3] Despite the advances in the management of AHF in the last decades, 4% to 7% of the patients die during hospitalization, and half of them die within five years.[4,5] Therefore, early prediction of mortality is essential for the management of patients with AHF, and there are many clinical and laboratory variables that predict mortality in AHF.[6-8]

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Although kidney dysfunction has also been associated with increased mortality risk in AHF,[9] previous studies had conflicting findings regarding the importance of chronic renal disease in HFpEF compared to HfrEF,[10,11] and the significance of renal functions in HFmrEF is unclear. Increased urinary albumin excretion, which might be a marker of inflammation, endothelial dysfunction, and activated the renin-angiotensin system, is a predictor of mortality and adverse events in the general population,[12] in patients with diabetes,[13] and hypertension.[14] The urinary albumin/creatinine ratio (UACR) in a random urine specimen is accepted as a more helpful method for evaluating renal functions. It avoids limitations of other tests such as glomerular filtration rate.[15] In chronic heart failure, even mild renal dysfunction, determined by the presence of microalbuminuria (defined as urinary albumin levels of more than or equal to 30-300 mg in 24 h urine collection or UACR of >30-300 mg/g in random spot urine sample), is an associated with adverse outcomes.[16] There are, however, few reports that examined the prognostic effect of UACRin patients with AHF. Furthermore, the prevalence and significance of microalbuminuria have not been compared in HFrEF, HFmrEF, and HFpEF. Therefore,

we aimed to examine the prevalence and importance of microalbuminuria in patients with AHF secondary to HFrEF, HFmrEF, and HFpEF.

Methods

Data of consecutive patients hospitalized through EDdue to AHF between June 2016 and June 2019 were retrospectively recorded. This study was conducted in Muğla Sıtkı Koçman University Hospital, and approved by the local institutional review board.

Inclusion Criteria

All adult patients (≥ 18 years)admitted to our ED with signs and/or symptoms of AHF and with increased N-Terminal pro-B-Type Natriuretic Peptide (NT-proBNP) levels were included.

Exclusion Criteria

Patients who did not have UACR, LVEF, or NT-proBNP evaluation at admission, patients aged <18 years, dialysis patients, and patients discharged to home were excluded.

Data Collection and Definitions

Patients were divided into three groups according to LVEF; patients with an LVEF <50% were defined as HFrEF, patients with an LVEF 40-49% were described as HFmrEF and patients with an LVEF < 40% were defined as HFpEF. In addition, echocardiographic criteria of diastolic dysfunction or structural heart disease were also required to determine HFpEF.

Patients'demographic characteristics and comorbidities were collected and noted from the hospital database. Definitions of demographic variables are given in the Table 1. In addition, blood and urine samples were obtained at admission, including NT-proBNP and estimated glomerular filtration rate (eGFR) levels.[17]

The albuminuria was defined according to the urine albumin to creatinine ratio: normoalbuminuria: <30 mg/g, microalbuminuria: 30 -299 mg/g, and macroalbuminuria: > 300 mg/g).The primary end point was in-hospital mortality.

Statistical analysis

Data were analyzed using SPSS for Windows (version 24; SPSS Inc, Chicago, IL). A p-value of ≤0.05 was considered significant. The univariate and multivariate regression analyses were performed to study the effect of various risk factors, including microalbuminuria and macroalbuminuria, on the primary outcome.

Results

A total of 586 adult AHF patients were admitted to our ED during the study period. However, 24 patients without LVEF data, 56 patients without NT-proBNPor UACR data, 64 patients who were discharged to home, and 16 patients with end-stage renal disease were excluded from the study

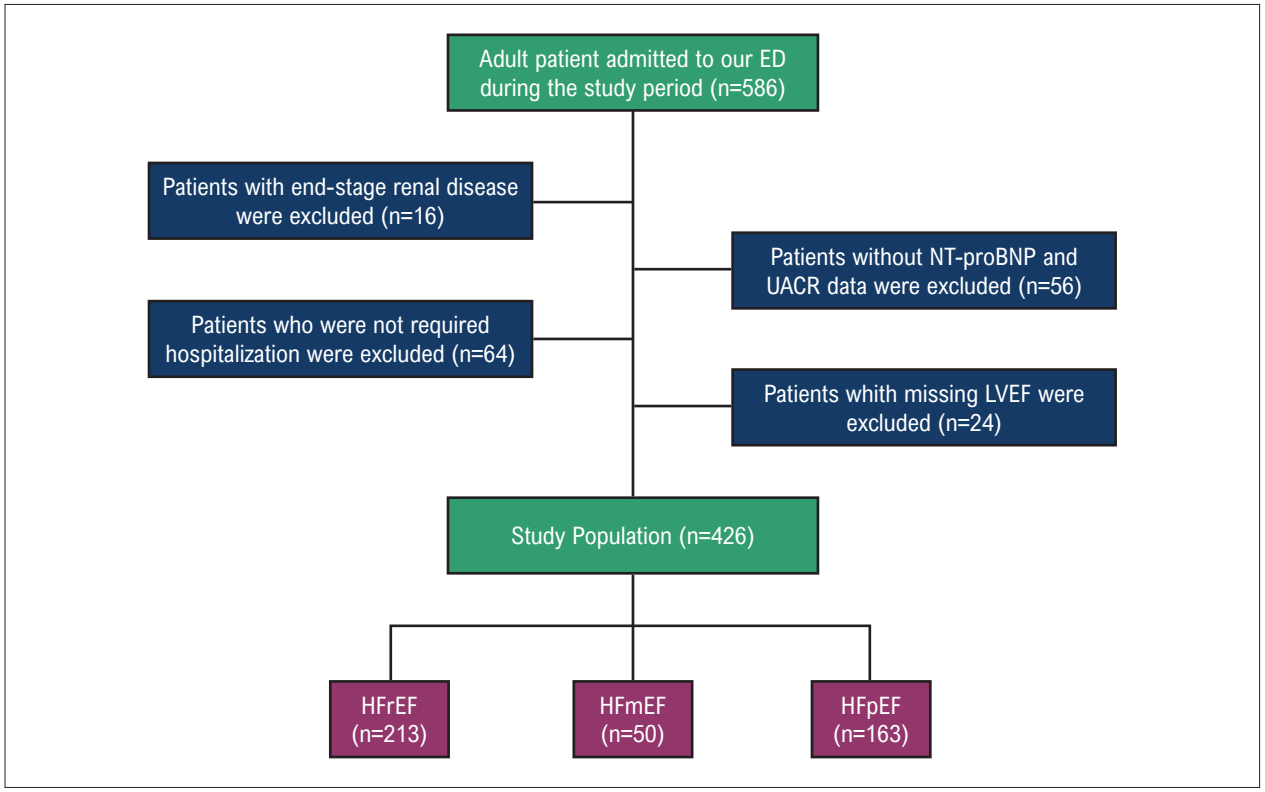

Figure 1 – *Participant flow chart. NT-proBNP: N-Terminal proB-Type Natriuretic Peptide; UACR: urinary albumin/creatinine ratio; LVEF: left ventricular ejection fraction; HfrEF: heart failure with reduced ejection fraction; HfmrEF: heart failure with mid-range ejection fraction; HfpEF: heart failure with preserved ejection fraction*

(Figure 1). The final study population included 426 patients (mean age 70.64 ± 10.03 years, 53.3 % female).

Comparison of baseline characteristics in heart failure subgroups

Among the study population, 50% had HFrEF, 38.3% had HFpEF, and 11.7% had HFmrEF.

The baseline characteristics of the patients are shown in Table 1. Patients with HFpEF were older, had a higher body mass index, and were more likely to be female. Patients with HFrEF were younger, had significantly higher admission NT-pro-BNP and UACR levels, had lower systolic blood pressures but higher heart rates at presentation. Patients with HFmrEF had an intermediate biomarker profile and intermediate phenotype for comorbid diseases. Patients with HFmrEF differed from HFpEF and HFrEF,

Table 1 – Patient demographics and characteristics

	HFrEF (n=213)	HFmrEF (n=50)	HFpEF (n=163)	p value
Female sex	110 (51.6)	22 (44.0)	95 (58.3)	<0.001
Age, years	68.09 ± 9.58	70.85 ± 10.15	72.83 ± 10.70	0.015
Smoking	40 (18.8)	10 (20.0)	30 (18.4)	0.344
Alcohol use	10 (4.7)	3 (6.0)	8 (4.9)	0.632
Body mass index, kg/m²	27.56 ± 5.66	28.98 ± 5.92	29.43 ± 6.24	0.004
Comorbidities				
Atrial fibrillation	65 (30.5)	15 (30.0)	50 (30.7)	0.845
Hypertension	160 (75.2)	38 (76.0)	121 (74.2)	0.921
Diabetes mellitus	63 (29.6)	14 (28.0)	45 (27.6)	0.814
Chronic renal disease	25 (11.7)	5 (10.0)	16 (9.8)	0.623
Coronary artery disease	95 (44.6)	26 (52.0)	66 (40.5)	0.014
Cerebrovascular disease	10 (4.7)	3 (6.0)	12 (7.4)	0.131
COPD	21 (9.9)	5 (10.0)	15 (9.2)	0.755
Signs and Symptoms				
Dyspnea, NYHA class III/IV	171 (80.3)	42 (84.0)	134 (82.2)	0.510
Palpitation	130 (61.1)	30 (60.0)	105 (64.4)	0.212
Ankle swelling	70 (32.9)	15 (30.0)	51 (31.3)	0.815
Chest pain	60 (28.2)	20 (40.0)	43 (26.4)	0.004
Physical Exam				
Systolic blood pressure, mmHg	122.5 ± 15.41	131.22 ± 20.66	132.30 ± 20.11	0.001
Diastolic blood pressure, mmHg	79.12 ± 11.96	80.10 ± 12.07	80.65 ± 11.86	0.109
Heart rate, bpm	88.75 ± 18.23	82.36 ± 18.05	82.55± 17.98	<0.001
Pulmonary crepitations	160 (75.2)	37 (74.0)	119 (73.0)	0.081
Laboratory				
NT-proBNP, pg/ml	5859 (1896 - 11857)	3421 (1104 - 8455)	2544 (986 - 5487)	<0.001
Glucose, mg/dl	118 (94 - 158)	120 (96 - 161)	119 (95 - 159)	0.742
BUN, mg/dl	22 (18 - 37)	23 (17 - 35)	22 (16 - 36)	0.291
Serum creatinine, mg/dl	1.2 (0.8 - 1.7)	1.2 (0.8 - 1.8)	1.1 (0.7 - 1.7)	0.366
Hemoglobin, g/dl	12.5 (10.1 - 14.5)	12.6 (10.5 - 13.5)	12.4 (10.8 - 14.2)	0.113
UACR	12.5 (5.9 - 1357.7)	10.3 (2.9 - 725.7)	10.1 (4.5 - 878.7)	0.001
eGFR (mL/min/1.73 m²)	68.7 ± 21.6	70.9 ± 21.3	70.7 ± 22.5	0.032
Hospitalstay,median,days	8	7	7	0.106
In-hospital mortality	14 (6.6)	1 (2.0)	4 (2.5)	0.003

Data are presented as mean ± standard deviation, number (%), or median and interquartile range. HfrEF: heart failure with reduced ejection fraction; HfmrEF: heart failure with mid-range ejection fraction; HfpEF: heart failure with preserved ejection fraction; NYHA: New York Heart Association; COPD: chronic obstructive pulmonary disease; NT-proBNP: N-terminal pro B-type natriuretic peptide; BUN: blood urea nitrogen; UACR: urinary albumin/creatinine ratio; eGFR: estimated glomerular filtration rate.

as they were more often male and more likely to have a history of coronary artery disease.

Of the 426 patients, 185 (43.4%) had increased UACR at admission; 136 patients had (31.9%) microalbuminuria, 49 patients had macroalbuminuria (11.5%), and 241 (56.6%) patients had normoalbuminuria. There were no significant differences in the prevalence of normo-, micro- and macroalbuminuria in patients with HFpEF and HFrEF. However, compared with HFpEF and HFmrEF, HFrEF patients were more likely to have micro- and macroalbuminuria and were less likely to normoalbuminuria (Figure 2). The prevalence of microalbuminuria was 35.2%, 28.8%, and 28.0% in HFrEF, HFpEF, and HFmrEF, respectively. The prevalence of microalbuminuria was 13.1%, 9.8%, and 10% in HFrEF, HFpEF, and HFmrEF, respectively.

Comparison of Outcomes

There was no difference in length of hospital stay between patients with HFpEF, HFmrEF or HFrEF.A total of 19 (4.5%) patients died during the in-hospital course, and in-hospital mortality was higher in HFrEF patients (6.6%) compared to patients with HFpEF (2.5%), and HFmrEF (2.0%) (p = 0.004).

Predictors of In-hospital Mortality

Multivariate analysis showed that NT-pro-BNP and macroalbuminuria had been associated with in-hospital mortality in all LVEF groups (Table 2). Coronary artery disease, male gender, and diabetes mellitus predicted in-hospital mortality only in patients with HFmrEF, whereas atrial fibrillation predicted in-hospital mortality only in patients with HFrEF. Older age was an independent predictor of in-hospital mortality in patients with HFrEF and HFpEF.

Microalbuminuria and Prognosis

The presence of microalbuminuria on admission has been associated with in-hospital mortality in HFrEF and HFmrEF, but

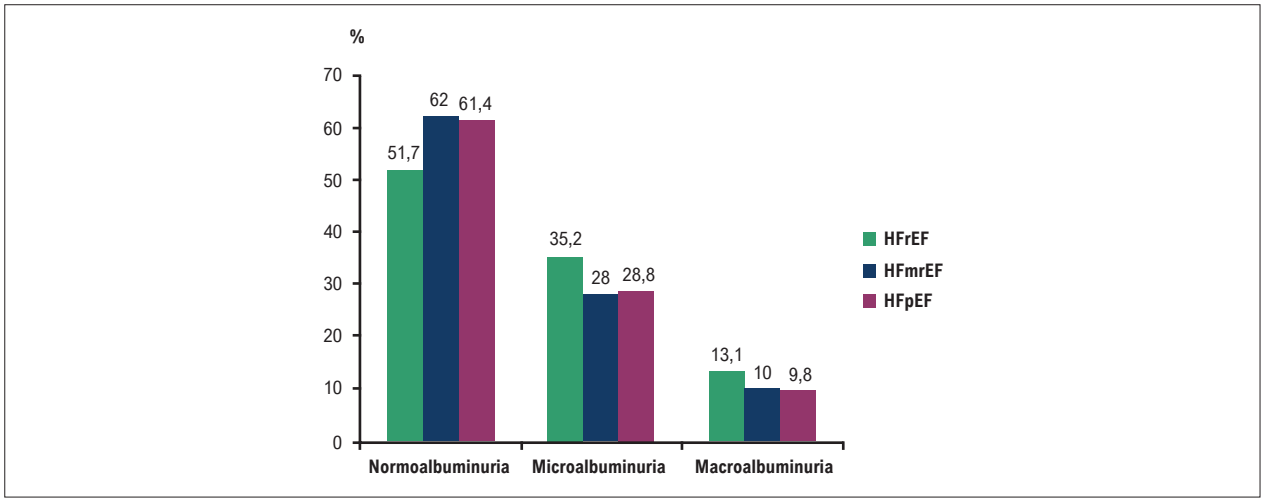

Figure 2 – *Comparison of the prevalence of normo-, micro- and macroalbuminuria in relation to heart failure subtypes. HfrEF: heart failure with reduced ejection fraction; HfmrEF: heart failure with mid-range ejection fraction; HfpEF: heart failure with preserved ejection fraction*

Table 2 – Predictors of in-hospital mortality in HF subtypes

	HFrEF		HFmrEF		HFpEF	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Coronary artery disease	2.10 (1.55-3.04)	0.065	3.45 (1.23-5.67)	0.043	1.49 (1.14-5.34)	0.089
NT-proBNP	2.68 (1.23-7.75)	<0.001	2.12 (1.34-3.45)	0.011	2.01 (0.09-3.23)	0.022
Age (per 10 years)	1.75 (1.13-3.45)	0.016	1.13 (0.80-1.51)	0.076	3.12 (1.38-4.81)	0.019
Diabetes mellitus	1.21 (0.81-1.43)	0.121	2.34 (1.03-4.16)	0.043	1.20 (0.89-2.55)	0.291
Microalbuminuria	1.94 (0.91-4.21)	<0.001	1.56 (1.19-3.45)	0.001	1.25 (1.12-1.68)	0.124
Macroalbuminuria	2.45 (1.34-5.65)	<0.001	1.92 (1.23-2.98)	0.024	1.66 (1.34-3.84)	0.032
Chronic renal disease	1.15 (1.01-1.33)	0.293	1.32 (1.11-2.77)	0.101	1.23 (0.82-1.56)	0.451
Atrial fibrillation	1.07 (0.83-1.42)	0.013	1.23 (0.89-1.55)	0.234	1.33 (1.18-2.01)	0.098
Male gender	1.22 (0.83-1.88)	0.462	3.31 (1.13-4.23)	0.001	0.89 (0.66-1.39)	0.453

HFrEF: heart failure with reduced ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction; NT-proBNP: N-terminal pro B-type natriuretic peptide.

not in HFpEF patients. Patients with microalbuminuria and macroalbuminuria had 1.94-, and 2.45-fold higher risk, respectively, for in-hospital mortality compared to patients with normoalbuminuria in HFrEF.Compared to patients with normoalbuminuria, patients with microalbuminuria and macroalbuminuria had 1.56-, and 1.92-fold higher risk for in-hospital mortality in HFrmEF, respectively.

Discussion

Our study has several important clinical implications: (i) Of the hospitalized AHF patients, 50% had HFrEF, 11.7% hadHFmrEF, and 38.3% had HFpEF. (ii) 43.4 % of the patients had abnormal UACR at admission to the ED. (iii) The NT-proBNP and UACRvalues and in-hospital mortality rates were the highest in HFrEF patients. (iv) The prevalence of micro- and macroalbuminuria in HFpEF was similar to HFmrEF's and lower than HFrEF's. (v) The prevalence of microalbuminuria was 35.2%, 28.8%, and 28.0% in HFrEF, HFpEF, and HFmrEF, respectively. (vi) The microalbuminuria predicted in-hospital mortality in HFmREF and HFrEF, but not in HFpEF.

Cardiovascular and renal diseases share similar comorbidities and risk factors. Extensive cohort studies have shown that increased UACR is associated with the development of HF in the general population.[18-20] However, most studies have described the significance of UACR in HFrEF, and studies examining the HF subtypes separately have divergent findings.[21,22] In a community-based study, Nayor et al.[21] found that microalbuminuria was associated with an increased risk of incident HFrEF but not HFpEF.[21] In contrast, the PREVEND cohort study showed that higher UACR was more strongly associated with incident HFpEF than HFrEF.[22] In a recent survey of 24433 patients, the association between UACR and HFpEF was greater than HFrEF after 9.3 years of follow-up.[23]

Renal function tests are also associated with adverse outcomes regardless of the severity of the disease in patients with established HF. However, studies investigating the impact of renal dysfunction on prognosis in the different LVEF groups also have conflicting results.[24,25] In a meta-analysis, Damman et al.[24] showed that chronic renal dysfunction was a stronger predictor of mortality in HFpEF than in HfrEF.[24] In contrast, in a meta-analysis of twenty-five prospective studies, renal dysfunction was a stronger predictor of mortality in patients with HFrEF than in HFpEF.[25] Both meta-analyses defined chronic renal disease as an eGFR of less than 60 ml/min/1.73m^2, and studies examining the prognostic value of microalbuminuria or UACR in chronic HF patients with different LVEF groups are much more limited.[26-30] In a cross-sectional study, 72 chronic HF were enrolled, and microalbuminuria was observed in 40% of HFpEF and 24% of HFrEF patients ($p = 0.04$).[26] However, the prognostic impact of microalbuminuria was not evaluated in this study. In the CHARM study, which included chronic HF patients, the prevalence of micro- and macroalbuminuria was 30% and 11%, respectively.[27] When stratifying into different LVEF groups, 31% of the patients with an LVEF ≤40% had microalbuminuria, and 10% had macroalbuminuria. Of the patients with an LVEF >40%, 29% had microalbuminuria, and 12% had macroalbuminuria. The findings of the CHARM study also revealed that albuminuria was a predictor of mortality. The risk associated with UACR was similar in patients with low and preserved LVEF.[27] In the GISSI-HF trial, micro- and macroalbuminuria were observed in 19.9% and 5.4% of the patients, respectively. UACR independently predicted mortality in patients with chronic HF.[28] Nevertheless, as 90.8% of the GISSI-HF patients had an LVEF≤ 40%, a separate analysis for different LVEF groups was not performed. In the CHART-2 study, 2039 chronic HF patients were enrolled.[29] The authors showed that not only microalbuminuria but also subclinical microalbuminuria, which was defined as UACR 10.2–27.3 mg/g, was significantly associated with adverse cardiovascular events as compared with normoalbuminuria, particularly in patients with preserved or mildly reduced eGFR.[28] TOPCAT study included only HFpEF patients to investigate the benefit of spironolactone therapy.[30] In a subgroup analysis of the TOPCAT study, micro- and macroalbuminuria conferred a 1.47- and 1.67-fold increased risk for primary outcomes in HFpEF.[30]

Although the prevalence of renal dysfunction is expected to be higher in AHF patients than patients with chronic HF, few studies assessed albuminuria in the AHF setting. In a prospective study of 115 AHF patients, Koyama et al.[31] showed that 69% of the patients had abnormal UACR at admission (27% had macroalbuminuria, 42% had microalbuminuria).[31] However, on day 7, 10% of the patients had macroalbuminuria, and 30% had microalbuminuria. The resolution of UACR was associated with decreases in NT-pro BNP levels.[31] The frequency of abnormal UACR at admission was 43.4% in our study, lower than the Koyama and colleagues' study. This difference may be due to younger age and lower comorbidity burden in our study.

Our study demonstrated that the microalbuminuria at admission to ED is an independent predictor of in-hospital mortality in HFmREF and HFrEF, but not in HFpEF.In HFpEF, the prognosis may be more related to comorbidities than in HFmrEF, and HFrEF, where progressive HF with subsequent renal dysfunction may be more pronounced. The relationship between HF and albuminuria is complex. It has a bidirectional nature, and the mechanisms responsible for the relation of microalbuminuria and prognosis in HFrEF and HFmrEF warrant further investigation.

Study Limitations

Our study is limited by its retrospective design and by having been conducted at a single center. Because daily changes in UACR were not recorded, we could not examine the relationship between alterations in UACR and prognosis. A single spot urine sample was used to determine UACR, which may fluctuate.

Conclusions

In patients with AHF, microalbuminuria on admission is associated with increased in-hospital mortality in HFmrEF and

HFrEF. Further prospective studies are required to explore the role of UACR as a prognostic marker in AHF.

Author Contributions

Conception and design of the research: Alataş OD; Acquisition of data: Alataş OD, Demir A, Yıldırım B, Acar E, Gökçek K, Gökçek A; Analysis and interpretation of the data: Alataş OD, Gökçek K; Statistical analysis: Biteker M, Acar E; Writing of the manuscript and Critical revision of the manuscript for intellectual content: Alataş OD, Biteker M.

Potential Conflict of Interest

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

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Microalbuminuria and the Risk of Mortality in Patients with Acute Heart Failure

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Short Editorial related to the article: Microalbuminuria and its Prognostic Significance in Patients with Acute Heart Failure With Preserved, Mid-Range, and Reduced Ejection Fraction

Heart failure (HF) is a chronic and progressive clinical syndrome resulting from structural or functional abnormalities of the heart. The most common symptoms and signs of HF are dyspnea, fatigue, pulmonary and peripheral congestion or edema and jugular vein distension. The prevalence of HF continuously increases due to improved treatment and reduced short-term mortality in patients with acute coronary syndromes, congenital heart disease, population aging and improved survival of patients with already developed heart failure by the widespread application of modern disease-modifying medications and devices.[1]

Acute heart failure (AHF) is recognized when symptoms of HF appear in the patient without the history of previous HF (de novo HF) or when symptoms and signs are rapidly exacerbating in a patient with previously recognized HF (decompensated HF). Acute heart failure is the most common cause of unplanned hospital admissions in older patients. The pathophysiology of both conditions is similar, but de novo HF requires a more detailed diagnostic approach to find the underlying pathology. The initial treatment of AHF includes intravenous diuretics and short-acting vasodilators. The minority of patients with AHF present with cardiogenic shock associated with low blood pressure and severely compromised perfusion of peripheral tissues; the cardiogenic shock is associated with much higher mortality than AHF without shock. Whereas the treatment of chronic HF has substantially improved survival rates, the outcome of AHF is still poor, with high mortality and hospital readmission rates. Currently used therapy is directed to reduce the pre and afterload of the heart and does not target the specific underlying pathology in a given patient, which may explain unsatisfactory progress of clinical outcomes. Therefore, individualized therapy is highly appreciated, requiring establishing specific markers.[2,3]

In this issue of the *Brazilian Archives of Cardiology*, Alataş et al. published an interesting study about microalbuminuria as a marker of mortality in AHF.[4] They analyzed the data of adult patients admitted to the emergency department with signs and symptoms of AHF and increased N-terminal pro-brain natriuretic peptide (NT-proBNP). Patients were divided into three groups according to left ventricular ejection fraction (LVEF): preserved (LVEF >50%, HFpEF), mid-range (LVEF 40-49%, HFmrEF) and reduced (LVEF<40%, HFrEF) including 213, 50 and 63 patients, respectively. Demographic characteristics and comorbidities were collected from the hospital database. Albuminuria was defined according to urinary albumin-to-creatinine ratio (UACR): normoalbuminuria <30 mg/g, microalbuminuria 30-299 mg/g and macroalbuminuria >300 mg/g. The mean age of patients was 70.6 years, and 53.3% of them were females. Patients with HFrEF had higher NT-proBNP and UACR values than patients with HFmrEF or HFpEF. There were no significant differences in the prevalence of norm-, micro and macroalbuminuria between groups with HFpEF and HFmrEF; however, both micro and macroalbuminuria were more frequent in FHrEF than in the two remaining groups. There was no difference in length of hospital stay between groups. In-hospital mortality was higher in HFrEF (6.6%) than in either HFmrEF (2.0%) or HFpEF (2.5%). According to multivariate analysis, NT-proBNP and macroalbuminuria were associated with in-hospital mortality in the whole group. Microalbuminuria was associated with in-hospital mortality in HFrEF and HFmrEF groups but not in the HFpEF group. The risk of in-hospital mortality in patients with HFrEF was 1,94- and 2.45-fold higher in those with micro and macroalbuminuria, respectively, than in those with normoalbuminuria. Micro and macroalbuminuria were associated with 1.56- and 1.92-fold higher mortality in patients with HFmrEF.

Albuminuria is associated with incident HF in the general population and higher mortality among patients with established HF.[5] However, the relationship between microalbuminuria and HF subtypes with preserved and reduced EF is more controversial. Even less is known about microalbuminuria as a marker in AHF. In 2013 Koyama et al.[6] examined the evolution of UACR during hospitalization in 115 patients with decompensated HF.[6] They observed a decrease in the prevalence of microalbuminuria and the mean UACR between days 1 and 7 of hospitalization, and this decrease was correlated with the decrease in NT-proBNP and serum bilirubin. There was no difference in LVEF between subgroups with normo, micro and macroalbuminuria; however, NT-proBNP was significantly correlated with baseline UACR. Nevertheless, the relationship between microalbuminuria and mortality was not reported. Recently, Wang et al.[7] examined the relationship between urinary albumin concentration and outcomes in 1818 patients admitted to the hospital due to ADHF. The patients were followed for a median period of 937.5 days. The compound

Keywords

Heart Failure/physiopathology; Albuminuria/physiopathology; Diuretics/therapeutic use; Natriuretic Peptides/therapeutic use; Stroke Volume.

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rate of mortality, heart transplantation, and left ventricular assist device implantation was 1.42- and 1.74-fold higher in patients with micro and macroalbuminuria, respectively, than in those without albuminuria. A multivariate Cox regression model including all variables significantly associated with prognosis demonstrated that micro and macroalbuminuria were still the significant predictors of mortality (hazard ratio 1.27 and 1.36, respectively). Subgroup analysis demonstrated that albuminuria predicted a higher risk of all-cause death in patients with LVEF>40% but not those with LV<40%. Thus, although microalbuminuria was associated with a worse prognosis in both studies.[6,7] this relationship was stronger in patients with low EF in the study of Alataş et al.[4] and in those with higher EF in the study of Wang et al.[7] The reason for this discrepancy is unclear, however, several differences between these studies should be highlighted. The study of Alataş et al.[4] included patients with acute heart failure (both de novo and decompensated), older age (mean 70 years) and lower eGFR)mean about 70 ml/min) and assessed in-hospital mortality. In contrast, Wang et al.[7] examined only patients with decompensated HF, younger age (median 57 years), higher eGFR (mean about 90 ml/min) and assessed the outcome within the median period of almost 3 years. In addition, UACR was reported in the study of Alataş et al.[4] whereas only absolute urinary creatinine concentration was measured by Wang et al.[7] Very recently, Matsumoto et al.[8] demonstrated that the risk of early (within 1 year) rehospitalization was higher in patients with ADHF and micro- or macroalbuminuria than in those with normoalbuminuria. In multivariate analysis, UACR and BNP were the independent predictors of rehospitalization. However, the predictive value of UACR in subgroups categorized according to EF was not examined.[8]

In conclusion, microalbuminuria emerges as a novel promising marker in patients with acute heart failure. The study of Alataş et al.[4] suggests that microalbuminuria is a predictor of in-hospital mortality, especially in those with reduced ejection fraction. Although the precise relationship between UACR, EF and other markers such as cardiac natriuretic peptides may differ depending on the characteristics of patients and outcomes of interest, this study[4] and other recent ones[7,8] open a new interesting area of research and individualized clinical approach.

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Role of Sodium Levels on Atrial Fibrillation in Heart Failure: Active Player or a Bystander?

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Abstract

Background: The coexistence of hyponatremia and atrial fibrillation (AF) increases morbidity and mortality in patients with heart failure (HF). However, it is not established whether hyponatremia is related to AF or not.

Objective: Our study aims to seek a potential association of hyponatremia with AF in patients with reduced ejection fraction heart failure (HFrEF).

Methods: This observational cross-sectional single-center study included 280 consecutive outpatients diagnosed with HFrEF with 40% or less. Based on sodium concentrations ≤135 mEq/L or higher, the patients were classified into hyponatremia (n=66) and normonatremia (n=214). A p-value <0.05 was considered significant.

Results: Mean age was 67.6±10.5 years, 202 of them (72.2%) were male, mean blood sodium level was 138±3.6 mEq/L, and mean ejection fraction was 30±4%. Of those, 195 (69.6%) patients were diagnosed with coronary artery disease. AF was detected in 124 (44.3%) patients. AF rate was higher in patients with hyponatremia compared to those with normonatremia (n=39 [59.1%] vs. n=85 [39.7%), p= 0.020). In the logistic regression analysis, hyponatremia was not related to AF (OR=1.022, 95% CI=0.785–1.330, p=0.871). Advanced age (OR=1.046, 95% CI=1.016–1.177, p=0.003), presence of CAD (OR=2.058, 95% CI=1.122–3.777, p=0.020), resting heart rate (OR=1.041, 95% CI=1.023–1.060, p<0.001), and left atrium diameter (OR=1.049, 95% CI=1.011–1.616, p=0.002) were found to be predictors of AF.

Conclusion: AF was higher in outpatients with HFrEF and hyponatremia. However, there is no association between sodium levels and AF in patients with HFrEF.

Keywords: Hyponatremia; Atrial Fibrillation; Heart Failure.

Introduction

Heart failure (HF) is categorized based on ejection fraction (EF) as reduced EF≤0.40 (HFrEF), preserved EF≥ 0.50 (HFpEF), or midrange EF (<0.50 but >0.40). Its rate is gradually increasing and is related to high rates of hospitalization and mortality.[1,2]

Anemia, infection, myocardial ischemia, renal failure, atrial fibrillation (AF), and electrolyte abnormalities are common predisposing factors for HF worsening and may contribute to the development of clinical symptoms of HF such as dyspnea, fatigue, and edema or limited activity.

Although sodium abnormalities, at least theoretically, may contribute to the risk of arrhythmia, disorders of electrolyte balance in potassium, calcium, and magnesium are well-known to have triggered arrhythmias. Hyponatremia is defined as serum sodium concentration ≤135 mEq/L, one of the most common electrolyte abnormalities, associated with poor outcome in patients with HF with a prevalence of about 13.8%–33.7%.[3-5]

The prevalence of AF in patients with HFrEF ranges from <10% to 50%.[5-7] AF in HF is a common disabling arrhythmia associated with severity of disease, high morbidity, and mortality. AF leads to HF and vice versa.[1,8-10] Although the relationship between AF and electrolyte imbalance is theoretically well-known, the association of hyponatremia with AF development in HF has not been well-documented in the literature. For the first time, a causative association between hyponatremia and AF development was claimed in a recent study by Cavusoglu et al.[11] Some skepticism, however, still exists about the role of low sodium concentration on AF development in HF, which demonstrated the need for further studies.[12]

Considering this potential relationship, we aimed to investigate whether there is an independent association or reciprocal predisposition between hyponatremia and of AF in our patients with HFrEF.

Methods

In this cross-sectional study, patients under the New York Heart Association (NYHA) functional classes I–IV admitted

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to the outpatient clinic with diagnosis of chronic systolic HF with ejection fraction (EF) of 40% or less were consecutively recruited. The study protocol was approved by the local Ethics committee (2019.152.09.12). All of the subjects provided written informed consent before enrolling in the study.

Patients younger than 18 years, patients with congenital heart disease, moderate to severe valvular disease, active myocarditis, acute coronary syndromes within the last 3 months, inflammatory disorders, malignancies, severe hepatic or chronic kidney disease with an estimated glomerular filtration rate (eGFR) ≤30 mL/min, hypertrophic cardiomyopathy, thyroid disorders, chronic obstructive pulmonary disease, severe anemia, and those with HFpEF or acutely decompensated symptoms showing NYHA class IV who would require inotropic support within the previous month were excluded.

Patients were divided into 2 groups based on sodium levels (≤135 mEq/L and >135 mEq/L: hyponatremia, and normonatremia. The study used 280 patients (202 males and 78 females). Power analysis was performed according to the comparison of hyponatremia and normonatremia groups in the presence of AF. The power of the study was 83.7% with 95% reliability. Therefore, the study sample size was suitable to validate the results.

Age, gender, current smoking status, presence of diabetes mellitus (DM), hypertension (HT), or hyperlipidemia (HL), medications used and disease duration were recorded for all subjects at the first medical consultation. A 12-lead resting electrocardiogram (ECG) was used to determine resting heart rate and sinus rhythm or atrial fibrillation. All patients with normal sinus rhythm in resting ECG were investigated using a 24-hour three-channel ambulatory ECG recorder (MT-200, Schiller A.G, Baar, Switzerland) to rule out paroxysmal AF.

All patients underwent detailed transthoracic echocardiography (GE Vingmed Ultrasound AS, Horten, Norway) as part of the study protocol. The modified Simpson method was used to calculate left ventricular EF. Left ventricle diastolic (LV) and left atrium systolic (LA) diameters were measured. Tricuspid regurgitation velocities were determined by continuous-wave Doppler echocardiography, and systolic pulmonary artery pressure (sPAP) was calculated according to the recommendations of current guidelines.[13]

Diagnosis of hypertension (HT) was established as systolic pressure ≥140 mm Hg and/or diastolic pressure ≥90 mm Hg on more than two occasions or use of any antihypertensive medication. DM was diagnosed as fasting blood glucose higher than 126 mg/dL or being on antidiabetic medication. Coronary artery disease (CAD) was defined based on a coronary angiogram as diameter narrowing ≥50% in an epicardial coronary artery.

Fasting venous blood samples were collected in the morning hours to determine fasting glucose, creatinine, low-density lipoprotein (LDL) cholesterol, uric acid, sodium, potassium, high-sensitivity C-reactive protein (hs-CRP), and hemoglobin levels. Serum osmolality (milliosmoles per kilogram) was calculated as (2 × Na) + (BUN/2.8) + (glucose/18) as described previously.[14]

Serum N terminal pro B-type natriuretic peptide (NT-pro-BNP) concentration was measured using Elecsys proBNP sandwich immunoassay (Elecsys 2010, Roche Diagnostics). The analytical range was between 5 to 35000 pg/mL. Interassay and intraassay coefficients of variation (CV) of NT-proBNP in the low and high ranges were reported as 8.8%–11.6% and 9.9–12.2%, respectively. Human hsCRP kit (High-Sensitivity C-Reactive Protein ELISA kit, DRG International Inc, NJ, USA) included inter-assay and intra-assay CV% <4.1% and <7.5%; the minimum detectable dose of hs-CRP was 0.01 mg/L.

Statistical analysis

Statistical analysis was performed using the predictive Analysis Software Statistics 18 (SPSS Inch, Chicago, Illinois, USA). The variables were tested to check the normality of distribution by the Kolmogorov–Smirnov test. Variables with normal distributions were presented as mean±standard deviation (SD), those without normal distributions were presented as median and interquartile range. Two independent sample t-tests were used to compare normally distributed data and the Mann-Whitney U test was used to compare non-normally distributed data. The categorical variables were presented as numbers (percentage). Comparisons between the categorical variables of the two groups were made by chi-square test. We performed logistic univariate and multivariate regression analyses to assess the predictors of AF. For the multivariate analysis, variables with p values <0.1 were entered into the model by a forward stepwise method. To verify the best cut-off sodium value point of sensitivity and specificity for the prediction of AF, receiver operator characteristic (ROC) curve analysis was used. A two-tailed $p<0.05$ was considered significant.

Results

Of 376 consecutive outpatients diagnosed with HF, 96 with characteristics satisfying the exclusion criteria were not included in the study. The reasons for exclusion were acute coronary syndrome in 20, chronic obstructive pulmonary disease in 10, eGFR ≤30 mL/min in 49, inflammatory disorders in 17 patients, and no prior coronary angiography to define the etiology in eight patients. Therefore, the sample size consisted of patients classified into two groups according to their sodium concentrations, as follows: the hyponatremia group included 66 patients, and the normonatremia group included 214 patients.

Demographic data and characteristics of the study population are presented in Table 1. In the overall study population, the mean age was 67.6±10.5 years; mean blood sodium level was 138±3.6 mEq/L, and the number (%) of patients with AF was 124 (44.3%). Of patients with AF, 96 patients had permanent AF while 28 patients (22.5%) were determined to have paroxysmal AF. Sodium levels in the hyponatremia group and in the normonatremia group were 132±3.7 and 140±2.7 mEq/L, respectively.

Table 1 – Clinical characteristics, laboratory and echocardiographic variables, and medications

Variables	All Patients n=280	Hyponatremia Group n=66	Normonatremia Group n=214	p-value
Age, years	67.6±10.5	67±11	68±10	0.820
Male, n (%)	202 (72.2)	47 (71.2)	155 (72.4)	0.847
Hypertension, n (%)	185 (66.1)	41 (62.1)	144 (67.3)	0.438
Diabetes mellitus, n (%)	96 (34.3)	32 (48.5)	64 (29.9)	0.005
Coronary artery disease, n (%)	195 (69.6)	44 (66.7)	151 (70.6)	0.548
Atrial fibrillation, n (%)	124 (44.3)	39 (59.1)	85 (39.7)	0.020
NYHA class I-II n (%)	176 (62.9)	45 (68.2)	131 (61.2)	0.306
NYHA class I-II +AF, n (%)		23/45 (51.1) [a]	42/131 (32.1) [c]	0.022
NYHA class III-IV, n (%)	104 (37.1)	21 (31.8)	83 (38.8)	0.306
NYHA class III- IV +AF, n (%)		15/21 (71.4) [b]	44/83 (53%) [d]	0.028
Disease duration (years)	5.5 (3–12)	5.1 (4–11)	5.4 (3–9)	0.546
Resting heart rate (bpm)	82.5 ±19	82±12	84±19	0.215
Laboratory measurements				
Fasting glucose (mg/dL)	125±55	136±61	121±52	0.041
Creatinine (mg/dL)	124±0.3	1.25±0.30	1.24±0.3	0.662
LDL cholesterol (mg/dL)	103±42	106±49	102±40	0.461
Uric acid (mg/dL)	7.4±2.4	7.6±2.4	7.3±2.3	0.294
Sodium (mEq/L)	138±3.6	132±3.7	140±2.7	<0.001
Potassium (mEq/L)	4.4±0.5	4.5±0.5	4.3±0.5	0.513
Hs-CRP (mg/dL)	3.8 (1.5–7.3)	4.2 (1.8–6.7)	3.6 (1.2–7.8)	0.367
NT-proBNP, pg/mL	2605 (903–6825)	2916 (1170–9566)	2378 (867–6015)	0.199
Osmolality (mOsm/kg)	291±9	283±9	294±7	<0.001
Hemoglobin (g/dL)	12.8±2	12.5±1.7	12.9±1.9	0.393
Echocardiographic parameters				
LA diameter (mm)	46±7	45±6	46±7	0.546
LV diastolic diameter (mm)	59±7	59±7	60±8	0.634
Ejection fraction (%)	30±4	29±4	31±4	0.518
sPAP (mmHg)	42±14	40±13	42±14	0.343
Medications				
ACEI/ARB, n (%)	184 (65.7)	38 (57.6)	146 (68.2)	0.070
MRA, n (%)	157 (56.1)	44 (66.7)	113 (52.8)	0.021
Diuretic, n (%)	208 (74.3)	48 (72.7)	160 (74.8)	0.194
Betablockers, n (%)	236 (84.3)	54 (81.8)	182 (85)	0.183
Digoxin, n (%)	56 (20)	19 (28.8)	37 (17.3)	0.022

ACEI: Angiotensinogen-converting enzyme inhibitor; ARB: Angiotensin receptor blocker; AF: Atrial fibrillation; Hs-CRP: High-sensitivity C-reactive protein; LA: Left atrium; LDL: Low-density lipoprotein; LV: Left ventricle; mOsm/kg: Milliosmoles per kilogram, MRA: Mineralocorticoid receptor antagonist; NT-proBNP: N terminal pro B-type natriuretic peptide; sPAP: Systolic pulmonary artery pressure. Between a and b, p=0120; between c and d, p=0.002.

The hyponatremia group had a higher ratio of AF and DM than the normonatremia group. The ratios of patients with hypertension, CAD, diabetes mellitus, and NYHA functional class III–IV were similar within the two groups. Fasting glucose, rates of mineralocorticoid receptor antagonist (MRA), and digoxin use were higher in the hyponatremia group compared to the normonatremia group. Osmolality was lower in the hyponatremia group, as naturally expected. Age, gender, disease duration, resting heart rate, creatinine, LDL cholesterol, uric acid, potassium, hs-CRP, NT-proBNP, hemoglobin, LA and LV diastolic diameter, EF (%), and sPAP values were similar in the two groups. Patients with AF had

lower sodium levels compared to those without AF (136±4.3 vs. 138±3.0 mEg/L, p=0.001) (Figure 1A) (Table 1).

In patients with hyponatremia, the rates of AF were found to be significantly higher in patients with higher NYHA functional classes. Although there was no difference in terms of AF rates between NYHA class I–II and III–IV in patients with hyponatremia and HFrEF, AF rates showed statistically significant difference in patients with normonatremia and HF (Table 1).

The results of univariate and multivariate logistic regression analysis to show the independent predictors of AF revealed advanced age, resting heart rate, and LA diameter. Diuretic and digoxin usage were found to be strongly correlated with the presence of AF (Table 2).

The ROC analysis (AUC=0.458, 95% CI=0.397–0.527) revealed that blood sodium levels ≤135 mEq/L have poor diagnostic sensitivity (55%) and specificity (41%) for predicting AF. If the cut-off value of sodium level was adjusted to ≤130 mEq/L, higher sensitivity (70%) and poor specificity (31%) values were found (Figure 1B).

Discussion

We report that the prevalence of AF was higher in outpatients with HFrEF and hyponatremia than in those with

Table 2 – Univariate and multivariate logistic regression analyses for the presence of atrial fibrillation

		Odds ratio	95% CI	p-value
Univariate analyses				
Age	0.076±0.023	1.079	1.031–1.170	0.001
Male gender	0.652±0.270	1.919	1.131–3.256	0.016
Hypertension	0.336±0.432	1.399	0.600–3.260	0.437
Diabetes mellitus	0.246±0.487	1.279	0.492–3.325	0.613
Coronary artery disease	-0.805±0.451	0.447	0.185–1.081	0.074
Functional capacity	0.026±0.419	1.027	0.451–2.334	0.950
Disease duration	1.196±0.576	0.827	0.271–2.493	0.729
Resting heart rate	0.041±0.008	1.042	1.026–1.059	<0.001
Fasting glucose	0.001±0.005	1.001	0.990–1.011	0.924
Creatinine	-0.025±0.182	0.976	0.682–1.395	0.892
LDL cholesterol	-0.007±0.003	0.993	0.987–0.999	0.024
Uric acid	0.172±0.100	1.188	0.976–1.446	0.086
Sodium levels	0.022±0.134	1.022	0.785–1.330	0.871
Potassium	-0.727±0.391	0.483	0.225–1.039	0.063
Hs-CRP	-0.012±0.016	0.988	0.958–1.020	0.461
NT-proBNP	0.001±0.001	1.000	0.999–1.001	0.071
Osmolality	-0.065±0.060	0.937	0.834–1.054	0.279
Hemoglobin	-0.174±0.131	0.840	0.650–1.086	0.183
LA diameter	0.046±0.013	1.047	1.021–1.516	<0.001
ACEI/ARB	-0.047±0.288	0.954	0.543–1.677	0.870
MRA	-0.163±0.290	0.850	0.481–1.501	0.575
Diuretic	1.448±0.364	4.256	2.086–8.685	<0.001
Beta-blockers	-0.165±0.388	0.848	0.396–1.814	0.671
Digoxin	1.876±0.365	6.526	3.193–13.340	<0.001
Multivariate analysis				
Age	0.045±0.015	1.046	1.016–1.177	0.003
Coronary artery disease	-0.805±0.451	2.058	1.122–3.777	0.020
Resting heart rate	0.041±0.009	1.041	1.023–1.060	<0.001
LA diameter	0.044±0.017	1.049	1.011–1.616	<0.001

ACEI: Angiotensinogen-converting enzyme inhibitor; ARB: Angiotensin receptor blocker; Hs-CRP: High-sensitivity C-reactive protein; LA: Left atrium; LDL: Low-density lipoprotein; NT-proBNP: N terminal pro B-type natriuretic peptide; MRA: Mineralocorticoid receptor antagonist.

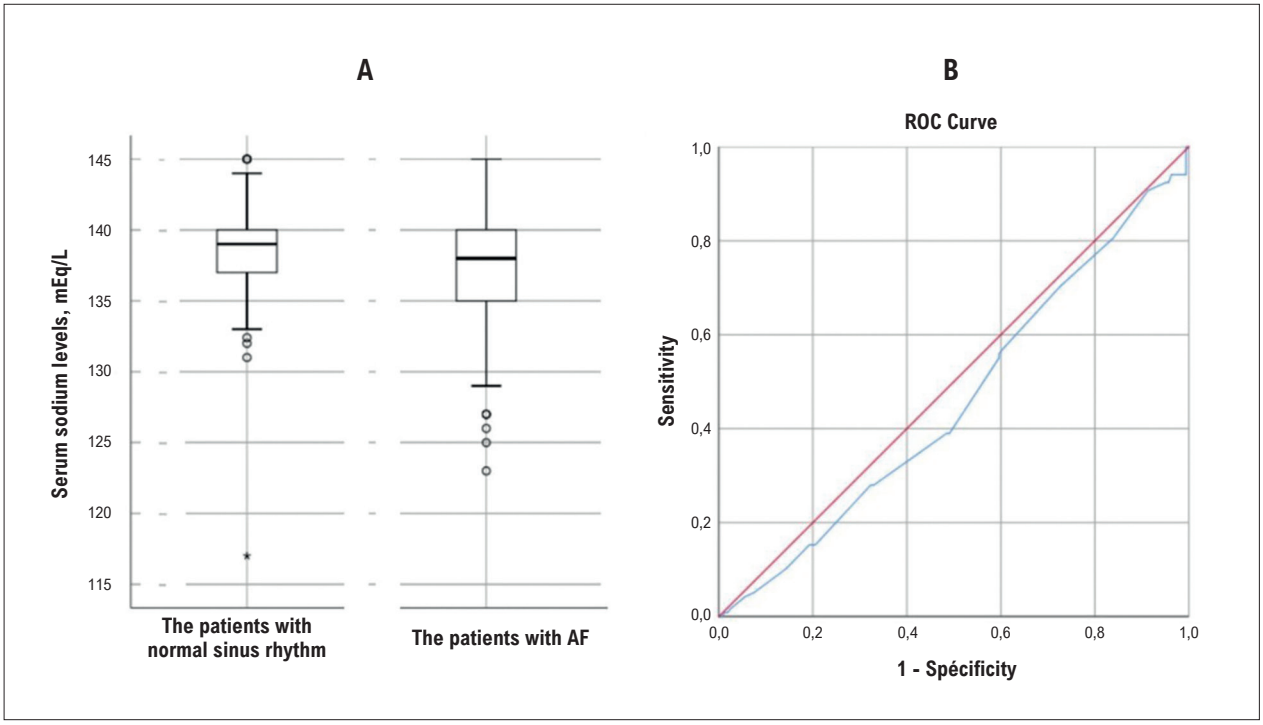

Figure 1 – *A) Shows the comparison of sodium levels between the patients who have heart failure with normal sinus and atrial fibrillation. B) Demonstrates the ROC analysis that shows a poor diagnostic sensitivity and specificity of sodium levels to predict the possibility.*

HFrEF and normonatremia, irrespective of the plasma osmolality levels and other confounding factors. There have been two studies in the literature showing higher rates of AF in patients with HFrEF and hyponatremia,[5,11] which our results were concordant with. However, hyponatremia was not an inciting factor for the development of AF in the study. Hyponatremia is mild-moderate sensitive, but not specific for predicting the development of AF. Namely, AF is not present in every patient with hyponatremia and HFrEF.

The important predisposing and determining factors for AF development were advanced age, presence of CAD, increased resting heart rate and LA dimension, which were established as predictors of AF by previous studies.[9,10,15] We reported a higher AF rate in patients with hyponatremia, irrespective of their NHYA functional class, as documented previously.[5,11] Therefore, the coexistence of hyponatremia and AF may demonstrate HF severity. The rates of AF in the patients with normonatremia and HF were higher in NHYA class III–IV, which also means that the presence of NYHA III–IV status is an important reason for diuretic usage. Therefore, hyponatremia appears to be only a bystander variable.

The most common reasons for hyponatremia in patients with HF are diuretic usage and neurohormonal response, including an autonomic imbalance in favor of the sympathetic nervous system or renin-angiotensin system (RAS) activation.[1,5,16]

Many factors have been responsible for the association of hyponatremia with an increased risk for AF. Heart failure reduces stroke volume and arterial filling, which results in stimulation of arterial baroreceptors, arginine vasopressin (AVP) release, and RAS activation. RAS activation leads to increased aldosterone and angiotensin II levels. Angiotensin II alerts the thirst center of the brain and stimulates AVP release. The subsequent increase levels of aldosterone, angiotensin II, sympathetic system, and AVP release induce reduced renal blood flow, enhanced water retention, and sodium reabsorption.[4,17] As a result of these neurohormonal changes, hypervolemia and hyponatremia occur. Some studies have shown increased levels of renin, angiotensin II, aldosterone, epinephrine, norepinephrine, and dopamine in patients with HF and hyponatremia compared to those with HF and normonatremia.[16-19]

Hyponatremia may also be a predictor of higher neurohormonal activation that suggests HF severity.[4] Diuretics, especially thiazides, often result in hyponatremia, which promotes water retention due to enhanced AVP activation in the distal tubules.[20,21] Hypervolemia leads not only to hyponatremia but also to atrial myocardial stretch, cardiac chamber, and pulmonary vein dilatation.[22] Hyponatremia, theoretically, may also contribute to the development of AF, causing electrophysiological changes in the myocyte action potential.[23] However, in clinical practice, it appears not to be a determinant of AF.

AF-induced rapid heart rate deleteriously affects left ventricle function, facilitates tachycardia, and predisposes apoptosis and myocardial fibrosis. Irrespective of the presence of HF, irregular heart rate and loss of atrial contraction results in a significant 7–9% and 20% reduction in cardiac output, respectively.[24] When HF and AF co-exist, two intertwined entities make cardiac output decrease synergistically, and mortality increases.[8] There is a common cause-effect relationship between these two entities.

Hyponatremia is frequently seen in patients with acute decompensated HF due to high diuretic usage and high sympathetic tonus triggering RAS activation.[5,21] Our findings are not concordant with that of the study of Cavusoglu et al. showing hyponatremia with a prevalence of 24%, and AF with a prevalence of 33%.[11] Bavishi et al. found that the prevalence of hyponatremia and AF in outpatients with HFrEF was 14.8% and 37.6%, respectively.[5] Our study has presented a hyponatremia rate of 23.5%, but a higher AF rate of 44.3%, because we performed ambulatory Holter ECG to find the presence of paroxysmal or persistent AF. AF rates are higher than we expected in ambulatory 24-hour Holter ECG monitoring in patients with HF.[2,15]

Study limitations

We presented missing data related to diuretic doses and albumin levels, which could affect the sodium levels.

Conclusion

Current findings yield insights on the pathogenesis of AF in patients with established HF. Although hyponatremia plays a key role in the deterioration of HF status, we found that low serum sodium concentration ≤135 mEq/L is not related to the probability of AF.

Author Contributions

Conception and design of the research: Akyüz A, Baykız D, Gökçek S, Efe MM, Alpsoy S; Acquisition of data and Analysis and interpretation of the data: Akyüz A, Baykız D, Gur DO, Gökçek S, Efe MM, Alpsoy S; Statistical analysis: Akyüz A, Gur DO; Obtaining financing: Akyüz A, Baykız D, Gökçek S, Efe MM; Writing of the manuscript: Akyüz A, Baykız D, Gur DO; Critical revision of the manuscript for intellectual content: Akyüz A, Baykız D, Gur DO, Alpsoy S.

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.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Hospital Namık Kemal University Medical Faculty, Tekirdağ under the protocol number 2019.152.09.12. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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Hypertension and Associated Lipid, Glucose, and Adiposity Parameters in School-Aged Adolescents in the Federal District, Brazil

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Abstract

Background: **The prevalence of hypertension and other metabolic disorders has increased in young individuals. However, no representative studies have been conducted in the population of the Federal District, Brazil.**

Objective: **To estimate the prevalence of hypertension and its association with lipid, glucose, and adiposity markers in school-aged adolescents living in the Federal District.**

Methods: **This cross-sectional study included participants of the Study of Cardiovascular Risks in Adolescents (Portuguese acronym, ERICA). Blood pressure, blood glucose, glycated hemoglobin, insulin, homeostatic model assessment for insulin resistance (HOMA-IR), triglycerides, total cholesterol, high-density lipoprotein, low-density lipoprotein, body mass index (BMI), waist circumference, and economic, demographic, and sexual maturity variables were assessed. The data were analyzed in Stata, and the analysis was divided into different stages: descriptive, crude, and adjusted. Significant results were set at $p < 0.05$.**

Results: **In total, 1,200 adolescents were included, and their mean age was 14.8 years. The prevalence of hypertension was 8% (95% confidence interval: 6.3; 9.9). Most parameters were associated with blood pressure in crude analysis. In adjusted analysis, glucose, lipid, and adiposity markers maintained the associations, and the highest magnitudes were those of BMI and HOMA-IR.**

Conclusion: **The study revealed a high prevalence of hypertension in adolescents living in the Federal District, and blood pressure levels were associated with other markers of lipid, glucose, and adiposity profile. The findings indicate the relevance of health surveillance for planning effective actions aimed at reversing this situation and preventing new cases.**

Keywords: **Hypertension; Adolescent; Adiposity; Blood Glucose; Lipids.**

Introduction

Noncommunicable diseases (NCDs) have become a public health problem of great relevance, playing a leading role in the global epidemiological setting together with acute cardiovascular diseases.[1] One of the most prevalent NCDs in the world is hypertension, a clinical condition characterized by high and sustained levels of blood pressure. It is known as an important risk factor for cardiovascular disease, in addition to being frequently associated with other metabolic disorders such as obesity, dyslipidemia, and glucose intolerance.[2]

The World Health Organization (WHO) reported in 2010 that an estimated 600 million people had a diagnosis of hypertension, predicting a 60% global increase in the number of cases by 2025.[3] In Brazil, 2013 National Health Survey data showed a prevalence of 21.4% for hypertension in the adult population.[4] A concomitant change in the demographic profile of individuals with chronic diseases has been observed, and their presence in children and adolescents is increasingly common.[5]

The first stages of life are important for human development, and early metabolic changes can have a negative impact on adulthood, increasing the risk of developing diseases and comorbidities over the years.[6] The Study of Cardiovascular Risks in Adolescents (Portuguese acronym, ERICA), which evaluated students from all Brazilian regions during 2013 and 2014, reported an estimated prevalence of 9.6% for hypertension.[7] A 2016 systematic review with meta-analysis[5] described an estimated prevalence of 8% for hypertension in Brazilian adolescents.

Given the importance of monitoring the health status of the adolescent population to assist health care

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decision-making and the lack of representative studies on hypertension and associated metabolic parameters in the adolescent population of the Federal District, Brazil, this study aimed to estimate the prevalence of hypertension and investigate its association with lipid, glucose, and adiposity parameters in Federal District school-aged adolescents.

Methods

Study design and setting

This cross-sectional study included participants of the ERICA study, conducted during 2013 and 2014.[8]

Eligibility criteria

Adolescents aged 12 to 17 years attending the final three years of middle school and high school in public and private institutions located in rural and urban areas, without any temporary or permanent disability, who had never become pregnant, and who agreed to participate in blood specimen collections were defined as eligible.

Sample size and participant selection

ERICA was representative of the adolescent population in large and medium-sized municipalities at the national, regional, and capital levels. Further details about the national sample and the representativeness of the study can be found in Vasconcellos et al.[9]

In the Federal District, blood samples were collected for laboratory testing at 33 schools. The adequacy of sample size for this study was ascertained by calculations including a total of 233,399 students in the Federal District attending in 2009 the final three years of middle school and the three years of high school,[10] a prevalence of 9% for hypertension in the Brazilian school-aged adolescent population,[7] an acceptable error of 1.7%, and a 95% confidence level. Thus, the minimum number of adolescents was 1,084.

Variables

Blood pressure

Systolic and diastolic blood pressure (SBP and DBP, respectively) measurements were defined as outcome variables. Omron® 705-IT, an automatic oscillometric device validated for adolescents, was used.[11]

Three measurements were taken, with a 3-minute interval between each one, but only the mean of the second and third measurements was used.[8] Adolescents were classified according to SBP and DBP values in relation to height, sex, and age, and those with values ≥ 95th percentile were defined as having hypertension.[12]

Bood samples collection for laboratory testing

Blood samples were collected by venipuncture after a 12-hour fasting period for determination of biochemical markers.[13] Blood glucose was determined by the hexokinase method, and values ≥ 100 mg/dL were defined as high.[14] Glycated hemoglobin (HbA1c) was measured by ion exchange chromatography, and concentrations ≥ 5.8%, corresponding to the 90th percentile for the study population, were defined as high. Insulin was determined by the chemiluminescence method and defined as high if ≥ 15 mU/L.[15]

Homeostatic model assessment for insulin resistance (HOMA-IR) was used to characterize insulin resistance (IR)[16] and calculated as follows: fasting insulin (mU/L) x (fasting glucose (mg/dL) x 0.0555)/22.5. HOMA-IR values ≥ 2.80 were defined as high.[17]

Total cholesterol (TC) and triglycerides (TG) were determined by an enzyme kinetic assay, and TC ≥ 170 mg/dL and TG ≥ 90 mg/dL were defined as high.[18] Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were assessed with an enzymatic colorimetric assay, and LDL ≥ 110 mg/dL and HDL ≤ 45 mg/dL were defined as abnormal.[18]

Adiposity markers

An electronic scale (Líder®) with a capacity of 200 kg and a precision of 50 g was used to measure weight, and a portable stadiometer (Alturexata®) with a precision of 1 mm and a range of up to 213 cm was used to measure height. Height was measured twice, and the maximum variation between the two measurements should be 0.5 cm. Mean was calculated automatically by a system developed for use in a personal digital assistant (PDA).[8]

Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m). WHO references[19] were used to calculate BMI-for-age z-scores adjusted for sex. The following cutoff points were used: z-score < -2, underweight; z-score ≥ -2 and < 1, normal weight; z-score ≥ 1 and < 2, overweight; and z-score ≥ 2, obesity.

A measuring tape with a resolution in millimeters and 1.5 m in length (Sanny®) was used to measure waist circumference (WC) at the midpoint between the iliac crest and the lowest rib. Measures were collected twice, and means were calculated.[8] Cutoff points were taken as values ≥ 90th percentile for the study population.

Demographic and economic variables

The variables were self-reported and categorized as follows: sex (female or male), age (< 15 or ≥ 15 years), and skin color or ethnicity (White, Brown, Black, Indigenous, Asian, or not reported). The schools were classified according to setting (rural or urban) and type (public or private), and the latter was used as a proxy for the family's economic class.

Sexual maturity

Adolescents were rated at different stages of sexual maturity according to the Tanner staging scale.[20] The most developed characteristic was used for categorization, and

stages 4 and 5 were defined as pubescent and the others as prepubescent.

Data analysis

In the descriptive stage, the prevalence and distribution of characteristics of interest in the study population were calculated, as well as the prevalence of hypertension in relation to these characteristics. Also, the prevalence of differences in biochemical and anthropometric parameters was compared between adolescents with and without hypertension. The results were accompanied by their respective 95% confidence intervals (CIs).

In the analytical stage, linear regression was used to investigate the associations between SBP and DBP (dependent variables) and laboratory and anthropometric markers (independent variables). This stage was subdivided into crude analysis and adjusted analysis, and the following variables were used for adjustment: sex, age, sexual maturity stage, skin color or ethnicity, obesity, and type of school. When the independent variable referred to BMI or WC, no adjustment was made for obesity status. The results were reported as β coefficients with their respective 95% CIs. Adjusted analysis was performed only when crude analysis presented $p < 0.20$, and $p < 0.05$ was defined as significant.

The complex sample design and the respective sample weights referring to the Federal District school-aged adolescent population were accounted for. Stata version 14.2 was used for all analyses.

Ethical aspects

The project was approved by the Human Research Ethics Committee of Faculty of Medicine, Universidade de Brasília (certificate number 05185212.2.2005.5540). The participants were previously informed of the study objectives and procedures and were evaluated only after assent and consent forms were signed by the students and by their parents or guardians, respectively.

Results

In total, 1,200 adolescents studying at 33 public and private schools in the Federal District were evaluated. The mean age was 14.8 years, and the prevalence of hypertension was 8.0% (95% CI: 6.3; 9.9). Hypertension was more frequently found in male students, in those aged ≥ 15 years, and in those studying at rural schools.

The analysis of blood markers revealed that hyperglycemia was the least prevalent inadequacy. The most prevalent inadequacy was low HDL value. Other characteristics are described in Table 1.

There was a higher prevalence of hyperinsulinemia in adolescents with hypertension. Adiposity parameters were higher in students with hypertension compared with those without hypertension (Table 2).

Most parameters were associated with SBP and DBP in crude analysis. In adjusted analysis, glucose, lipid, and adiposity parameters maintained the associations, and the highest magnitudes were those of BMI and HOMA-IR (Table 3).

Discussion

To our knowledge, this is the first study to investigate hypertension in school-aged adolescents in the Federal District. The estimated prevalence was similar to that found for the Brazilian regions of Midwest (8.7, 95% CI: 7.9; 9.6), North (8.4, 95% CI: 7.7; 9.2), Northeast (8.4, 95% CI: 7.6; 9.2), Southeast (9.8, 95% CI: 8.8; 11.0), and for the national sample of ERICA (9.6, 95% CI: 9.0; 10.3). It was lower than the estimated prevalence for the South (12.5, 95% CI: 11.0; 14.2) only.[7] A high prevalence of alterations other biochemical and adiposity markers was described, and the reported associations may potentiate cardiovascular risk in this population.

Similar to the findings of ERICA,[7] although most adolescents studied in urban areas, hypertension was more common in rural schools. An explanatory hypothesis is that rural environments often have limited access to health care services, which hinders the diagnosis and treatment of chronic diseases such as hypertension.[21]

The evaluation of glucose metabolism markers in adolescents showed that high levels in fasting blood glucose were less prevalent than those in other markers. However, the evaluation of blood glucose alone is insufficient to rule out metabolic changes because, at the onset of IR, blood glucose may remain within normal levels as a consequence of a possible hyperinsulinemia.[14] Changes in HbA1c, which were more prevalent in these students, may be a better marker in the evaluation of glycemic control as they reflect blood glucose changes in the long term.[22]

High blood glucose levels favor hypertension through an increase in cardiac output caused by hyperosmolality induced by hyperglycemia.[23] Increased blood glucose can also lead to an excessive generation of reactive oxygen species (ROS), which contributes to endothelial dysfunction.[24] When prolonged, hyperglycemia can also contribute to the generation of advanced glycation end-products, which intensify oxidative stress by activating a proinflammatory cascade. This increases the expression of ROS and contributes to the inhibition or reduction of nitric oxide production, leading to peripheral vascular resistance by vasoconstriction.[24,25]

In addition, there was an increase in insulin and HOMA-IR levels. Andrade et al.[26] suggest, however, that IR may be related to development during adolescence, involving hormonal and body composition changes in the early stages of puberty. They believed this could be reversed after the growth spurt, which does not explain the present results because most of the study population was in the final stage of puberty. Other studies have also reported higher insulin levels and HOMA-IR changes in adolescents with hypertension compared with other groups.[27,28]

Adolescents with hypertension also had higher BMI and WC, which is consistent with the findings of other studies.[29,30] High adiposity contributes to hypertension, among other mechanisms, by favoring oxidative stress with the onset of a proinflammatory state, with increased expression of cytokines such as interleukin 6 (IL-6) and tumor necrosis factor-alpha.[31] Inflammation is an important mediator both

Table 1 – Profile of school-aged adolescents and prevalence of hypertension. Study of Cardiovascular Risks in Adolescents, Federal District, Brazil, 2013-2014

	Total sample		With hypertension	
Characteristic	**%**	**95% CI**	**%**	**95% CI**
School setting				
Urban area	97.0	81.4; 99.5	7.4	5.9; 9.0
Rural area	3.0	0.4; 18.5	27.7	24.6; 30.8
Type of school				
Public	55.2	37.5; 71.5	8.3	5.8; 11.7
Private	44.8	28.4; 62.4	7.6	5.7; 9.8
Sex				
Female	50.4	-	4.3	2.8; 6.2
Male	49.6	-	11.8	9.3; 14.7
Age				
< 15 years	47.6	-	5.4	3.8; 7.6
≥ 15 years	52.4	-	10.3	7.7; 13.5
Skin color/ethnicity				
White	35.6	30.1; 41.3	8.4	5.3; 13.1
Brown	53.5	48.4; 58.4	7.9	6.1; 10.1
Black	6.0	4.4; 8.2	8.9	3.9; 19.1
Indigenous	0.2	0.0; 0.6	16.7	1.5; 71.5
Asian	2.7	1.7; 4.1	3.5	0.8; 13.6
Not reported	2.0	1.3; 3.1	2.8	0.3; 18.7
Sexual maturity stage*				
Pubescent	81.9	78.2; 85.0	8.0	6.3; 10.1
Prepubescent	18.1	14.9; 21.7	7.7	3.6; 15.5
Blood glucose†				
≥ 100 mg/dL	1.5	0.7; 3.2	28.1	8.4; 62.4
HbA1c‡				
≥ 5.8% (≥ 90th pctl)	13.6	10.7; 17.1	7.5	3.6; 14.8
Insulin§				
≥ 15 mU/L	11.3	8.2; 15.5	17.6	11.6; 25.8
HOMA-IR//				
≥ 2.80	18.2	13.9; 23.5	15.7	10.7; 22.4
Triglycerides¶				
≥ 90 mg/dL	30.5	27.4; 33.8	9.4	6.3; 14.0
Total cholesterol¶				
≥ 170 mg/dL	30.6	27.6; 33.7	9.2	5.9; 13.8
LDL¶				
≥ 110 mg/dL	21.3	19.0; 23.7	7.2	4.7; 10.8
HDL¶				
≤ 45 mg/dL	41.8	38.1; 45.4	9.5	7.2; 12.5
BMI#				
Underweight and normal weight	77.0	73.8; 79.9	4.2	2.9; 6.0
Overweight	14.7	12.3; 17.4	16.0	10.8; 23.0
Obesity	8.3	6.3; 10.6	28.6	18.2; 41.9
Waist circumference**				
Not increased (< 90th pctl)	88.4	84.8; 91.1	6.2	4.8; 7.8
Increased (≥ 90th pctl)	11.6	8.8; 15.1	21.6	14.2; 31.3

*BMI: body mass index; CI: confidence interval; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; HOMA-IR: homeostatic model assessment for insulin resistance; LDL: low-density lipoprotein; pctl: percentile. * Tanner, 1962; † SBD, 2019; ‡ Values ≥ 5.8% (corresponding to the 90th percentile for the study population); § SBC, 2005; // Chissini et al., 2019; ¶ SBC, 2017; # WHO, 2007; ** Values ≥ 80.8 cm (female) or ≥ 86.3 cm (male).*

Table 2 – Prevalence of biochemical and nutritional status changes in adolescents with and without hypertension. Study of Cardiovascular Risks in Adolescents, Federal District, Brazil, 2013-2014

	Adolescents with hypertension		Adolescents without hypertension	
Parameter	**%**	**95% CI**	**%**	**95% CI**
Blood glucose*				
≥ 100 mg/dL	5.4	1.3; 18.8	1.1	0.5; 2.5
HbA1c†				
≥ 5.8% (≥ 90th pctl)	12.7	6.0; 25.1	13.7	10.9; 17.1
Insulin‡				
≥ 15 mU/L	25.4	13.8; 41.8	10.2	7.5; 13.6
HOMA-IR§				
≥ 2.80	35.9	23.8; 50.1	16.7	12.7; 21.6
Triglycerides//				
≥ 90 mg/dL	36.3	26.3; 47.6	30.0	27.0; 33.2
Total cholesterol//				
≥ 170 mg/dL	35.1	23.8; 48.4	30.2	27.1; 33.4
LDL//				
≥ 110 mg/dL	19.2	12.2; 28.9	21.4	19.2; 23.9
HDL//				
≤ 45 mg/dL	50.0	36.3; 63.8	41.0	37.6; 44.5
BMI¶				
Underweight and normal weight	40.7	29.1; 53.4	80.1	77.0; 82.9
Overweight	29.6	20.3; 41.0	13.4	11.0; 16.1
Obesity	29.6	18.5; 43.8	6.4	4.7; 8.5
Waist circumference#				
Increased (≥ 90th pctl)	31.5	20.0; 45.8	9.9	7.5; 12.8

BMI: body mass index; CI: HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; HOMA-IR: homeostatic model assessment for insulin resistance; LDL: low-density lipoprotein; pctl: percentile
**SBD, 2019; †values ≥ 5,8% (corresponding to the 90th percentile for the study population); ‡SBC, 2005; §Chissini et al., 2019; //SBC, 2017; ¶WHO, 2007; #Values ≥ 80,8 cm for female or ≥ 86,3 cm for male (corresponding to the 90th percentile for the study population).*

in the onset and in the maintenance of high blood pressure levels because it may cause vascular and renal damage.[32] In addition, obese individuals may show a greater degree of sympathetic activation, with an increased renal production of norepinephrine and a consequent increase in renal tubular reabsorption of sodium.[33] Sympathetic activation can be further stimulated by an excessive production of leptin, which is common in those with high adiposity.[34] The presence of nonfunctional adipose tissue in obesity can also affect the renin-angiotensin system by increasing the circulating levels of angiotensin II and aldosterone; this causes hemodynamic changes that also contribute to increased blood pressure blood pressure,[35] which explain the association observed in this study.

The presence of inflammation and endothelial dysfunction markers is also typical of dyslipidemias.[36] Changes in lipid profile and presence of inflammatory cytokines such as IL-6 are related to increased arterial stiffness and thus to blood pressure, favoring the onset of hypertension and increasing the risk of developing cardiovascular diseases.[37]

The results of studies conducted in other regions of Brazil[29,38] and the world[39,40] corroborate the prevalences and associations found in the Federal District, and these findings reinforce the high frequency of cardiovascular risk factors at increasingly earlier ages.[41] The unhealthy lifestyle of the Brazilian adolescent population, consisting especially of low food quality,[42,43] sedentary behavior, high screen time,[44] and emotional stress,[45] potentiate the risks.

The interpretation of the results of this study is conditioned to some limitations of ERICA. The prevalence of hypertension may have been overestimated because blood pressure was measured on a single day.[12] However, in large cross-sectional studies such as ERICA, this is a common limitation as increasing the number of visits implies greater financial and logistics investments. It is worth noting that the methodology adopted on the day of collection may have reduced this bias.[8]

Despite these limitations, ERICA had appropriate quality monitoring processes for fieldwork and great methodological rigor for statistical analysis. These steps

Table 3 – Association between biochemical and nutritional status parameters and systolic and diastolic blood pressure in adolescents. Study of Cardiovascular Risks in Adolescents, Federal District, Brazil, 2013-2014

	Crude analysis		Adjusted analysis*	
Parameter	**β coefficient**	**95% CI**	**β coefficient**	**95% CI**
SBP				
Glucose (mg/dL)	0.26§	0.13; 0.39	0.16‡	0.04; 0.27
HbA1c (%)	1.77†	-0.84; 4.39	0.68	-1.41; 2.78
Insulin (U/L)	0.24‡	0.03; 0.45	0.23‡	0.06; 0.40
HOMA-IR	1.19‡	0.17; 2.21	1.07‡	0.25; 1.88
TG (mg/dL)	0.04§	0.02; 0.06	0.03‡	0.01; 0.04
TC (mg/dL)	-0.001	-0.03; 0.02		
LDL (mg/dL)	0.008	-0.02; 0.03		
HDL (mg/dL)	-0.18§	-0.27; -0.09	-0.06‡	-0.12; -0.002
BMI (kg/m^2)	1.49§	1.28; 1.71	1.41§	1.20; 1.63
WC (cm)	0.68§	0.58; 0.79	0.58§	0.48; 0.69
DBP				
Glucose (mg/dL)	0.10‡	0.01; 0.19	0.08‡	0.002; 0.17
HbA1c (%)	1.09†	-0.56; 2.76	0.65	-0.77; 2.07
Insulin (U/L)	0.13†	-0.05; 0.31	0.09	-0.05; 0.24
HOMA-IR	0.62†	-0.26; 1.51	0.45	-0.25; 1.16
TG (mg/dL)	0.02‡	0.01; 0.03	0.01‡	0.005; 0.02
TC (mg/dL)	0.02‡	0.00; 0.03	0.02‡	0.007; 0.03
LDL (mg/dL)	0.01‡	0.00; 0.03	0.01‡	0.002; 0.03
HDL (mg/dL)	-0.01	-0.06; 0.02		
BMI (kg/m^2)	0.57§	0.43; 0.72	0.56§	0.40; 0.72
WC (cm)	0.27§	0.20; 0.33	0.25§	0.18; 0.33

*BMI: body mass index; CI: confidence interval; DBP: diastolic blood pressure; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; HOMA-IR: homeostatic model assessment for insulin resistance; LDL: low-density lipoprotein; SBP: systolic blood pressure; TG: triglycerides; TC: total cholesterol; WC: waist circumference. † p < 0.20; ‡ p < 0.05; § p < 0.001. * Analysis was adjusted for sex, age, sexual maturity stage, skin color or ethnicity, type of school, and presence or absence of obesity.*

contributed to the robustness of the study and the reliability of the results.

Conclusion

The estimated prevalence of hypertension in Federal District school-aged adolescents was 8%, associated with metabolic and adiposity markers. These findings highlight the metabolic links that may be present in hypertension. Within this context, health promotion and disease prevention actions are crucial to avoid epidemiological background reported in the Federal District and to contribute to improve the population's quality of life and lower the burden to the health care system.

Author Contributions

Conception and design of the research: Carvalho KMB, Dutra ES, Gonçalves VSS; Acquisition of data: Carvalho KMB, Dutra ES, Gonçalves VSS; Analysis and interpretation of the data and Writing of the manuscript: Lima LR, Okamura AB, Gonçalves VSS; Statistical analysis: Lima LR, Gonçalves VSS; Critical revision of the manuscript for intellectual content: Lima LR, Okamura AB, Carvalho KMB, Dutra ES, Gonçalves VSS.

Potential Conflict of Interest

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

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Study Association

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

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Hypertension in Adolescence, a Direct Relationship to Obesity and Insulin Resistance

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Short Editorial related to the article: Hypertension and Associated Lipid, Glucose, and Adiposity Parameters in School-Aged Adolescents in the Federal District, Brazil

Hypertension is the main modifiable risk factor for developing cardiovascular diseases, and its occurrence at an earlier age favors accelerated vascular aging in the following years.[1] The increase in blood pressure in adolescence does not usually occur in isolation and is associated with other risk factors such as excessive salt intake, reduced physical activity, and, especially, overweight/obesity.[2,3] Considering that a high-fat percentage in childhood and adolescence has early adverse effects on blood pressure, adequate body fat measurements can determine more accurate markers of higher adiposity and predictors of the incidence of hypertension in younger individuals.

Major advances in technology are present in adolescents' daily lives and generally favor physical inactivity and weight gain, which are directly related to blood pressure levels. In addition to a sedentary lifestyle strongly associated with hypertension in adolescence, physical exercise plays a protective role, reducing blood pressure by several mechanisms. A cross-sectional study with children and adolescents aged 11 to 17 years showed the association of male gender and central obesity with hypertension in these students. On the other hand, the same study pointed to moderate and vigorous physical activities as an effective way to prevent the increase in diastolic blood pressure in young people of this age.[4]

Autonomic imbalance seems to be one of the initial mechanisms for blood pressure elevation in adolescents. In this group of young individuals, the autonomic imbalance is mainly represented by sympathetic hyperactivity, which is also associated with obesity, changes in sleep patterns and, consequently, an increased risk of cardiovascular events. A recent study has shown that adolescents already have autonomic dysfunction assessed by heart rate variability, even in the pre-hypertension range.[5]

In the study carried out by Lima et al.,[6] published in this issue of *Arquivos Brasileiros de Cardiologia*, the authors intended to determine the prevalence of hypertension and its association with lipid, glucose and adiposity profiles. The originality of this project is that it was carried out in a population of 1200 adolescents, aged 12 to 17 years, from the Federal District who participated in the Study of Cardiovascular Risks in Adolescents (ERICA).[7] The 8% prevalence of hypertension found among adolescents in the Federal District was similar to the other geographic regions of the country evaluated in the same ERICA study, except for the southern region with a prevalence of 12.5%, well above the current study and the other regions. Hypertensive adolescents had higher adiposity parameters and a higher occurrence of hyperinsulinemia, but the most common alteration was low levels of HDL-cholesterol. Most variables correlated with systolic and diastolic blood pressure levels and, even after adjustments, body mass index (BMI) and the homeostatic model assessment for insulin resistance (HOMA-IR) were the parameters with the highest association strength.

Assessment of insulin resistance in adolescents is a major challenge. In this age group, insulin levels tend to be higher and associated with other hormonal changes related to body modifications. However, this does not seem to be the reason for the hyperinsulinemia reported in the present study, as the authors indicate that most adolescents were already at the end of puberty. In addition, non-hypertensive adolescents had substantially lower insulin levels than those with hypertension, suggesting a more direct relationship. In this case, insulin resistance may be the biochemical confirmation of the metabolic syndrome, a condition increasing in frequency in childhood and adolescence, leading to a greater risk of developing chronic diseases in adulthood.[8]

Most clinical trials with hypertension involve the adult and/or elderly participants. In Brazil and worldwide, there are few publications regarding hypertension in adolescents. This reinforces the importance of this study, as we need national data that will form the basis for our future guidelines in this area. Certainly, the Federal District does not represent the reality of our entire country, which limits the external validity and, therefore, we cannot extrapolate the current results. On the other hand, the findings indicate important information that adds to other studies in Brazil, and, in this way, we can build a more reliable national panel.

Keywords

Adolescent; Hypertension; Obesity; Risk Factors; Insulin Resistance; Sedentarism; Epidemiology.

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Neutrophil-To-Lymphocyte Ratio and Abdominal Aortic Atherosclerosis among Asymptomatic Individuals

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Abstract

Background: Neutrophil-to-lymphocyte ratio (NLR) has been proposed as an inflammatory marker that might be associated with coronary atherosclerosis, although most of the current data is restricted to the acute setting. Additionally, the association of NLR with extracoronary atherosclerosis and stable disease remains unclear.

Objective: To analyze the association between NLR and abdominal aortic atherosclerosis (AAAt).

Methods: We included asymptomatic individuals who underwent a health screening program. AAAt was measured by ultrasound. Absolute leukocyte and lymphocyte counts were used to calculate the NLR. The level of significance for statistical analysis was 0.05.

Results: Among 36,985 individuals (age: 42±10 years, 72% male), AAAt was identified in 7%. Those with AAAt were older and more likely to be male and diabetic. Presence of AAAt was associated with increased NLR (odds ratio [OR] 1.17; 95% confidence interval [CI] 1.13-1.21). However, this association was no longer significant when the analysis was adjusted for risk factors (OR 1.02; 95% CI 0.97-1.06), mostly due to the inclusion of age in the model. When neutrophils and lymphocytes were analyzed separately, the negative association between lymphocytes and AAAt was inverted once age was accounted for, suggesting a strong confounding effect of age on the relationship between lymphocytes and atherosclerosis. Finally, the association of neutrophils and AAAt lost significance after an additional adjustment for traditional risk factors, but not age alone.

Conclusion: Although the NLR was associated with AAAt, this was largely due to the confounding effect of age. Overall, the results suggest a limited role of leukocyte measurements as biomarkers of AAAt.

Keywords: Atherosclerosis; Biomarkers; Lymphocytes; Neutrophils; Risk Factors.

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide.[1] Combinations of risk factors, such as diabetes, hypertension, dyslipidemia, obesity, and smoking, can lead to the development of atherosclerosis. In the early stages of atheroma plaque formation, circulating low-density lipoproteins (LDLs), in the context of endothelial dysfunction, penetrate and accumulate in the tunica intima of the arteries. When oxidized, LDL particles may initiate an inflammatory response that culminates with the recruitment of monocytes/macrophages to the plaque region and activate innate and adaptive immunity. Therefore, the growth and complications of atherosclerotic plaques are an immune-mediated inflammatory response.[2]

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Many studies have noted the relationship between white blood cell (WBC) count and coronary artery disease (CAD) risk.[3] The systemic inflammatory state leads to an increase in neutrophils, and the acute stress caused by complications of atherosclerotic plaques leads to a decrease in lymphocytes.[4-6] Neutrophils were also associated with a higher chance of events,[7] while lymphocytes were significantly lower in patients with cardiac events and who still had a higher risk of future events (eg, CAD, unstable angina, cardiac death).[8,9] The neutrophil-to-lymphocyte ratio (NLR) is an inflammatory marker that has been extensively studied in recent years and appears to play an important role not only in predicting cardiovascular events but also in predicting clinical outcomes in the setting of cerebral hemorrhages,[10,11] major cardiac events,[12] and sepsis and infectious diseases.[13] Therefore, this simple index, derived from an inexpensive and easily reproducible test, may contain significant information regarding the risk of cardiovascular outcomes.[14]

The association between NLR and prognosis in different settings of cardiovascular disease, such as acute coronary syndromes, cardiac arrhythmias, congestive heart failure decompensation, transcatheter aortic valve replacement, and valvular heart diseases,[15] has been reported by several

authors. However, some studies still have limitations in multivariate analyses that do not always consider all the confounding factors, impairing the results of the true association between NLR and cardiovascular risk. Moreover, there are no data available correlating NLR with subclinical atherosclerotic cardiovascular disease and showing the application of NLR in cardiovascular risk stratification. Thus, the objectives of this study were to correlate the presence of subclinical atherosclerosis with NLR and to assess whether NLR adds discrimination to traditional risk factors.

Methods

Study population

We included all individuals who underwent a health screening program at the Preventive Medicine Center of Hospital Israelita Albert Einstein in São Paulo, Brazil, between 2006 and 2015. This program consists of an extensive clinical and laboratory evaluation and abdominal ultrasonography. The study protocol was approved by the local Institutional Review Board and was granted a waiver of informed consent.

Clinical and laboratory assessment

Demographics, medical history, and medication use were routinely recorded by standardized questionnaires. Smoking status was categorized as current smoker (at least 1 cigarette during the last 30 days) versus former smoker and nonsmoker. Height (m) and weight (kg) were measured with a stadiometer and a standard physician's scale, respectively, to calculate body mass index (BMI, kg/m^2). Blood pressure was measured three times in sitting position with an aneroid sphygmomanometer according to the standard method recommended by the American Heart Association.[16] Hypertension was defined as average blood pressure values ≥ 140/90 mm Hg during the evaluation or use of blood pressure-lowering medications. Diabetes mellitus was defined as fasting blood glucose level ≥ 5.55 mmol/L or use of drug treatment for hyperglycemia. Dyslipidemia was defined as elevated triglyceride (TG) level (≥ 1.7 mmol/L); elevated LDL-cholesterol level (4.12 mmol/L); and low high-density lipoprotein (HDL)-cholesterol level (< 1.02 mmol/L for men or < 1.28 mmol/L for women) or use of lipid-lowering medications. Blood samples were collected after recommended 12-h fasting and processed at the Central Laboratory of the Preventive Medicine Unit of Hospital Israelita Albert Einstein. Total cholesterol, TG, HDL-cholesterol, glucose, and blood cells were determined with standardized automated laboratory tests.[17] When TG < 4.5 mmol/L, LDL-cholesterol was calculated by the Friedewald formula.[18] When TG ≥ 4.5 mmol/L, LDL-cholesterol was measured directly.

Abdominal ultrasonography

Abdominal ultrasonography was performed by certified radiologists using a standardized approach. The abdominal aorta was systematically evaluated for the presence of atherosclerosis. Abdominal aortic atherosclerosis (AAAt) was defined by the presence of atheroma or lipid plaque in the abdominal ultrasound report.

Statistical analysis

Continuous variables are presented as means and standard deviations or medians and interquartile ranges, as appropriate. Normality was assessed by visual inspection of histograms. Categorical variables are presented as absolute counts and percentages. Differences in baseline characteristics of individuals according to NLR quintiles and the presence of AAAt were evaluated with independent-sample t-test, one-way analysis of variance (ANOVA) for continuous variables with a normal distribution, and Wilcoxon rank-sum or Kruskal Wallis test for those known not to be normally distributed. Chi-square test was used for categorical variables. The associations between NLR, neutrophils, lymphocytes, and AAAt were tested first in a univariate analysis and then adjusted for age and gender with logistic regression models. Additional multivariate analyses included age, gender, smoking status, hypertension, diabetes, and dyslipidemia. Tests were conducted at a significance level of 5%. All analyses were performed with Stata version 13.0.

Results

The study sample consisted of 36,985 individuals (men: 71.5%; mean age: 42.3±9.9 years). Their baseline demographic, clinical, and laboratory characteristics are presented in Table 1, for all the patients and according to NLR quintiles.

There was no difference across NLR quintiles for levels of total cholesterol and LDL-cholesterol. Patients in the highest NRL quintile were older and more likely to have diabetes and hypertension ($p < 0.001$ for all). They had both the highest neutrophil and lowest lymphocyte counts ($p < 0.001$ for both). Patients in the lowest NRL quintile had the lowest BMI ($p = 0.027$), the lowest TG level ($p < 0.001$), and the highest HDL-cholesterol level ($p < 0.001$). This group also had the lowest neutrophil and highest lymphocyte counts ($p < 0.001$ for both).

AAAt was identified by ultrasound in 7% of the patients. Compared with participants without AAAt, those with AAAt were older, more frequently male and former or current smokers, and more often had a diagnosis of diabetes, hypertension, or dyslipidemia (Table 2).

The NLR was higher in patients with AAAt compared with those without AAAt. After multivariate analysis, higher NLR levels were directly associated with atherosclerosis. When analyzed separately, neutrophils were directly associated with AAAt, whereas lymphocytes were negatively associated with it. However, the association between NLR and atherosclerosis was lost when adjusted for sex, age, and risk factors. This occurred mainly because of the inclusion of age in multivariate analysis. The negative association between lymphocytes and AAAt was reversed when age was included in the model, suggesting a confounding effect. The association between neutrophils and AAAt lost significance after adjustment for traditional risk factors, but not age alone.

Table 1 – Baseline characteristics of study participants and comparison between neutrophil-to-lymphocyte ratio quintiles

	Total	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	p
Age (years)	42.3±9.9	40.3±9.8	41.6±9.7	42.4±9.7	42.8±9.7	44.4±10.3	< 0.001
Male (%)	26248 (71.5%)	5144 (70%)	5430 (74%)	5365 (73%)	5317 (72%)	4992 (68%)	< 0.001
Smoking (%)							0.042
Former	4,790 (13.1%)	958 (13.1%)	963 (13.1%)	956 (13%)	965 (13.2%)	948 (12.9%)	
Current	3759 (10.3%)	726 (9.9%)	732 (10%)	720 (9.8%)	738 (10.1%)	843 (11.5%)	
Diabetes mellitus (%)	936 (2.6%)	116 (2%)	157 (2%)	178 (2%)	200 (3%)	285 (4%)	< 0.001
Hypertension (%)	4819 (13.1%)	782 (11%)	841 (11%)	963 (13%)	999 (14%)	1234 (17%)	< 0.001
BMI (kg/m²)	26.5±4.3	26.2±4.2	26.4±4.3	26.7±4.3	26.8±4.4	26.5±4.3	0.027
Dyslipidemia (%)	9927 (27%)	1878 (26%)	1990 (27%)	2093 (28%)	1960 (27%)	2006 (27%)	0.002
Triglycerides* (mg/dL)	112 (79-161)	107 (77-156)	113 (79-163)	112 (81-162)	115 (80-163)	112 (79-158)	< 0.001
Cholesterol (mg/dL)	196.9±37.6	198.2±37.7	199±37.3	197.7±38	196.8±37.5	193±37.2	0.328
HDL-cholesterol (mg/dL)	49.1±13.6	50.6±14.7	49.1±13.5	48.7±13.3	48.2±13	49±13.6	< 0.001
LDL-cholesterol (mg/dL)	121.7±34	121.9±34.3	123.1±33.7	122.8±34.2	122.1±34	118.6±33.4	0.122
Leukocytes (/mm³)	6472±1,575	5918±1368	6202±1364	6344±1397	6609±1502	7286±1840	< 0.001
Neutrophils (/mm³)	3600±1195	2616±680	3166±718	3494±789	3906±918	4818±1399	< 0.001
Lymphocytes (/mm³)	2117±580	2554±615	2285±515	2101±473	1949±452	1696±430	< 0.001
C-reactive protein*	0.12 (0.06-0.27)	0.10 (0.05-0.22)	0.11 (0.06-0.23)	0.12 (0.06-0.26)	0.13 (0.07-0.28)	0.17 (0.08-0.38)	< 0.001

**Median (interquartile range). BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein. Note: One-way analysis of variance (ANOVA) was used for continuous variables. Kruskal-Wallis test was used for triglycerides. Chi-square test was used for categorical variables.*

Table 2 – Characteristics of patients according to the presence of atherosclerosis on abdominal ultrasonography

	Atherosclerosis	No atherosclerosis	p
Age (years)	57.2±8.3	41.2±9.1	< 0.001
Male (%)	2132 (82%)	24476 (71%)	< 0.001
Smoking (%)			< 0.001
Former	810 (31.2%)	4042 (11.7%)	
Current	379 (14.6%)	3444 (10%)	
Diabetes mellitus (%)	253 (10%)	700 (2%)	< 0.001
Hypertension (%)	1004 (39%)	3896 (11%)	< 0.001
BMI (kg/m²)	27.3±3.8	26.4±4.3	< 0.001
Dyslipidemia (%)	1419 (55%)	8655 (25%)	< 0.001
Triglycerides* (mg/dL)	128 (91-178)	110 (78-159)	< 0.001
Cholesterol (mg/dL)	196.3±42	196.9±37.3	0.21
HDL-cholesterol (mg/dL)	46.5±12.7	49.2±13.7	< 0.001
LDL-cholesterol (mg/dL)	120.7±37.8	121.7±33.7	0.07
Leukocytes (/mm³)	6611.7±1775.5	6474.8±1665.2	< 0.001
Neutrophils (/mm³)	3732±1248.6	3590.7±1191.1	< 0.001
Lymphocytes (/mm³)	2077.8±822.5	2126.6±592.9	< 0.001

**Median (interquartile range). BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein. Note: T-test was used for continuous variables. Mann-Whitney test was used for triglycerides. Chi-square test was used for categorical variables.*

Discussion

This study demonstrated that no association between NLR and aortic atherosclerosis is noted once known confounders are accounted for. Despite the significant association noted in univariate analysis, these effects seemed to be largely related to the confounding effect of age, as NLR strongly correlated with age in our population. Collectively, our study suggests there is no role for NLR as a marker of atherosclerosis in asymptomatic patients who participated in a health screening program.

We already know that inflammation biomarkers are associated with an increased risk of cardiovascular events and some anti-inflammatory therapies are able to prevent them.[19] Identifying the patients who are at highest risk is key for the best therapy to be explored, and an important biomarker to be identified in these patients could be NLR, which is associated with prognosis in atherosclerotic diseases, as well as its prevalence, as shown in Figure 1.

The association between NLR as a predictor of mortality and acute coronary outcomes has been demonstrated by many studies. In acute diseases, the results are associated with elevated levels of neutrophils,[12] the mediators of myocardial injury responses such as myocardial infarction. This has also been demonstrated in studies addressing stable coronary disease. The relative lymphocyte count is associated with the survival of patients with CAD,[9] while different biomarkers, such as C-reactive protein (CRP) and leukocytes, are associated with chronic and acute outcomes.[20] CRP, like NLR, is a biomarker associated with inflammation and prediction of mortality risk. In study models including only NLR or CRP, each parameter alone was able to predict risk. When both were applied, however, there was a significant improvement in prediction.[21] Our results, however, do not support that. While results from other studies claim that NLR is an independent predictor of cardiovascular mortality, our analyses showed that there is a strong confounding factor when age is included in the model.

Since NLR correlates with the patient's age, the study analysis should be adjusted for it. In no study to date, however, we have observed such an adjustment. All analyses are based on risk factors and prognosis. Because age is an important point of comparison between patients, adjustment is extremely necessary.

There are differences between our study population and those of other studies. We approached a younger group from a large population, and this group had good socioeconomic conditions and consisted mostly of men and White people. Most studies address populations from the Northern Hemisphere, while our population lives in a tropical country in Latin America. Also, our study included the systematic evaluation of risk factors and laboratory tests. In statistical analysis, we made detailed adjustments for confounding factors and performed separate analyses for NLR, neutrophils, and lymphocytes (Table 3).

Our study must, however, be read within the context of its design. Our data are cross-sectional, so we were unable to infer causality. The selected population showed a higher prevalence of men, mostly young, which leads to a low prevalence of the disease and may attenuate the ability to perceive associations. In addition, our study focused on the evaluation of aortic atherosclerosis, which does not necessarily have the same pathophysiological process of atherosclerosis in other territories, such as the coronary artery.

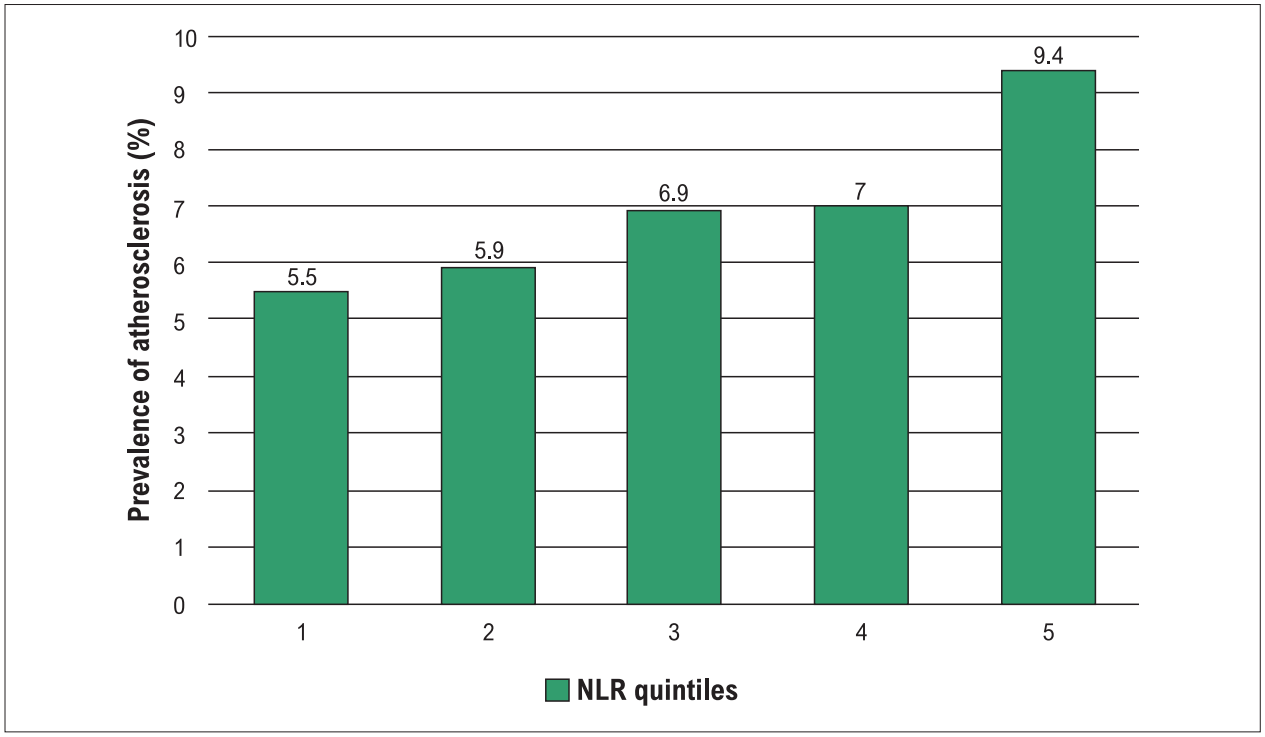

Figure 1 – *Prevalence of atherosclerosis according to neutrophil-to-lymphocyte ratio (NLR) quintiles (p < 0.001).*

Table 3 – Multivariate analysis of the relationship between neutrophil-to-lymphocyte ratio, neutrophils, or lymphocytes and abdominal atherosclerosis

	Odds ratio for atherosclerosis (95% confidence interval)		
	Unadjusted	Model 1	Model 2
NLR	1.17 (1.13-1.21)	1.00 (0.96-1.05)	1.00 (0.95-1.04)
Neutrophil	1.07 (1.03-1.11)	1.05 (1.01-1.10)	0.99 (0.95-1.04)
Lymphocyte	0.91 (0.87-0.95)	1.06 (1.02-1.11)	1.01 (0.97-1.05)

NLR: neutrophil-to-lymphocyte ratio. Model 1: Adjusted for age and gender. Model 2: Adjusted for age, gender, smoking status, hypertension, diabetes, and dyslipidemia.

Conclusion

Although atherosclerosis was associated with NLR, this was largely due to the confounding effect of age. The association of neutrophils and lymphocytes with atherosclerosis lost significance once these were included in multivariate models. The results suggest a limited role of the biomarker in the evaluation of subclinical atherosclerosis.

Author Contributions

Conception and design of the research: Cesena F, Laurinavicius AG, Santos RD, Bittencourt MS; Acquisition of data: Bittencourt MS; Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content: Marin BS, Cesena F, Laurinavicius AG, Santos RD, Bittencourt MS; Statistical analysis and Writing of the manuscript: Marin BS, Bittencourt MS.

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.

Ethics approval and consent to participate

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

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Short Editorial Neutrophil-To-Lymphocyte Ratio and Abdominal Aortic Atherosclerosis Among Asymptomatic Individuals

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Short Editorial related to the article: Neutrophil-To-Lymphocyte Ratio and Abdominal Aortic Atherosclerosis among Asymptomatic Individuals

In the article,[1] the authors evaluate the role of the neutrophil-to-lymphocyte ratio (NLR) in abdominal aortic atherosclerosis (AAAt). The backbone of this paper is the knowledge that the development and complications of atherosclerotic plaques are part of an immunological response. They have used the neutrophil-to-lymphocyte ratio (NLR) as an inflammatory marker and have used abdominal aortic ultrasound to evaluate subclinical atherosclerosis through the findings of aortic atheroma or lipid plaque (AAAt). The RNL and acute coronary syndrome results were well established, with the inflammatory state leading to a rise in neutrophils and the acute stress of plaque rupture or obstruction leading to a fall in the lymphocyte count.[2] In a group of 779 patients with ST-elevation myocardial infarction (STEMI), Machado et al. have shown a strong correlation between NLR at 48-72 hours and early and late death.3 Bozkurt et al. have studied 39 patients with Hemophagocytic syndrome (HPS) and heart failure with preserved ejection fraction. HPS is a state with high lethality, of severe systemic hyper inflammation with increased T lymphocytes and high levels of cytokines. In those patients, NRL was a strong predictor of mortality.4

The authors have studied 36.985 individuals through abdominal ultrasound and have found abdominal aortic atherosclerosis in 7% of them. In this group of abdominal aorta asymptomatic atherosclerosis, many individuals had confounding factors for NLR analysis as an atherosclerosis marker, namely age, smoking habit, diabetes, arterial hypertension, and dyslipidemia.

Through multivariate analysis adjusted for age, sex and risk factors for atherosclerosis, they have observed no relationship between NLR and AAAt. The quest for information on asymptomatic atherosclerotic individuals through a simple and low-cost exam, like NRL, was tempting. But multivariate analysis has not shown a relationship between NRL and subclinical atherosclerosis

The inclusion of age was important to define the role of NLR as an AAAt marker in asymptomatic individuals. In AAAt positive individuals, the age was 57,2+/-8,3 years, and in the AAAt negative individuals, the age was 41.2 +/- 9.1 years ($p<0.001$). It is very important to include age in a study model like this one.

The most important information of this research is the need for complete data collection to have a correct statistical analysis. If age were not included in the research, NLR would be considered a marker for asymptomatic atherosclerosis, with all the consequences from this information.

The choice of AAAt in this study would deserve some discussion. Li et al.[5] have studied abdominal aorta atherosclerotic plaques in a group of 1667 patients submitted to coronagraphy. Of those, 1268 had coronary artery disease, and 399 had not. There was more prevalence of atherosclerotic plaques in the coronary artery disease group than in the no coronary artery disease group (37,3% vs. 17%, $p<0,001$). In multivariate analysis, abdominal aortic plaques were an independent factor associated with coronary artery disease. This paper brings validation to the choice of AAAt to analyze the role of NLR in asymptomatic atherosclerosis

Keywords

Interleukin-8; Neutrophil Activation; Plaque, Atherosclerotic/complications; Atherosclerosis; Coronary Artery Disease; Inflammation; Aorta Abdominal/diagnostic imaging; Lymphohistiocytosis, Hemophagocytic

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Short Editorial

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The Relationship between Epicardial Fat and Atrial Fibrillation Cannot Be Fully Explained by Left Atrial Fibrosis

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Abstract

Background: **Epicardial adipose tissue (EAT) has been associated with atrial fibrillation (AF), but its pathophysiological mechanisms remain unclear.**

Objectives: **To measure the correlation between EAT and left atrium (LA) fibrosis, and to assess their ability to predict relapse after pulmonary vein isolation (PVI).**

Methods: **Patients with AF enrolled for a first PVI procedure underwent both cardiac computerized tomography (CT) and cardiac magnetic resonance (CMR) imaging within less than 48 hours. EAT_{LM} was quantified on contrast-enhanced CT images at the level of the left main. LA fibrosis was quantified on isotropic 1.5 mm 3D delayed enhancement CMR. After pulmonary vein isolation (PVI), patients were followed up for AF relapse. Statistical significance was set at $p<0.05$.**

Results: **Most of the 68 patients (46 men, age 61±12 years) had paroxysmal AF (71%, n=48). Patients had a median EAT_{LM} volume of 2.4 cm^3/m^2 (interquartile range [IQR] 1.6–3.2 cm^3/m^2), and a median amount of LA fibrosis of 8.9 g (IQR 5–15 g). The correlation between EAT_{LM} and LA fibrosis was statistically significant but weak (Spearman's R=0.40, p=0.001). During a median follow-up of 22 months (IQR 12–31), 31 patients (46%) had AF relapse. Multivariate analysis yielded two independent predictors of AF relapse: EAT_{LM} (HR 2.05, 95% CI 1.51–2.79, p<0.001), and non-paroxysmal AF (HR 2.36, 95% CI 1.08–5.16, p=0.031).**

Conclusion: **The weak correlation between EAT and LA suggests that LA fibrosis is not the main mechanism linking EAT and AF. EAT was more strongly associated with AF relapse than LA fibrosis, supporting the existence of other more important mediators of EAT and AF.**

Keywords: **Atrial fibrillation; Atrial Fibrosis; Epicardial Fat; Pulmonary Vein Isolation.**

Introduction

Epicardial adipose tissue (EAT) has recently been shown to be associated with the presence, severity, and relapse of atrial fibrillation (AF).[1] Although the pathophysiological mechanisms underlying this association remain to be established, several hypotheses have been put forward, including direct adipocyte infiltration, oxidative stress, and the secretion of adipokines causing inflammation and fibrosis of atrial tissue.[1] Establishing whether this relationship is causal, and ascertaining its underlying processes may prove useful to better understand AF and identify potential therapeutic targets. Thus far, the evidence linking EAT and atrial fibrosis has come mostly from histological and biochemical analyses of samples obtained from cardiac surgery,[2] but both of these features can be assessed non-invasively. In this study, we aimed to measure the correlation between the volume of EAT and the amount of left atrium (LA) fibrosis assessed by non-invasive imaging, and to assess their ability to predict time to relapse after pulmonary vein isolation (PVI).

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Methods

Study population

All consecutive patients with symptomatic drug-refractory AF undergoing cardiac computed tomography (CT) prior to percutaneous PVI at Hospital Santa Cruz (Carnaxide, Portugal) between November 2015 and December 2017 who underwent both cardiac computerized tomography (CT) and cardiac magnetic resonance (CMR) imaging within less than 48 hours were included in an observational registry used for this retrospective study. Patients with moderate or severe valvular heart disease, left atrial thrombus, abnormal thyroid function, or contraindication to anticoagulation were excluded. Atrial fibrillation was categorized as paroxysmal if self-terminated in less than 7 days, persistent if episodes lasted ≥7 days or required cardioversion, or long-standing persistent if AF was maintained for more than 12 months. This observational registry conforms to the ethical guidelines of the declaration of Helsinki and was approved by the institutional review board. All patients signed a an informed consent form.

Cardiac CT and CMR protocols

All patients underwent cardiac CT and CMR imaging less than 72 hours before the ablation procedure for the assessment of pulmonary vein anatomy, measurement of LA volume, exclusion of thrombi and integration with electroanatomical mapping.

CT scans were performed on a dual-source 64-slice scanner (Somatom Definition®, Siemens Healthineers®, Erlangen, Germany) with injection of 90 mL nonionic contrast medium (400 mg I/mL iomeprol Bracco®) at a flow rate of 5 mL/s followed by 30–50 mL of saline solution. Scan parameters included detector collimation of 2x32x0.6 mm, slice acquisition of 64x0.6 mm, gantry rotation time of 330 ms, automatic exposure control, and tube potential of 100 kV (except if body mass index greater than 30 kg/m^2 and bodyweight over 90 kg, where 120 kV were used).

Prospective ECG tube current modulation was systematically used to minimize radiation exposure. Image reconstruction was performed with a slice thickness of 0.75 mm.

CMR images were acquired on a 1.5 T scanner (Magnetom Avanto®, Siemens Healthineers). The scan protocol included an isotropic 1.5 mm 3D inversion-recovery gradient-recalled-echo sequence with fat saturation and respiratory navigator, acquired 15 to 20 min after administration of 0.2 mmol/kg intravenous gadobutrol. Inversion time was chosen individually in order to nullify normal myocardium, using a TI scout sequence.

ECG gating was used to set the timing of image acquisition to late ventricular systole (LA diastole).

Image Analysis

The CT quantification of EAT was performed semi-automatically on axial images using a TeraRecon Aquarius® Workstation (version 4.4.12, TeraRecon®, San Mateo, CA, USA). Four contiguous slices centered on the ostium of the left main (LM) were selected for analysis. The pericardium was manually traced in the first and last images, and automatically interpolated in the two middle slices, which were then checked for accuracy and adjusted if necessary. EAT_{LM} volume was defined as the total volume of tissue within the pericardial sac in this 4-slice region of interest with attenuation values between -250 and -30 Hounsfield units.[1] Left atrial volume was calculated by tracing the LA borders on CT images, excluding the pulmonary veins and the left atrial appendage.[3]

CMR post-processing for the quantification of LA fibrosis was performed with ADAS® software (version 2.3.3, Galgo Medical). LA wall contours were drawn manually, excluding the mitral valve and pulmonary veins from the analysis. The signal intensity of the LA wall was normalized using an image intensity ratio (IIR) calculated as the ratio between the signal intensity of each pixel and the mean blood pool intensity. IIR >1.20 was considered to represent LA fibrosis.[4] Quantifications of LA fibrosis and EAT were performed only after the ablation procedure (without knowing its outcome). Examples of EAT and LA fibrosis quantifications are presented in Figure 1.

Pulmonary vein isolation protocol

Pulmonary vein isolation was guided by electroanatomical mapping, using either NavX® (St Jude Medical®, St Paul, MN, USA) or CARTO® (Biosense Webster®, Diamond Bar, CA, USA) systems. The right femoral vein was used as the preferred vascular access, through which three catheter electrodes were introduced: (i) a decapolar catheter, advanced through the coronary sinus; (ii) a variable circular mapping catheter, placed in the pulmonary veins; and (iii) an irrigated contact force-sensing ablation catheter. Left atrial access was established by transseptal puncture. Radiofrequency ablation was performed more than 5 mm from the pulmonary vein ostia, with continuous lesions enclosing the left and right pairs of pulmonary veins.[5]

Treatment was considered successful if bidirectional block was achieved. When required, electrical cardioversion was performed at the end of the procedure. Oral anticoagulation was resumed 6 hours after the ablation, maintained for 6 months, and then withdrawn or continued according to CHA_2DS_2-VASc criteria. Class I/III antiarrhythmic drugs were generally maintained in all of the patients for the first 3 months after the procedure, then withdrawn if there was no AF relapse. A proton pump inhibitor was prescribed for the first month after the ablation.

Study endpoint and patient follow-up

The study endpoint was AF relapse, defined as symptomatic or documented AF and/or other atrial arrhythmias, after a 3-month blanking period. Symptomatic AF was defined as the presence of symptoms likely due to AF episodes. Documented AF was defined by the presence of at least one episode of AF lasting more than 30 seconds in an ECG, 24-hour Holter monitoring, or event-loop recording. The follow-up protocol consisted of outpatient visits with 12-lead ECG and 24-h Holter monitoring at the assistant physicians' discretion (typically at 6 and 12 months, and yearly thereafter). If the clinical records were insufficient, a structured telephone interview was conducted. Patients kept on antiarrhythmic drugs after the third month of follow-up were not considered as failed ablation.

Statistical analysis

Normally and non-normally distributed continuous variables were expressed as mean±standard deviation and median and interquartile range, respectively, while categorical variables were expressed as frequencies and percentages. Statistical significance was set at $p<0.05$. Shapiro-Wilk test was employed to assess the population's normality. Unpaired Student's t-test was used to assess statistically significant differences between normally distributed continuous variables, while Mann-Whitney U test was used for non-normally distributed continuous variables. Chi-square test was used to analyze categorical variables. Spearman's correlation coefficient was used for gauging the correlation between EAT_{LM} volume and LA fibrosis. Univariate proportional-hazards Cox regression was used to identify predictors of time to AF

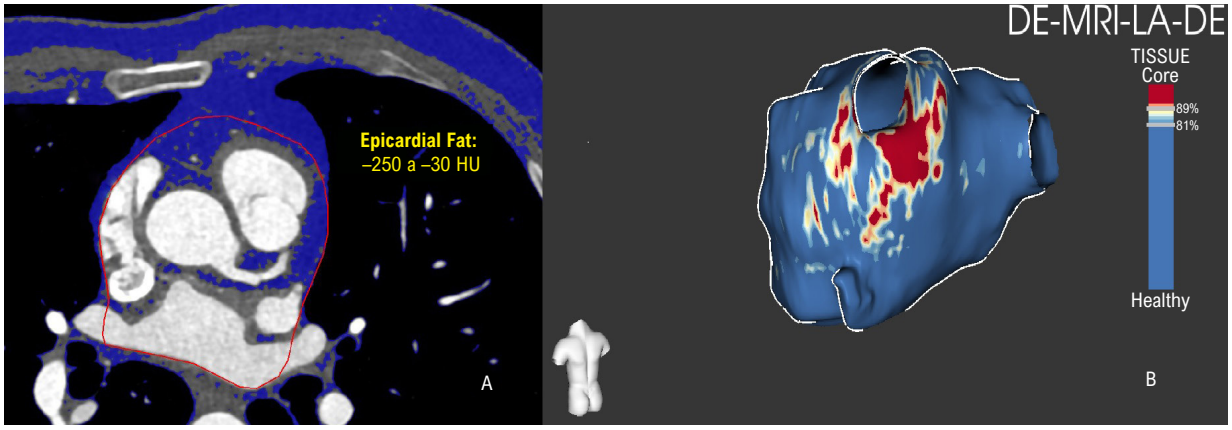

Figure 1 – *Epicardial fat (A) measured with computed tomography and left atrium fibrosis (B) measured with cardiac magnetic resonance.*

relapse. Variables with p-value ≤0.10 in univariate analysis were selected for a multivariate Cox regression model and considered statistically significant if $p<0.05$. A 95% confidence level was used in our statistical analysis. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL).

Results

The baseline characteristics of the study population are presented in Table 1. Overall, patients had a median EAT_{LM} volume of 2.4 cm^3/m^2 (interquartile range [IQR] 1.6–3.2 cm^3/m^2), and a median estimated amount of LA fibrosis of 8.9 g (IQR 5–15 g), corresponding to 8% (IQR 5–11%) of the total LA wall mass.

The correlation between EAT_{LM} and LA fibrosis was statistically significant but weak (Spearman's correlation coefficient = 0.40, p=0.001) – Figure 2.

During a median follow-up of 22 months (IQR 12–31), 31 patients (46%) had AF relapse. Patients who had AF relapse were more likely to have non-paroxysmal AF, and had higher LA volumes, higher amounts of EAT_{LM} and LA fibrosis. On assessing the time to AF relapse, these four predictors were identified in univariate Cox regression. Multivariate analysis yielded two independent predictors of time to AF relapse: EAT_{LM} and non-paroxysmal AF (Table 2).

Discussion

The main findings of this study are essentially twofold: 1) epicardial adipose tissue and LA fibrosis are weakly correlated; and 2) epicardial adipose tissue seems to be a more powerful predictor of AF relapse than LA fibrosis. Epicardial adipose tissue has been shown to be metabolically active, with endocrine and paracrine activity.[6] Specifically, the secretome from human epicardial fat, but not from subcutaneous adipose tissue, has pro-fibrotic effects on the atrial myocardium of rats.[7] EAT is also known to secrete activin A, a member of the TGF-β class capable of inducing atrial fibrosis.[6] A recent study also showed an association between EAT and slow atrial conduction, greater electrogram fractionation and increased atrial fibrosis.[8] Fat-induced atrial fibrosis would therefore seem to be a reasonable explanatory mechanism for the link between EAT and AF. Thus far, supporting evidence for this "fibrogenic hypothesis" has come from histological and biochemical analyses of samples obtained from cardiac surgery.[2] To the best of our knowledge, our study is the first *in vivo* assessment of the relationship between EAT volume and the amount of LA fibrosis in patients with AF. The weak correlation we found between these two parameters does not disprove a pathophysiological connection but suggests that LA fibrosis is not the sole or main mechanism by which EAT and AF are linked. The fact that EAT was more strongly associated with AF relapse than LA fibrosis itself further supports the existence of other more important mediators between epicardial adiposity and this arrhythmia. These may include pro-inflammatory action of cytokines secreted by EAT including C-reactive protein, interleukins 1β, 6 and 8 and tumor necrosis factor α, which may have arrhythmogenic effects.[9-12] Fatty infiltration is another possible mechanism, with some studies showing that increased EAT volume is associated with direct infiltration of the atrial myocardium,[13] possibly causing prolongation of P-wave indices. This delay in atrial tissue conduction may be a potential mechanism for initiation and maintenance of AF.[14]

For this study, we used a modification of the CT method proposed by Tran et al.[15] to measure EAT. This method uses a single slice measure of EAT at the level of the left main coronary artery, yielding results that are highly correlated with total epicardial adipose tissue.[15] This EAT quantification method was selected for its simplicity and good reproducibility, but it should be noted that there is currently no consensus on the best methodology to measure epicardial fat, a step that will be crucial if this parameter is to be used in clinical practice. A similar problem occurs with the *in vivo* measurement of LA fibrosis, where the use of standardized protocols is necessary to ensure uniformity of image acquisition and processing.[16]

Table 1 – Baseline characteristics of the study population

Baseline characteristics	Total (n=68)	Without AF relapse (n=37)	With AF relapse (n=31)	p-value
Age, years	61±12	61±11	61±12	0.968
Male sex, n (%)	46 (67.6)	22	24	0.312
Weight, kg	81±13	79±12	82±13	0.097
Body mass index, kg/m^2	28±4	27±4	29±4	0.091
Type of AF				0.003
paroxysmal, n (%)	48 (70.6)	32	16	
non-paroxysmal, n (%)	20 (29.4)	5	15	
Hypertension, n (%)	41 (60.3)	21	20	0.621
Diabetes, n (%)	6 (8.8)	5	1	0.209
Active smoking, n (%)	4 (5.9)	1	3	0.304
LV systolic dysfunction, n (%)	0 (0)	0	0	1.000
Known CAD, n (%)	6 (8.8)	4	2	0.366
CHA_2DS_2-VASc, median (IQR)	2 (1–3)	2 (1–3)	2 (1–3)	0.578
LA volume on cardiac CT, mL/m^2	56±15	52±12	60±17	0.025
EAT_{LM} volume, mL/m^2	2.4±1.2	1.9±0.7	3.1±1.2	<0.001
LA fibrosis, g, median (IQR)	8.9 (5–15)	6.7 (4–13)	11.2 (6–17)	0.049
LA fibrosis, % of LA mass, median (IQR)	7.5 (5–11)	6.9 (4–11)	8.8 (6–12)	0.170

AF: atrial fibrillation; LV: left ventricle; LA: left atrium; CT: computed tomography; EAT_{LM}: epicardial adipose tissue.

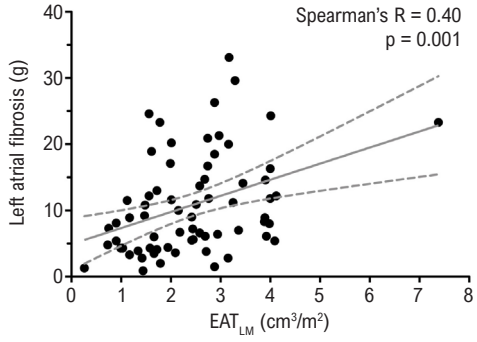

Figure 2 – *Left atrial fibrosis and epicardial fat correlation graphic. EAT_{LM}: epicardial adipose tissue.*

Table 2 – Univariate and multivariate Cox regression of AF-relapse predictors

Predictors of AF relapse	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
EAT_{LM}	2.19	1.65–2.91	<0.001	2.05	1.51–2.79	<0.001
Non-paroxysmal AF	3.36	1.64–6.87	0.001	2.36	1.08–5.16	0.031
LA fibrosis	1.05	1.01–1.09	0.033	–	–	0.881
LA volume (indexed to BSA)	1.03	1.01–1.06	0.006	–	–	0.153

AF: atrial fibrillation; BSA: body surface area; EAT_{LM}: epicardial adipose tissue; LA: left atrium; HR: hazard ratio; CI: confidence interval.

Limitations

Several limitations of this study should be acknowledged. We used a convenience sample of patients undergoing AF ablation, who may not be representative of the global AF population. AF relapse may be underreported, since the follow-up protocol did not include continuous ECG monitoring. On the other hand, symptomatic undocumented episodes may not represent true AF relapse and thus result in overestimation of relapse. Also, we did not measure total epicardial fat, but only a limited portion of it. Despite these limitations, our findings may contribute to the ongoing efforts to uncover the pathophysiological links between AF, EAT and LA fibrosis.

Conclusion

The weak correlation between EAT and LA fibrosis suggests that the latter is not the main mechanism by which EAT and AF are linked. EAT was more strongly associated with AF relapse than LA fibrosis, further supporting the existence of other more important mediators between EAT and AF.

Author Contributions

Conception and design of the research, Acquisition of data, Analysis and interpretation of the data, Statistical analysis, Obtaining financing, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Matos D, Ferreira A, Freitas P,[2] Rodrigues G, Carmo J, Costa F, Abecasis J, Carmo P, Saraiva C, Cavaco D, Morgado F, Mendes M, Adragao P.

Potential Conflict of Interest

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

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Study Association

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

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Obesity and Epicardial Fat Associated with Higher Atrial Fibrillation Recurrence After Ablation: Just Coincidence?

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Short Editrial related to the article: The Relationship between Epicardial Fat and Atrial Fibrillation Cannot Be Fully Explained by Left Atrial Fibrosis

Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice, and its prevalence increases with age.[1] It is a condition resulting from multiple pathophysiological aspects, not exclusively electrophysiological, from extrasystoles triggered by the pulmonary veins. It is associated with different cardiovascular and systemic diseases, obesity being a known risk factor.

Overweight and obese individuals are at greater risk for the occurrence of AF. For each unit of increment in BMI, the corrected risk for incidence of AF increases from 3 to 7% concerning individuals with a BMI below 25.[2,3] The mechanisms that justify this observation are not fully understood and are probably multifactorial. Obese patients often have arterial hypertension[4] and associated obstructive sleep apnea, factors already considered predisposing to AF.[5] A recent study demonstrated that implementing a weight control program has an important impact on the clinical control of this arrhythmia.[6]

Thanassoulis et al. highlighted an association between the extent of epicardial fat and greater occurrence of AF (odds ratio = 1.28 per standard deviation of epicardial fat volume). However, this observation does not apply to the amount of intrathoracic and abdominal fat.[7] Therefore, this association cannot be considered exclusively by the existence of obesity and its consequences. One of the justifications is the atrial inflammation induced by epicardial fat. AF is an arrhythmia associated with increased inflammatory markers, such as C-reactive protein, interleukins and tumor necrosis factor.[8] These substances can be produced by the atrial adipose tissue, causing inflammation in the adjacent myocardium. Epicardial fat contains higher inflammatory cells levels than subcutaneous fat, including mast cells, macrophages, and lymphocytes.[9,10]

In an experimental study, 10 sheep fed a hypercaloric diet for 36 weeks were compared with a control group with conventional feeding. The animals were submitted to an electrophysiological study and electroanatomical mapping of the left atrium. Obese sheep presented greater LA volume and pressure, greater electrical conduction heterogeneity, increased amount of fractionated atrial electrograms, reduced voltage of electrograms in the posterior LA wall and greater voltage heterogeneity, suggesting the presence of an atrial fibrotic substrate such as a possible determinant of this association between obesity and AF.[11]

In this issue of *Arquivos Brasileiros de Cardiologia*, Matos et al.,[12] studied 68 patients who had undergone coronary CT angiography, cardiac magnetic resonance imaging with 3D enhancement (using ADAS 3D software - Galgo Medical) before a first ablation procedure of atrial fibrillation. They observed a weak but statistically significant correlation between atrial fibrosis on electroanatomical mapping and epicardial adipose tissue measured on tomography in a slice at the left main coronary artery (TAE_{TC}) - Spearman correlation coefficient = 0.40, p=0.001.

Patients were followed up for a median period of 22 months (IQI 12-31), with AF recurrence in 31 patients (46%). Those who had recurrence were more likely to have non-paroxysmal AF, had larger atrial volume and larger volumes of adipose tissue and atrial fibrosis. However, in the multivariate analysis, only TAE_{TC} and non-paroxysmal AF were associated with greater recurrence. This observation is interesting and differs from previous studies,[13-15] in which recurrence was related to the extent of atrial fibrosis.

These findings corroborate the importance of epicardial fat not only in the generation of fibrosis but also as an inflammatory factor. Furthermore, autonomic influences could contribute to a greater arrhythmia recurrence, as the ganglion plexuses are located in the epicardial fat, which can even be targeted in AF ablation.[16-18]

Despite the growing understanding of the pathophysiological mechanisms involved in the occurrence of AF and the constant technical improvement of catheter ablation procedures, many aspects are still not well understood. Among them is its association with obesity and atrial epicardial fat extension.

Keywords

Atrial Fibrillation; Obesity; Catheter Ablation.

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The Impact of COVID-19 on Diagnosis of Heart Disease in Latin America an INCAPS COVID Sub-analysis

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Original Article

Abstract

Background: **The COVID-19 pandemic has disrupted the delivery of care for cardiovascular diseases in Latin America. However, the effect of the pandemic on the cardiac diagnostic procedure volumes has not been quantified.**

Objective: **To assess (1) the impact of COVID-19 on cardiac diagnostic volumes in Latin America and (2) determine its relationship with COVID-19 case incidence and social distancing measures.**

Methods: **The International Atomic Energy Agency conducted a worldwide survey assessing changes in cardiac diagnostic volumes resulting from COVID-19. Cardiac diagnostic volumes were obtained from participating sites for March and April 2020 and compared to March 2019. Social distancing data were collected from Google COVID-19 community mobility reports and COVID-19 incidence per country from the Our World in Data.**

Results: **Surveys were conducted in 194 centers performing cardiac diagnostic procedures, in 19 countries in Latin America. Procedure volumes decreased 36% from March 2019 to March 2020, and 82% from March 2019 to April 2020. The greatest decreases occurred in echocardiogram stress tests (91%), exercise treadmill tests (88%), and computed tomography calcium scores (87%), with slight variations between sub-regions of Latin America. Changes in social distancing patterns ($p < 0.001$) were more strongly associated with volume reduction than COVID-19 incidence ($p = 0.003$).**

Conclusions: **COVID-19 was associated with a significant reduction in cardiac diagnostic procedures in Latin America, which was more related to social distancing than to the COVID-19 incidence. Better balance and timing of social distancing measures and planning to maintain access to medical care is warranted during a pandemic surge, especially in regions with high cardiovascular mortality.**

Keywords: **Cardiac Testing; Coronavírus; COVID-19; Cardiovascular Disease; Global Health.**

Summary statement

The COVID-19 pandemic was associated with a significant reduction in cardiac diagnostic procedures in Latin America in April 2020, which was more related to social distancing measures than to the COVID-19 incidence.

Key points

- Cardiac diagnostic procedure volumes decreased 36% from March 2019 to March 2020, and 82% from March 2019 to April 2020.
- Changes in social distancing patterns ($p < 0.001$) were more strongly associated with volume reduction than COVID-19 incidence ($p = 0.003$).
- A better timing of social distancing measures and planning to maintain access to medical care is warranted during a pandemic surge, especially in high cardiovascular mortality regions.

Introduction

Cardiovascular disease (CVD) remains the leading cause of mortality worldwide, including in Latin America (LATAM).[1,2] While mortality rates have progressively decreased over the past four decades in most high-income countries, the same phenomenon has not been observed in low- and middle-income countries, many of them in the LATAM region.[3]

A comprehensive approach to managing CVD and reducing associated mortality involves adequate prevention, including control of risk factors, appropriate use of tests to diagnose and guide treatment, and the establishment of appropriate therapies. The World Health Organization recently called attention to the worldwide disruption of healthcare caused by the COVID-19 pandemic, which unfortunately imposes an additional burden to CVD patient care in regions such as LATAM that have been severely affected by COVID-19.[4]

The International Atomic Energy Agency (IAEA) Division of Human Health aims to support member states to combat CVD, cancer, malnutrition, and other diseases through the use of appropriate prevention, diagnostic testing and treatment. Accordingly, the IAEA coordinated a worldwide survey of cardiovascular imaging centers (the IAEA Noninvasive Cardiology Protocols Study of COVID-19, INCAPS COVID survey), in order to assess the impact of the pandemic on the diagnostic evaluation of CVD.

The objectives of this study were: (1) to assess the impact of COVID-19 on cardiac diagnostic procedural volumes in LATAM and (2) to determine its relationship with COVID-19 case incidence, temporal presentation, and social distancing interventions. Understanding the relationship of the pandemic phases, social distancing measures, and the provision of CVD diagnosis in LATAM is crucial to being better prepared for similar situations in the future.

Methods

Study design

Data for this study were collected as part of the IAEA survey on the impact of COVID-19 on cardiac imaging (INCAPS COVID) and correlated with publicly available social distancing metrics from Google Community Mobility Reports and monthly COVID-19 incidence from Our World

in Data database in LATAM.[5-7] The INCAPS COVID survey included questions regarding the healthcare facility, healthcare professionals, personal protective equipment, strategic plans for reopening, and changes in procedural volumes for a range of cardiovascular diagnostic procedures (Appendix).

Data collection

Based on the IAEA standardized methodology, an electronic data entry system was designed to collect data, employing a secure software platform, the International Research Integration System (IRIS, https://iris.iaea.org). In INCAPS COVID no patient-specific or confidential data were collected, and participation by study sites was voluntary; therefore, no external ethics committee review was required.

Participants were asked to provide estimates of cardiac diagnostic procedure volumes from March 2019, March 2020, and April 2020, including the following: transthoracic and transesophageal echocardiography, cardiac magnetic resonance (CMR), stress testing (exercise treadmill test, echocardiography stress test, single-photon emission computed tomography [SPECT], positron emission tomography [PET], and CMR), PET infection studies, computed tomography calcium score, coronary computed tomography angiography, and invasive coronary angiography. For analysis purposes, we divided LATAM into the following sub-regions: South America (Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, and Uruguay); Central America and Mexico (Costa Rica, Guatemala, Honduras, Mexico, Nicaragua, Panama, and El Salvador); and The Caribbean (Cuba, Dominican Republic, and Jamaica).

COVID-19 cases by country

Numbers of COVID-19 cases for each LATAM country were downloaded from the open access website Our World in Data (https://ourworldindata.org/coronavirus-source-data), which collects data from different official sources worldwide.[5] Our World in Data is a collaborative effort between researchers at the University of Oxford based at the Oxford Martin Programme on Global Development, who are the scientific editors of the website content, and the non-profit organization Global Change Data Lab, which publishes and maintains the website and the data tools. Data collected ranged from February 2020 to July 2020 to better reflect the pandemic evolution in LATAM. The number of new cases per million inhabitants per month per country was used for analysis.

Mobility data by country

Mobility data was downloaded from Google Community Mobility Reports (https://www.google.com/covid19/mobility/) that collect mobility trends in 6 different categories: retail and recreation, grocery and pharmacy, parks, transit stations, workplaces, and residential. The baseline was the median value, for the corresponding day of the week, during the 5-week period January 3 to February 6, 2020.[6] Google calculates these insights based on data from users who have opted in to location history for their Google account, so the data represents a sample of all users. We used the change in time spent at home (residential) by month as an "immobility index" variable, from February 2020 to July 2020, which reflects changes in social distancing patterns during that period of time. Mobility data from Cuba was not available and, therefore, was not used in these analyses.

Statistical analysis

Survey question responses are presented as numbers and percentages. Total procedures per center are presented as median and interquartile range. Percentage change in procedure volume was compared between March 2019 and March or April 2020 using the nonparametric Kruskal-Wallis test with asymptotic two-sided p values. In order to evaluate the association of the changes in cardiac procedure volumes with changes in mobility and COVID-19 incidence, we built a generalized estimating equation using countries as individual units and the monthly exam numbers as the outcome with month as a time variable. Statistical analysis was performed in Stata (version 15.1, Stata Corporation, LLC, College Station, Texas), Microsoft Excel (2016), and choropleth maps were constructed in R (version 4.0.1, R Development Core Team, Vienna, Austria) using tmap and rnaturalearth packages.

Results

Centers and procedure reduction

Data were obtained from 194 inpatient and outpatient centers in 19 countries in LATAM. The larger regional countries Brazil, Argentina, and Mexico were also the countries that contributed data from the largest number of centers: 70, 54, and 23 centers respectively. Characteristics of all centers are summarized in Table 1. A total of 198,597 cardiac diagnostic procedures were performed at participating sites during the three months considered.

In LATAM, cardiac diagnostic procedure volumes decreased by 36% from March 2019 to March 2020, and 82% from March 2019 to April 2020 (Figure 1 – Map). There was some variation between LATAM regions and by type of cardiac procedure, with the greatest decreases from March 2019 to April 2020 in echocardiography stress test (91%), exercise treadmill test (88%), and computed tomography calcium score (87%) (Table 1). The smallest decreases were reported for invasive coronary angiography (67%) and cardiac PET (65%). Procedure volumes also markedly decreased from March 2020 to April 2020. These decreases were significant ($p < 0.001$) in combination (Figure 2) and for each procedure. Separate generalized linear models for LATAM regions and overall found significant declines in procedural volume ($p < 0.001$), using regression models weighted by 2019 procedural volume. In the 194 facilities in our study, an estimated 129,030 cardiac diagnostic procedures, which would have been performed based on the procedure rates from March 2019, were not performed during these two months of the pandemic.

Mobility data, COVID-19 incidence, and reduction in procedure volume

The greatest increase in time spent at home (immobility index) occurred during the month of April 2020 in most

Table 1 – Characteristics of centers in Latin America

	South America	Central America and Mexico	The Caribbean
Countries	9	7	3
Number of centers	155	31	8
Teaching institution (n, %)	69 (44.5)	21 (67.7)	4 (50)
Hospital beds (median, IQR)	202.5 (120 - 400)	167 (100 - 300)	168 (80 – 412.5)
Type of institution (n, %)			
Hospital, inpatient only	3 (1.9)	5 (16.1)	0 (0)
Hospital, outpatient only	3 (1.9)	0(0)	0 (0)
Hospital, inpatient and outpatient	91 (58.7)	19 (61.3)	4 (50)
Outpatient imaging center	45 (29.0)	2 (6.4)	2 (25)
Outpatient physician practice	13 (8.4)	5 (16.1)	2 (25)
Total procedures per center (median, IQR)			
March 2019	157 (67 - 502)	91 (38 - 430)	173 (53.5 - 559.5)
March 2020	89 (31 - 253)	35 (19 - 143)	147.5 (24.5 - 343.5)
April 2020	32 (7 - 97)	20 (1 - 53)	36 (2.5 - 117)
% Reduction from March 2019 to April 2020			
Transthoracic echocardiogram	80.1	54.0	87.0
Transesophageal echocardiogram	81.6	88.0	89.6
Cardiac magnetic resonance	77.2	80.9	100
Computer tomography calcium score	81.7	96.1	99.5
Coronary computed tomography	73.0	84.8	85.9
Invasive coronary angiography	63.8	77.7	70.8
Exercise treadmill test	88.4	84.9	95.6
Echo stress test	91.1	94.5	76.9
SPECT	84.0	81.0	97.8
PET	62.0	90.9	NA
Stress cardiac magnetic resonance	76.3	89.2	NA

IQR: interquartile range; PET: positron emission tomography; SPECT: single-photon emission computed tomography.

countries. There was a mean 26.7% increase in April that dropped to 18.8% in July, when compared to baseline. Exceptions were Nicaragua and Chile, where immobility increased until June (Figure 3).

However, the COVID-19 pandemic was still in its early stages in most countries in April 2020, with a total of 199,277 cases by the end of the month. In most countries, the new monthly COVID-19 cases per million inhabitants continued rising and had not yet peaked by July 2020 (Figure 3). Exceptions were Cuba and Jamaica, which peaked in April. By the end of July the number of cases rose 23.5 times, totaling 4,681,377 confirmed COVID-19 cases.[5]

Both the reduction in mobility and the increase in COVID-19 incidence were associated with the reduction in cardiac diagnostic procedures in the generalized models ($p < 0.001$). When fitting a multivariable model, mobility ($p < 0.001$) was more strongly associated with volume reduction than COVID-19 incidence ($p = 0.003$).

Discussion

The results show a significant decrease in the number of cardiac diagnostic procedures performed in LATAM during the COVID-19 pandemic. The greatest decrease in the number of diagnostic procedures occurred in the month with the lowest mobility (April), which in turn coincided with the strictest quarantine periods in each country.

In most LATAM countries, social isolation was introduced in March, even without a significant number of COVID-19 cases. In March and April, there was a greater decrease in the number of cardiac diagnostic procedures conducted in LATAM, compared to Western Europe (46% decrease in March and 69% in April) and to US/Canada (39% decrease in March and 68% in April), despite the fact that these regions were experiencing the first peak of the pandemic, whereas LATAM was barely in the early stages.[7] By the end of March, fewer than 250 cases had been reported in 9 countries in the region, and, since that time, the number of cases has continued to increase. By late August

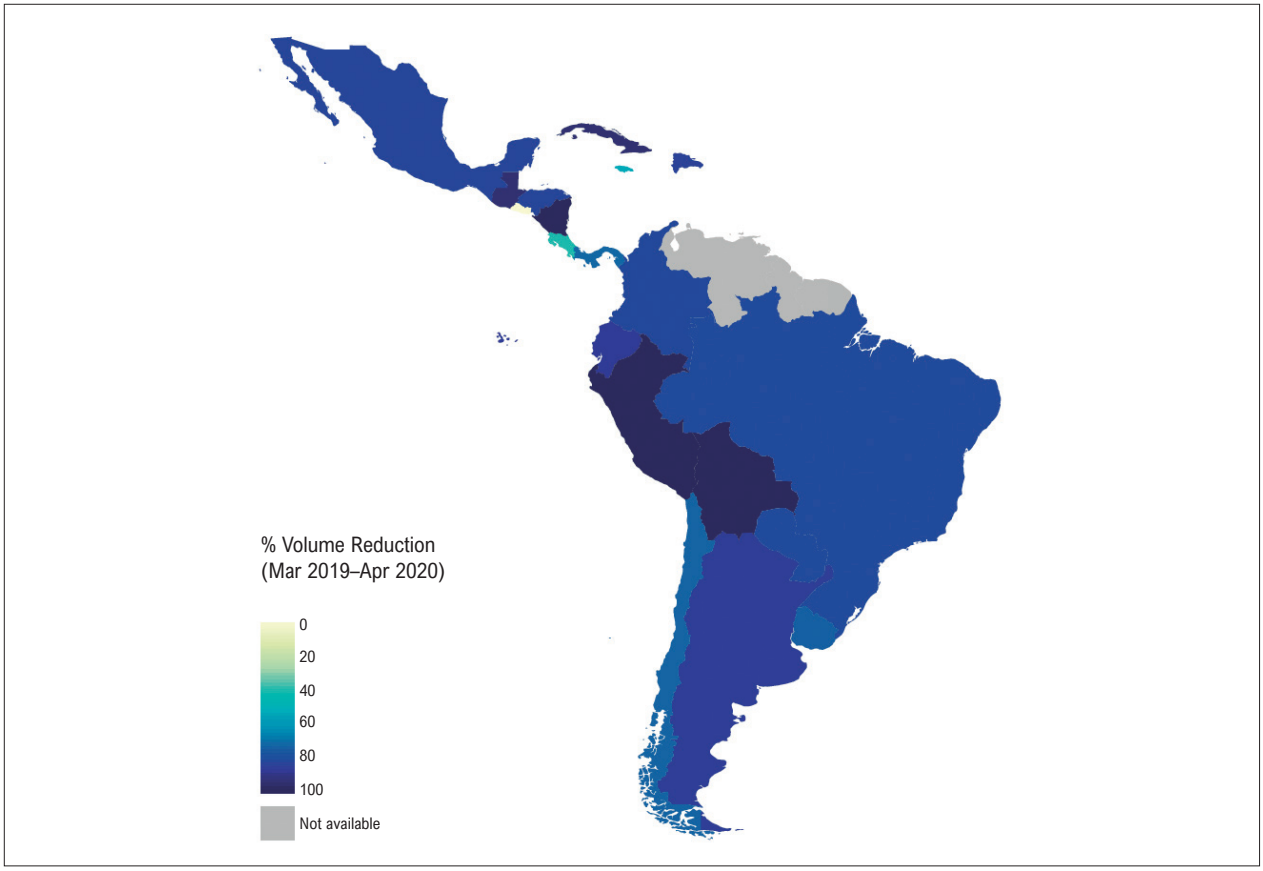

Figure 1 – *Color-coded map of Latin America displaying the reduction in total cardiac diagnostic procedure volumes by country from March 2019 to April 2020, in the beginning of the COVID-19 pandemic.*

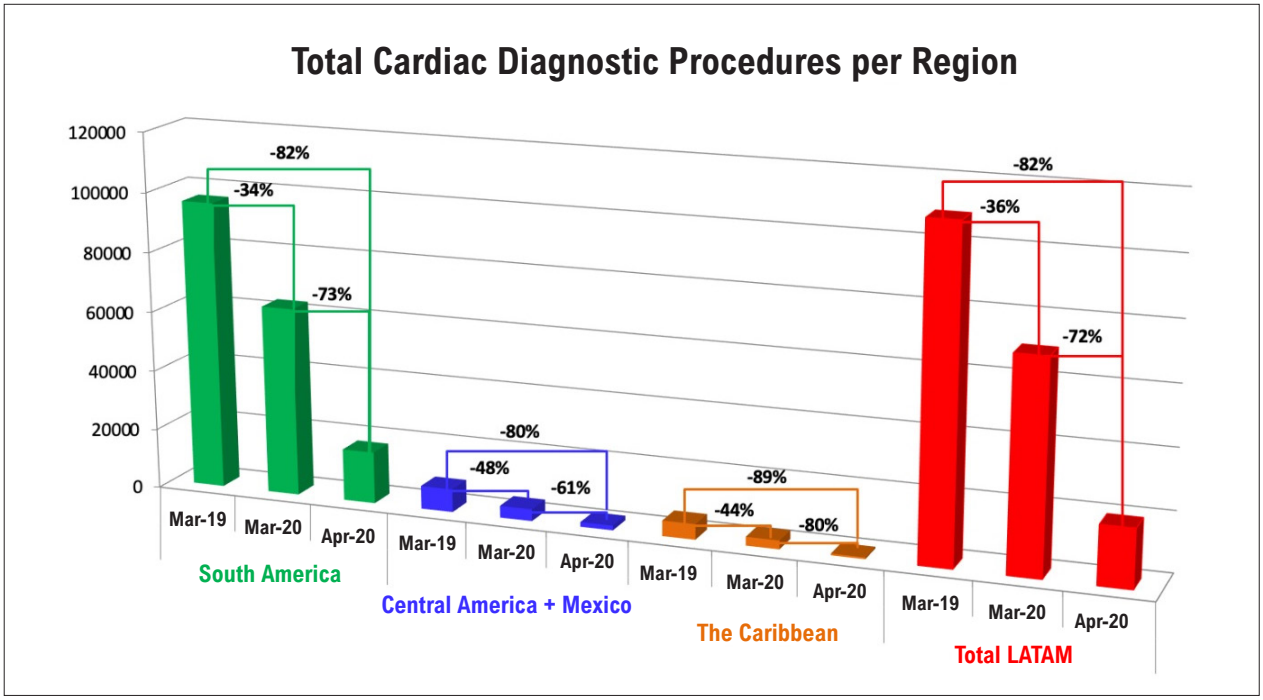

Figure 2 – *Reduction in total cardiac diagnostic procedure volumes in sub-regions of Latin America: South America; Central America and Mexico; and The Caribbean from March 2019 to March 2020 and April 2020, in the beginning of the COVID-19 pandemic.*

Number of new COVID-19 cases per million per month

	February	March	April	May	June	July
Brazil	0	22	346	1,977	4,092	5,843
Chile	0	128	651	4,184	9,476	4,056
Panama	0	249	1,229	1,539	4,581	7,279
Peru	0	29	1,000	3,692	3,843	3,795
Dominican Republic	0	83	530	945	1,374	3,328
Colombia	0	16	106	433	1,313	3,753
Bolivia	0	9	86	727	1,930	3,693
Argentina	0	21	73	264	1,019	2,583
Mexico	0	9	130	541	1,033	1,517
Honduras	0	14	64	437	1,386	2,283
Ecuador	0	111	1,287	788	969	1,627
Costa Rica	0	65	75	66	436	2,752
Guatemala	0	2	31	232	707	1,754
El Salvador	0	5	53	330	564	1,551
Paraguay	0	9	26	100	172	423
Uruguay	0	93	88	55	32	90
Jamaica	0	13	121	63	40	56
Nicaragua	0	0	0	5	6	6
Cuba	0	15	115	46	28	23

Cases
0 — 10,000

Immobility index per month

	February	March	April	May	June	July
Brazil	1.67	8.00	17.27	15.65	13.93	12.43
Chile	1.00	11.74	23.50	24.87	27.00	26.43
Panama	1.27	17.39	39.67	35.52	29.67	29.86
Peru	0.00	19.29	37.97	34.10	28.90	24.29
Dominican Republic	1.40	12.90	29.17	22.71	15.70	12.86
Colombia	-0.60	13.03	31.53	25.23	20.70	18.86
Bolivia	1.93	16.00	37.70	33.45	27.60	27.00
Argentina	0.60	12.97	25.53	20.74	17.43	18.14
Mexico	-1.13	5.61	19.77	19.42	16.20	14.43
Honduras	-0.67	13.35	25.60	22.35	19.63	20.93
Ecuador	2.13	18.77	34.93	29.16	22.27	20.29
Costa Rica	-1.27	9.19	22.53	17.00	16.37	19.86
Guatemala	-0.60	12.61	25.57	24.74	22.60	23.64
El Salvador	-0.53	13.16	31.20	30.39	25.47	22.86
Paraguay	0.20	13.65	26.60	18.26	13.67	12.43
Uruguay	1.07	9.87	18.77	14.29	11.40	9.14
Jamaica	1.93	9.39	22.77	19.35	14.20	12.43
Nicaragua	-0.53	3.35	10.07	12.32	13.40	11.71
Cuba	N/A	N/A	N/A	N/A	N/A	N/A

Mobility
-1.27 — 60.00

Figure 3 – *Right panel: Number of new COVID-19 cases per million people per month in 19 countries in Latin America. Left panel: Change in time spent at home ("immobility index") per month, using the 5-week period from January 3 to February 6, 2020 as the baseline in 18 countries in Latin America (not including Cuba). Note that the greatest immobility occurred in April 2020 in most countries, in concordance with the abrupt drop in cardiac diagnostic procedures. On the other hand, the number of new COVID-19 cases per million people was still progressively rising from March to July 2020 in most countries.*

2020, SARS-CoV-2 had compromised all LATAM countries, with 7.15 million people affected.[5] The real number of cases may be even higher, since the number of tests per million remains low.[8,9]

Beyond the direct mortality caused by COVID-19, there were growing concerns regarding the consequences of the COVID-19 pandemic on health systems.[10-12] Fear of contagion in hospitals and health centers could have led to reluctance by patients to undergo cardiac diagnostic procedures. Additionally, elective interventions and consultations had to be postponed to prioritize COVID-19-related issues and avoid exposing patients to an unnecessary risk of infection in hospital or outpatient settings.[13]

LATAM frequently faces health problems that primarily affect the poor, added to health systems that are already fragile.[14,15] In such conditions, social distancing slows the peaking of the pandemic to allow countries with limited health resources to prepare for diagnosis and treatment of critically ill patients.[16,17] Nevertheless, according to Walker and colleagues, the LATAM countries that had the earliest first peak of the pandemic (Ecuador, Mexico, Brazil, Chile, Bolivia, Panama, Peru) or with unmitigated/limited health curves (Peru, Chile, Mexico, Ecuador) registered a higher death rate per million inhabitants.[18] This could be explained by the inability of these countries to prepare for the peak of the pandemic, finding their health systems vulnerable and producing high mortality figures. Similar events took place in European countries hit by the first wave of the COVID-19 pandemic, such as Italy and Spain.

Globally, approximately 70% of CVD deaths take place in low- and middle-income countries.[3,19] The long time interval between the start of social distancing measures and the first peak of the pandemic in LATAM countries limited patients' access to cardiac diagnostic procedures, further delaying diagnosis and timely treatment of CVD. In the LATAM population, this cascade of events may increase cardiovascular morbidity and mortality, as has been reported in Brazil.[20] This survey was not designed to collect outcome information, but the negative effect of diagnostic delays will likely be corroborated in future studies for this purpose. A similar conclusion was reached by the Latin American Society of Interventional Cardiology when surveying the practice of Interventional Cardiology during the COVID-19 pandemic, focusing on myocardial infarction.[21] They reported a 51.2% reduction in care for ST-elevation myocardial infarction

(STEMI), with the risk of increased mortality and/or morbidity following STEMI.

COVID-19 infection may be associated with cardiovascular events or mimic heart disease.[22-27] It is therefore essential, during the COVID-19 pandemic, to maintain the availability of all cardiac diagnostic modalities, in patients who are positive or negative for COVID-19.

There are several lessons we have learned for the future. We highlight five: 1) During a pandemic, access to cardiac diagnostic procedures should be maintained as much as possible for the entire population, regardless of the type of mobility restriction established in each country, strictly following the proper sanitary precautions. 2) Education campaigns should be established in the media and on social networks to explain to the community the importance of seeking help quickly in the face of warning signs of heart disease, while implementing measures to prevent the spread of COVID-19. 3) Non-COVID areas ("blue") for the care of non-COVID pathologies and COVID areas ("red") for infected patients should be established in health services. 4) It is necessary to guarantee worldwide access to health care supplies, from personal protective equipment to radiotracers.[28] 5) Governments should guarantee health services not only for COVID patients but also for non-COVID patients with CVD.

Limitations

The INCAPS COVID-19 survey evaluated data for the months of March and April 2020, when the pandemic was still in the initial phase in most LATAM countries. However, according to the evolution of lockdowns, the dates of economic reopening, and the mobility data for each country, April 2020 was the month with the least activity in these countries. The survey was carried out in a limited number of hospitals and diagnostic centers in each country, with variable participation, which could put the representability of the results in question. Nevertheless, the universal decrease in the number of non-invasive cardiac procedures carried out throughout LATAM suggests that our sample is representative. Finally, no long-term survey data to follow the entire pandemic curve was available at this point in time.

Conclusion

COVID-19 was associated with a significant and abrupt reduction in cardiac diagnostic procedures in LATAM, which was more related to social distancing measures than to the increase in disease incidence. Better balance and timing of social distancing measures and planning to maintain access to medical care in general and cardiovascular care in particular is warranted during a pandemic surge, especially in regions with high cardiovascular mortality.

Acknowledgements

The INCAPS COVID Investigators Group, listed by name in the Appendix, thanks cardiology and imaging professional societies worldwide for their assistance in disseminating the survey to their memberships. These include, alphabetically, but are not limited to, American Society of Nuclear Cardiology, Arab Society of Nuclear Medicine, Australasian Association of Nuclear Medicine Specialists, Australian and New Zealand Society of Nuclear Medicine, Belgian Society of Nuclear Medicine, Brazilian Nuclear Medicine Society, British Society of Cardiovascular Imaging, Conjoint Committee for the Recognition of Training in CT Coronary Angiography, Consortium of Universities and Institutions in Japan, Danish Society of Cardiology, Gruppo Italiano di Cardiologia Nucleare, Indonesian Society of Nuclear Medicine, Japanese Society of Nuclear Cardiology, Moscow Regional Department of the Russian Nuclear Medicine Society, Philippine Society of Nuclear Medicine, Russian Society of Radiology, Sociedad Española de Medicina Nuclear e Imagen Molecular, Society of Cardiovascular Computed Tomography, and Thailand Society of Nuclear Medicine.

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*Supplemental Materials

For additional information, please click here.

Confinement and Cardiovascular Diagnosis in a Pandemic Season: The Difficult Balance on the Razor's Edge

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Short Editorial related to the article: The Impact of COVID-19 on Diagnosis of Heart Disease in Latin America an INCAPS COVID Sub-analysis

The COVID-19 pandemic has had a strong impact on healthcare delivery worldwide. Cardiovascular diseases, including their acute forms, were no exception. In the first weeks of the pandemic, at the end of the first quarter of 2020, there was a clear reduction in resources to health services, both in scheduled care and in admissions for acute coronary syndromes, with a strong impact on the immediate prognosis and with future consequences not fully documented.[1,2]

In addition to the direct impact of the virus on the myocardium, which causes considerable morbidity and mortality,[3-6] there is also a reduction in access to health care - which occurred both as a result of a massive channeling of resources from the different health systems to the fighting the pandemic, as well as the population's fear of resorting to these services during the pandemic – is having an important impact on the diagnosis and treatment of cardiovascular disease. Although the real dimension of this impact on the prognosis of patients with and without COVID has not yet been established, its importance likely exceeds the effects directly determined by the pandemic.[7]

Early on, the cardiology community realized the impact that both the pandemic, more or less directly, and the measures used to combat it – namely mandatory confinements and social distancing – would have on the diagnosis of cardiovascular diseases.[8]

All over the world, the scientific community organized itself to promote a survey of the new reality triggered by the COVID-19 pandemic in terms of access to health care in the cardiovascular area. In addition to quantifying its real impact on the quality of services provided, this collective effort had the additional objective of identifying the most relevant factors conditioning health access. This knowledge allows a better understanding of the phenomenon and lays the foundations for developing strategies that can minimize its effects both in the next waves of this pandemic and in similar situations that may occur in the future.

Among the different groups that organized themselves in a few weeks to carry out this survey worldwide, the International Atomic Energy Agency stood out. Based on its pre-existing international network of clinical research – INCAPS – expanded its scope and implementation, having achieved a truly surprising turnout. With the active involvement of cardiologists, radiologists and physicians from other specialties dedicated to cardiovascular diagnosis from around the world, the INCAPS COVID consortium was able to obtain data regarding cardiac diagnostic volumes at the beginning of the pandemic (March and April 2020) as well as the March 2019 – which served as a representative comparison base for the pandemic loss. This data was integrated with social distancing data from Google's Community Mobility Reports and data on COVID-19 incidence by country from "Our World in Data." Using this strategy, it was possible to survey the reality at a global and regional level, allowing the assessment of the impacts felt in the different diagnostic modalities in cardiology, according to the region[8-11] and, comparing these data between different geographic areas.[12]

In this issue of ABC journal, Cerci RJ et al.[13] publish data from this group regarding the impact of the COVID-19 pandemic on the provision of care for cardiovascular diseases in Latin America. Cardiac diagnostic volumes were evaluated, and their relationship to COVID-19 case incidence and social distancing measures was determined using data from 194 centers in 19 Latin American countries.

Compared to March 2019, volumes of cardiac diagnostic procedures declined sharply (36% in March and 82% in April 2020), with small variations across Latin American subregions. The drop was more related to social distancing than to the increase in the incidence of COVID-19, with a greater reduction in the number of diagnostic procedures in the month of lower mobility (April) – which coincided with the strictest periods of confinement for each country. Interestingly, this percentage drop was higher than that seen in the same period in Western Europe (46% and 69%, respectively) and North America (39% and 68%, respectively). However, these regions were experiencing the first peak of incidence, while in Latin America, the pandemic just begining. This seems to be justified because most Latin American countries introduced social isolation as early as March 2020, despite the low number of cases. These data reinforce the importance of a better balance between social distancing measures and access to medical care during a pandemic outbreak. Reduced access to health care exacerbates pre-existing inequalities in the quality of services provided and can be particularly relevant in regions with high cardiovascular mortality.[14,15]

Keywords

Cardiovascular Diseases/physiopathology; COVID-19; Pandemics; Needs Assessment/statistics & numerical data; Research/trends; Medical Care/methods; Health Status Disparities

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Original Article

Sonothrombolysis Promotes Improvement in Left Ventricular Wall Motion and Perfusion Scores after Acute Myocardial Infarction

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Abstract

Background: **It has recently been demonstrated that the application of high-energy ultrasound and microbubbles, in a technique known as sonothrombolysis, dissolves intravascular thrombi and increases the angiographic recanalization rate in patients with ST-segment–elevation myocardial infarction (STEMI).**

Objective: **To evaluate the effects of sonothrombolysis on left ventricular wall motion and myocardial perfusion in patients with STEMI, using real-time myocardial perfusion echocardiography (RTMPE).**

Methods: **One hundred patients with STEMI were randomized into the following 2 groups: therapy (50 patients treated with sonothrombolysis and primary coronary angioplasty) and control (50 patients treated with primary coronary angioplasty). The patients underwent RTMPE for analysis of left ventricular ejection fraction (LVEF), wall motion score index (WMSI), and number of segments with myocardial perfusion defects 72 hours after STEMI and at 6 months of follow-up. $P < 0.05$ was considered statistically significant.**

Results: **Patients treated with sonothrombolysis had higher LVEF than the control group at 72 hours (50% ± 10% versus 44% ± 10%; $p = 0.006$), and this difference was maintained at 6 months of follow-up (53% ± 10% versus 48% ± 12%; $p = 0.008$). The WMSI was similar in the therapy and control groups at 72 hours (1.62 ± 0.39 versus 1.75 ± 0.40; $p = 0.09$), but it was lower in the therapy group at 6 months (1.46 ± 0.36 versus 1.64 ± 0.44; $p = 0.02$). The number of segments with perfusion defects on RTMPE was similar in therapy and control group at 72 hours (5.92 ± 3.47 versus 6.94 ± 3.39; $p = 0.15$), but it was lower in the therapy group at 6 months (4.64 ± 3.31 versus 6.57 ± 4.29; $p = 0.01$).**

Conclusion: **Sonothrombolysis in patients with STEMI resulted in improved wall motion and ventricular perfusion scores over time.**

Keywords: **Myocardial Infarction; Sonothrombolysis; Microbubbles; Contrast Media; Ventricular Function Left; Pulmonary Embolism.**

Introduction

In Brazil, cardiovascular diseases are responsible for approximately 28% of all deaths annually, half of which are due to acute coronary syndromes.[1] The therapies that are currently available for recanalization in acute myocardial infarction (AMI) include pharmacological fibrinolysis and percutaneous coronary intervention, which show improved prognosis of patients with AMI. Unfortunately, in Brazil, these techniques are only available to approximately 40% of the population. Nevertheless, if the patient is treated by one of these choice therapies, the phenomenon of no-reflow (extensive cellular death in the infarcted area) occurs in approximately 60% of patients treated.[2]

Restoring the patency of the coronary artery as quickly as possible is decisive, and it has important consequences in the results of improved quality of life and longevity, reduced hospitalization, and reduced costs to the health system.[3-6]

Sonothrombolysis is an innovative therapy, which consists of continuous intravenous infusion of microbubbles associated with intermittent application of high-energy ultrasound, resulting in rupture of the microbubbles and lysis of the intravascular thrombus.[7-9] A potential application of sonothrombolysis that has been demonstrated in experimental studies is recanalization of the coronary artery in the context of AMI.[9] In spite of a wide array of studies in animals, few studies have attempted to demonstrate the efficacy of sonothrombolysis in human beings. An initial attempt took place with the use of ultrasound alone in the recanalization of epicardial arteries in patients with AMI, which was

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unsuccessfully tested in the Perfusion by Thrombolytic and Ultrasound (PLUS) trial.[10] In an initial experience in a restricted number of patients, Slikkerveer et al. also demonstrated its feasibility and absence of complications in patients with AMI.[11] In a groundbreaking manner, our group has demonstrated, in 30 patients with ST-segment–elevation myocardial infarction (STEMI), that sonothrombolysis is a safe therapy and that it results in increased angiographic recanalization and improved coronary microcirculation.[12] More recently, we carried out the Microvascular Recovery with Ultrasound in Acute Myocardial Infarction (MRUSMI) trial, designed to investigate the clinical effects of the application of diagnostic ultrasound with a high mechanical index (MI), associated with microbubbles in 100 patients with STEMI, who were randomized into a control group and a therapy group of patients who received sonothrombolysis. This recently published study[13] demonstrated that the patients who were treated with sonothrombolysis before and immediately after primary coronary angioplasty showed greater pre-angioplasty coronary recanalization rate and reduced infarct size, as demonstrated by magnetic resonance.

Real-time myocardial perfusion echocardiography (RTMPE) is a technique that allows for simultaneous analysis of left ventricular wall motion and perfusion, which has been used for diagnosis and prognostic evaluation of patients with coronary artery disease.[14-17] As the effects of sonothrombolysis on long-term wall motion and perfusion scores have not yet been studied, we proposed an evaluation of the effect of sonothrombolysis on scores that measure wall motion and number of segments with myocardial perfusion defects 72 hours and 6 months after treatment of patients with STEMI, using RTMPE.

Methods

Study Protocol

The 100 patients in this study are part of the MRUSMI trial (ClinicalTrials.gov # NCT02410330), which was designed to investigate whether the application of high-MI impulses, from a diagnostic ultrasound transducer, during the infusion of commercially available microbubbles, in patients with STEMI, would increase early epicardial patency rates and microvascular flow.[13] This is a randomized, prospective clinical trial. Exclusion criteria for the study were prior AMI, known cardiomyopathy, significant valve disease, use of fibrinolytic therapy before arrival at the emergency department, allergy to Definity® echocardiographic contrast, and chest pain lasting more than 12 hours upon arrival.

From May 2014 to July 2018, 3479 patients with STEMI arrived at the emergency department of our institution. Of these, 303 met the inclusion criteria for the study protocol, and 100 arrived at a time when it was possible to apply the emergency diagnostic ultrasound before and after percutaneous coronary intervention (7:00 am to 7:00 pm, Monday through Friday), as shown in Figure 1. The 100 patients with STEMI were randomized, using a randomization website (www.random.org, randomization plan # 4544). Simple randomization was used. It was kept under the exclusive care of the nurse who coordinated the study, and it was unknown to all participants until the moment the patients accepted to participate in the study.

All patients received medical treatment in accordance with the institutional protocol, which follows STEMI treatment guidelines.[6] Patients in the therapy group (n = 50) received diagnostic ultrasound with multiple image-guided, high-MI impulses (1.8 MHz; MI 1.1 to 1.3; 3 μsec pulse duration) applied in the apical 4-, 2-, and 3-chamber views. The frame rate was 25 Hz. The ultrasound was performed with an infusion of commercially available microbubbles (5% Definity®) at 1.5 ml/min. The high-MI pulses were applied repeatedly during brief intervals, after low-MI images had detected microbubbles in the myocardial microvasculature. The intervals between high-MI impulses ranged from 5 to 15 seconds, depending on the time required for myocardial contrast replenishment. The patients in the control group (n = 50) underwent echocardiography with diagnostic images using a 1.8-MHz diagnostic ultrasound transducer with low-MI (0.18) images, frame rate of 25 Hz, and limited (no more than 3), high-MI diagnostic impulses to evaluate regional wall motion and microvascular perfusion before and after percutaneous coronary intervention. Ultrasound was performed with an infusion of commercially available microbubbles (5% Definity®) at 1.5 ml/min.

In order to evaluate wall motion score index (WMSI) and the number of segments with myocardial perfusion defects over time, all patients underwent RTMPE 72 hours after randomization and at 6 months of follow-up (Figure 2). Figure 3 illustrates an example of an image of the left ventricle in 2- chamber view with apical perfusion defect before the application of sonothrombolysis. After 15 minutes of sonothrombolysis, the myocardial perfusion defect disappeared.

This study was approved by the Research Ethics Committee of the Faculty of Medicine of the University of São Paulo (CAPPesq, acronym in Portuguese), under protocol number 0578/11. All procedures involved in this study are in accordance with the Declaration of Helsinki of 1975, updated in 2013. Informed consent was obtained from all participants included in the study.

Real-time myocardial perfusion echocardiography (RTMPE)

Echocardiography study was conducted with IE 33 equipment (Philips Medical Systems, Bothell, WA, USA), equipped with wide-band, 2-to-5–MHz, transthoracic transducers and myocardial perfusion software. In all studies, focus was set at the level of the mitral valve. The left ventricle was evaluated in 3 standard echocardiographic planes, namely, apical 4-, 2-, and 3-chamber views, defining 17 segments, as recommended by the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association.[18] All echocardiograms were analyzed on Q-Station 3.2.2 specific software (Philips Medical Systems, Bothell, WA, USA) after appropriate digital storage. For analysis of myocardial perfusion, echocardiography images were acquired with specific imaging software with

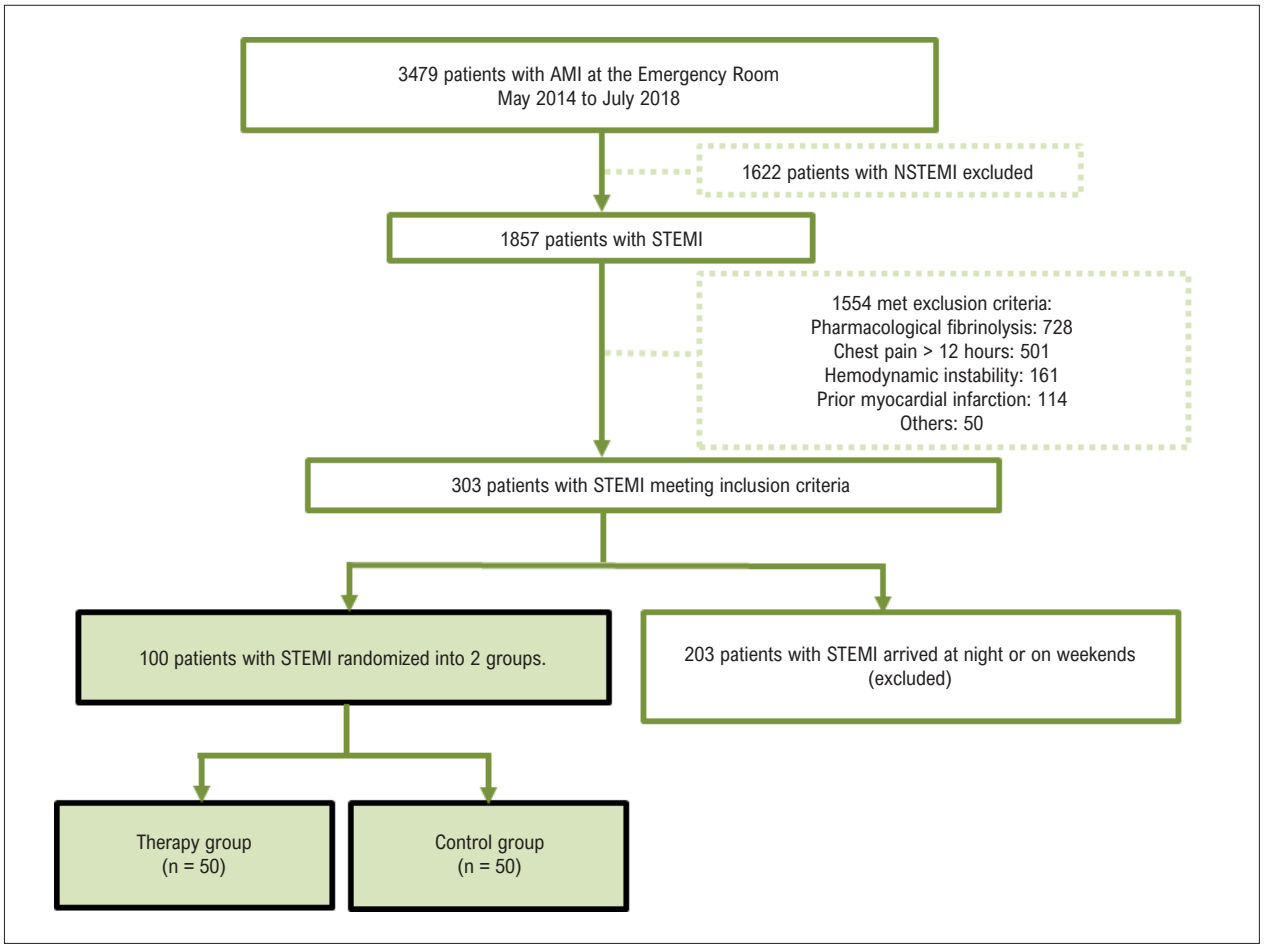

Figure 1 – *Flowchart of the Microvascular Recovery with Ultrasound in Acute Myocardial Infarction (MRUSMI) trial. AMI: acute myocardial infarction; NSTEMI: non–ST-segment–elevation myocardial infarction; STEMI: ST-segment–elevation myocardial infarction.*

real-time myocardial perfusion. The images were adjusted before injection of contrast to minimize the artifacts due to cardiac mobility. A sequence of ultrasound pulses, with the use of elevated MI, greater than 1.0 (flash), were manually fired at the peak of contrast intensity to destroy microbubbles within the myocardium. Then, images with low MI (0.1) were analyzed for at least 15 consecutive cardiac cycles to allow for subsequent myocardial replenishment. The patient showed angiographic recanalization. To measure signal strength via RTMPE, representative sequences of images preceding and following the flash image were digitally captured, stored on an optical disk, and analyzed at a later moment. Diagnostic low-MI images with ultrasound contrast were used to assess microvascular perfusion, regional wall motion, and left ventricular ejection fraction (LVEF), 72 hours after randomization and at 6 months of follow-up (Figure 2).

Evaluation of myocardial wall motion and perfusion

The contrasted images were used to calculate measurements of LVEF, end-diastolic volume, and end-systolic volume, using Simpson's biplane method, in accordance with the guidelines of the American Society of Echocardiography.[19] The WMSI was evaluated by analyzing the wall thickening of each myocardial segment in all 3 apical windows, highlighted by contrast. This score was calculated by summing the values attributed to each segment (1 = normal kinesia, 2 = hypokinesia, 3 = akinesia, and 4 = dyskinesia), divided by the total number of segments analyzed. Analysis of myocardial perfusion was carried out using a scoring system, where myocardial contrast replenishment within 4 seconds of application of high-MI impulse was assigned a value of 1; when complete replenishment in the risk area took more than 4 seconds after the application of high-MI impulse, a value of 2 (mild reduction) was assigned; and, when there was practically no myocardial contrast replenishment for 10 seconds after the high-MI impulse, a value of 3 was assigned. A score of 3 was considered microvascular obstruction.[16] For comparative analysis between the therapy and control groups, the number of myocardial segments with scores of 2 or 3 was evaluated at 72 hours after treatment and at 6 months of follow-up.

All evaluations of LVEF, wall motion, and microvascular perfusion were carried out by an experienced, independent echocardiographic reviewer (WMJ), who was blinded to

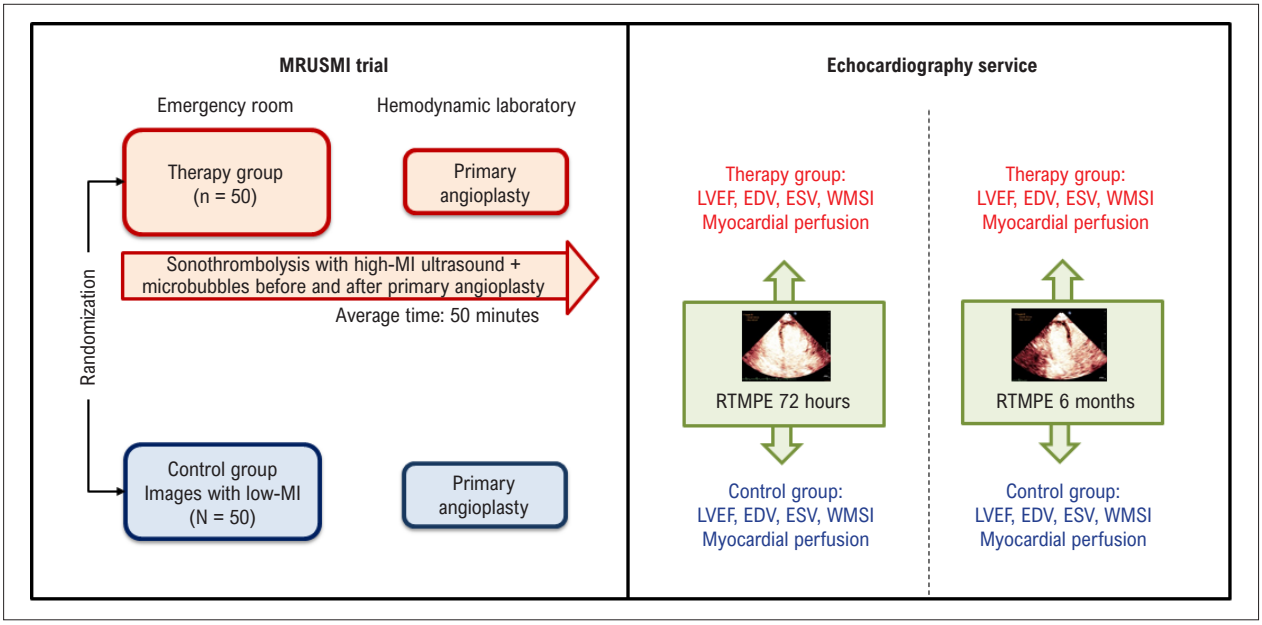

Figure 2 – *Study protocol. The evaluated patients participated in the Microvascular Recovery with Ultrasound in Acute Myocardial Infarction (MRUSMI) trial, randomized to receive treatment with sonothrombolysis associated with primary coronary angioplasty (therapy group) or conventional treatment with primary coronary angioplasty (control group). Patients in both groups underwent real-time myocardial perfusion echocardiography (RTMPE) 72 hours and 6 months after randomization, for evaluation of ventricular volumes, systolic function, and myocardial perfusion. EDV: end-diastolic volume; ESV: end-systolic volume; LVEF: left ventricular ejection fraction; MI: mechanical index; WMSI: wall motion score index.*

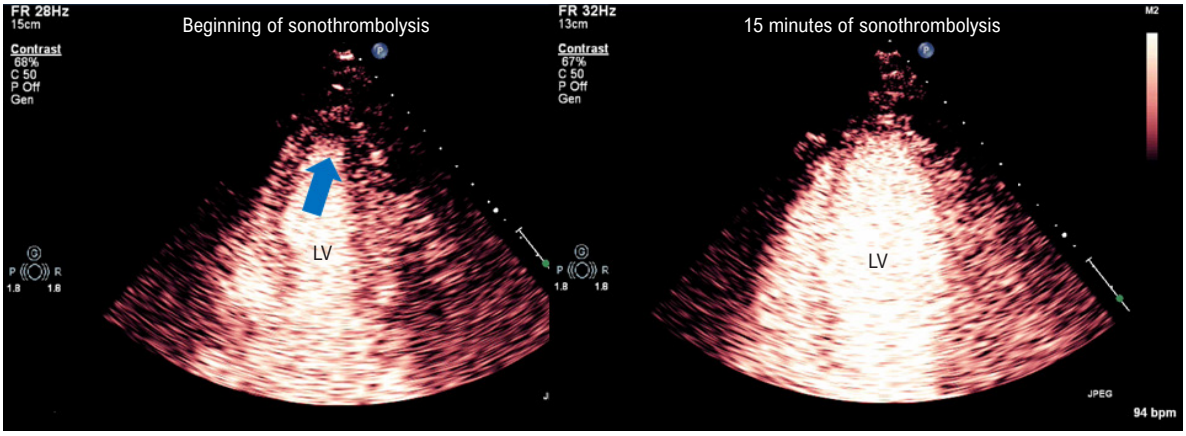

Figure 3 – *Real-time myocardial perfusion imaging demonstrating perfusion defect in the apical region of the left ventricle of a patient with ST-segment–elevation myocardial infarction before initiating sonothrombolysis (left image, arrow). After 15 minutes of sonothrombolysis, the myocardial perfusion defects disappeared. The patient showed angiographic recanalization with sonothrombolysis. LV: left ventricle.*

the treatment given at the moment of the measurements. The reviewer knew nothing about the randomization sequence, which was opened only after the conclusion of analyses of LVEF, wall motion, and microvascular perfusion. In a previously published study, intraobserver variability was validated for measurements of end-diastolic volume (intraclass correlation of 0.949; $p < 0.001$), end-systolic volume (intraclass correlation of 0.987; $p < 0.001$), and LVEF (intraclass correlation of 0.817; $p < 0.001$).[13]

Statistical Analysis

The sample calculation was based on data from the pilot study.[12] A sample size of 100 patients was calculated, including 20% possible losses, to achieve statistical significance of $p < 0.05$ and power of 80%, using comparative assumptions between the therapy and control groups, ST-segment resolution of 80% versus 50%, an increase in early angiographic patency of at least 50% versus 20%, and a 30% reduction in the infarcted area on magnetic resonance imaging.

Categorical variables were shown in tables describing their absolute (n) and relative (%) frequency, and chi-square test or Fisher's exact test was used to evaluate their association. Continuous variables were shown in tables describing their means and standard deviation. The Kolmogorov-Smirnov test evaluated whether distribution was normal. In both groups of randomized patients, changes in WMSI, number of segments with perfusion defects, and LVEF between 72 hours and 6 months were compared using the unpaired t test. Comparisons between 6 months and 72 hours, in the therapy and control groups, were made by paired Student t test. All analyses were carried out with the assistance of SPSS 17.0 for Windows. $P < 0.05$ was considered statistically significant.

Results

Average age of randomized patients was 59 years, and there were no differences in relation to sex between the study groups. There were also no differences regarding prevalence of diabetes, high blood pressure, dyslipidemia, and tobacco use (Table 1). The distribution of the STEMI arterial territory was similar in the control and therapy groups (Table 2).

Table 3 shows the values of ventricular volumes and LVEF in the total population and in the control and therapy groups, at 72 hours and 6 months after randomization. The group that received sonothrombolysis (therapy group) had lower end-diastolic and end-systolic volumes and higher LVEF than the control group 72 hours after STEMI. All patients underwent RTMPE at follow-up, and this difference was maintained at 6 months of follow-up.

There were no significant differences between the therapy and control groups in relation to WMSI at 72 hours (1.62 ± 0.39 versus 1.75 ± 0.40; $p = 0.09$), but, after 6 months of follow-up, the therapy group evolved with lower WMSI than the control group (1.46 ± 0.36 versus 1.64 ± 0.44; $p = 0.02$), as shown in Figure 4. The decreased WMSI values demonstrate improved left ventricular function. In relation to myocardial perfusion obtained by RTMPE, no differences were observed between the number of segments with perfusion defects between the therapy and control groups 72 hours after STEMI (5.92 ± 3.47 versus 6.94 ± 3.39; $p = 0.15$), but, at 6 months of follow-up, the therapy group showed a lower number of segments with perfusion defects than the control group (4.64 ± 3.31 versus 6.57 ± 4.29; $p = 0.01$), as shown in Figure 5. In the mean period of 17 months, 8 patients (16%) died in the control group, and 8 patients (16%) died in the therapy group.

Discussion

This is the first study in human beings to evaluate the effects of sonothrombolysis on left ventricular function and perfusion at 6 months of follow-up after STEMI. Using RTMPE, we have demonstrated that patients with STEMI treated with this novel therapy showed improvements over time with respect to WMSI and the number of segments with myocardial perfusion defects. The results of the MRUSMI trial demonstrated that door-to-balloon times were not different between the control and therapy groups (78 ± 32 minutes versus 77 ± 26 minutes, respectively; $p = 0.42$). Recanalization of the culprit vessel at first angiography before primary angioplasty was observed in 24/50 (48%) patients of the therapy group in comparison with 10/50 (20%) in the control group ($p < 0.001$). On magnetic resonance conducted 72 hours after STEMI, the therapy group showed lower infarct size than the control group (29 ± 22 grams versus 40 ± 20 grams; $p = 0.026$).[13]

These beneficial effects were evident on the microvascular level, with improved capillary flow observed immediately after percutaneous coronary intervention. Prior to this, the behavior of the WMSI and the number of segments with myocardial perfusion defects over time had not been evaluated. Our results confirm that the early recanalization and improved coronary microcirculation obtained with sonothrombolysis have additional benefits for patients with STEMI, when compared to patients who received conventional treatment with primary angioplasty.

High-energy transthoracic ultrasound has been studied as an adjunctive treatment to fibrinolytic drugs in the approach to arterial thrombi and as an isolated method for treating vascular thrombi.[20,21] A proposed mechanism for explaining how ultrasound dissolves thrombi is by inducing cavitation.[22,23] Cavitation is the ultrasonic generation of gas bodies that expand and retract. This leads to shear forces that disrupt the environment, with the potential to rupture thrombi. Studies using catheter-based systems capable of releasing ultrasound into the coronary artery have proven that they were able to dissolve thrombi without the use of a fibrinolytic agent. This type of high-energy, low-frequency ultrasonic system, with 45 kHz, delivered through the tip of a 1.6-mm catheter has been shown to successfully recanalize the anterior descending artery of patients who had AMI of the anterior wall.[24] In order to overcome the limitations of ultrasound in acute coronary syndromes, experimental studies have demonstrated that the association of administration of microbubbles under the effect of ultrasound can accelerate thrombi dissolution. Gas microbubbles are small microspheres that have specific acoustic properties that make them highly useful as ultrasound contrast agents for diagnostic imaging. Because they act as cavitation nuclei, microbubbles reduce the peak negative pressure threshold required to induce cavitation. In this manner, the destruction of microbubbles mediated by ultrasound may further accelerate thrombi dissolution. In animal models of thrombosed iliofemoral arteries, low-frequency transcutaneous ultrasound associated with intravenously injected microbubbles produced recanalization rates above 90%, without requiring a thrombolytic agent.[25] In a preclinical study of 45 pigs, it was demonstrated that, during a continuous intravenous infusion of microbubbles containing perfluorocarbon gas, the ultrasonic energy emitted by a diagnostic ultrasound transducer was able to restore microcirculation flow and improve coronary artery recanalization rates.[26] The randomized clinical PLUS trial, which attempted to evaluate the additional value of therapeutic ultrasound alone, without microbubbles, in patients with AMI, was interrupted.[10] Interruption of the study was recommended in July 2003 due to the low likelihood of significant differences in coronary flow grade on the Trombolysis in Myocardial Infarction score or ST-segment resolution with treatment by

Table 1 – Clinical characteristics of patients in the control and therapy groups

Variables	Total	Groups		p
		Control	Therapy	
Age (years)	59.06 ± 10.39	59.04 ± 11.01	59.08 ± 9.85	0.985(1)
Height (cm)	167.70 ± 8.47	169.04 ± 8.30	166.36 ± 8.51	0.114(1)
Weight (kg)	75.49 ± 16.23	76.61 ± 16.32	74.40 ± 16.24	0.501(1)
BSA (m2)	1.84 ± 0.22	1.87 ± 0.22	1.82 ± 0.22	0.313(1)
Male sex	72 (72.0%)	40 (80.0%)	32 (64.0%)	0.075(2)
Prior PCI	8 (8.0%)	3 (6.0%)	5 (10.0%)	0.715(3)
Tobacco use	44 (44.0%)	20 (40.0%)	24 (48.0%)	0.20(2)
Dyslipidemia	35 (35.0%)	15 (30.0%)	20 (40.0%)	0.295(2)
Diabetes	32 (32.0%)	11 (22.0%)	21 (42.0%)	0.032(2)
Hypertension	56 (56.0%)	28 (56.0%)	28 (56.0%)	1.000(2)
Medication use				
Aspirin	98 (98.0%)	50 (100.0%)	48 (96.0%)	0.495(3)
Statins	33 (33.0%)	14 (28.0%)	19 (38.0%)	0.288(2)
Nitrate	52 (52.0%)	25 (50.0%)	27 (54.0%)	0.689(2)
Betablocker	19 (19.0%)	5 (10.0%)	14 (28.0%)	0.022(2)
Calcium channel blocker	9 (9.0%)	4 (8.0%)	5 (10.0%)	1.000(3)
ACEI	20 (20.0%)	9 (18.0%)	11 (22.0%)	0.617(2)

Variables expressed as mean ± standard deviation or number (%). (1)Unpaired Student's t test; (2)Chi-square test; (3)Fisher's exact test. ACEI: angiotensin-converting enzyme inhibitors; BSA: body surface area; PCI: percutaneous coronary intervention.

Table 2 – Distribution of the ST-segment–elevation myocardial infarction arterial territory

Variables	Control group	Therapy group	p value
ADA	26 (52%)	26 (52%)	0.83(1)
RCA	14 (28%)	17 (34%)	
CXA	10 (20%)	7 (14%)	

Variables expressed as number (%). (1)Chi-square test. ADA; anterior descending coronary artery; CXA: circumflex artery; RCA: right coronary artery.

ultrasound. We now know that a possible cause of the study's failure was the fact that it did not associate intermittent ultrasound with microbubbles. Without the latter, there is not enough tissue inertial cavitation and release of nitric oxide to promote sonothrombolysis and reduce no-reflow effectively.[10] Mathias et al.,[12] in 2016, published a pilot study evaluating 30 patients, demonstrating the safety and feasibility of the application of high-MI ultrasound and continuous infusion of microbubbles for early recanalization and improved coronary microcirculation in patients with STEMI.[12] These findings were confirmed in the MRUSMI trial, increasing the population to 100 patients.[13] The high-MI impulses used to improve epicardial and microvascular recanalization in this study are part of a standard resource in an ultrasound system that is normally used to evaluate myocardial perfusion and regional wall motion.[14-17] These high-MI impulses cause cavitation in microbubbles (increase and collapse) during the period of insonation that finally ruptures them.[9] This growth and collapse cause shear tension in regions close to the microbubbles, which, in the case of a thrombus, results in its dissolution.

The reasons as to why sonothrombolysis can result in improved WMSI and myocardial perfusion in 6 months can be associated with several factors that are not yet fully known. The main factor seems to be the early recanalization of the coronary arteries, before performing percutaneous coronary intervention, as observed in the MRUSMI trial (48% in the therapy group versus 20% in the control group). Moreover, a smaller infarcted area was observed on magnetic resonance at 72 hours in the therapy group. Another possible effect could be related to the induction of nitric oxide release.[27] Future multicenter studies are needed to clarify the pathophysiological mechanisms and to prove the benefits of sonothrombolysis in patients with acute coronary syndromes. It is worth underscoring the potential of this new therapeutic option for treating acute thrombotic conditions.[28,29]

Table 3 – Volumes and ejection fraction obtained by real-time myocardial perfusion echocardiography at 72 hours and 6 months after randomization

Variables	Total	Groups		p (between control and therapy groups)
		Control	Therapy	
72 hours				
EDV (mL)	108 ± 35	114 ± 40	102 ± 29	0.096(1)
ESV (mL)	59 ± 30	66 ± 34	53±23	0.022(1)
LVEF (%)	47 ± 11	44 ± 11	50±10	0.006(1)
WMSI	1.68 ± 0.39	1.75 ± 0.40	1.62 ± 0.39	0.09(1)
Number of segments with perfusion defects	6.42 ± 3.49	5.92 ± 3.47	6.94 ± 3.39	0.15(1)
6 months				
EDV (mL)	122 ± 47	136 ± 52*	109 ± 36	0.003(1)
ESV (mL)	66 ± 39	76 ± 45*	55 ± 29	0.006(1)
LVEF (%)	50 ± 12	47 ± 12*	53 ± 10*	0.008(1)
WMSI	1.52 ± 0.37	1.64 ± 0.44*	1.46 ± 0.36*	0.02(1)
Number of segments with perfusion defects	5.86 ± 3.84	6.57 ± 4.29	4.64 ± 3.31*	0.01(1)

*Variables expressed as mean ± standard deviation. (1)Unpaired Student's t test between control and therapy groups. *p < 0.05 on paired Student's t test (comparison of parameters between 6 months and 72 hours). EDV: end-diastolic volume; ESV: end-systolic volume; LVEF: left ventricular ejection fraction; WMSI: wall motion score index.*

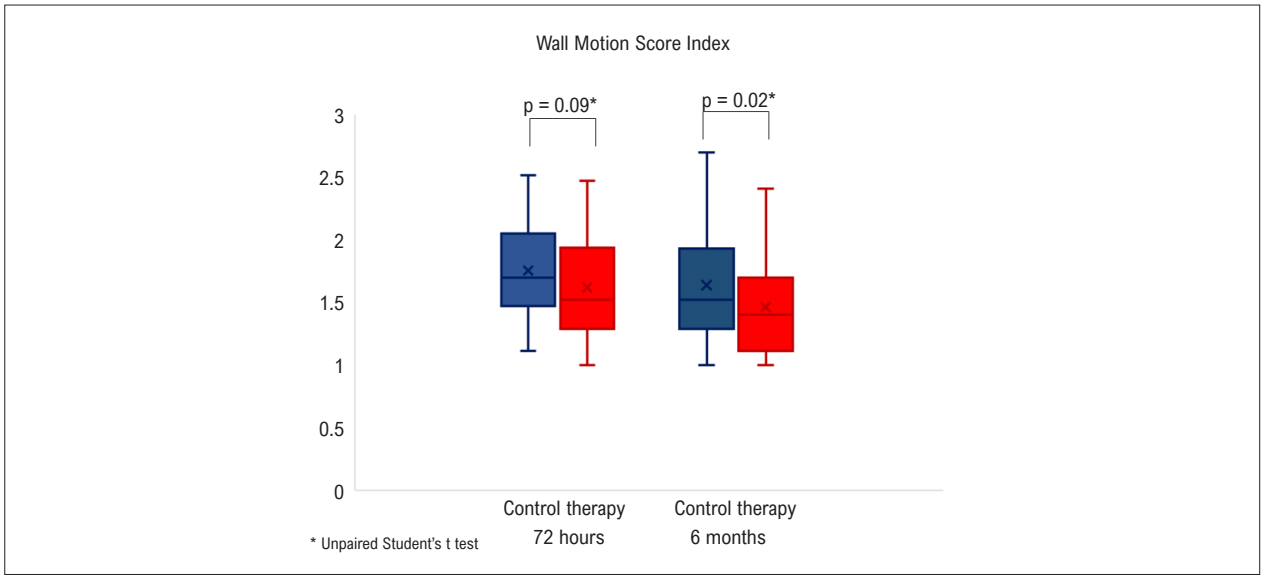

Figure 4 – *Wall motion score index in the control and therapy groups evaluated by real-time myocardial perfusion echocardiography 72 hours and 6 months after randomization.*

Study limitations

Given that this study was a sub-analysis of the MRUSMI trial, the remaining data pertaining to results related to angiography, electrocardiography, and cardiac biomarkers have been previously reported. Our results were limited to the findings of RTMPE, with a focus on analysis of WMSI and the number of segments with myocardial perfusion defects. That notwithstanding, we emphasize the novelty of these findings, as well as the importance of these effects at 6 months of follow-up in patients with STEMI who were treated by sonothrombolysis. This is a single-center study, with a small number of patients, and it should be extended to multicenter evaluations in order to confirm the findings of this pioneering study. Another point that could be raised as a study limitation is regarding the fact that analysis of wall motion and myocardial perfusion may be considered to be subjective; however, we highlight the widespread application of these scores in routine echocardiography

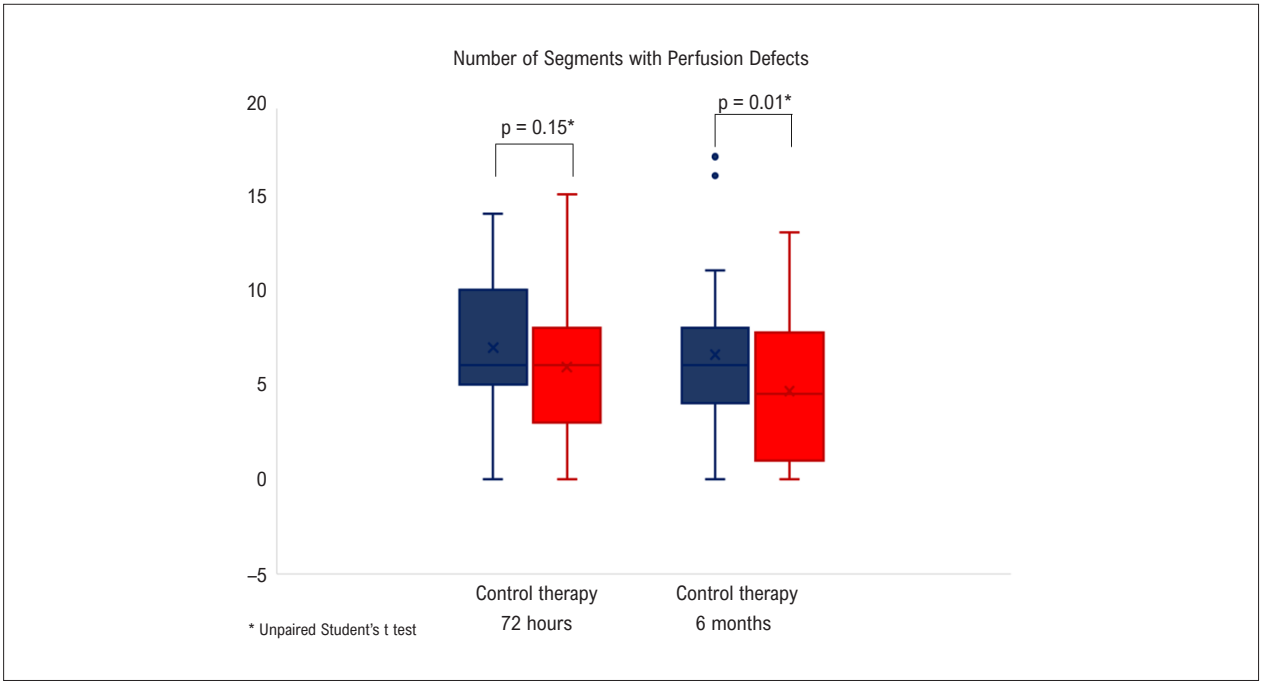

Figure 5 – *Number of segments with perfusion defects in the control and therapy groups evaluated by real-time myocardial perfusion echocardiography 72 hours and 6 months after randomization.*

practice, as well as the fact that the researchers involved in this study have ample experience with the technique of RTMPE.[30-32]

Conclusion

Sonothrombolysis is a new therapeutic approach for treating patients with STEMI that results in improved left ventricular wall motion score and reduced perfusion defects over time.

Author Contributions

Conception and design of the research: Tsutsui J, Sbano J, Ramirez J, Kalil Filho R, Mathias W; Acquisition of data: Tavares BG, Aguiar MO, Oliveira M, Soeiro AM, Nicolau J, Ribeiro H, PoChiang H; Analysis and interpretation of the data: Tavares BG, Sbano J, Rochitte CE, Lopes B, Mathias W; Obtaining financing: Mathias W; Writing of the manuscript: Tavares BG, Tsutsui J, Soeiro AM, Mathias W; Critical revision of the manuscript for intellectual contente: Tsutsui J, Nicolau J, Ribeiro H, Ramirez J, Kalil Filho R, Mathias W.

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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Therapeutic Echocardiography

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Short Editorial related to the article: Sonothrombolysis Promotes Improvement in Left Ventricular Wall Motion and Perfusion Scores after Acute Myocardial Infarction

Since its introduction by Edler and Hertz, echocardiography has become the main test of cardiologic propedeutic. Over the last 50 years, its evolution has been remarkable, from A-mode, M-mode, two-dimensional and Doppler images to modern three-dimensional reconstructions and softwares for studying myocardial deformation currently used. For this reason, echocardiography can provide diagnostic data and important prognostic information in virtually all heart diseases.[1]

One of the major advances in echocardiography was the recognition of substances capable of increasing the ultrasound signal for better visualization of cardiac structures, popularly known as "echocardiographic contrasts." Its beginning dates back to the late 1960s, with the first experience reported on the use of agitated saline solution associated with cardiac ultrasound.[2] This technique (agitation of a saline solution so that it is aerated, allowing an increase in sound reflection) is still used to detect intracardiac communications. Still, it has as drawbacks the poor intravascular stability of the solution and the fact that air bubbles are eliminated from the body during the passage of blood through the lungs, thus preventing its use for visualization of structures of the left heart when the agitated saline solution is administered in a peripheral vein.[3]

However, the emergence of industrially manufactured second-generation echocardiographic contrast media consolidated the use of these substances in echocardiographic practice. These compounds correspond to stabilized microparticles of gas, which, once inside the intravascular environment, do not change blood circulation, as they are smaller than red blood cells and capillaries. In addition, they are stable and can be injected into a peripheral vein, passing intact through the pulmonary circulation, allowing left ventricular opacification and the detection of myocardial perfusion.[4-6] These characteristics give high security to ultrasound contrast media, associated with a great improvement in image quality, increasing diagnostic accuracy.[7]

The ability of current echocardiographic contrast media to allow clearer visualization of the separation between the blood and the adjacent myocardium is due to a property of these molecules, which undergo volumetric oscillation when subjected to ultrasonic energy. This fact accentuates the reflection of sound through the return to the emitting source of sound waves in their fundamental frequency and harmonic frequencies. On the other hand, high-intensityultrasonic energy pulses applied to these contrasts lead to inertial cavitation, that is, violent expansion and collapseof these microparticles, generating local pressure oscillationand strong shock waves in the intravascular environment. Inertial cavitation is the physical basis for the phenomenon of sonothrombolysis, which corresponds to the capacityof high-energy ultrasonic pulses, associated with the presence of echocardiographic contrast, to lead to thrombus dissolution.[8] This possibility was first demonstrated in animal models.[9,10] Subsequently, it was shown that sonothrombolysis helped dissolve thrombi and acted on the vascular endothelium and microcirculation, releasing vasodilating substances, including nitric oxide.[11] This knowledge was the foundation for using this technique in clinical studies.

The group of Professor Mathias and his collaborators pioneered the development of randomized trials to test sonothrombolysis as an adjuvant treatment in acute myocardial infarction - AMI.[12-14] These studies showed that it was possible to use commercially available ultrasound equipment and echocardiographic contrast media in patients with ST-segment elevation AMI randomized to receive or not sonothrombolysis, as an adjuvant treatment to primary angioplasty, to reduce the phenomenon of microvascular obstruction,[12] increase the rate of recanalization of epicardial vessels and decrease the size of the necrotic area,[13] as well as decrease ventricular remodeling and improve long-term myocardial function.[14]

In this edition of the *Arquivos*, a new piece of knowledge was introduced by this same group after the publication by Tavares B. et al.[15] Using a group of 100 patients randomized 1:1 to primary angioplasty or angioplasty and sonothrombolysis, the authors demonstrated an improvement in the group undergoing sonothrombolysis in the echocardiographic index of motility and left ventricular perfusion in an echocardiographic evaluation performed 6 months after the AMI. These two echocardiographic indices used as endpoints are known to define the prognosis in coronary heart disease, and their improvement in the group undergoing sonothrombolysis reflects the method's ability to help the dissolution of thrombi in epicardial coronary vessels, but also to improve microcirculation.[16,17] An important aspect that we must emphasize is that the work by Tavares B. et al.[15] has the unique characteristic of demonstrating the usefulness of using echocardiographic

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contrast in all its range of functions: ventricular opacification, myocardial perfusion and, finally, antithrombotic therapy.

Sonothrombolysis breaks a paradigm of cardiology. Certainly, not even Edler and Hertz imagined the scope that echocardiography would reach, which now leaves the merely diagnostic scope, to become an important therapeutic adjuvant. In a country that suffers intensely from the impact of obstructive coronary disease (AMI corresponded to 7.06% of total deaths in 2017)[18] and where reperfusion therapies that are known to be effective (angioplasty and fibrinolysis) are not yet available to a large part of the population in an adequate time (about a quarter of patients with ST-segment AMI arrive at the hospital with more than 6 hours of pain),[19] the emergence of a new adjuvant therapeutic alternative can have a strong impact on the health of the population. Certainly, many questions still need to be answered: what is the impact of sonothrombolysis on mortality after AMI? Is there a benefit also for patients who underwent only fibrinolysis? Is there a benefit for coronary syndromes without ST-segment elevation? Can sonothrombolysis be started immediately after pain, still in the pre-hospital transport service? This path of knowledge will be long and will take some years. However, the work of Tavares B.,[15] published in this edition of the *Arquivos* represents an important contribution.

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Review Article

Plasma Ceramides in Cardiovascular Disease Risk Stratification

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Abstract

Ceramide production takes place throughout the body and plays a key role in the maintenance of normal physiology. However, ceramide levels are altered during disease states, particularly considering the development of diabetes and dyslipidemia.

Ceramide production is also associated with atherosclerotic plaque instability. Recent studies revealed that patients with unstable coronary artery disease (CAD) presented increased plasma ceramide levels (especially C16, C18, and C24:1). These molecules are currently considered emerging biomarkers of cardiovascular diseases (CVD), being used for predicting atherosclerotic plaque instability and adverse cardiovascular events independently from traditional risk factors.

With the aim of describing and discussing the role of ceramides in the stratification of cardiovascular diseases, this narrative review contextualizes the importance of this biomarker in the present cardiology scenario.

Introduction

Data from the World Health Organization (WHO) show that, out of the 50 million deaths recorded in the last decade, cardiovascular diseases (CVD) were responsible for a significant percentage: around 17 million people.[1] This mortality is particularly high in the acute phase following an acute myocardial infarction (AMI), with a 10%–15% recurrence of ischemic events within a year and cumulative rates of up to 50% in 10 years.[2]

Approximately 50% of the patients who undergo primary percutaneous coronary intervention have multivessel disease, usually manifested as a progressive chronic condition with high mortality rates. Currently, it is not possible to precisely prevent the recurrence of acute ischemic events, clearly demonstrating the need for biomarkers that may predict coronary atherosclerotic plaque instability.[3]

Recent studies have highlighted the pathophysiological role of classes of lipids other than LDL-cholesterol in atherosclerosis and AMI, including ceramides, sphingomyelin, phosphatidylcholines, and cholesterol esters.[4,5] Ceramides participate in multiple pathways involved in cellular damage signaling, liberating proinflammatory cytokines that directly modulate apoptosis via the expression of proapoptotic proteins.[6]

Our group has worked, throught mass spectometry, among other molecular techniques, for developing a plasma biomarker capable of diagnosing atherosclerotic plaque instability and predicting reinfartion and progression to Heart Failure (HF) in patients with Acute Coronary Syndrome (ACS).

Ceramides are lipid biomarkers with an emerging role in early diagnosis and risk stratification, acting as marker of primary and secondary cardiovascular (CV) events in patients with clinical and subclinical atherosclerosis who are susceptible to acute ischemic events.[5,7]

Ceramide: a brief review of the physiology of this new lipid biomarker

Ceramides and LDL-cholesterol are structural lipids that maintain membrane fluidity and integrity through the formation of selective pores, modulating the movement of compounds between intra and extracellular spaces. Ceramides are sphingolipids formed by a sphingosine molecule and a fatty acid, being key components in the formation of cellular membranes[8] and acting as an

Keywords

Cardiovascular Diseases; Coronary Artery Disease; Ceramides/therapeutic use; Diseases Stratification; Diabetes Mellitus Dyslipidemias; Biomarkers

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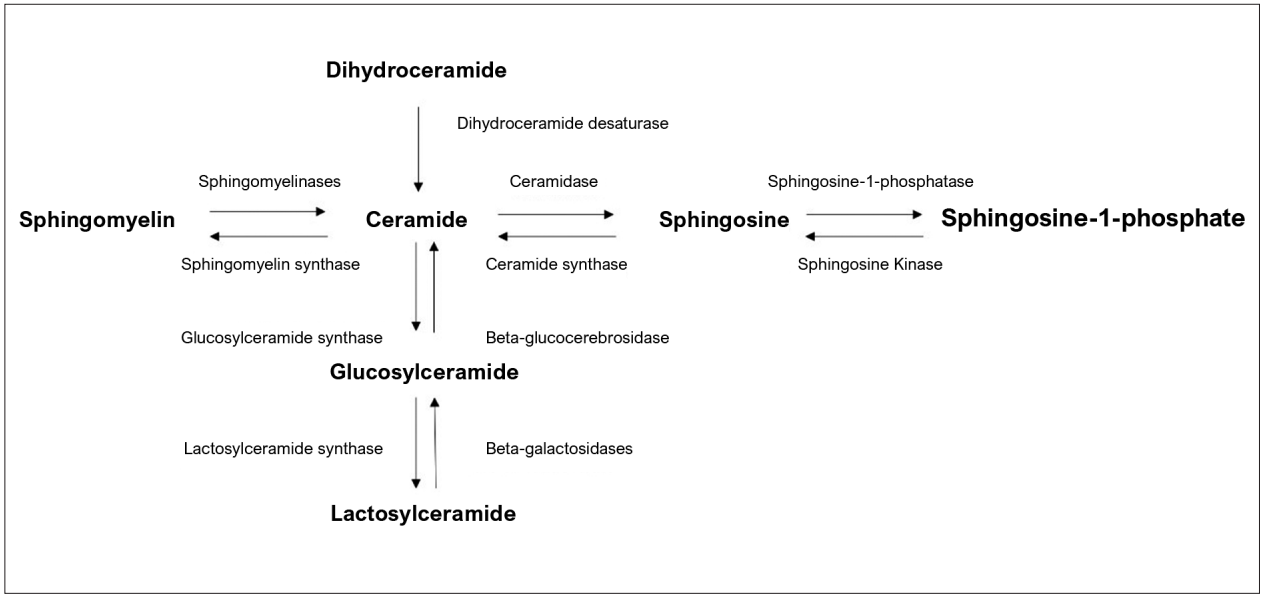

Figure 1 – *Sphingolipid metabolic pathways.*

important signaling intermediate in processes that regulate cell homeostasis, such as inflammation, apoptosis, and cellular stress response.[9]

Ceramides accumulate in the coronary atheroma,[10] and their glycosylated forms glucosylceramides and lactosylceramides (Figure 1) are abundant in the developing plaque.[11,12] Moreover, data from our group showed that the myocardial tissue itself may produce ceramides in a direct response to ischemia and reperfusion.[13]

Ceramidase is an enzyme that cleaves fatty acids from ceramide for producing sphingosine, which is in turn phosphorylated by sphingosine-1-phosphatase for producing sphingosine-1-phosphate. Ceramide synthesis may occur through the hydrolysis of sphingomyelin (degradation), the salvage (recycling) pathway where sphingosine is phosphorylated, or the de novo pathway where dihydroceramides are desaturated.[9]

Ceramides: a link between atherosclerosis, diabetes, and dyslipidemia

Ceramides constitute approximately 30% of circulating LDL-cholesterol. An increase in ceramide concentration alters cell membrane permeability, enabling the accumulation of LDL-cholesterol on blood vessel walls. This build-up amplifies the inflammatory process on vessel walls, promoting the apoptosis of vascular smooth muscle cells and endothelial dysfunction, which leads to atherosclerotic plaque instability and rupture[14] (Figure 2).

In addition to the atherosclerotic plaque, this build-up occurs in smooth and skeletal muscles, interfering with the expression of glucose transporter type 4 (GLUT4); this causes a defect in muscle glucose uptake and hinders glycogen synthesis.[15] Ceramides also stimulate the apoptosis of pancreatic β cells, directly reducing insulin production.[16] Major et al.[17] demonstrated that ceramides can mimic the cytotoxic effects of TNF-α, IL-1β, and IFN-γ in pancreatic β-cells, triggering inflammation and apoptosis.[17] Ceramide accumulation in tissues results in metabolic dysfunction in multiple organs and diabetes complications. Figure 3 shows the main tissues affected by ceramides.[18,19]

Therefore, the measurement of these molecules helps the physician determine not only the degree of dyslipidemia and risk of atherosclerosis through LDL-cholesterol levels, but also insulin resistance and β-cell apoptosis through ceramide levels.

Clinical implications led to a patent of this biomarker in the United States and Europe, being currently available for clinical use at referral hospitals such as Mayo Clinic.[20]

Are screening examinations necessary for the follow-up of subclinical atherosclerosis?

There is a significant gap in the detection of subclinical atherosclerosis and the cost-effective triage and follow-up of this entity, with various noninvasive tests being routinely requested in populations at different levels of CV risk. This is a reality worldwide, and despite the increasing advances in noninvasive tests for detecting atherosclerosis, risk stratification remains imperfect.[21] Considering the escalating costs of health care, this stereotyped medical practice should thus be reevaluated.

The association of clinical and imaging data for supporting the development of protocols that incorporate classical risk scores such as Framingham and Interheart has been proposed for improving CV risk stratification.

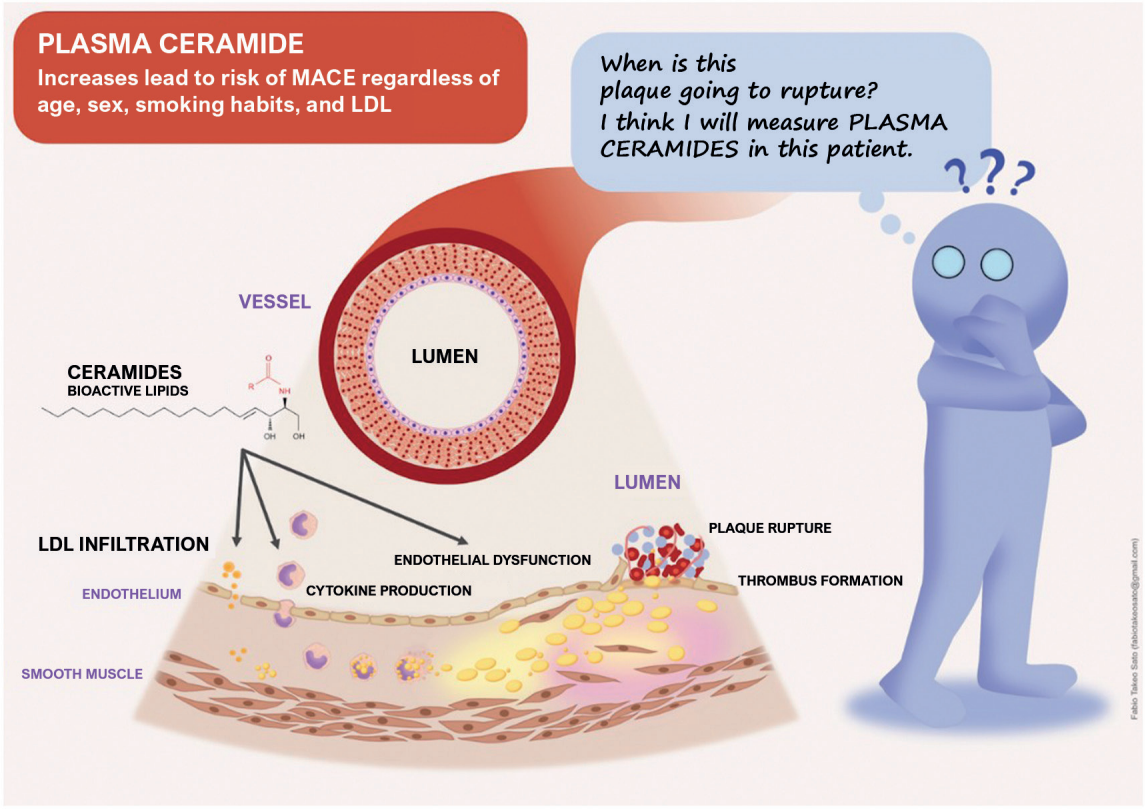

Figure 2 – *Plasma ceramides and plaque rupture. Source: author's collection.*

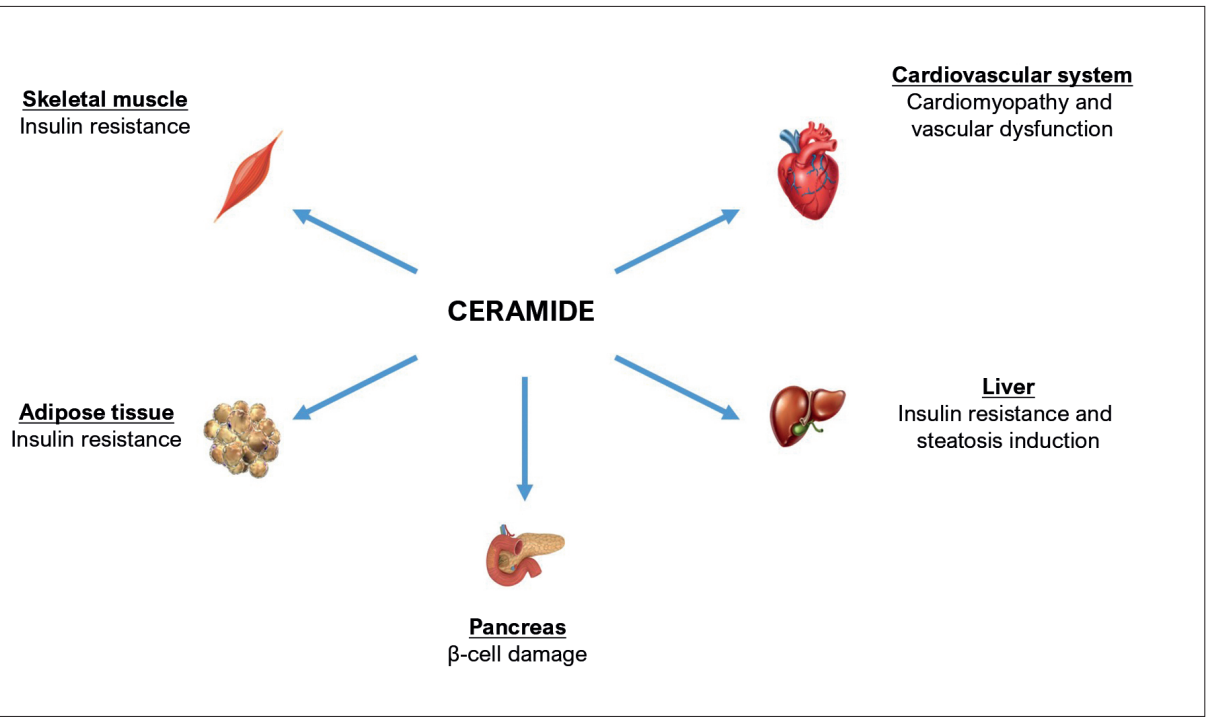

Figure 3 – *Effect of ceramides on different organs.*

However, these methods, when applied to the general population without a triage step, have limited capacity for assessing CV risk due to aspects related to logistics and costs.[22]

The Multi-Ethnic Study of Atherosclerosis (MESA) study, one of the most relevant in this area, brought important contributions for comprehending the development and progression of CVD from subclinical to clinical stage. In a sub analysis of the MESA study, a large protein biomarker panel was evaluated in the search for predictive markers of CVD progression. When compared to classical CV risk factors, protein biomarkers with various specific panels for inflammation, insulin resistance, lipids, hemostasia, fibrinolysis, oxidative damage, and endothelial stress, among others, had incremental predictive values for long-term adverse CV events that were only borderline (area under the receiver operating characteristic curve [AUC]: 0.768 vs 0.776, p = 0.003) and did not reach an incremental predictive value in the prediction of medium-term adverse CV events (AUC: 0.795 vs 0.796, p = 0.627). Considering these similar ROC curve values, new screening and risk stratification methods are thus required to improve early detection of plaque instability.[23]

Once developed, such a biomarker would enable interventions at the beginning of atherosclerotic plaque progression, being essential for avoiding the large additional costs of atherosclerosis' symptomatic stage. These considerations are important and should be evaluated when developing platforms for population health screening and seeking adequate cost-effectiveness.

Should we measure ceramides and LDL-cholesterol?

Subclinical atherosclerosis precedes most CV events, and its detection may improve CV risk stratification.[11,12] However, an incompatibility has been reported between apparently benign conventional risk factor profiles and subclinical atherosclerosis detected by coronary artery calcification (CAC) or intima thickness measured at the carotid ultrasound.[13,14]

Studies have identified subclinical atherosclerosis in almost 60% of middle-aged individuals at low CV risk according to traditional risk scores, and multiple affected vessel sites were found in 41% of these individuals.[15] These findings suggest that variables other than conventional CV risk factors may play relevant roles in atherogenesis.

Patients with atherosclerosis consist in a very heterogeneous population, with complex risk stratification, and should not be all considered as being at similar risk for acute events. The use of CV risk scores is currently recommended by guidelines as a stratification tool; however, due to limitations in predictive precision especially in patients at high CV risk, the optimization of risk tools by recalibrating scores or their association with biomarkers is frequently needed for a higher predictive accuracy in different populations.

LDL-cholesterol is a risk factor directly involved in the development of atherosclerotic plaque, therefore being an important therapeutic target in clinical practice. Dieting, lifestyle changes, and use of medications can result in significant and sustained reductions in plasma LDL-cholesterol levels. However, CVD are still one of the main causes of death worldwide,[24] suggesting that conventional LDL-cholesterol control is not enough; the early detection and prevention of atherosclerotic plaque instability may thus open the way for significantly reducing disease progression.

Current guidelines endorse LDL-cholesterol control and the measurement of nonspecific inflammatory markers such as C-reactive protein (CRP) in CV risk stratification. However, the pathophysiology of atherosclerosis involves the complex intersection of dyslipidemia, inflammation, endothelial dysfunction, platelet activation, and other factors.[25] Recent data demonstrate possible associations between each of these pathways and plasma ceramide levels, indicating an association and plausible causality of this biomarker in acute CVD and atherosclerotic plaque instability.

Moreover, plasma LDL-cholesterol does not precisely predict major adverse cardiac and cerebrovascular events (MACCE), requiring a thorough medical assessment and a series of noninvasive tests in clinical practice for following up on the progression of atherosclerotic plaques.[26] On the other hand, plasma ceramide concentrations are increased in various conditions related to cardiac diseases, in addition to their biochemical role in the progression of atherosclerosis, which has also been studied by our group. Preliminary studies have showed an increase in ceramides in the acute phase of AMI and in vulnerable plaques in humans, correlating these findings with pre-clinical data that showed the upregulation of ceramide-producing enzymes in the myocardium, with an increase in plasma ceramide levels within the first 24 hours of an acute ischemic event.[5]

Ceramides are also associated with a higher risk of disease progression in patients with HF. Among 423 patients with acute HF, plasma ceramide concentrations were independently associated with death and worsening of left ventricular function during hospitalization.[27]

Searching for evidence: ceramides and CV risk prediction

Primary prevention:

The FINRISK study was performed in patients with no previous history of CV events and showed that circulating levels of specific ceramides (16:0, 18:0, and 24:1) were significantly associated with subsequent major CV events when compared to individuals who remained asymptomatic. Significant univariate associations between ceramides and fatal events suggest they play a fundamental role in atherosclerotic plaque rupture in this population.[7]

Still considering primary prevention, Petterson et al.[28] demonstrated that the proportion of ceramides C24:0/C16:0 and the plasma concentration of ceramide C24:0 were inversely associated with coronary risk factors such as age and smoking habits, in addition to the development of CAD and HF.[28]

CAD:

In a post-hoc analysis of the Systolic Blood Pressure Intervention Trial (SPRINT) study (N = 9631), Nguyen et al. demonstrated that plasma LDL-cholesterol levels were not associated with the primary composite outcome (myocardial infarction, stroke, acute decompensated HF, and CV death).[29] In addition, when evaluating patients in secondary prevention of CV events (N = 1562), they observed that LDL-cholesterol was only marginally associated with the incidence of CV events (adjusted hazard ratio 1.005 [95% confidence interval, CI = 1.002–1.009], p = 0.005 [1 mg/dl increase], with poor discrimination for MACE [AUC = 0.54, p = 0.087]).[29]

Although studies with PCSK9 inhibitors such as Odyssey Outcomes and Fourier[30] reinforce the "the lower, the better" principle, meaning there is an association between low LDL-cholesterol levels and a low risk of clinical atherosclerosis, no perfect risk correlation is observed. In order to fill this gap, the measurement of metabolites with metabolomic techniques has been increasingly employed since it presents advantages over classical methods such as a more comprehensive analysis and the acquisition of a metabolic profile of the target tissue considering the disease of interest.

An undirected metabolomic analysis identified 3 plasma ceramides that were significantly associated with CV mortality in a cohort with angiography-confirmed CAD. Ceramides associated with high CV mortality in this study were C16:0, C18:0, and C24:1, and their association was independent from age, body mass index (BMI), smoking habits, use of statins, triglycerides, LDL-cholesterol, and total cholesterol.[14]

Kaasenbrood et al.,31 using the Secondary Manifestations of Arterial Disease (SMART) risk score, attempted to improve the prediction of acute events in this group of patients.[31] This risk score comprehends clinical and laboratory variables (total cholesterol, HDL-cholesterol, estimated glomerular filtration rate [eGFR], CRP) and was tested in various cohorts; based on the obtained results, the authors suggested new algorithms for estimating CV risk in order to individually and more precisely stratify this population, demonstrating the possibility of improving risk scores by incorporating biomarkers.[31]

In the Prevención con Dieta Mediterránea (Mediterranean Diet Prevention, PREDIMED) study, a prospective cohort study with patients at high CV risk, ceramides C24:0, C22:0, and C16:0 were associated with CVD.4 The odds radios comparing the extreme quartiles of plasma ceramides C16:0, C22:0, C24:0, and C24:1 were 2.39 (1.49–3,83; p < 0.001), 1.91 (1.21–3.01; p = 0.003), 1.97 (1.21–3.01; p = 0.004), and 1.73 (1.09–2.74; p = 0.011), respectively. In another prospective study with approximately 500 patients who underwent elective coronary angiography, Meeusen et al. reported that plasma C16:0, C18:0, and C24:1 levels were independently associated with increased risk of MACCE in a mean follow-up of 4 years.32 The risk associated with ceramides was also independent from traditional risk factors, including age, sex, BMI, smoking habits, and cholesterol. Moreover, the predictive value remained significant after additional adjustments for serum glucose and family history of CAD. These results suggest that, when plasma ceramide levels are high in patients with or without significant stenosis of the coronary artery, the risk of death is high in both groups.[32]

Another risk score involving ceramides is the Coronary Event Risk Test (CERT2), which was developed in the Western Norway Coronary Angiography Cohort (WECAC) study and validated by the Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) and Langzeiterfolge der Kardiologischen Anschlussheilbehandlung (KAROLA) studies.[33] Results showed that the CV risk estimation tool incorporating ceramide measurement could reliably stratify MACE in patients with stable CAD. Additional evidence obtained by the WECAC and LIPID studies demonstrated that these biomarkers alone were able to accurately stratify primary CV risk in patients with and without diabetes; in individuals with diabetes, the only significant predictors in this study were the CERT2 score and high-sensitivity troponin.

ACS

In the European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis - Intravascular Ultrasound (favor verificar se está correto.) (ATHEROREMO-IVUS) study (N = 600 patients), Cheng et al. demonstrated that plasma C16:0, C18:0, and C24:1 levels were significantly associated with vulnerable coronary plaque in individuals with ACS. Increased plasma levels of these ceramides were also significantly associated with higher angiographic severity of coronary stenosis,[14,34] as well as a lower perfusion of the myocardial wall after stress in patients with established or suspected CAD who underwent myocardial perfusion scintigraphy.[35,36] These findings suggest causality between increased ceramides in the atherosclerotic plaque and its instability or severity.

Using optical coherence tomography (OCT) in patients with ST-segment–elevation myocardial infarction (STEMI), Bo Yu et al. recently observed increased plasma C16:0, C18:0, and C24:0 levels when compared to individuals with no coronary disease or with stable CAD (p < 0.001, p < 0.001, p < 0.001, p < 0.001, respectively). This was the first study using OCT that proved a positive independent association between plasma ceramide concentrations and plaque rupture, suggesting that plasma ceramide concentrations may act as potential biomarkers of plaque rupture.[37]

Table 1 – Relative risk scores involving ceramides in different cohorts

Score	Category	BECAC (5-year risk)[5]			SPUM-ACS (1-year risk)[5]		
		Deaths (n)	%	Relative Risk	Morte (n)	%	Relative Risk
0-2	Low	15/549	2.7%	1.0	9/575	1.6%	1.0
3-6	Moderate	29/601	4.8%	1.8	16/611	2.6%	1.7
7-9	Increased	20/288	6.9%	2.5	9/270	3.3%	2.1
10-12	Higher	17/149	11.4%	4.2	17/181	9.4%	6.0

Source: adapted from Mayo Clinic. https://www.mayoclinic.org/

Additional evidence of this association was obtained by Laasksonen et al.[5] in a prospective cohort of patients with stable CAD; increased serum ceramide levels were observed in 81 out of 1580 patients (Table 1), who later presented MACE in 4.6 years of follow-up. This proportion was maintained even after adjustment for treatment with statins. Ceramides were predictive in both cases, with comparable odds ratios (OR) in patients using statins or not: 1.68 (1.31–2.15) vs 1.7 (1.33–2.17). In this study, plasma LDL-cholesterol was not significantly predictive of MACE.[5]

Laasksonen et al.[5] analyzed the Special Program University Medicine-Inflammation in Acute Coronary Syndromes (SPUM-ACS) study (N = 1637), performed with patients with ACS, where ceramides were also predictors of MACE regardless of CV risk. In 51 patients who died within a year of a cardiac event, plasma ceramides were found at significantly higher levels when compared to patients who survived during follow-up.[5]

Finally, De Carvalho et al.[13] assessed patients with AMI in 2 cohorts of patients subjected to invasive stratification, comparing MACCE-free survival rates in high-risk patients as defined by the Global Registry of Acute Coronary Events (GRACE) score adjusted to the local population. In this study, the GRACE score was less capable of predicting event-free survival when compared to an association of 12 plasma ceramides measured in the acute phase of AMI.[13] This study included Chinese, Malay, and Indian people, ethnicities that represent a considerable proportion of the global population; external revalidation of the predictive value of these biomarkers was performed in a Caucasian population in New Zealand, demonstrating the development of a potentially universal biomarker. These data were also corroborated by the molecular biology analysis of atherosclerotic plaque biopsies obtained from patients subjected to heart surgery who had experienced or not recent infarctions, confirming the increase in ceramide production in patients with vulnerable atherosclerotic plaque.

The main studies evaluating the association between ceramides and risk of acute CV events are demonstrated on Table 2.

Conclusion

Plasma ceramides are elevated in patients with MACCE, and pre-clinical and clinical studies demonstrate an association between these lipids and atherosclerotic plaque instability.

Their measurement has incremental value for risk stratification, in addition to the classic risk factors both in primary and secondary CV prevention; consecutive measurements may have higher incremental predictive value than other biomarkers considering future adverse events. However, we still need further evidence from randomized studies to assess the impact of this marker on prognosis and of treatment escalation guided by plasma ceramide levels.

Author Contributions

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

Table 2 – Main studies evaluating the association between ceramides and risk of acute cardiovascular events (by year of publication)

Author/reference	Study characteristics	Primary outcome	Adjustment	Main result
Laaksonen et al.[5] European Heart Journal 2016;37, 1967-1976	Prospective cohort study with N = 1580 adults (62 years old; 59% male; BMI 25 kg/m2; LDL-cholesterol 2.8 mmol/l, triglycerides 1.4mmol/l; statin use 62.6%) who underwent elective coronary angiography due to stable CAD and were recruited at the Haukeland University Hospital in Bergen (BECAC study) with 4.6 years of follow-up, in addition to 1637 patients (63 years old; 78% male, BMI 26 kg/m2, LDL-cholesterol 2.6 mmol/l, triglycerides 1 mmol/l, statin use 27.2%) with an ACS diagnosis who underwent invasive treatment in 4 Swiss university hospitals (SPUM-ACS study), with 1-year follow-up	Cardiovascular death	Total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, age, sex, smoking habits, previous acute myocardial infarction, diabetes mellitus, hypertension, previous stroke	Cer (d18:1/16:0) and Cer (d18:1/24:1) were associated with an increased risk of cardiovascular death in all cohorts. OR Cer (d18:1/16:0)/Cer(d18:1/24:0) was 4.49 (95% CI, 2.24–8.98), 1.64 (1.29–2.08), and 1.77 (1.41–2.23) for Corogene, SPUM-ACS, and BECAC studies, respectively
Havulinna et al.[7] Arteriosclerer Thromb Vasc Biol 2016;36: 2424-2430	Populational cohort study with N = 8101 healthy patients (48 years old; 47% male, BMI 26 kg/m2, LDL-cholesterol 3.3 mmol/l, triglycerides 1.3 mmol/l) from FINRISK 2002	Major cardiac and cerebrovascular adverse events	Total cholesterol, HDL-cholesterol, arterial pressure, diabetes mellitus, and smoking habits	Cer (d18:1/16:0), Cer (d18:1/18:0), and Cer (d18:1/24:1) levels were significantly higher in patients with adverse cardiovascular progression when compared to asymptomatic individuals. Serum concentrations of high-risk ceramides predicting cardiovascular death in patients with CAD were also higher in FINRISK MACE cases when compared to asymptomatic individuals, as follows: Cer (d18:1/16:0), Cer (d18:1/18:0), and Cer (d18:1/24:1) 11.4%, 21.3%, and 17.0%, respectively ($p < 0.001$ for all)
Wang et al.[4] Circulation 2017; 135: 2028-2040	Cohort study nested in the PREDIMED randomized study with N = 980 participants (68 years old; 45% male, BMI 30 kg/m2, LDL-cholesterol 3.4 mmol/l, triglycerides 1.6 mmol/l), including 230 cases of CVD and 767 randomly selected participants. The sub cohort included 37 overlapping CVD cases. Two participants with undetectable plasma ceramide concentrations were excluded. Follow-up: 4.5 years	MACE	IAge, sex, BMI, family history of premature CAD, smoking habits, history of hypertension, dyslipidemia, and type 2 diabetes.	Among the high-risk ceramides identified, the upper quartiles of plasma Cer (d18:1/16:0), Cer (d18:1/22:0), Cer (d18:1/24:0), and Cer (d18:1/24:1) levels were associated with an adverse cardiovascular outcome. The multivariable hazard ratios comparing the extreme quartiles of plasma C:16, C22:0, C24:0, and C24:1 concentrations were 2.39 (1.49–3.83, $p < 0.001$), 1.91 (1.21–3.01, $p = 0.003$), 1.97 (1.21–3.01, $p = 0.004$), and 1.73 (1.09–2.74, $p = 0.011$), respectively.

De Carvalho et al.[13] JACC Basic Transl Sci 2018;3:163-175	Prospective longitudinal study with N = 327 patients from a primary cohort (57 years old; 90% male, BMI 26 kg/m2, LDL-cholesterol 3.1 mmol/l, triglycerides 1.2 mmol/l) and 119 patients in the validation cohort (66 years old; 72% male, BMI 29 kg/m2, LDL-cholesterol 3.2 mmol/l) with ACS who underwent invasive stratification with plasma measurements performed before and after stratification; 1-year follow-up.	Major cardiac and cerebrovascular adverse events	GRACE	Among the high-risk ceramides previously identified, the plasma Cer (d18:1/16:0), Cer (d18:1/18:0), and Cer (d18:1/24:1) levels were associated with adverse cardiovascular events
Meeusen et al.[32] Arterioscler Thromb Vasc Biol. 2018; 38: 1933-1939	Cross-sectional study: 495 participants (60 years old; 62% male, BMI 28kg/m2, LDL-cholesterol 3.1 mmol/l, triglycerides 1.7 mmol/l, statin use 28.5%) before nonurgent coronary angiography. Follow-up: 4 years	MACE (myocardial infarction, percutaneous intervention, myocardial revascularization surgery, stroke, or death).	Age, sex, BMI, hypertension, smoking habits, LDL-cholesterol, HDL-cholesterol, triglycerides, glycemia, family history of CAD	Among the high-risk ceramides previously identified, plasma Cer (d18:1/16:0), Cer (d18:1/18:0), and Cer (d18:1/24:1) levels were associated with adverse cardiovascular events. Adjusted hazard ratios per standard deviation (95% CI) were 1.50 (1.16–1.93) for Cer (16:0), 1.42 (1.11–1.83) for Cer (18:0), and 1.43 (1.08–1.89) for Cer (24:1)
Peterson et al.[28] J Am Heart Assoc. 2018;7: e007931	Community-based study: 2642 participants from the Framingham Heart Study (FHS; 66 years old; 46% male, BMI 28 kg/m2, LDL-cholesterol 2.7 mmol/l, triglycerides 1.3 mmol/l, statin use 42.7%) and 3134 participants from the Study of Health in Pomerania (SHIP; 54 years old, 48% male, BMI 28 kg/m2, LDL-cholesterol 5.5 mmol/l, triglycerides 1.8 mmol/l, statin use 14.5%) were followed up for 6 and 8 years, respectively	MACE (fatal and non-fatal cardiovascular events)	Age, sex, BMI, hypertension, diabetes mellitus, smoking habits, anti-hypertensives, total cholesterol/HDL-cholesterol ratio, triglycerides, and lipid-lowering drugs	Among the high-risk ceramides previously identified, only Cer (d18:1/24:0) were associated with adverse cardiovascular outcomes. In the meta-analysis of both cohorts and after adjusting risk factors for CAD, C24:0/C16:0 ratios were inversely associated with CAD (hazard ratio per mean standard deviation increase, 0.79; 95% CI, 0.71–0.89; p < 0.0001) and inversely associated with HF (hazard ratio, 0.78; 95% CI, 0.61–1,00; p = 0.046).
Hilvo et al.[33] European Heart Journal 2019, in press	Longitudinal study; 3 large cohort studies: 3789 patients (62 years old; 72% male, LDL-cholesterol 2.9 mmol/l, triglycerides 1.5 mmol/l, statin use 72.6%) from the Western Norway Coronary Angiography Cohort (WECAC); 5991 patients (65 years old; 83% male, LDL-cholesterol 3.9 mmol/l, triglycerides 1.6 mmol/l, statin use 49.9%) from the Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) study; and 1023 patients (62 years old; 84% male, LDL-cholesterol 3 mmol/l, triglycerides 1.6 mmol/l, statin use 75.6%) from the Langzeiterfolge der Kardiologischen Anschlussheilbehandlung (KAROLA) study. Follow-up: 6 years	MACE (composite endpoint including death due to CV events, MI, and stroke)	IAge, sex, treatment with statins (WECAC, KAROLA), diabetes mellitus, hypertension, current smoking habit, previous MI, previous stroke, stratified according to vitamin B intervention (WECAC) and treatment group (LIPID).	A simple risk score, based on ceramides and phosphatidylcholines with the best prognostic characteristics, was developed by the WECAC study and validated in the other 2 cohorts. This score was highly significant for predicting mortality due to CVD (multi-adjusted hazard ratios [95% CI] per standard deviation were 1.44 [1.28–1.63] at the WECAC, 1.47 [1.34–1.61] at the LIPID study, and 1.69 [1.31– 2.17] at the KAROLA study). Moreover, a combination of the risk score with high-sensitivity troponin T increased hazard ratios to 1.63 (1.44–1.85) and 2.04 (1.57–2.64) in the WECAC and KAROLA cohorts, respectively.

BECAC: Bergen Coronary Angiography Cohort; DAC: doença arterial coronariana; FINRISK: population-based risk factor survey ; IC95%: intervalo de confiança de 95%; KAROLA: Langzeiterfolge der KARdiOLogischen Anschlussheilbehandlung; LIPID: Intervenção a Longo Prazo com Pravastatina em Doença Isquêmica; MACCE: eventos adversos cardíacos e cerebrovasculares maiores; MACE: eventos adversos cardíacos maiores; RR: razão de risco; SPUM-ACS: Special Program University Medicine-Inflammation in Acute Coronary Syndromes ; WECAC: The Western Norway Coronary Angiography Cohort.

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Non-Classical Secretion: A Possible Mechanism to Explain Cardiac Troponin Elevations in the Absence of Acute Myocardial Infarction

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"It is important to realize that if certain areas of science appear to be quite mature, others are in the process of development, and yet others remain to be born."

Santiago Ramón y Cajal, Advice for a young investigator

Introduction

Nowadays, high-sensitivity cardiac troponin (hs-cTn) assays are available for clinical use and are part of the definition of acute myocardial infarction (AMI).[1,2] However, troponin elevation is not limited to AMI, since other conditions related to oxygen demand mismatch, direct myocardial damage, increased myocardial strain, systemic processes (i.e. sepsis), neurological disease and renal failure can also result in its increase.[2] Troponin can now also be detected in atypical scenarios, such as in strenuous endurance exercise, rapid atrial pacing, and dobutamine stress echocardiography, as well as in 50% to 100% of healthy subjects.[2,3]

From a physiological point of view, troponin is a protein complex that regulates myofibrillar function, consisting of three subunits: I, T and C. In the case of troponin I and troponin T, there are three different tissue-specific isoforms: fast-twitch skeletal, slow-twitch skeletal and cardiac specific (fsTn, ssTn and cTn).[4] Conversely, TnC has two isoforms, one present in fast-twitch skeletal muscle (fsTnC) and one that expresses in both slow-twitch skeletal and cardiac muscle (ssTnC/cTnC).[4]

Apart from cell necrosis, several alternative release mechanisms have been postulated for the cardiac isoforms of troponin.[3,4] However, none of them concisely explains why troponin is released in cases unrelated to myocardial infarction or whether this is indicative of reversible or permanent damage to the cardiac cell. In light of the current lack of knowledge, our main objective was to explore the feasibility of a novel mechanism for cardiac troponin release using a bioinformatical approach.

Keywords

Troponin; non-classical secretion; high-sensitivity cardiac troponin assays; exosomes; microvesicles

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Materials and Methods

Review of the existing evidence

Before starting the analysis, a comprehensive review of the literature was conducted in search of articles related to troponin secretion. The searched databases were PubMed, bioRxiv and OpenGrey. Articles were first assessed based on their title. If it mentioned troponin and a secretory process, the abstract was read. Articles were selected if the abstract mentioned troponin and a secretory process or pathway. Purely clinical articles that did not investigate a mechanism of troponin release were excluded. The screening and review processes were performed by all authors. Disagreements were solved by consensus during regular meetings.

Sequence analysis

To assess for non-classical secretion of troponin, the *SecretomeP 2.0* server was used (http://www.cbs.dtu.dk/services/SecretomeP/).[5] This tool is applied to predict whether a certain protein undergoes secretion without a signal peptide. It is a sequence-based method that, with the help of neural networks, detects specific features common to extracellular/secreted proteins.[5]

The canonical sequences from the three subunits of troponin from fast-skeletal, slow-skeletal and cardiac muscle were retrieved from the UniProtKB database (Supplementary File 1). The proteins were first assessed with *SignalP 5.0*, a commonly used method for the recognition of signal peptides based on neural networks (http://www.cbs.dtu.dk/services/SignalP/).[6] This initial step was performed to rule out the classical secretory pathway. Afterwards, the sequences were analyzed with *SecretomeP 2.0*. Finally, to assess the possibility of type IV non-classical secretion (used by transmembrane proteins that bypass the Golgi apparatus), the sequences were evaluated using *TMHMM 2.0*, a program designed to detect transmembrane helices (http://www.cbs.dtu.dk/services/TMHMM/).[7] A graphical representation of the followed bioinformatic pipeline can be found in Supplementary Figure 1.

Results

Review of the existing evidence

Nearly 19,900 articles were reviewed, of which 31 were read in full. After abstract and full text assessment, no articles were found to be related to troponin secretion. Hence, to the best of our knowledge, this is the first paper reporting evidence of troponin non-classical secretion.

Sequence analysis

After analyzing the troponin isoforms, *SecretomeP 2.0* predicted cTnT, fsTnI, ssTnI, fsTnT and fsTnC to be non-classically secreted. In the 5 cases, the proteins achieved a neural network score (NN-score) greater than 0.6, which is the minimum threshold for mammalian sequences. Additionally, none of the eight troponin isoforms were found to contain a signal peptide or a transmembrane helix, according to *SignalP 5.0* and *TMHMM 2.0*, respectively. The summarized results are shown in Table 1. The complete results can be found in Supplementary Table 1.

Discussion

Non-classical secretion

Non-classical or unconventional secretion is a pathway of protein release. Contrary to classical secretion, it is independent of the Endoplasmic Reticulum (ER)/Golgi apparatus.[8] Consequently, it does not require a signal peptide, which is a short sequence of amino acids that leads the protein through the classical (ER/Golgi-mediated) secretory process.[9] Instead, non-classically secreted proteins are released through a myriad of mechanisms that can be classified into 4 groups: type I (pore-dependent transport), type II (ABC transporter-mediated release), type III (released from endosomes/autophagosomes) and type IV (Golgi bypass by transmembrane proteins).[8] Moreover, other mechanisms, such as exosomes and blebs, have also been recognized to take part in non-classical secretion.[9] Interestingly, most of the scenarios of unconventional secretion are triggered by cell stress, such as inflammation. Some examples of proteins that use the non-classical pathway are IL-1β/IL-1α, FGF-1, FGF-2, and galectins.[8,9]

Clinical implications of troponin non-classical secretion

Clinically, cTnT non-classical secretion could help to solve the debate around hs-cTn assays. An established secretion pathway for cTn potentially explains why troponins are detected in healthy subjects. In addition, cTn non-classical release might contribute to better define the pathological basis of "myocardial injury". This term was included in the fourth universal definition of myocardial infarction, with a troponin value above the upper reference limit being the required condition for its diagnosis.[1] It is not unreasonable to think that an activity condition that generates stress in the cardiac cell results in the release of troponin through a non-classical secretory process (Figure 1). In this regard, troponin elevations might be the result of systemic conditions reflecting on the heart through an inflammatory or cell stress mechanism. This could be the case of patients with sepsis, anemia, cancer, stroke, seizures, or after strenuous exercise.[1,4] Interestingly, cTnT, but not I, has shown a circadian pattern of release.[2] cTnT release by tumor cells, possibly by extracellular vesicles, has also been recently reported.[10]

Proposal limitations

The main limitation of our proposal is that both cardiac troponin I and troponin T are elevated in myocardial injury.[1] A possible explanation could be that cardiac troponin T, the structural subunit of the troponin complex, carries the other subunits in the form of a dimer or trimer along the non-classical secretory process; however, this

Table 1 – Summarized results obtained with *SignalP 5.0*, *SecretomeP 2.0* and *TMHMM 2.0*

Troponin isoform	UniProtKB sequence identifier	*SignalP 5.0*	*SecretomeP 2.0*	*TMHMM 2.0*
Cardiac Troponin T – cTnT (TNNT2)	P45379-1	No signal peptide detected	**NN-score = 0.746**	No transmembrane helices detected
Fast-twitch skeletal Troponin I – fsTnI (TNNI2)	P48788-1	No signal peptide detected	**NN-score = 0.611**	No transmembrane helices detected
Slow-twitch skeletal Troponin I – ssTnI (TNNI1)	P19237-1	No signal peptide detected	**NN-score = 0.727**	No transmembrane helices detected
Fast-twitch skeletal Troponin T – fsTnT (TNNT3)	P45378-1	No signal peptide detected	**NN-score = 0.689**	No transmembrane helices detected
Fast-twitch skeletal Troponin C – fsTnC (TNNC2)	P02585-1	No signal peptide detected	**NN-score = 0.670**	No transmembrane helices detected

The troponin isoforms with a NN-score above the 0.6 threshold for mammalian sequences are shown. NN-score: neural network score.

is a hypothetical assumption and remains an open but intriguing question.

SecretomeP 2.0 limitations

SecretomeP 2.0 was created in 2004, and after more than 15 years it remains a popular method for the assessment of non-classical secretion.[5] However, and as it happens with all computational methods, the results obtained are predictions. Thus, they should be interpreted together with the existing experimental body of evidence.

Evidence supporting cTn non-classical secretion

It is worth mentioning that there is some literature supporting our findings. Bleb formation by cardiomyocytes during ischemia, as well as troponin release through microvesicles in multiple cell lines (in both protein and mRNA form) have been previously demonstrated. Available evidence is summarized in Table 2.

Future perspectives

Further research should aim at demonstrating circulating troponin within microvesicles. This could be tested in patients with acute myocardial infarction (where necrosis should be the leading release mechanism) versus atypical scenarios showing troponin elevation such as strenuous exercise, sepsis and stroke (where non-classical secretion should drive troponin release). After the isolation of plasma microvesicles, a possible approach to validate the non-classical secretion pathway could be the use of mass spectrometry followed by confirmation through specific antibodies.

Conclusion

High-sensitivity assays have brought considerable uncertainty about cTn elevations. Troponin liberation due to non-AMI related causes remains elusive and its exact prognostic significance continues to be investigated. Since our proposal is based on *in silico* evidence, it should be

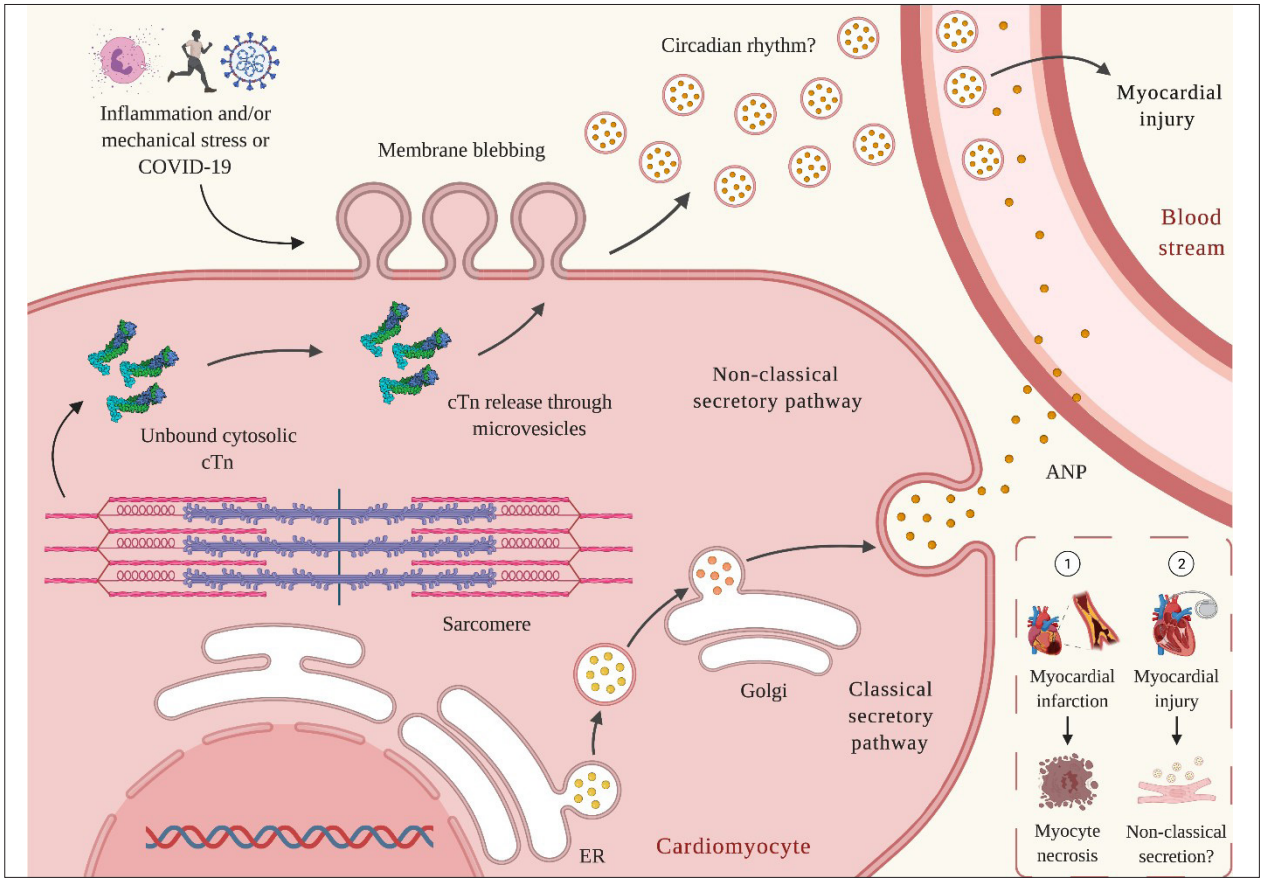

Figure 1 – *Illustration of the cytosol of a cardiomyocyte. The classical pathway of secretion is depicted by the vesicles traveling from the endoplasmic reticulum to the Golgi apparatus; the vesicles arrive at the plasma membrane, where they release their load. The proposed non-classical pathway of secretion is also shown. It starts with the formation of membranous blebs as a result of inflammation and/or cell stress, affecting the cardiomyocyte. Subsequently, unbound cytosolic cardiac troponin (cTn) (representing around 2-4% and 6-8% of the total cTnI and cTnT, respectively)[1] enters these blebs and is released as microvesicles. These microvesicles enter the bloodstream, where they can be detected by high-sensitivity cardiac troponin assays. The box on the lower right corner shows the physiopathology of two different conditions where troponin increases. In the first case, a type 1 myocardial infarction case, an acute ischemic event leads to irreversible myocyte necrosis and troponin release. In the second case, rapid atrial pacing induces troponin release. Whether non-classical secretion participates in this process remains an open question. Created with BioRender.com. The 3D structure of the troponin complex was created with data from Protein Data Bank ID 1J1E.*

Table 2 – Experimental evidence supporting cardiac troponin non-classical secretion. *Vesiclepedia* is an electronic compendium of biomolecules identified in extracellular vesicles

Experimental evidence	Reference
Troponin-like protein secreted by Meloidogyne incognita, a root-knot nematode	Jaubert S, Laffaire JB, Piotte C, Abad P, Rosso M-N, Ledger TN. Direct identification of stylet secreted proteins from root-knot nematodes by a proteomic approach. Molecular and Biochemical Parasitology 121: 205–211, 2002. doi: 10.1016/S0166-6851(02)00034-8
Human cardiomyocytes form membranous blebs triggered by anoxia. Reversible cytosolic enzyme release by means of blebs has also been shown.	Hickman PE, Potter JM, Aroney C, Koerbin G, Southcott E, Wu AHB, Roberts MS. Cardiac troponin may be released by ischemia alone, without necrosis. Clinica Chimica Acta 411: 318–323, 2010. doi: 10.1016/j.cca.2009.12.009
Identification of an uncharacterized protein in the exosomes released by rat cardiomyocytes under different stressors (ethanol and hypoxia/reoxygenation). The uncharacterized protein UniProt ID (E9PTA1) turned out to be the secondary accession number of Tnnc1 (cardiac troponin C of Rattus norvegicus).	Malik ZA, Kott KS, Poe AJ, Kuo T, Chen L, Ferrara KW, Knowlton AA. Cardiac myocyte exosomes: stability, HSP60, and proteomics. American Journal of Physiology-Heart and Circulatory Physiology 304: H954–H965, 2013. doi: 10.1152/ajpheart.00835.2012
Evidence from Vesiclepedia	
Troponin I type 3 (cardiac) Homo sapiens mRNA and protein identified in colorectal cancer cells (microvesicles), T cells (exosomes) and urine (extracellular vesicles)	PubMed IDs: 19930720, 23463506, 25138791
Troponin C type 2 (fast-twitch skeletal muscle) Homo sapiens protein identified in ovarian cancer cells (exosomes) and urine (extracellular vesicles)	PubMed IDs: 24434149, 25138791
Troponin C type 2 (fast-twitch skeletal muscle) Mus musculus protein identified in melanoma cells (extracellular vesicles)	PubMed ID: 29907695
Troponin C type 1 (slow-twitch skeletal muscle) Homo sapiens protein and mRNA identified in brain cancer cells (extracellular vesicles), colorectal cancer cells (microvesicles and extracellular vesicles), kidney cancer cells (extracellular vesicles), leukemia cells (extracellular vesicles), lung cancer cells (extracellular vesicles), melanoma cells (extracellular vesicles) and ovarian cancer cells (extracellular vesicles)	PubMed IDs: 27894104, 19930720
Troponin I type 2 (fast-twitch skeletal muscle) Homo sapiens protein identified in urine (exosomes)	PubMed ID: 22418980
Troponin T type 1 (slow-twitch skeletal muscle) Homo sapiens mRNA identified in colorectal cancer cells (microvesicles) and glioblastoma cells (microvesicles)	PubMed IDs: 19930720, 19011622
Troponin T type 3 (fast-twitch skeletal muscle) Homo sapiens protein identified in brain cancer cells (extracellular vesicles), breast cancer cells (extracellular vesicles), colorectal cancer cells (extracellular vesicles), kidney cancer cells (extracellular vesicles), melanoma cells (extracellular vesicles) and ovarian cancer cells (extracellular vesicles)	PubMed ID: 27894104

More information can be found here: Pathan M, Fonseka P, Chitti SV, et al. Vesiclepedia 2019: a compendium of RNA, proteins, lipids and metabolites in extracellular vesicles. Nucleic Acids Res. 2019;47(D1):D516-D519. doi:10.1093/nar/gky1029.

experimentally confirmed before its exact clinical significance can be ascertained. Whether other troponin variants also enter the non-classical secretory pathway is also an open question. Nonetheless, it is fair to say that troponin non-classical secretion is a promising research field in cardiology that awaits to be explored.

Author Contributions

Conception and design of the research: Gonzalez-Rayas JM, Rayas-Gomez AL; Acquisition of data and Statistical analysis: Gonzalez-Rayas JM; Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content: Gonzalez-Rayas JM, Hernandez-Hernandez JA, Lopez-Sanchez RC, Rayas-Gomez AL, Gonzalez-Yanez JM; Writing of the manuscript: Gonzalez-Rayas JM, Hernandez-Hernandez JA, Lopez-Sanchez RC, Rayas-Gomez AL, Gonzalez-Yanez JM.

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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.

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***Supplemental Materials**

For additional information, please click here.

Acute Myocarditis Following mRNA COVID-19 Vaccine

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Introduction

Vaccination is one of the most important breakthroughs of modern medicine and constitute a major advance in the prevention of infectious diseases.[1] Overall, vaccines are effective and have an excellent general safety profile.[2] In fact, reports of severe adverse effects following vaccination are extremely rare and idiosyncratic.[2]

As the vaccine's mechanism of action is based on the host immune response, a close relationship with autoimmunity cannot be disregarded.[3] Cases of immunologic reactogenicity, such as Guillain-Barré syndrome and acute myocarditis following vaccination have been previously reported.[4,5]

We report the case of a young male patient who developed acute myocarditis following the mRNA vaccine against SARS-CoV-2.

Case report

A 32-year-old male individual was admitted with presyncope and oppressive retrosternal chest pain. The pain lasted for two hours, did not radiate and was not modified by respiratory movements or position. He had had fever (39ºC) and generalized myalgia for two days, starting one day after the administration of the second dose of the mRNA COVID-19 vaccine. The patient was hemodynamically stable and his physical examination at admission was unremarkable, except for the presence of fever. He denied any recent episodes of chest pain, respiratory tract or gastrointestinal infection. He was not taking any drugs or medications and no occupational or recreational risk factors were identified.

The patient was otherwise healthy except for a history of idiopathic myopericarditis, which occurred 13 years before. At that time, the cardiac magnetic resonance (CMR) assessment performed in the acute phase revealed subepicardial late gadolinium enhancement in the lateral wall. The patient was discharged and remained stable, under regular clinical follow-up. Complete resolution of these findings was observed in a CMR at one year of follow-up.

Given the characteristics of the chest pain in a young patient with a concomitant viral syndrome, myocarditis was considered as a likely diagnosis. The patient had elevated inflammatory parameters (leukocytosis and C-reactive protein 4.6mg/dL) and myocardial biomarkers (high-sensitive cardiac troponin T 834ng/L and NT-proBNP 433pg/mL) on blood analysis. The chest radiography was normal. The ECG showed diffuse concave ST-segment elevation (figure 1A). On transthoracic echocardiography, left ventricular ejection fraction was preserved (58%) and no abnormalities were observed in segmental contractility, although the global longitudinal strain was mildly reduced (-17%). There was no pericardial effusion. The CMR revealed subepicardial late gadolinium enhancement in the mid anterior, lateral and inferior walls (figure 2A) accompanied by increased native T1 and T2 in the mid anterior and lateral segments (figures 2B and 2C). No inflammation signs were observed in the pericardium. Naso- and oropharyngeal swab polymerase chain reaction (PCR) tests for SARS-CoV-2 were negative on two different occasions. Given the high clinical suspicion and a CMR pattern consistent with acute myocarditis in a patient without any known cardiovascular risk factors, coronary angiography was not performed.

The diagnosis of acute myocarditis was assumed. The patient was discharged three days after admission and advised against intense physical activity during a three to six-month period. An ECG was repeated after clinical improvement and revealed normalization of the ST-segment elevation (figure 1B). A CMR assessment at three months of follow-up demonstrated significant improvement in the subepicardial late gadolinium enhance pattern (figure 2D) and normalization of the previously observed abnormalities in mapping T1 and T2 sequences (figures 2E and 2F).

Discussion

Myocarditis is an inflammatory disease of the myocardium caused by a variety of infectious and non-infectious conditions.6 Its clinical presentation varies widely, ranging from mild chest pain to cardiogenic shock or life-threatening ventricular arrhythmias.[6,7] Although the cardiac biopsy remains the gold standard, it is not routinely performed in clinical practice for most patients and, therefore, the CMR, by meeting the modified Lake Louise Criteria, is extremely useful in establishing the diagnosis.[6-8]

Keywords

Coronavirus-19; COVID-19; Vaccine/adverse effects; Immunogenicity Vaccine; Molecular Mimicry; Myocarditis; Diagnostic Imaging

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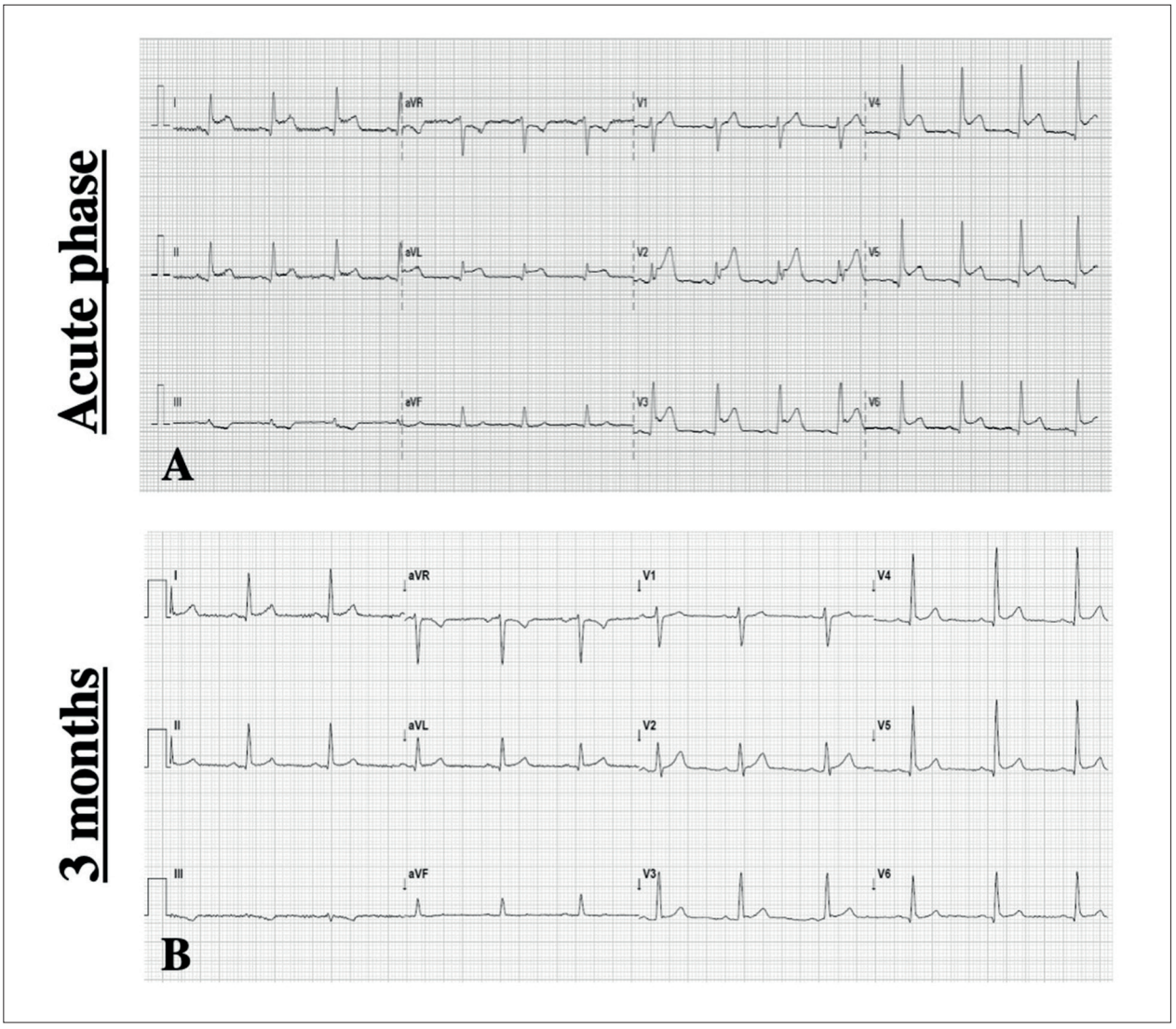

Figure 1 – *ECG at admission demonstrating diffuse concave ST-segment elevation (figure 1A) and ECG at three months of follow-up showing resolution of ST-segment abnormalities (figure 1B).*

There are only a few reports of myocarditis following vaccination. Although there were some initial concerns about the development of inflammatory cardiac disease in live viral vaccine recipients, more recent studies suggest its overall risk is not increased.[5,9] In fact, in a cohort including over 41000 patients, only one case of definitive pericarditis and none of myocarditis were diagnosed in the first 42 days after vaccination.[5]

The introduction of vaccines against SARS-CoV-2 is a key element in controlling the spread of this pandemic. Among those receiving mRNA COVID-19 vaccine in large-scale clinical trials, it proved to be highly effective and safe, with no reports of significant adverse cardiovascular effects.[10] Systemic symptoms related to immunologic reactogenicity were common, mostly mild to moderate ones, and more frequently observed following the second dose, with a median onset of 1-2 days after vaccine application.[11]

We report a case of a 32-year-old man who developed self-limited acute myocarditis after COVID-19 immunization. This clinical case is in line with recently published ones.[12-14] As we described, acute myocarditis following COVID-19 vaccination appears to be a potentially rare and self-limited complication, affecting mostly young and healthy male patients two to three days after receiving the second dose.

The exact immunological mechanisms linking the vaccine to the development of acute myocarditis is not completely clear. Autoinflammatory syndrome, cross-reactivity, molecular mimicry, and autoantibody generation in susceptible or predisposed individuals have been suggested to be implicated in pathogenesis.[13] In fact, previous reports have elicited the role of cross-reaction and mimicry in post vaccination autoimmune phenomena.[15]

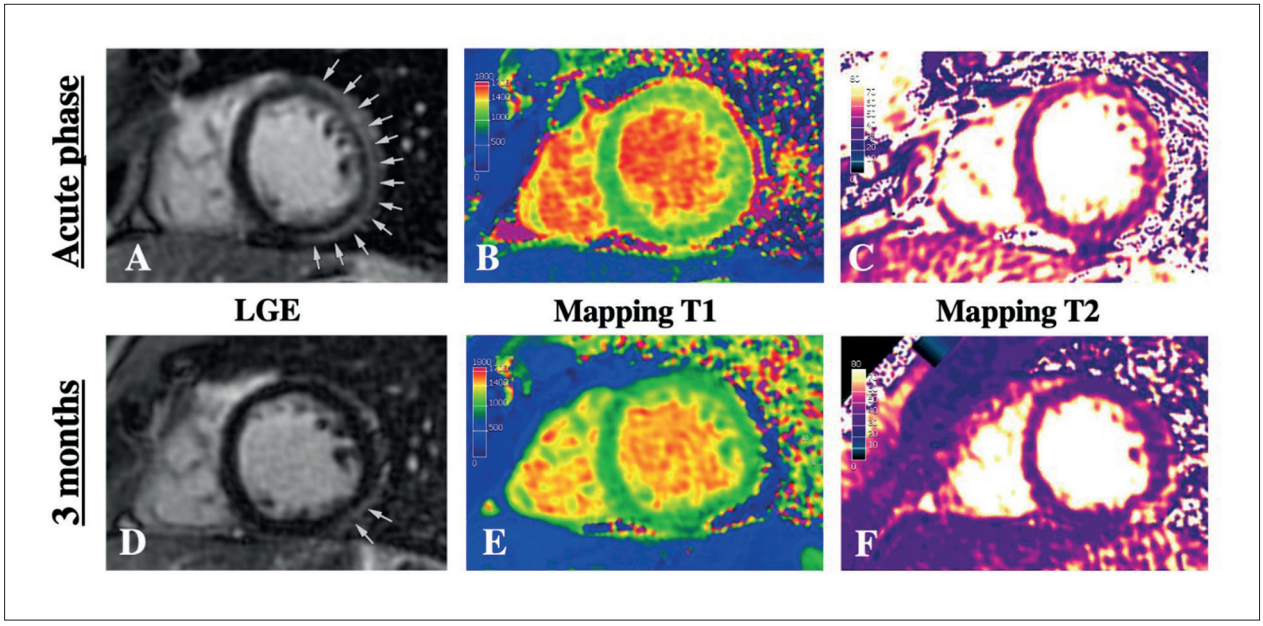

Figure 2 – *Cardiac magnetic resonance (CMR) at admission demonstrating subepicardial late gadolinium enhancement (LGE) in the mid anterior, lateral and inferior walls (figure 2A), and increased native T1 (figure 2B) and T2 (figure 2C). The CMR at three months of follow-up revealed improvement in the subepicardial late gadolinium enhancement pattern (figure 2D), and normalization of native T1 (figure 2E) and T2 (figure 2F).*

Although other etiologies such as viral myocarditis coincidental with the vaccination timing cannot be definitively excluded, given the temporal association, we can hypothesize that the immune response to the vaccine may have triggered the recurrence of myocarditis in this patient.

More studies are needed to further clarify the epidemiology, pathophysiology, and long-term clinical outcomes of these patients. Future research on this subject should focus on: (1) exploring predisposing factors and pathophysiological mechanisms for the development of myocardial injury following COVID-19 vaccination (including molecular mimicry, autoantibody formation, and the role of specific immune cell populations); (2) characterizing myocardial ultrastructural and functional changes, as well as cardiac biomarkers and cardiac function; (3) prospectively characterizing these patients' clinical presentation, clinical course and long-term outcomes.

Conclusions

Self-limited acute myocarditis may be a potential and rare adverse effect of mRNA COVID-19 vaccines. While clinicians must be aware of this possibility, by no means it should discourage vaccination, as the risk-benefit analysis regarding COVID-19 immunization shows a consistent beneficial effect across all groups.[14,16] The vaccine is currently recommended for everyone aged ≥ 12 years.[16]

Author Contributions

Conception and design of the research: Gomes DA, Ferreira J, Trabulo M; Acquisition of data: Gomes DA, Santos RR, Freitas P, Paiva MS; Analysis and interpretation of the data and Critical revision of the manuscript for intellectual content: Gomes DA, Santos RR, Freitas P, Paiva MS, Ferreira J, Trabulo M; Writing of the manuscript: Gomes DA.

Potential Conflict of Interest

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Ethics approval and consent to participate

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Prevalence and Associated Factors of SARS caused by Covid-19 in Adults and Aged People with Chronic Cardiovascular Disease: A Critical Analysis

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Dear Editor,

The study by Paiva et al. evaluated the incidence of patients infected with the COVID-19 virus, associated with cardiovascular diseases (CVD), in Brazil. It is concluded that the high prevalence of severe acute respiratory syndrome (SARS) in adults and in the elderly is related to sociodemographic and clinical characteristics, signs and symptoms. In view of that, the importance of primary health care is reiterated – in order to maintain regular medical visits aiming at controlling the disease and symptoms, while the presence of cardiovascular comorbidities increases in severe COVID-19 cases.[1]

The study included 116,343 patients, of whom 61.9% were diagnosed with SARS caused by COVID-19. At the same time, the study demonstrates that the presence of chronic diseases can be considered a risk factor for infection by COVID-19 due to greater vulnerability and morbimortality. Therefore, patients with previous CVD are more likely to develop more severe conditions. However, in females, there was a lower prevalence of SARS by COVID-19 because there is a variation between the immune response and the susceptibility to viral infections between sexes, which generates differences in disease severity and evolution.[1]

In Wuhan, China, a meta-analysis with 46,248 infected patients analyzed the most prevalent comorbidities, with CVD (5±4%) in third place. Wang et al., 2020 evaluated only hospitalized patients affected by viral infection, which has shown a higher prevalence – 19.6% – CVD, which reinforces the fact that the comorbidity of CVD contributes an increased severity of COVID-19, given the evident need for hospitalization. In addition, the patients evolved with higher levels of hypoxemia and urgent hospitalization in ICUs.[2] In the study conducted by Melo, the results were similar to those found in a study in Italy – both analyzed over seven days in March 2020; it found a decrease of 13% of patients with acute myocardial infarction (AMI) associated in the same week of 2019. On the other hand, even though there was a reduction in AMI cases and in the rate of hospital deaths, there was an increase in the in-hospital lethality rate in hospitalizations for CVD. Both studies demonstrated the relationship of COVID-19 with the high prevalence of cardiac lesions and a great potential for COVID-19 severity in CVD, in which mortality of hospitalized patients with CVD reached the most economically active portion of the population – from 20 to 59 years of age.[3]

In view of that, the importance of medical follow-up of patients with chronic diseases is highlighted, since, according to Askin et al., in 2020, there was a marked increase in myocardial damage in patients with COVID-19, increasing the risk of morbimortality. Therefore, the appreciation of CVD as a complication associated with the COVID-19 virus, due to the increase of the disease symptoms, is of extreme significance for primary health care.[4]

The current situation requires strategies aimed at preventing complications associated with chronic diseases, such as CVD. Therefore, current data demonstrate the need for special attention to patients at high risk as well as proper management of cardiovascular complications, aiming at quickly identifying and applying adequate treatment. Furthermore, it is recommended that patients with CVD get vaccinated – due to the risk of secondary bacterial infection by SARS-CoV-2 – and adopt a proper diet, regular sleep and physical activity, avoiding smoking and alcohol consumption.

Keywords

COVID-19; Coronavirus; Pandemic; Risk factors; Cardiovascular disease

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Letter to the Editor

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Reply

We would like to thank the authors for the analysis "Prevalence and Factors Associated with SARS caused by COVID-19 in Adults and Aged People with Chronic Cardiovascular Disease: A Critical Analysis", especially due to the exposure of data reinforcing the findings presented in our study.[1] The analysis is also relevant because it includes information regarding the increase of in-hospital mortality rates in hospitalizations due to cardiovascular diseases (CVD) as a factor that further calls for the need to draw special attention to this group at risk in the analysis of complications associated with COVID-19.[2,3]

CVDs are established as risk markers in the Brazilian primary health care (PHC) in order to guarantee the longitudinality of user care and the development of preventive strategies aimed at the integral care of the community,[4] but it is essential to question the applicability of these actions in the family health strategy (FHS). The challenge of monitoring the users as a way of managing CVD suggests overcoming the imperative of actions that support the diagnostic and therapeutic system in the care and management of chronic conditions.[5] A recently published study with more than 100 million participants from around the world highlights the importance of prevention, detection and control in primary care, including in low- and middle-income countries, with proposals for actions that include sending text messages to check on users with risk factors.[6]

In the current context of facing the COVID-19 pandemic, this discussion is even more relevant because, as emphasized by the authors who conducted the critical analysis, the worsening of the symptoms of COVID-19 places CVDs as an important risk factor that affect screening, management and early intervention actions.

Studies suggest the creation of proposals for models of care for chronic conditions, such as CVD, involving focus groups with positive results in the community, improvement of clinical indicators during follow-up, adoption of self-care practices and better analysis of the priorities in health planning.[6] Similarly, the World Health Organization (WHO) released an implementation guide for CVD management in primary care, with proposals for risk screening, assessment and management at this level of health care and education for professionals regarding the screening of risk factors, lifestyle interventions and the required referrals.[7] Especially regarding the management of users with CVD and persistent symptoms after COVID-19, the clinical and psychosocial complexity provided by the coexistence of these conditions calls for long-term multidisciplinary care. To conclude, we would like to express our gratitude for the possibility of reinforcing the need to review primary health care actions, especially in our current health context.

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Position Statement on Cardiovascular Safety of Vaccines Against COVID-19 - 2022

Development: Work group on Cardiovascular Safety of Vaccines against COVID-19 of the Scientific Committee of the Brazilian Society of Cardiology

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Note: These statements 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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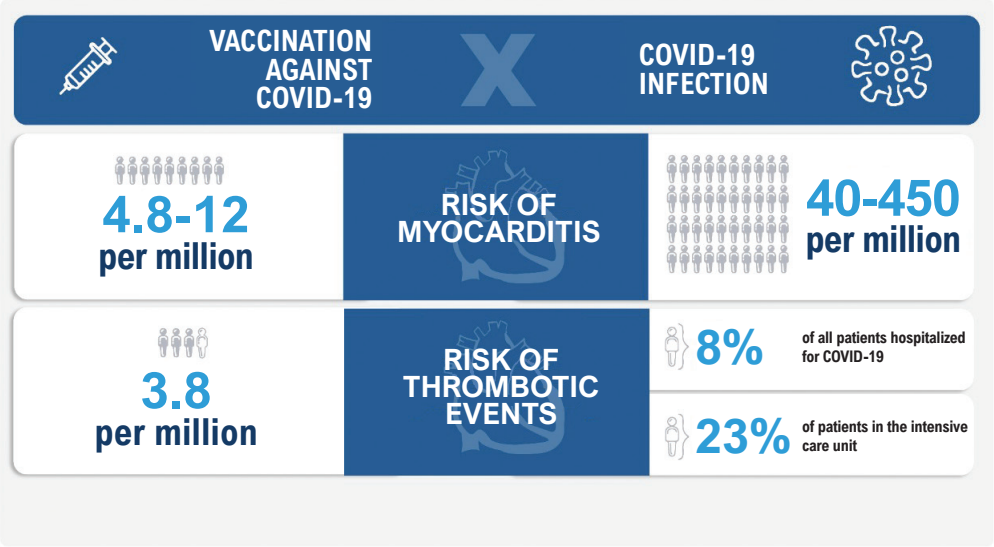
Position Statement on Cardiovascular Safety of Vaccines Against COVID-19 – 2022	
The report below lists declarations of interest as reported to the SBC by the experts during the period of the development of these statement, 2022.	
Expert	**Type of relationship with industry**
Bruno Pereira Valdigem	Nothing to be declared
Carisi Anne Polanczyk	Financial declaration A - Economically relevant payments of any kind made to (i) you, (ii) your spouse/partner or any other person living with you, (iii) any legal person in which any of these is either a direct or indirect controlling owner, business partner, shareholder or participant; any payments received for lectures, lessons, training instruction, compensation, fees paid for participation in advisory boards, investigative boards or other committees, etc. From the brazilian or international pharmaceutical, orthosis, prosthesis, equipment and implants industry: - Bayer, Pfizer, Novartis, Roche, Amgen, Bristol. Other relationships Funding of continuing medical education activities, including travel, accommodation and registration in conferences and courses, from the brazilian or international pharmaceutical, orthosis, prosthesis, equipment and implants industry: - Bayer, Roche.
Cristiane Nunes Martins	Nothing to be declared
Humberto Graner Moreira	Financial declaration A - Economically relevant payments of any kind made to (i) you, (ii) your spouse/partner or any other person living with you, (iii) any legal person in which any of these is either a direct or indirect controlling owner, business partner, shareholder or participant; any payments received for lectures, lessons, training instruction, compensation, fees paid for participation in advisory boards, investigative boards or other committees, etc. From the brazilian or international pharmaceutical, orthosis, prosthesis, equipment and implants industry: - Novartis: Entresto; Bayer: Xarelto; Pfizer: Eliquis; Libbs: Plenance Eze.
Mucio Tavares de Oliveira Júnior	Financial declaration B - Research funding under your direct/personal responsibility (directed to the department or institution) from the brazilian or international pharmaceutical, orthosis, prosthesis, equipment and implants industry: - Sanofi Pasteur: FLUZONE Senior; Torrent: experimental drug. Other relationships Funding of continuing medical education activities, including travel, accommodation and registration in conferences and courses, from the brazilian or international pharmaceutical, orthosis, prosthesis, equipment and implants industry: - Astra Zeneca, Boehringer, Novartis, Torrent Pharma, Sanofi Pasteur, Merck, Biolab.

Central Illustration: Position Statement on Cardiovascular Safety of Vaccines Against COVID-19 - 2022



Arq Bras Cardiol. 2022; 118(4):789-796.

Introduction

The Scientific Committee of the Brazilian Society of Cardiology, by determination of its Administrative Council, has convened a work group to monitor and set up scientific evidence on cardiovascular safety of vaccines against COVID-19 in a continuous and systematic way. This group aims to reproduce scientifically solid data, summarize currently available evidence, and develop recommendations to Brazilian cardiologists in the form of positions of the Brazilian Society of Cardiology.

Vaccines to prevent SARS-CoV-2 infection are considered the most effective strategy to control the pandemic. Despite the short time for vaccine development, each vaccine approved has gone through all preclinical and clinical stages (phase I and II) of clinical research.

The strict standards of safety applied to these studies are maintained during the so-called "phase IV", or post-marketing surveillance. This stage is crucial for evaluating the occurrence of rare adverse events whose causal relationship with vaccines can only be established after they were administered to a large number of people.

Like other vaccines, adverse events, including those related to the circulatory system, have been observed during this surveillance phase of immunization programs against COVID-19. Here we review the evidence on two cadiovascular adverse effects – thrombosis with thrombocytopenia and vaccine-induced myocarditis.

Vaccine-induced immune thrombotic thrombocytopenia (VITT)

In February 2021, a post-thrombotic syndrome was described in some vaccinated individualsand the syndrome was named vaccine-induced immune thrombotic thrombocytopenia (VITT). Two adenovirus-vectored vaccines have been implicated in the cause of VITT:

- ChAdOx1S nCoV-19 (Oxford/AstraZeneca and Serum Institute of India);
- Ad26.COV2.S (Janssen; Johnson & Johnson)

Although recognized as a vaccine adverse event, the real **incidence** of VITT is still unknown, and evidence has suggested it as a rare complication. Most reports have described a small number of cases among tens of millions of vaccinated people.[1-4] In January 2022, a report of the Vaccine Adverse Event Reporting System (VAERS) identified 54 cases of thrombosis among more than 14 million Ad26.COV2.S recipients, an incidence of 3.83 per million (approximately 1 in 263,000).[2,3] Close surveillance of these outcomes has been made, and suggested high reliability of the reports.

The **risk factors** for VITT are still unknown. Female sex, obesity, and age between 30 and 50 years have been proposed as risk factors based on initial reports, although they may merely reflect the demography of early-vaccinated populations.[1,3-5] In the United States, the risk of VITT after Ad26.COV2.S vaccination was estimated at 3.8 cases per million of doses in the general population, and between nine and 10.6 cases per million of doses for women aged between 30 and 49 years.[2,3,6]

Although thrombosis is the most common **clinical presentation**,[1,4,7] thrombocytopenia alone may also occur.[7,8] Cerebral venous thrombosis is one of the most commonly described.

The **prognosis** of VITT depends on the site, extension and complication of thrombosis, and time for diagnosis. In a series of 220 individuals with definite or probable VITT, a mortality rate of 22% was reported.[5] Factors associated with increased risk of death include cerebral venous thrombosis, severe thrombocytopenia and concomitant bleeding complications. In the US, VITT-related mortality was 0.57 deaths per million doses of Ad26.COV2.S in total population, and 1.8-1.9 deaths per million doses among women aged between 30 and 49 years.[2,3] Comparatively, the overall mortality rate of COVID-19 is 1-2%. The incidence of thrombosis reaches 8% of all patients hospitalized for COVID-19, and 23% of intensive care unit patients.[9] In addition, there is evidence that the incidence of cerebral venous thrombosis in patients hospitalized for COVID-19 was 207 per million cases, much higher than the incidence of vaccine-induced thrombosis (0.9-3.8 per million cases).[10]

Therefore, there is a consensus that the benefits of vaccination **surpass the potential ri**sks of rare side effects of the vaccine, like VITT.[11]

Specific recommendations:

• Previous **history of venous thromboembolism** (VTE) or predisposition for VTE **are not contraindications** for COVID-19 vaccination, regardless of the type of vaccine. No study has shown an increased risk of VITT or other thrombotic complications after vaccination in these individuals;

• Individuals who received the first dose of ChAdOx1 nCoV-19 and **did not develop VITT** should complete the vaccination schedule of two doses. There is no evidence that the second dose (or even the booster) increases the risk of thrombotic complications. A review of AstraZeneca safety database in Europe and United Kingdom identified an incidence of 8.1 cases of VITT per million for the first doses, and of only 2.3 cases per million for the second doses;[12]

• Individuals who received an adenovirus-vectored vaccine and developed VITT should not receive a second dose. A transition to a mRNA vaccine schedule is recommended;

• Available evidence **does not** support the performance of any clinical, laboratory or imaging tests in **asymptomatic individuals** before or after vaccination.[13]

Vaccine-induced myocarditis

The association between myocarditis and vaccines has been described as a rare adverse event. Its incidence has been more commonly reported in smallpox, influenza, and hepatitis B vaccines. From 1990 to 2018, only 0.1% of more than 620 thousand notifications of post-vaccine adverse events were attributed to myopericarditis in the United States.[14]

In July 2021, the Centers for Disease Control and Prevention (CDC) reported a possible association between mRNA vaccines and SARS-CoV-2 in myocarditis and pericarditis. The two mRNA-based vaccines that have been associated with myocarditis are:

• BNT162b2 from Pfizer

• mRNA-1273 from Moderna

First, it was estimated an incidence of 32.4 cases per million doses, 66.7 cases per million doses in males aged 12–17 years following the second dose. The incidence significantly decreased with age and was markedly lower among women of all ages.[15]

After initial reports of myopericarditis in this age group (12-17 years old), greater attention has been paid to politics of safety and surveillance of this adverse event. In recent months, population-based studies on the occurrence of this event after vaccination against SARS-CoV-2 have been published. These studies will be described below and are summarized in Table 1.

• Witberg et al.[16] identified 54 cases that met the CDC criteria for myocarditis among more than 2.5 million vaccinated individuals, who were monitored by a health organization in Israel. Among the patients with myocarditis, 37 (69%) received the diagnosis between three and five days after the second vaccine dose. The estimated incidence of myocarditis (measured within 42 days after the first dose of the vaccine) was 2.13 cases per 100,000 persons. The highest incidence of myocarditis was reported in male patients aged between 16 and 29 years (10.7 cases per 100,000 persons). Most cases of myocarditis were described as mild (76%) or intermediate (22%) as intermediate; one case was associated with cardiogenic shock. Patients who had left ventricular dysfunction on echocardiography during admission (29%) had normal ventricular function after a median follow-up of 83 days.

• Mevorach et al.[17] reported 136 cases of myocarditis, defined according to the Brighton Collaboration and the CDC criteria among 5.1 million individuals vaccinated with two doses of BNT162b2 mRNA (Pfizer) in Israel. Of these, 117 (85%) presented myocarditis after the second dose, and 81% were hospitalized within seven days after vaccination. The incidence ratio was 0.35 cases per 100,000 within 21 days after the first dose, and 2.10 cases per 100,000 individuals after the second dose. The incidence increased from 1.3 to 15.1 per 100,000 individuals after the first and second dose, respectively, among male teenagers aged between 16 and 19 years. The ratio for the comparison of the incidence of myocarditis between vaccinated and unvaccinated persons after the second dose was 2.35 (95% CI, 1.1 to 5.0). Most (95%) of these cases were self-restricted and had a benign course, and one death has occurred. Recently, the same authors investigated the cases of hospitalization for myocarditis in adolescents aged between 12 and 15 years and found 13 cases possibly related to the vaccine based on a temporal criterion.[18] The risk of myocarditis among male adolescents was 0.56 cases per 100,000 after the first dose, and among female adolescents with the same age, the risk was 0 cases per 100,000 after the first dose and 0.69 cases per 100,000 after the second dose.

• Using information on vaccinations from the Danish Vaccination Register, Husby et al.[19] followed 4.9 million residents aged over 12 years between October 2020 and October 2021, and identified 269 new cases of myocarditis in the period. Of 3,482,295 individuals vaccinated with BNT162b2 (Pfizer), 48 developed myocarditis or myopericarditis within 28 days of vaccination. The absolute

Table 1 – Characteristics of population-based studies evaluating myocarditis or myopericarditis associated with mRNA vaccines against COVID-19

Authors	Country	Study population	Individuals vaccinated with mRNA vaccine	Number of cases of confirmed myocarditis	Event/ 100,000 vaccinated (absolute rate)	Deaths	BTN162b2 vaccine (Pfizer)	mRNA1273 vaccine (Moderna)
Witberg et al.[16]	Israel	Database of the health care organization in Israel, of members ≥ 16 years old	2,558,421	54	2.1	1	100%	
Mevorach et al.[17]	Israel	Database of the Israeli Ministry of Health, people ≥ 16 years old	5,125,635	136	2.4	1	100%	
Husby et al.[19]	Denmark	Population cohort of people ≥ 12 years old	3,981,109	69	1.7	0	87%	13%
Chua et al.[20]	Hong Kong	Population cohort including adolescents aged between 12 and 17 years	178,163	33	18.5	0	100%	
Patone et al.[21]	Great-Britain	Data from the English National Immunisation (NIMS) Database, people aged ≥ 16 years	17,999,580	169	0.9	25*	94%	6%
Oster et al.[23]	USA	Vaccine Adverse Event Reporting System (VAERS), people ≥ 12 years old	192,405,448	1626	0.85	0	70%	30%
Simone et al.[24]	USA	Data from the Kaiser Permanente Southern California – members ≥ 18 years old	2,392,924	15	0.58	0	50%	50%
Montgomery et al.[26]	USA	Data from the US Military Health Service, member aged between 20 and 51 years	2,810,000	23	0.8	0	Not available	

** Any death caused by myocarditis (among other causes) registered within 28 days after the first or the second dose of the vaccine.*

incidence was 1.4 per 100,000 vaccinated individuals. The risk of myocarditis was not significantly different between vaccinated and non-vaccinated individuals within 28 days after vaccination (adjusted hazard ratio [HR] 1.34 (95% confidence interval [95%CI] 0.90 to 2.00), but was significantly higher when using a shorter analysis time (14 days post exposure) (HR 1.89; 95%CI 1.23-2.90). Unlike other cohorts, the risk of myocarditis was higher among women than men. Among 498,814 individuals vaccinated with mRNA-1273 (Moderna), 21 developed myocarditis or myopericarditis within 28 days from vaccination date (incidence of 4.2 per 100,000 vaccinated individuals; HR 3.92, 95%CI 2.30-6.68). The adjusted hazard ratio among 12-39-year-old individuals was 1.48 (95%CI0.74-2.98) with BNT162b2, and 5.24 (95%CI 2.47-11.12) with mRNA-1273. .24 (2.47 to 11.12). Only one death occurred, and clinical outcomes were generally similar between vaccinated and unvaccinated individuals;

• In Hong Kong, Chua et al.[20] reported 33 cases of myocarditis and/or pericarditis among 178,163 adolescents from 12 to 17 years of age vaccinated with BNT162b2 (Pfizer). Twenty-nine were males and most cases (81.8%) developed acute myocarditis/pericarditis after the second dose. The overall incidence was 18.5 per 100,000 persons vaccinated. The incidence after the first and second doses were 5.57 and 37.32 per 100,000 persons vaccinated, respectively. All patients had mild diseases and recovered spontaneously;

• Patone et al.[21] evaluated the risks of myocarditis, pericarditis and arrhythmias associated with COVID-19 vaccination *versus* SARS-CoV2 infection. The authors evaluated more than 38.6 million adults in England and observed that 0.001% of individuals had myocarditis in the 28 days following the first or the second dose of the vaccine. In this period, there was one extra myocarditis event per million people vaccinated with BNT162b2 (Pfizer), and six extra myocarditis events per one million with mRNA-1273 (Moderna). Even the extra 10 extra myocarditis events per one million people vaccinated after a second dose of mRNA-1273 would be lower than the extra 40 myocarditis events per one million patients following a SARS-CoV-2 positive test. The same authors expanded their study to include 42 million vaccinated people in England (preprint data),[22] showing similar results following a booster of the vaccines. In men younger than 40 years, similar findings were obtained regarding the risk of myocarditis related to SARS-CoV-2 infection and vaccination, except for the mRNA-1273 vaccine, that posed a higher risk than that related to SARS-CoV-2 infection;

• More recently, in a detailed analysis of cases of myocarditis in the United States, Oster et al.[23] concluded that the risk of myocarditis following mRNA-based vaccines was higher after the second dose in adolescents and young male adults. Among 192 405 448 persons receiving a total of 354 100 845 mRNA-based COVID-19 vaccines, there were 1,626 cases of myocarditis, mostly (82%) in males. Regarding the BNT162b2

vaccine (Pfizer), the incidence of myocarditis was 70.6 per million doses in adolescent males aged 12 to 15 years, 105.9 per million doses in adolescent males aged 16 to 17 years, and 52.4 per million doses in young men aged 18 to 24 years. Most cases were mild or moderate, with a favorable course. Until publication, the were two death notifications potentially duet to myocarditis, still under investigation.

Other studies, also carried out in the United States, have reported an incidence of 0.58 cases per 100,000 within 10 days following the second dose of both mRNA vaccines,[24] and an estimate of 6.3 extra myocarditis event per one million of doses administered in the first three weeks of vaccination with mRNA vaccine in individuals between 12 and 39 years of age.[25] In members of the US Military, the incidence of myocarditis was 0.8 per 100,000 of doses administered among male military members.[26]

The direct comparison of these studies has limitations, due to peculiarities of each study, including differences in diagnostic criteria, study period, age range of populations, methods for risk calculation (absolute or excess risk). In addition, one variable has not been included in several analyses, which is the pre-existence of myocarditis, be it for COVID-19 or due to other causes. Altogether, the available evidence suggests that the risk of acute myocarditis associated with COVID-19 vaccination is real but has a very low incidence and is more commonly reported in young men.

The **pathophysiological mechanisms** of myocardial inflammation and injury described in mRNA vaccines against COVID-19 have not been well established, and they may be related to gene sequence that encodes the SARS-CoV-2 spike protein or to the immune response (e.g. hypersensitivity reactions) to these vaccines. The fact that the highest rates have been observed in young male individuals, and mainly following the second dose, supports the hypothesis of a maladaptive immune response, which may be influenced by sex hormones.

Clinical presentation is vaccine-induced myocarditis similar to the classical presentation of acute myocarditis and includes chest pain and dyspnea. Besides, troponin levels are increased in almost all cases, and nearly 70% have some electrocardiographic changes. Acute systolic dysfunction, with a drop in left ventricular ejection fraction, was reported in 6-12% of cases.[20,23,27,28]

The **prognosis** of vaccine-related myocarditis is very favorable; in most cases, it is self-limited, with resolution of symptoms and normalization of laboratory tests, electrocardiogram and echocardiogram over the follow-up period. In the most comprehensive review of published cases, Kohli et al.[27] reported that serious life-threatening complications due to vaccine-related myocarditis remain rare.

On the other hand, we must always consider the magnitude of the benefits of vaccination to the whole population in the analysis of potential adverse events. In the British study mentioned above, the rate of myocarditis associated with SARS-Cov2 within 28 days of vaccination was 30 cases per million in the general population, and 73 cases per million among men older than 40 years.[22] Thus, **based on most population-based studies currently available, the incidence of myocarditis associated with COVID-19 surpasses the incidence of myocarditis associated with vaccines. One exception is younger men, mainly adolescents, in whom the risk of vaccine-associated myocarditis exceeds that of myocarditis related to COVID-19 at the same age range.[21,22] Even so, as compared with mortality rates of SARS-CoV2 infection (0.1 to 1.0 per 100,000 individuals between 12 and 29 years of age), and with the risk of hospitalization, the overall benefits of vaccination overweigh the related risk of myocarditis.**

It is important to highlight that individuals infected with SARS-CoV2 are at increased risk for cardiovascular diseases, other than those recognized as vaccination adverse effects (thrombosis and myocarditis or myopericarditis). Abbasi et al. [29] estimated the risks and excess burden of cardiovascular outcomes attributed to COVID-19 in a 12-month-period and compared it to a control group (individuals that did not have COVID-19). For every 1,000 people, COVID-19 was associated with an extra: 45 cases of any cardiovascular event, 23 cases of major adverse cardiovascular events (myocardial infarction, stroke, and all-cause mortality), 20 cases of dysrhythmias and 11 cases of atrial fibrillation, 12 cases of heart failure, 10 cases of thromboembolic disorders (5.5 cases of pulmonary embolism and four of deep vein), seven cases of ischemic heart disease thrombosis (5.3 cases of acute coronary disease, three cases of myocardial infarction, and 2.5 incidents of angina), four cases of stroke, 1.23 cases of inflammatory disease of the heart or pericardium.[29]

Based on an analysis of epidemiological data, Gargano et al.[30] concluded the benefits of COVID-19 vaccination (prevention of SARS-CoV2 infection, and associated hospitalizations, intensive care unit [ICU] admissions and death) outweigh the risks of myocarditis after vaccination in all populations to whom vaccination was recommended. The balance between risk and benefit varied with age and sex. Per million males aged between 12 and 29 years receiving the second dose of mRNA COVID-19 vaccine, 11,000 COVID-19 cases, 560 hospitalizations, 138 ICU admissions, and six deaths due to COVID-19 could be prevented, compared with 39-47 expected myocarditis cases in this population.[31]

Also, evidence suggests that BNT162b2 (Pfizer) shows efficacy of 91% against multisystem inflammatory syndrome (MIS) in adolescents aged 12-18 years and prevents the progression to severe stages.[32] The incidence of MIS-C is 316 per million of SARS-CoV-2 infection cases,[33] affecting predominantly males,[34] frequently resulting in prolonged hospitalizations and need for intensive care, in contrast to most cases of vaccine-associated myocarditis.

Finally, even with the escalation of the Omicron variant of COVID-19, when new questions about the risks versus benefits of vaccination in children and young adolescents may arise, data from recently published studies have shown that vaccination with mRNA vaccines (particularly BNT162b2/Pfizer), especially when boosted with a third dose, remains highly effective against severe forms of COVID-19, including death.[35] Besides, the third dose is 82% effective in preventing the need for urgency and emergency care, and 90% effective in preventing hospitalizations.[36] Thus, a booster dose of the COVID-19 vaccine also provides additional protection against severe diseases caused by both Omicron and Delta,[30] which are still circulating in our environment. Table 1 describes characteristics

of population-based studies evaluating myocarditis or myopericarditis associated with mRNA-based vaccines against COVID-19.

Management of suspected myocarditis or myopericarditis associated with vaccines

Myocarditis or myopericarditis should be suspected in patients vaccinated with BNT162b2 (Pfizer) or mRNA-1273 (Moderna) who present symptoms of chest pain or discomfort (predominantly), dyspnea or tachypnea, fatigue, palpitations, syncope, inappetence and lethargy, and results of electrocardiogram, echocardiography, nuclear magnetic resonance excluding other suspected causes.[37]

None of the studies performed an analysis or a review comparing the types of treatment administered, and in almost all studies treatment was conservative. In addition to general care, most patients received ibuprofen, some received corticosteroids, and a minority received corticosteroids and immunoglobulins. We can infer that patients with systolic dysfunction induced by myocarditis received traditional treatment with angiotensin converting enzyme inhibitors or an angiotensin II receptor blocker or sacubitril/valsartan, combined with a mineralocorticoid receptor antagonist (perhaps a SGLT2 inhibitor).[23,24,29,38]

Conclusions

Vaccines against COVID-19 are safe and their benefits far outweigh the risks of associated adverse effects. The main cardiovascular adverse effects associated with these vaccines are VITT and myocarditis. While the former has been associated with adenovirus-vectored vaccines, the latter has been observed in mRNA vaccine recipients.

Vaccine-related myocarditis remains a rare adverse event, although its incidence among males can reach 107 cases per million doses, higher than myocarditis associated with COVID-19 in this same population.

However, since the course of myocarditis associated with vaccines is generally mild and self-limited, even in male adolescents, the overall protective effect of COVID-19 vaccines, particularly for the prevention of severe COVID-19, hospitalization, MIS-C and death, still overcomes the risk of vaccine-induced myocarditis.

As for children, the benefits are beyond those directly related to patient health, by decreasing the risk of direct transmission in this age group and, indirectly, to older individuals. Vaccination reduces the need of mitigation measures at schools, minimizes school interruptions and helps in the maintenance of well-being, health and safety of children.

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Joint Guideline on Venous Thromboembolism – 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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Joint Guideline on Venous Thromboembolism – 2022	
The report below lists declarations of interest as reported to the SBC by the experts during the period of the development of these statement, 2021/2022.	
Expert	**Type of relationship with industry**
Adriano José de Souza	Nothing to be declared
Alair Augusto Sarmet Moreira Damas dos Santos	Nothing to be declared
Alexandre Dias Mançano	Nothing to be declared
Ana Cláudia Gomes Pereira Petisco	Nothing to be declared
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Armando Luis Cantisano	Nothing to be declared
Artur Martins Novaes Coutinho	Nothing to be declared
Bruno de Lima Naves	Financial declaration A - Economically relevant payments of any kind made to (i) you, (ii) your spouse/partner or any other person living with you, (iii) any legal person in which any of these is either a direct or indirect controlling owner, business partner, shareholder or participant; any payments received for lectures, lessons, training instruction, compensation, fees paid for participation in advisory boards, investigative boards or other committees, etc. From the brazilian or international pharmaceutical, orthosis, prosthesis, equipment and implants industry: - Bayer: Xarelto. Other relationships Funding of continuing medical education activities, including travel, accommodation and registration in conferences and courses, from the brazilian or international pharmaceutical, orthosis, prosthesis, equipment and implants industry: - Bayer: Xarelto; Apsen: Dobeven; Servier: Daflon.
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Robson Barbosa de Miranda	Financial declaration A - Economically relevant payments of any kind made to (i) you, (ii) your spouse/partner or any other person living with you, (iii) any legal person in which any of these is either a direct or indirect controlling owner, business partner, shareholder or participant; any payments received for lectures, lessons, training instruction, compensation, fees paid for participation in advisory boards, investigative boards or other committees, etc. From the brazilian or international pharmaceutical, orthosis, prosthesis, equipment and implants industry: - Mindray Brasil: Diagnostic equipment. Other relationships Any economically relevant equity interest in companies in the healthcare or education industry or in any companies competing with or supplying to SBC: - Teaching Area: Vascular Ultrasound Course with Doppler.
Ronaldo de Souza Leão Lima	Nothing to be declared
Salomon Israel do Amaral	Nothing to be declared
Simone Nascimento dos Santos	Financial declaration A - Economically relevant payments of any kind made to (i) you, (ii) your spouse/partner or any other person living with you, (iii) any legal person in which any of these is either a direct or indirect controlling owner, business partner, shareholder or participant; any payments received for lectures, lessons, training instruction, compensation, fees paid for participation in advisory boards, investigative boards or other committees, etc. From the brazilian or international pharmaceutical, orthosis, prosthesis, equipment and implants industry: - Jonhson and Abbott: Intracardiac echo.
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Content

Introduction

Venous thromboembolism (VTE) manifests as pulmonary embolism (PE) and/or deep vein thrombosis (DVT), being part of the same disease spectrum and presenting the same risk factors. It is the third most common cause of acute cardiovascular syndrome worldwide, being potentially life-threatening.[1,2]

In Brazil, according to data from the Ministry of Health collected between 2010 and 2021, the number of hospitalizations for VTE exceeded 520,000, with a total of more than 67,000 deaths from 2010 to 2019.[3]

It has a high mortality rate. Approximately 34% of affected patients die suddenly or within a few hours of the first manifestation, that is, even before receiving any type of treatment.

Almost two-thirds of the cases of VTE manifest as DVT alone, mostly as proximal DVT of the lower extremities, and one-third as PE.[2]

In the literature, several studies have associated the presence of VTE with climate variation. In Brazil, Ohki et al.[4] reported a higher incidence of VTE in the southern states, where temperatures are lower. These states have a mean incidence of VTE of 2.86 cases per 100,000 population.[4]

This clinical syndrome exponentially increases with age, even with the implementation of prevention strategies.[5] Women are most commonly affected in their youth, especially in the postpartum period. During pregnancy, tobacco use, thrombophilia and/or a history of VTE increase the risk in this group of patients. Other conditions, such as prolonged immobility, obesity, cancer, major surgery with prolonged anesthesia, multiple trauma, lower-extremity varicose veins, hormone replacement therapy, and cardiovascular diseases, are considered at risk for VTE, although it has been recognized that there is variation in risk prediction between these causes.[2]

Ethnicity is considered a risk factor for VTE. The incidence of VTE is significantly higher among White and African American people and lower among Hispanic and Asian people. Overall, approximately 25%-50% of patients presenting with a first episode of VTE have an idiopathic condition, without an easily identifiable risk factor. Early mortality in VTE is strongly

associated with pulmonary involvement, in the form of PE, in addition to advanced age, cancer and underlying cardiovascular disease.[2]

The recurrence rate is relevant, and non-fatal VTE may have sequelae such as pulmonary hypertension in cases of chronic PE and post-thrombotic syndrome in DVT. Notably, there has been a reduction in deaths due to better diagnostic and therapeutic management after the publication of international position statements and guidelines on the topic.[4]

Diagnosis of VTE may not be straightforward. Therefore, it is necessary to focus on the diagnostic approach to PE and DVT, as failure to correctly diagnose the condition can be fatal or lead to permanent comorbidities.

In this context, the Department of Cardiovascular Imaging (DIC/SBC), the Brazilian College of Radiology (CBR), the Brazilian Society of Angiology and Vascular Surgery (SBACV), and the Brazilian Society of Nuclear Medicine (SBMN) jointly developed this document. The purpose is to address clinical and diagnostic aspects aiming to create a single recommendation for the 4 societies, which should serve as a source of information for Brazilian physicians and as a standard for the clinical, laboratory, and imaging diagnosis of VTE. The prophylactic and therapeutic approach to VTE is not within the scope of this document.

This recommendation was developed based on guidelines, on population-based studies and case reports published in the literature, and on the expertise of the participating members. Each work group compiled the data, drafted the writings, and then submitted the revised text for final adjustments. The most controversial issues were discussed at a meeting attended by the majority of the members. Corrections were made to the original text if necessary.

This recommendation is aimed at clinicians or surgeons and intends to provide the best information on the diagnostic approach to VTE, offering data on the accuracy of diagnostic tools used in daily practice. For imaging physicians, a detailed description of the imaging protocols can help them make a more accurate diagnosis, thus contributing to a better clinical management of suspected or confirmed cases.

1. Pathophysiology and Clinical and Laboratory Diagnosis of Venous Thromboembolism

1.1. Introduction

The spectrum of venous thromboembolism (VTE) comprises deep vein thrombosis (DVT) and pulmonary embolism (PE). Thromboembolic disease is the third most common acute cardiovascular disease, after ischemic heart syndromes and stroke. There is a broad spectrum of clinical manifestations for these diseases, from clinically silent to massive embolism, leading to death.[5] About one-third of all cases of PE are fatal, and cancer is one of the many disease states associated with an increased risk of thromboembolic disease. Approximately two-thirds of the cases of VTE are DVT, 85%-90% occurring in the lower extremities, and one-third are PE.[6,7] In this context, we must always remember that VTE is a serious but preventable disease.

1.2. Pathophysiology

A) Deep vein thrombosis (DVT):

DVT is a common and potentially fatal condition, with PE as the main complication. It can be considered the first cause of avoidable in-hospital morbidity and mortality. In addition, it can also lead to post-thrombotic syndrome (PTS) as a chronic complication, which has an important socioeconomic impact. These complications can occur despite proper diagnosis and therapy, but early diagnosis and prompt initiation of the correct treatment can reduce their potential deleterious effects.

DVT can occur in both inpatients and outpatients. The symptoms are nonspecific, varying from a completely asymptomatic to a catastrophic condition, such as in phlegmasia cerulea dolens (PCD). PCD is characterized by complete occlusion or massive subocclusion of the limb draining veins (iliofemoral system) and collaterals, which may extend to the microcirculation. Venous hypertension reaches a level that can obstruct arterial inflow.[7,8] The high hydrostatic pressure and interstitial fluid sequestration leads to the formation of hardened edema in the entire limb, which can accumulate 3 to 5 liters in volume and lead to the collapse of arterioles, skin blisters due to tissue ischemia, purpura/ecchymosis, cyanosis, loss of distal pulses, circulatory collapse, and hypovolemic shock.[9,10]

Importantly, the diagnosis of DVT begins with the clinical history. Attention should be paid to any past medical history that may potentiate or predispose the patient to an increased risk of developing venous thromboembolic phenomena, as postulated by Virchow:

- BLOOD FLOW CHANGES (stasis): age, immobility ≥3 days or physical inactivity such as in the postoperative period, stroke, knee or hip fracture, major general surgery (>45 min, the risk rises with increasing procedure duration), major trauma, spinal cord injury with limb paresis, immobilization of extremities with casts or orthoses, long-haul travel in a confined space, pregnancy, congestive heart failure, varicose veins, COPD, and burns, among others.[11]

- ENDOTHELIAL DAMAGE (trauma): advanced age (after 40 years of age, the risk doubles with each decade), smoking, known thromboembolic history, trauma, surgery, and central venous catheters, among others.[11,12]

- HYPERCOAGULABILITY: cancer and/or their treatment, inherited or acquired thrombophilia, obesity, use of estrogen-containing oral contraceptives and hormone replacement therapy, pregnancy, smoking, acute infectious diseases, nephrotic syndrome, and inflammatory bowel disease, among others.[9,11,13,14]

1.3. Clinical and laboratory diagnosis

Once the epidemiological risk for DVT has been identified, and it is mandatory, physical examination should be performed. The clinical diagnosis of DVT has low sensitivity and specificity, as only 20%-40% of patients with suggestive clinical features have the disease confirmed. The most common signs and symptoms of DVT are pain and edema. Despite the low

accuracy of clinical diagnosis, it is important to note that, at first, any asymmetric swelling in the lower extremities should be considered until the diagnostic evaluation is completed.

However, it should be also taken into account that, in a considerable number of cases (20%-50%), the patient may present with extensive DVT (even proximal) without any suggestive symptoms. Clinical suspicion is essential for the diagnosis in these cases, but one should keep in mind that the clinical diagnosis does not have satisfactory sensitivity/specificity. Therefore, the final diagnosis must be confirmed by complementary tests. Vascular Doppler ultrasound is the method of choice to confirm DVT due to its high accuracy, being easy to perform and harmless with good reproducibility. However, the availability of this diagnostic modality is limited in smaller medical centers and outpatient clinics as well as during night and weekend shifts.[15]

DVT is classified as proximal when it involves the femoral and/or popliteal veins, with or without the involvement of other leg veins, and as distal when it involves the deep infrapatellar veins.[16,17] Proximal DVT has the greatest potential for embolism. Other classifications that can determine the severity of DVT are based on the degree of thrombus extension. More extensive thrombi usually have greater consequences because they carry venous hypertension and the degree of partial and complete luminal obstruction. The degree of luminal narrowing, as well as its location and extension, will determine the clinical severity and prognosis of venous thrombosis.

In 1997, in an attempt to simplify the diagnostic approach to these patients, Wells et al.[18] developed a clinical prediction model to classify patients for DVT risk. The method proved to be feasible and useful in several studies when combined with complementary noninvasive tests. The same group implemented modifications in 2003, facilitating understanding for clinicians.[6] Several specialty and consensus societies recommend the use of clinical prediction models to estimate the probability of a DVT diagnosis before performing a vascular ultrasound (VUS) examination.[19] Among these tools, the most used one is the combination of Wells score and serum D-dimer levels.[7] (Chart 1).

– Simplified score: ≥ 2 DVT likely; ≤1 DVT unlikely

– The diagnostic accuracy of Wells score depends on the population assessed (outpatient x inpatient), extent of DVT (proximal x distal), and degree of probability (low x moderate x high), being more accurate in outpatients with proximal DVT who have a high probability score (>2). Silveira et al., in a population-based study, obtained an overall diagnostic accuracy of 0.56 (AUC – area under the curve) for inpatients. In outpatients, the effectiveness of the score can range from 11.9% to 79.5%.[6,20,21]

1.3.1. Measurement of D-dimer

Measurement of D-dimer (a degradation product of cross-linked fibrin clot) is widely used in the investigation of patients with suspected VTE.[22,23] The quantitative D-dimer assay, based on a rapid ELISA test, has high diagnostic sensitivity (close to 95%). However, the assay has low specificity (40%) because D-dimer levels may be increased in several conditions other than VTE, such as in acute myocardial infarction, stroke, inflammation, active cancer, and pregnancy. Specificity also decreases with age, and it can be as low as 10% in older adults. In a systematic review, the use of an adjusted cutoff value for patients over 50 years of age in order to rule out the presence of DVT (patient age in years [above 50] x 10 in μg/L) appeared to be as safe as the standard cutoff value, which was pointed out as a recommendation in the latest 2019 European guideline.[24,25]

Consequently, a negative quantitative D-dimer test has a high negative predictive value for VTE. The results of the studies reveal that the risk of development of PE in patients with low clinical probability who are not treated after a negative D-dimer test is <1% within 3 months of the initial evaluation. However, because of its low predictive value, a positive quantitative D-dimer test does not modify the pretest (clinical) probability and is therefore clinically useless. A highly sensitive negative D-dimer test combined with a low pretest probability can exclude DVT.[26]

D-dimer levels can also vary depending on the number of veins involved, thrombus extension and volume. More extensive proximal thromboses have higher D-dimer levels than those confined to the calf veins, which may be an

Chart 1 – Wells clinical model for predicting deep vein thrombosis (DVT)[6]

Clinical Findings	Score
Active cancer OR cancer treated within the previous 6 months.	1
Paresis, paralysis, or recent immobilization of the lower extremities.	1
Recently bedridden for 3 days OR major surgery within the previous 4 weeks.	1
Localized tenderness along the distribution of the deep venous system.	1
Entire leg swollen.	1
Calf swelling at least 3 cm larger than that on the contralateral side (measured 10 cm below the tibial tuberosity).	1
Pitting edema (positive Godet sign) confined to the symptomatic leg.	1
Collateral superficial veins (nonvaricose).	1
Previously documented DVT.	1
Presence of more likely differential diagnosis: lymphedema, cellulitis, joint changes, superficial thrombophlebitis, muscle rupture, Baker's cyst.	-2

important factor when assessing the burden of thromboembolic disease.[22,27-29] Recent evidence also suggests that very high D-dimer levels are associated with a 4-fold increase in the probability of PE.[22,30] However, when the clinical probability is high, regardless of D-dimer levels, VUS is mandatory to confirm or rule out the presence of DVT[31] (Chart 2).

B) Pulmonary embolism (PE):

PE is defined as the obstruction of one or more pulmonary arteries. In most cases, it is caused by blood clots that, most often, arise from DVT of the lower extremities and reach the pulmonary arteries.

Clinical signs of PE are nonspecific, such as dyspnea, chest pain, hemoptysis, and syncope or presyncope. In some cases, it may be asymptomatic and discovered incidentally. Syncope appears to be present in approximately 17% of cases and is associated with a high prevalence of hemodynamic instability and right ventricular failure. PE with hemodynamic instability is infrequent, but it may be suggestive of massive impairment of the pulmonary circulation.

If PE is suspected, it must be confirmed or ruled out to avoid the risk of overtreatment or undertreatment. The Wells or Geneva clinical risk scores are often used to classify the pretest probability.

The diagnostic accuracy of Wells scores for the diagnosis of PE was established by a meta-analysis of 11 studies, in which sensitivity ranged from 63.8% to 79.3% and specificity, from 48.8% to 90%, with an AUC of 0.778. For the Geneva score, however, sensitivity ranged from 55.3% to 73.6% and specificity, from 48.8% to 90.0%, with an AUC of 0.693.[32]

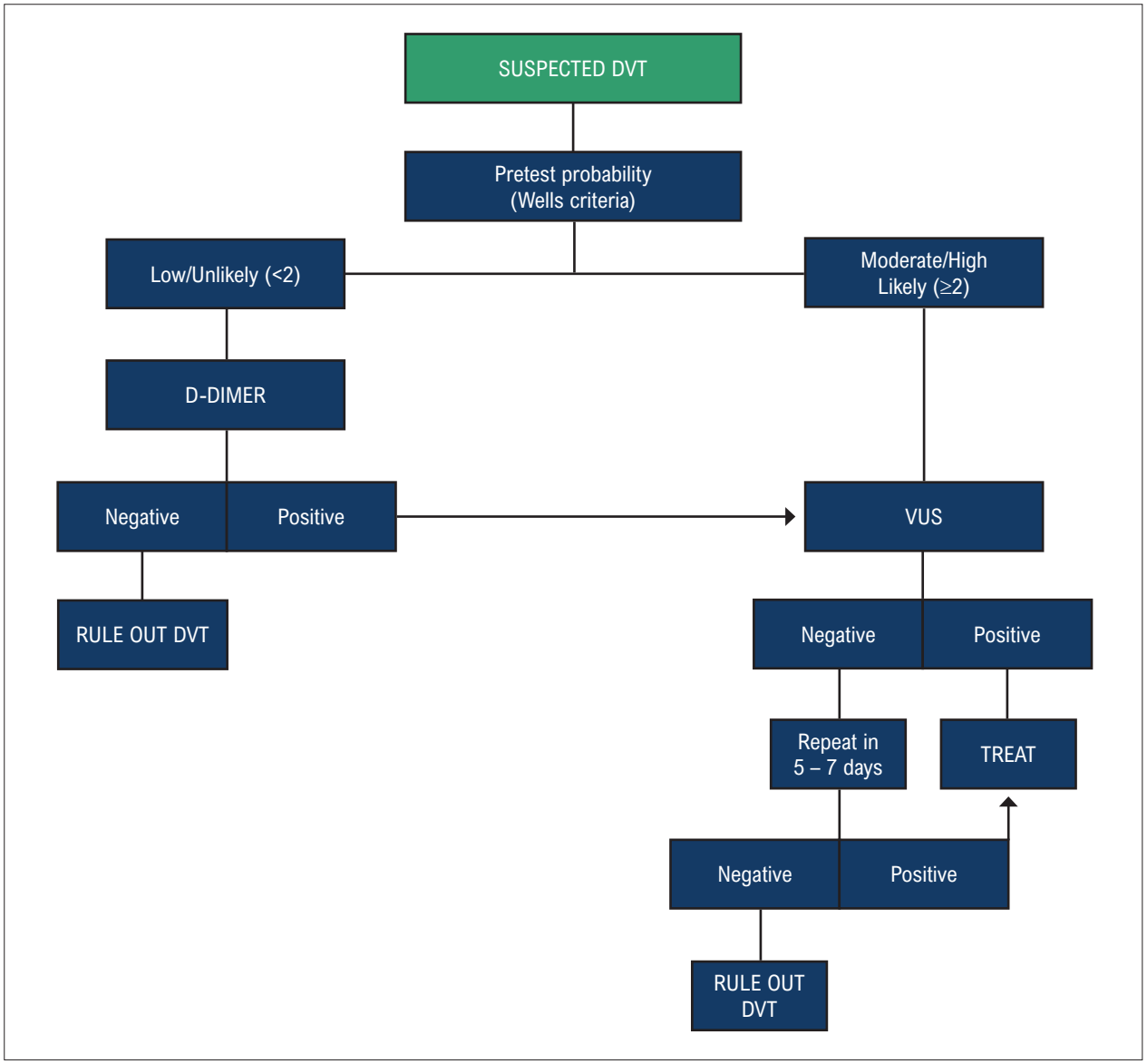


Chart 2 – *Diagnostic flowChart for the study of deep vein thrombosis (DVT)*

These scores, when combined with the measurement of D-dimer, as described earlier, have important implications for the diagnostic workup and for the indication of imaging tests (Charts 3 and 4).

In the assessment of the clinical probability of PE, diagnostic strategies will depend on the patient's hemodynamic stability. In addition to clinical features and D-dimer, complementary techniques recommended for the diagnosis of PE include computed tomography (CT) pulmonary angiography, V/Q SPECT scintigraphy, and echocardiography (Charts 5 and 6). Each of these techniques and the role of echocardiography in the diagnostic flowchart, when PE has hemodynamic repercussions, hypotension, or shock, will be discussed later.

2. Echocardiographic Changes in Pulmonary Embolism

2.1. Introduction

The usefulness of echocardiography (transthoracic and/or transesophageal) in acute pulmonary embolism (PE) lies in the investigation of right ventricular (RV) pressure overload (due to increased pulmonary vascular resistance, with consequent increase in RV afterload) and in functional assessment of the RV. In most cases, patients with thromboembolic episodes without hemodynamic repercussions have normal test results. In hemodynamically unstable patients, echocardiography plays an important supporting role in the stages of diagnosis and noninvasive monitoring of treatment response.

2.2. Echocardiography in Low-risk Pulmonary Embolism (PE)

Echocardiography is not a mandatory test in the diagnostic routine for hemodynamically stable patients with suspected PE in diagnostic algorithms.[1] The negative predictive value of echocardiography ranges from 40%-50%, and a normal result does not exclude PE.[35-37] Despite not contributing to the diagnosis, it represents an important tool for prognostic discrimination: the absence of changes in RV size or function indicate a good prognosis.[38-41] The test is also important to define differential diagnoses of acute dyspnea.

In the study of the RV, with its unique crescent shape, there are technical difficulties inherent in the asymmetric geometry of the cavity. However, the technique for a comprehensive examination was standardized in 2015 in an updated joint document from the American Society of Echocardiography and the European Association of Cardiovascular Imaging.[42] The general recommendations define the essential windows and views that provide the images required to obtain all the necessary data for quantification:

a) Left parasternal, long- and short-axis;

b) Apical 4-chamber;

c) RV-focused apical 4-chamber;

d) Left parasternal RV inflow;

e) Subcostal.

Chart 7 provides the recommendations for measurement of right cavity dimensions and the parameters for RV systolic function.

2.3. Echocardiography in High-risk Pulmonary Embolism (PE)

Echocardiography is mandatory for all hemodynamically unstable patients with clinically suspected PE, as it can reliably detect changes in the right cavities that reflect a sudden and severe increase in pulmonary vascular resistance (RV afterload), responsible for dysfunction and hypotension. In these patients, normal test results definitively rule out the hypothesis of massive PE.[39,43] Conversely, the detection of changes corresponding to RV pressure overload and dysfunction (in the absence of other obvious causes of differential diagnosis) allows the diagnosis of massive PE and emergency reperfusion therapy, even if chest CT angiography or ventilation and perfusion scintigraphy cannot be performed.[44]

The only pathognomonic echocardiographic finding of PE is the presence of mobile thrombi in the right cavities and/or pulmonary trunk or branch arteries (Figure 1). This finding is associated with high early mortality[45-49] and occurs in only 4% of overall cases of PE, reaching an 18% prevalence in patients with PE in intensive care units.[50,51]

Key changes related to severe acute PE[52] are:

a) RV dilatation;

b) RV dysfunction;

c) Pulmonary pressure overload. It occurs in 30%-40% of patients with PE and indicates worse prognosis.[53-55]

Chart 3 – Wells clinical model for predicting pulmonary embolism[33]

VARIABLES	SIMPLIFIED SCORE
Clinical signs of DVT	3
Heart rate > 100 bpm	1.5
Immobilization or recent surgery	1.5
Previous DVT or PE	1.5
Hemoptysis	1
Cancer	1
Alternative diagnosis less likely than PE	3
PE likely > 4 and PE unlikely ≤ 4	

Chart 4 – Revised Geneva clinical model for predicting pulmonary embolism (PE). Adapted from Le Gal et al.[34]

VARIABLES	CLINICAL POINTS EVALUATED	
	ORIGINAL	SIMPLIFIED
Previous PE or DVT	3	1
HEART RATE		
75 – 94 bpm	3	1
≥ 95 bpm	5	2
Surgery or fracture ≤ 1 month	2	1
Hemoptysis	2	1
Active cancer	2	1
Unilateral lower-limb pain	3	1
Pain on lower-limb deep venous palpation and unilateral edema	4	1
Age > 65 years	1	1
CLINICAL PROBABILITY		
3-level score		
Low	0 - 3	0 - 1
Intermediate	4 - 10	2 - 4
High	≥ 11	≥ 5
2-level score		
Unlikely	0 - 5	0 - 2
Likely	≥ 6	≥ 3

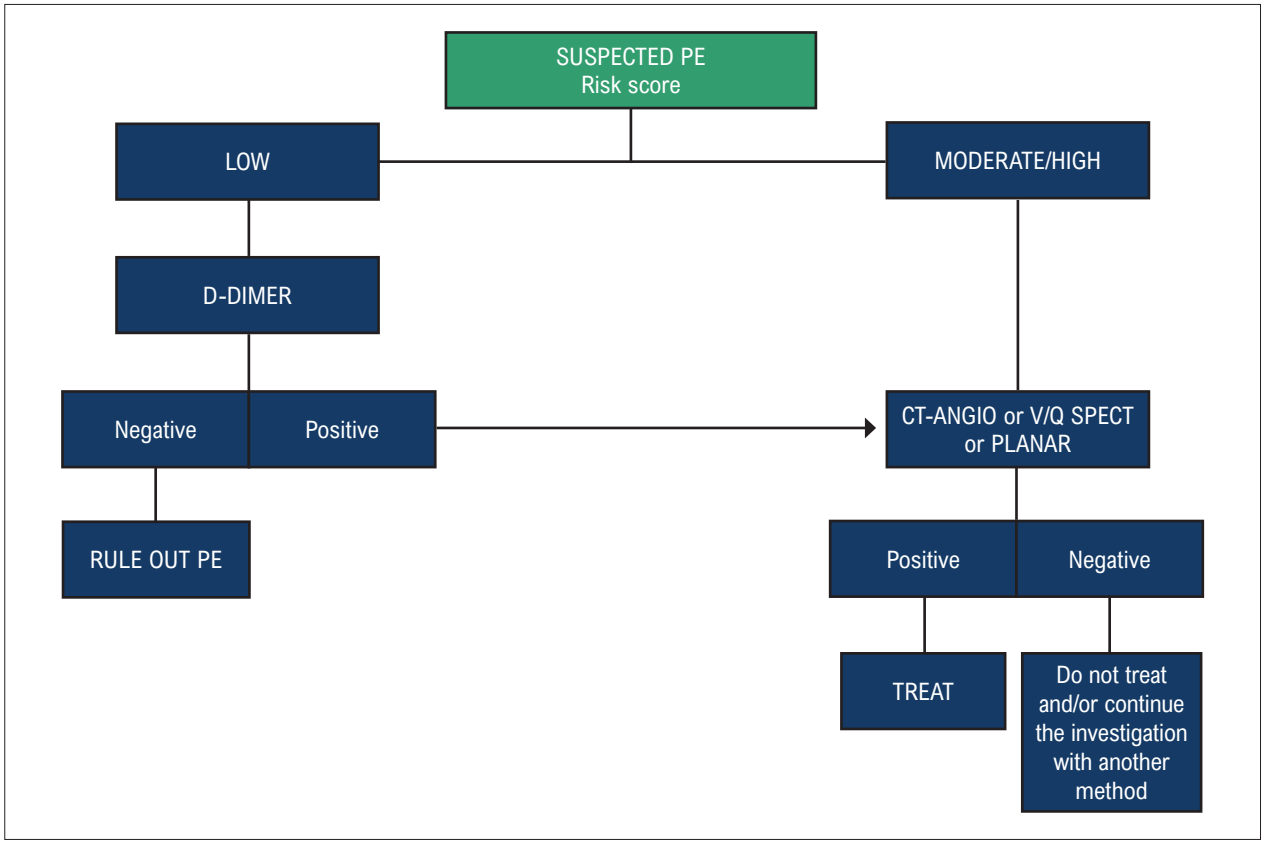


Chart 5 – *Diagnostic flowChart for the assessment of suspected pulmonary embolism (PE) without hemodynamic repercussions*

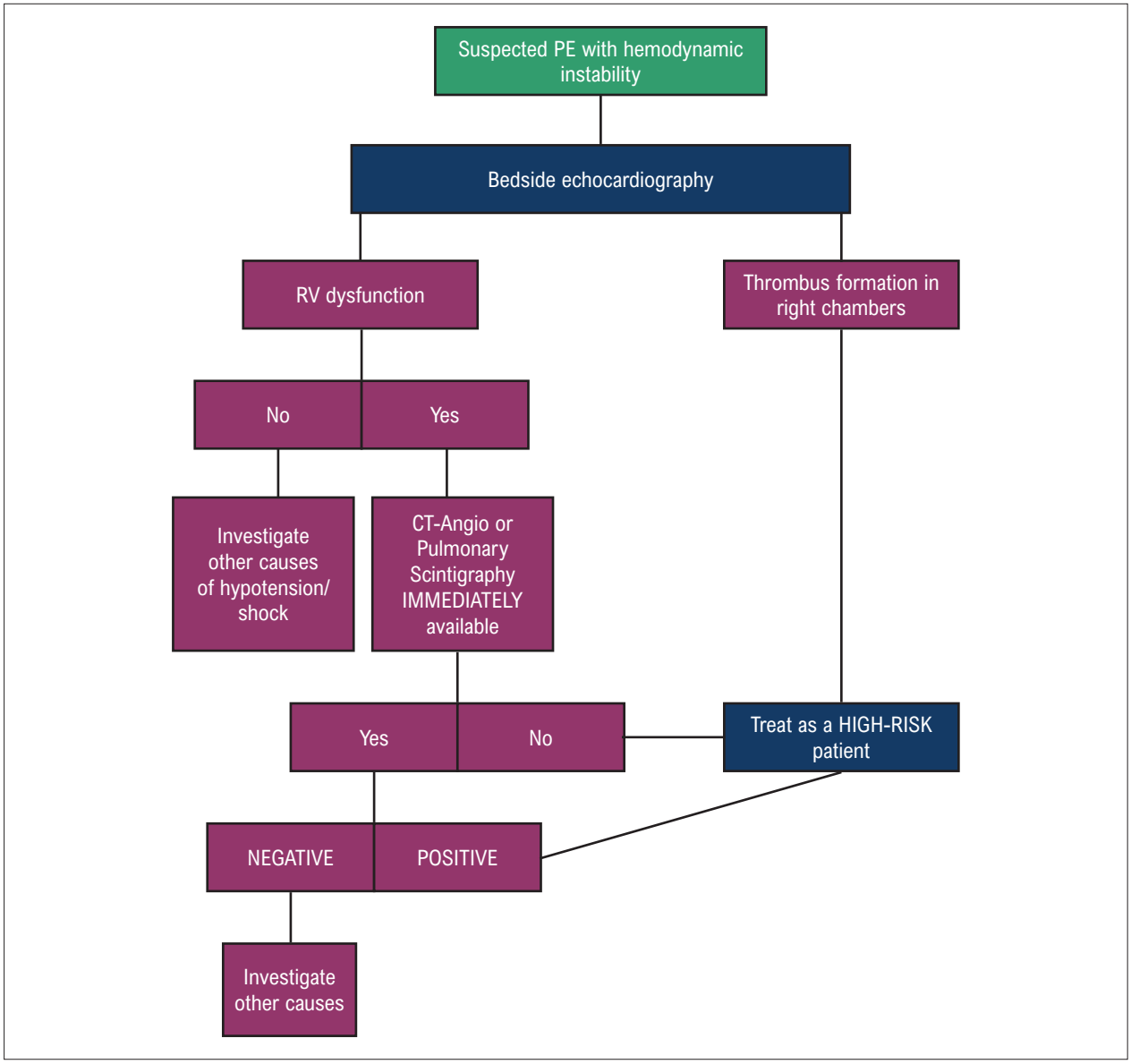


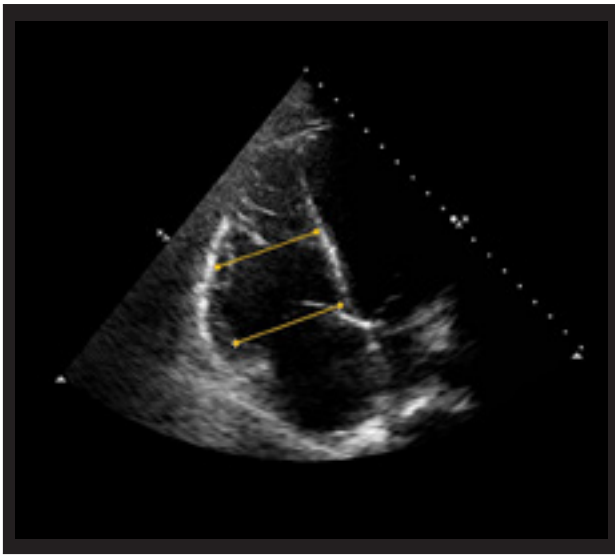
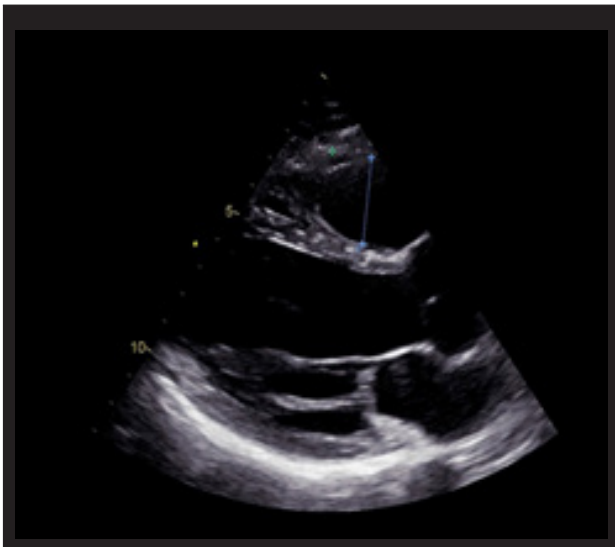
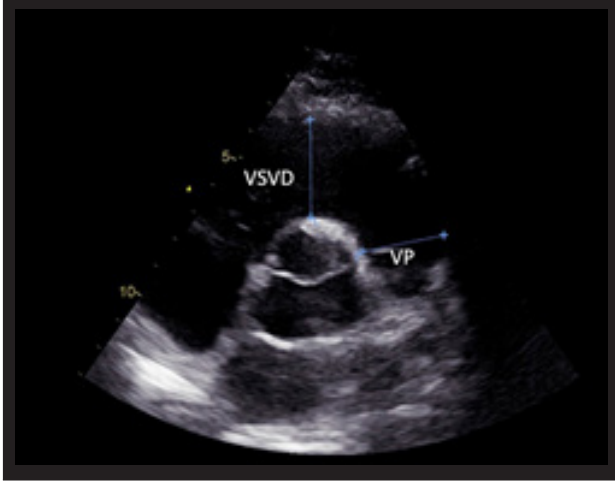
Chart 6 – *Diagnostic algorithm for acute pulmonary embolism (PE) in hemodynamically unstable patients*

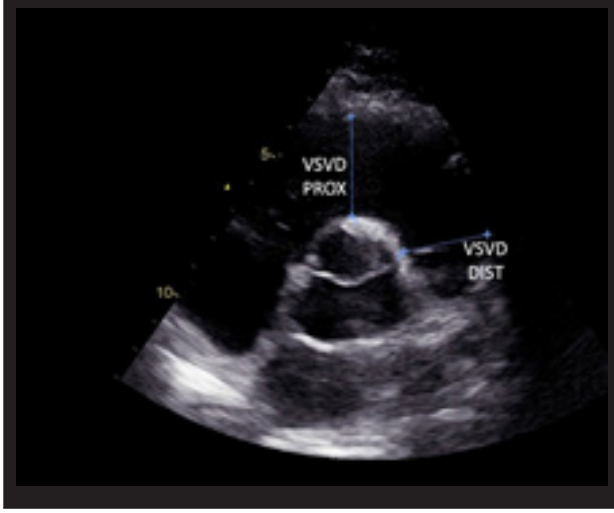
When ventricular dilatation (Figure 2) is associated with the 60/60 sign – combination of pulmonary ejection acceleration time of <60 ms and tricuspid valve peak systolic gradient <60 mm Hg –, or with McConnell sign – hypokinesia of basal and mid segments of the RV free wall and normokinesia of the apical segment (Figure 3) –, the positive predictive value for massive PE is high.[56] However, the 60/60 sign is present in only 12%, and McConnell sign in 20% of unselected patients. Another sign of increased RV afterload is reduced pulmonary acceleration time and the presence of mid-systolic deceleration (Figure 4). Signs of RV pressure overload help to differentiate hypokinesia or akinesia of the free wall due to PE from that caused by acute right ventricular infarction (which may mimic McConnell sign).[52,57]

Patients with PE may also have reduced tricuspid annular plane systolic excursion (TAPSE).[58,59] Tissue Doppler and parietal strain of the RV have low sensitivity as isolated findings in patients with acute PE.[60,61] In patients with suspected acute PE but with increased RV free wall thickness or tricuspid valve regurgitant jet velocities above >3.8 m/s or tricuspid valve peak systolic gradient >60 mm Hg, we should include differential diagnosis with chronic thromboembolic pulmonary hypertension.[38,62]

The 3D echocardiography can be used in laboratories with experience in the technique for an evolutionary analysis of patients with massive PE treated with thrombolysis. Figures 5 and 6 illustrate a case of massive PE, with significant hemodynamic repercussions, treated with a thrombolytic agent, progressing to an improvement in RV ejection fraction and a significant reduction in right cavity volumes.

Chart 7 – Recommendations for measurement of right cavity dimensions and the parameters for right ventricular (RV) systolic function.

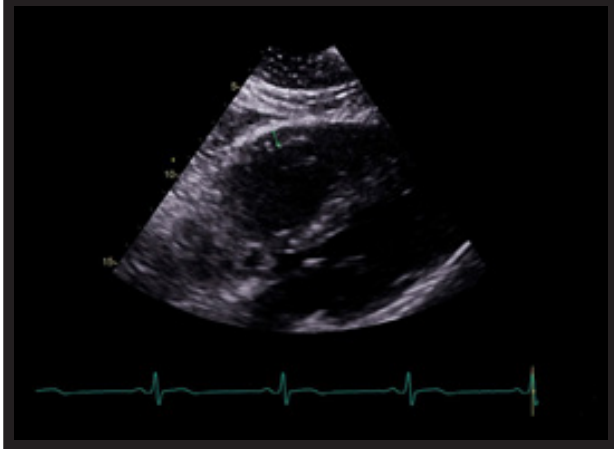
LINEAR MEASUREMENTS OF THE RIGHT VENTRICULAR (RV) INFLOW TRACT	
	**RVd1 =** basal RV linear diameter (maximal transversal dimension in the basal one-third of RV inflow at end-diastole in the RV-focused view). NV = 33 ± 4 mm25-41 **RVd2 =** mid-cavity RV linear diameter (transversal RV dimension in the middle third of RV inflow, approximately halfway between the maximal basal diameter and the apex, at the level of papillary muscles at end-diastole. NV = 27 ± 4 mm[19-35]
LINEAR MEASUREMENTS OF THE RIGHT VENTRICULAR (RV) OUTFLOW TRACT	
	**A)** Proximal RV outflow diameter (parasternal long-axis view) = linear dimension measured from the anterior RV wall to the interventricular septal-aortic junction at end-diastole. NV = 25 ± 2.5 mm[20-30]
	**B)** Proximal RV outflow diameter (parasternal short-axis view) = linear dimension measured from the anterior RV wall to the aortic valve at end-diastole. NV = 28 ± 3.5 mm[21-35]



C) Distal RV outflow diameter (parasternal short-axis view) = linear transversal dimension measured just proximal to the pulmonary valve at end-diastole.

NV = 22 ± 2.5 mm[17-27]

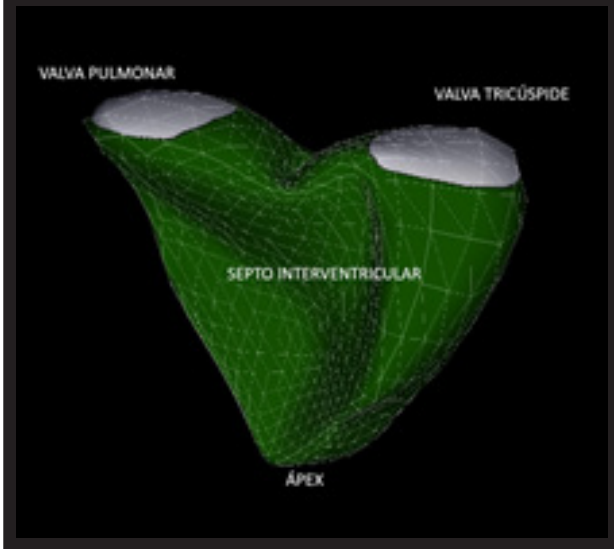
LINEAR MEASUREMENTS OF RV WALL THICKNESS



Linear measurement of RV wall thickness (either by one-dimensional or 2D echocardiography) is performed at end-diastole, below the tricuspid annulus at a distance approximating the length of anterior tricuspid leaflet (fully open and parallel to the RV free wall).

NV = 3 ± 1 mm[1-5]

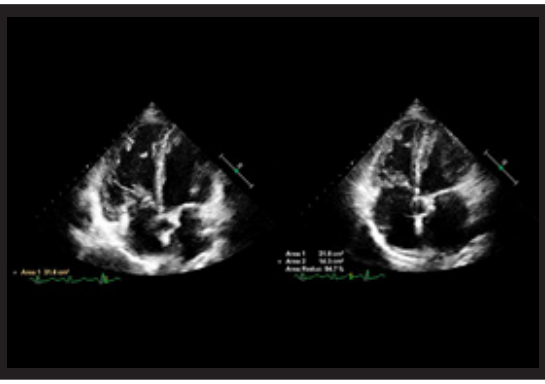
MEASUREMENTS OF RV VOLUME BY 3D ECHOCARDIOGRAPHY



RV size should be measured by conventional 2D echocardiography using multiple acoustic windows. However, in laboratories with experience in 3D echocardiography, 3D measurement of RV volumes is recommended.

NV for RV EDV: Men 61 ± 13 mL/m^2[235-87]
Women 53 ± 10.5 mL/m^2[232-74]
NV for RV ESV: Men 27 ± 8.5 mL/m^2[210-44]
Women 22 ± 7 mL/m^2[28-36]

RV GLOBAL SYSTOLIC FUNCTION ASSESSED BY FRACTIONAL AREA CHANGE (FAC)

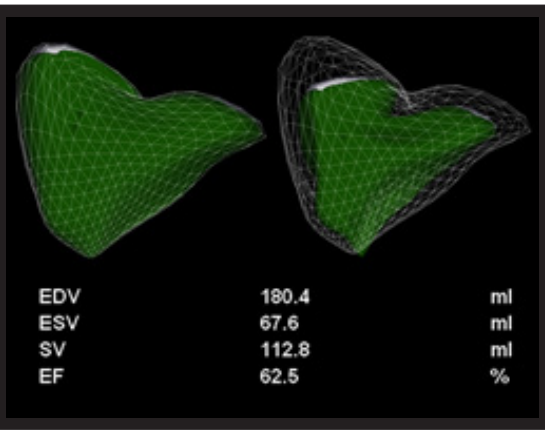


RV FAC (%) = 100 X (EDA – ESA)/EDA

RV-focused apical 4-chamber view. Manual tracing of RV endocardial border from the lateral tricuspid annulus along the free wall to the apex and back to medial tricuspid annulus, along the interventricular septum
at end-systole (ESA) and at end-diastole (EDA). Trabeculations, papillary muscles, and moderator band are included in the cavity area measurement.

NV = 49 ± 7% (>35%)

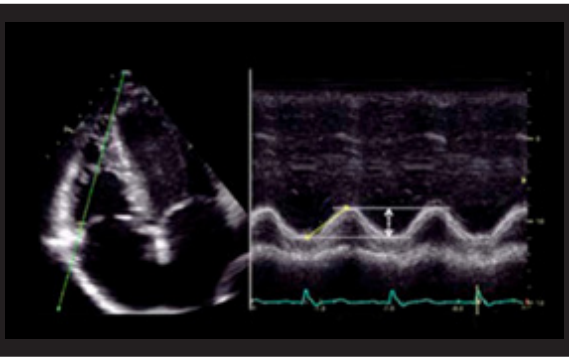
RV GLOBAL SYSTOLIC FUNCTION ASSESSED BY 3D ECHOCARDIOGRAPHY EJECTION FRACTION



Fractional RV volume change by 3D echocardiography [RV EF (%) = 100 X (EDV-ESV)/EDV]. When performed correctly, it correlates with ejection fraction calculated by cardiac magnetic resonance. In experienced laboratories, 3D measurement of RV ejection fraction is recommended.

NV = 58 ± 6.5% (>45%)

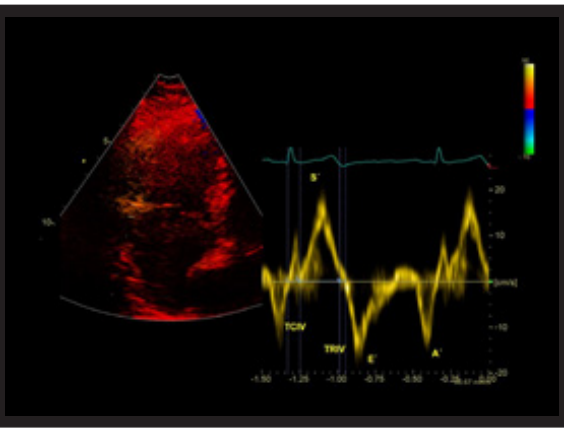
RV LONGITUDINAL SYSTOLIC FUNCTION ASSESSED BY TAPSE



Tricuspid annular longitudinal excursion measured by M-mode between end-diastole and peak systole. RV-focused apical 4-chamber view for proper alignment of M-mode cursor with the direction of RV longitudinal excursion (lateral tricuspid annulus).

NV = 24 ± 3.5 mm (>17mm)

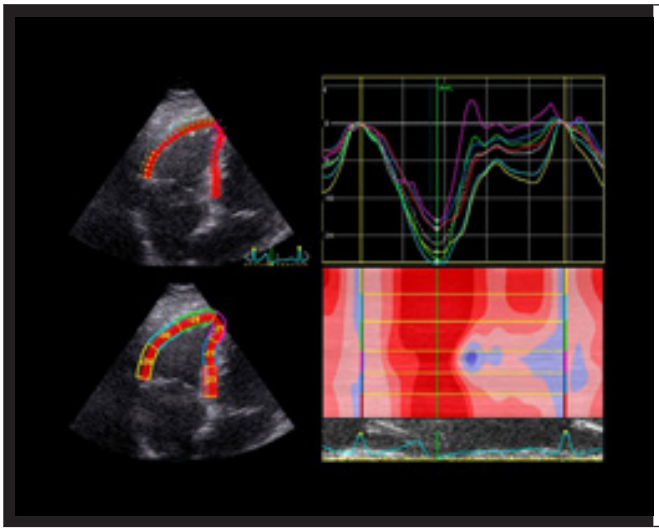
RV LONGITUDINAL SYSTOLIC FUNCTION ASSESSED BY PULSED TISSUE DOPPLER S WAVE



Peak systolic velocity of tricuspid annulus assessed by pulsed-wave tissue Doppler imaging (cm/s), obtained from the RV-focused apical 4-chamber view. It is important to keep the basal segment and the annulus aligned with the Doppler cursor to avoid velocity underestimation.

NV = 14.1 ± 2.3 cm/s (>9.5 cm/s)

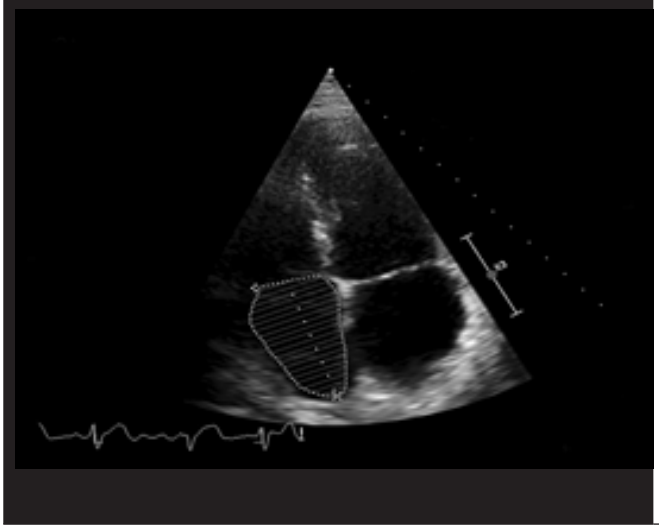
RV LONGITUDINAL SYSTOLIC FUNCTION ASSESSED BY GLOBAL LONGITUDINAL STRAIN



RV longitudinal strain should be measured in the RV-focused apical 4-chamber view. The panel demonstrates RV global longitudinal strain of the 6 segments (3 free wall and 3 septal segments); the values are averaged. RV longitudinal strain is often measured by software not dedicated to that chamber. Currently, there are software versions dedicated to the RV, which should be preferred as they provide data on RV free wall strain and on the strain that incorporates the interventricular septum.

NV: 29 ± 4.5 (20% in absolute value)

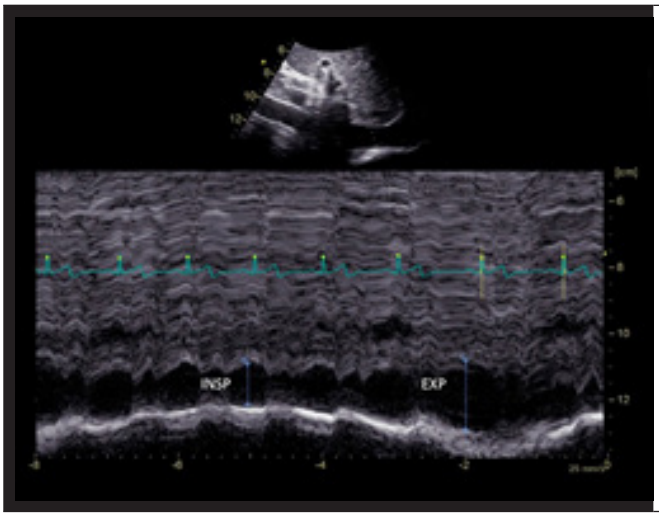
RIGHT ATRIAL (RA) 2D VOLUMETRIC MEASUREMENTS



The recommended parameter to assess RA size is RA volume, calculated using single-plane disk summation (2D) techniques in a dedicated apical 4-chamber view.

NV: Men 25 ± 7 mL/cm^2 [18-32]
Women 21 ± 6 mL/cm^2 [15-27]

MEASUREMENT OF THE INFERIOR VENA CAVA (IVC) DIMENSIONS



In the subcostal view with the patient in the supine position at 1-2 cm from the junction with the RA (perpendicular to the long axis).

The "collapsibility index (CI)" (% decrease in IVC diameter during inspiration) correlates with RA pressure.

IVC ≤ 21 mm and CI > 50% = normal RA pressure (3 mm Hg, ranging from 0-5 mm Hg).

IVC ≤ 21mm and CI < 50% = intermediate RA pressure (8 mm Hg, ranging from 5-10 mm Hg).

zIVC > 21 mm and CI < 50% = high RA pressure (15 mm Hg, ranging
from 10-20 mm Hg).

TSVD: trato de saída do VD; ASC: área de superfície corporal; VD: ventrículo direito; AD: átrio direito; VCI: veia cava inferior; VN: valor de normalidade.

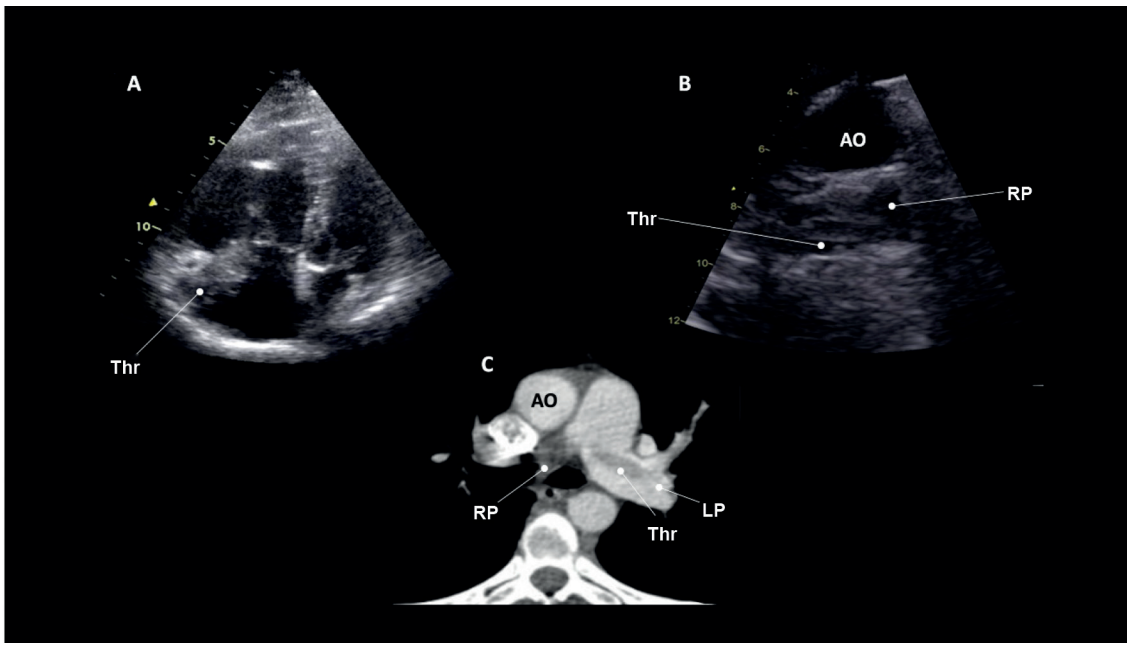


Figure 1 – *A-C: direct visualization of thrombus in the right cavities and left pulmonary artery. Thr: thrombus; Ao: aorta; LP: left pulmonary artery; RP: right pulmonary artery.*

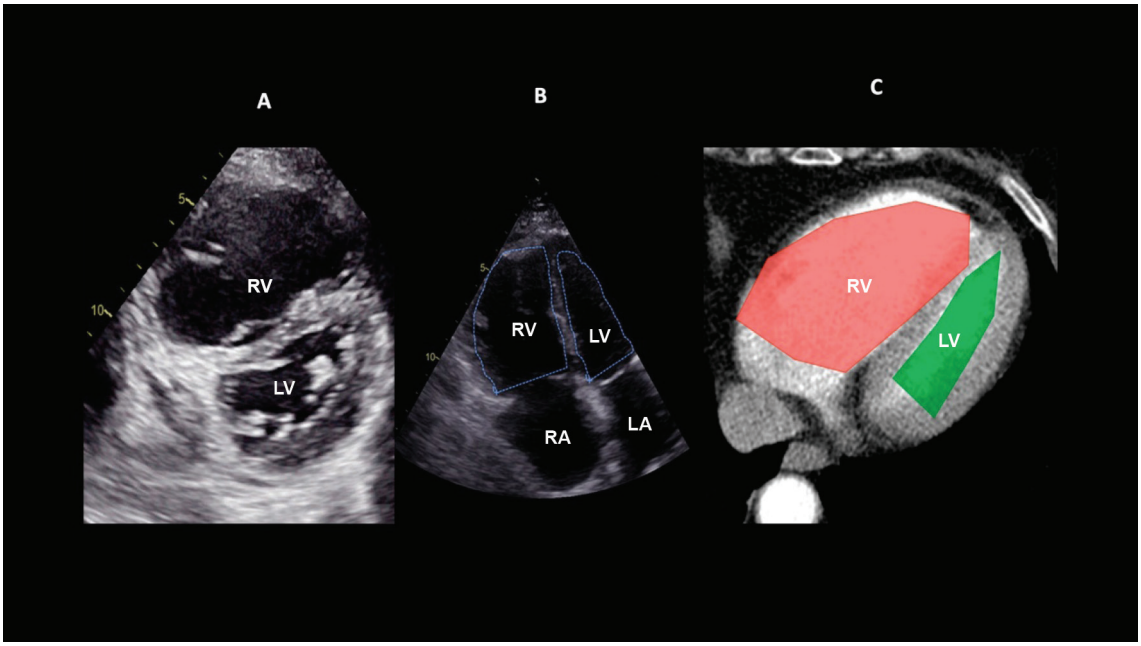


Figure 2 – *Right ventricular (RV) pressure overload and/or dysfunction A: straightening of the interventricular septum with RV dilatation in the parasternal cross-sectional view; B: RV dilatation assessed in the apical view by the RV:LV ratio at end-diastole (>0.6 mild; 1-2 important; >2 severe); C: equivalent to RV:LV ratio assessed by CT angiography. RV: right ventricle; LV: left ventricle; RA: right atrium; LA: left atrium.*

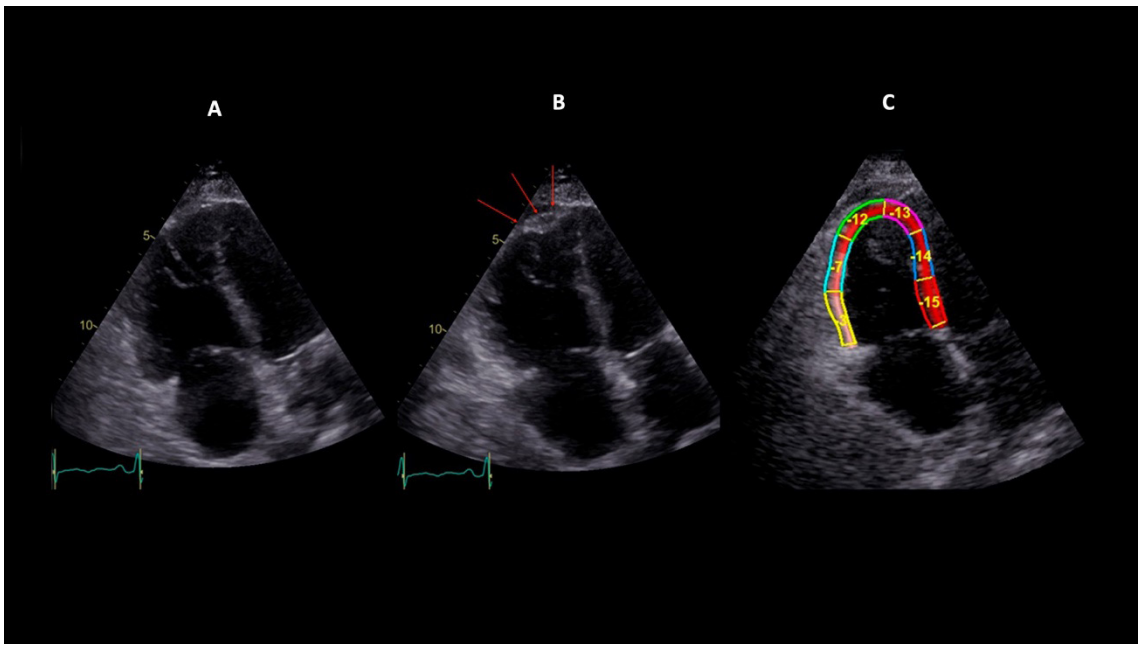


Figure 3 – *McConnell sign. Presence of right ventricular dilatation, free-wall hypokinesia, and preserved apical contractility. A: diastole; B: systole; C: longitudinal strain of the right ventricle (RV); left ventricle (LV); right atrium (RA); and left atrium (LA).*

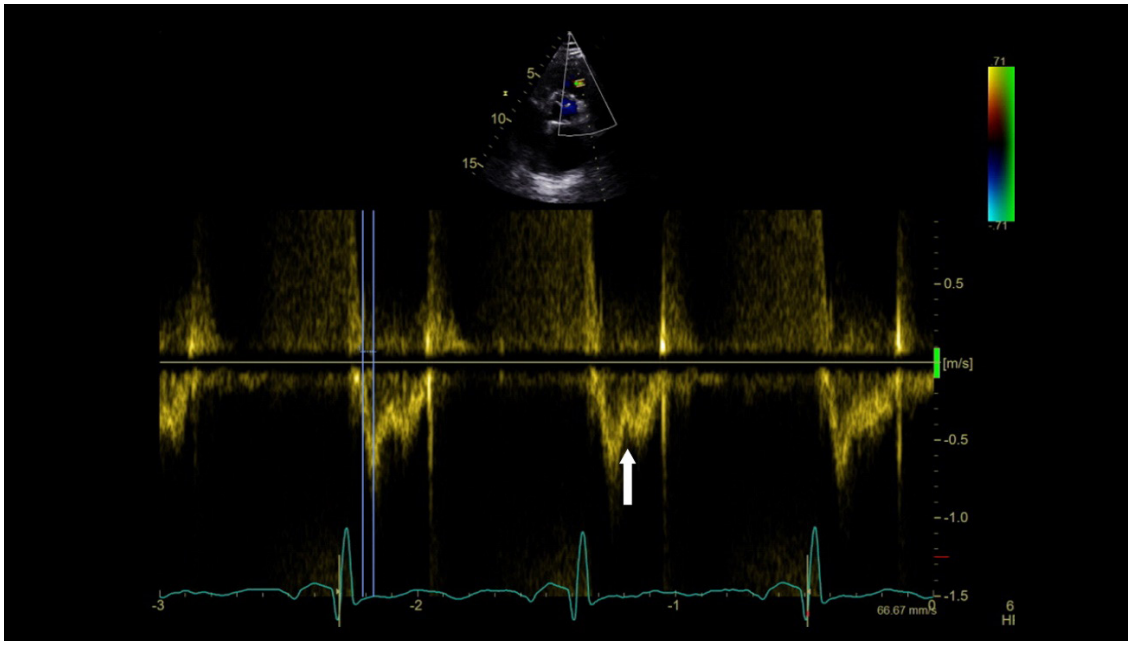


Figure 4 – *Hemodynamic profile of right ventricular outflow tract (RVOT) caused by increased RV afterload: reduction in acceleration time and presence of mid-systolic deceleration (notch – white arrow).*

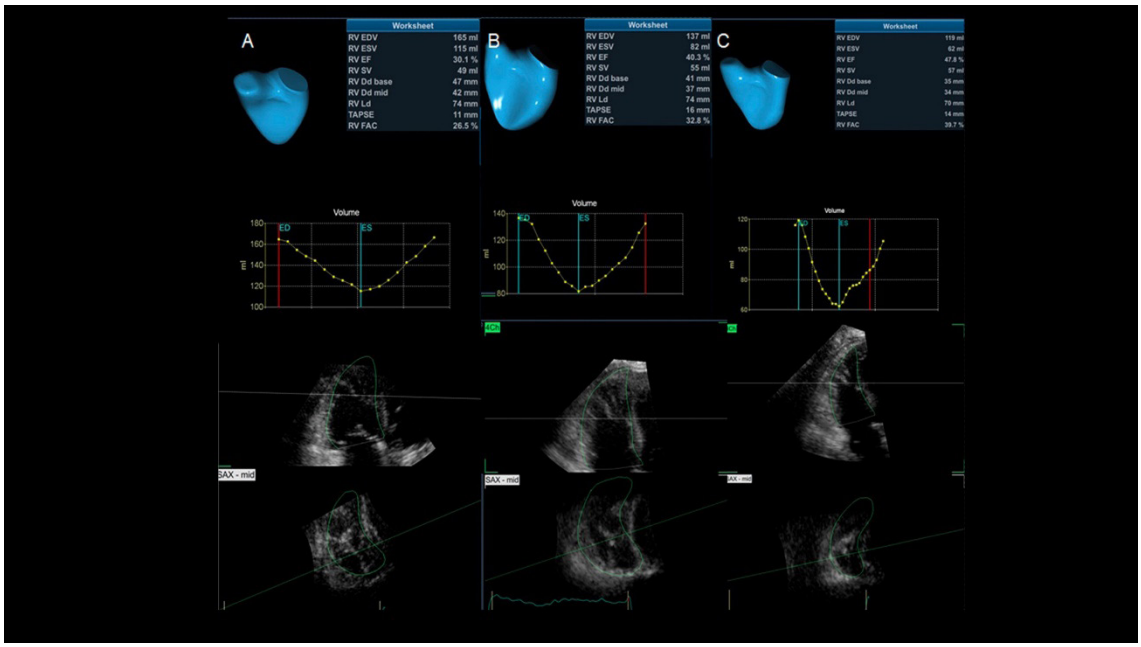


Figure 5 – *Follow-up3D echocardiographic analysis of the right ventricle (RV), with measurement of RV end-diastolic volume (EDV), end-systolic volume (ESV), and ejection fraction (EF). Patient with submassive pulmonary embolism at 3 time points: A: pre-thrombolysis (RV EDV = 165 mL, RV ESV = 115 mL, RV EF = 30%); B: day 2 after thrombolysis (RV EDV = 137 mL, RV ESV = 82 mL, RV EF = 40%); C: day 5 after thrombolysis (RV EDV = 113 mL, RV ESV = 62 mL, RV EF = 47.8%).*

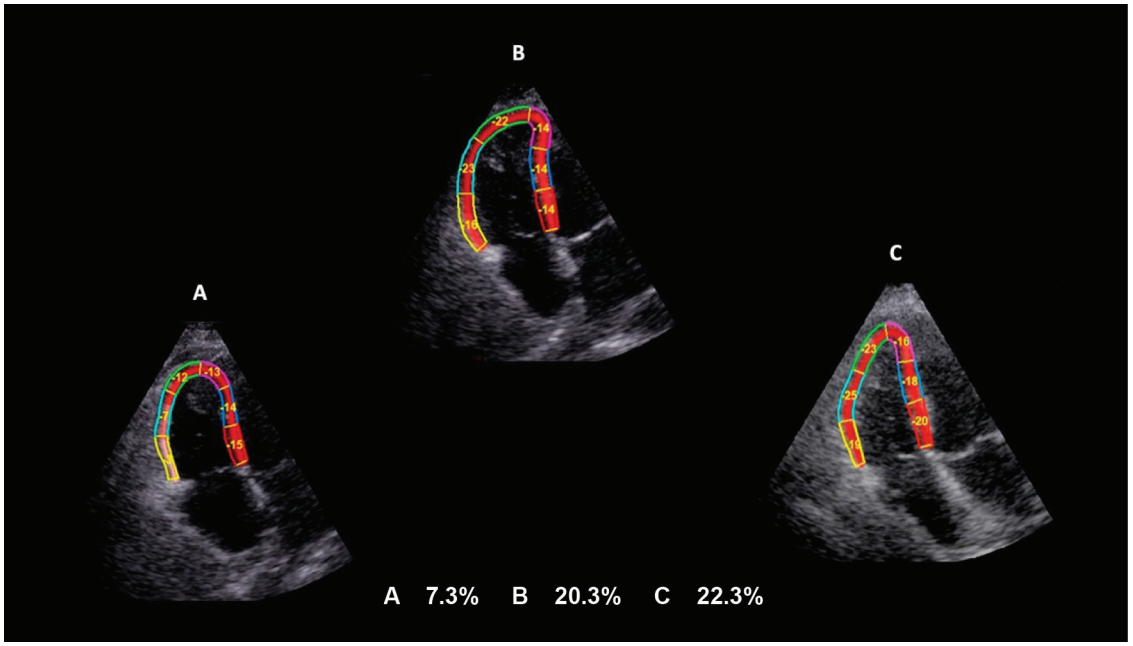


Figure 6 – *Figure 6 – Follow-up images analysis of the right ventricular (RV) free-wall longitudinal strain in absolute values and at 3 time points: A: pre-thrombolysis; B: day 2 after thrombolysis; C: day 5 after thrombolysis with normalized strain values.*

In the beginning of 2020, the new coronavirus pandemic (COVID-19) added a crucial new role for echocardiography. So far, findings reported in the literature indicate that the virus causes a significant increase in the risk of venous thrombosis (translated by increased D-dimer) and, consequently, in the occurrence of PE. Conversely, it seems to cause local thrombosis in pulmonary microcirculation vessels, leading to RV pressure overload. Both factors can result in RV dilatation and dysfunction, measured by various parameters, as described in Chart 8. These patients develop acute respiratory failure requiring invasive positive pressure ventilation, which also contributes to increased RV afterload. In these patients, echocardiographic changes are very similar (RV dilatation and dysfunction with pulmonary hypertension), and it is not always possible to differentiate the predominant cause. In a recent multicenter study of 870 patients hospitalized for COVID-19, RV free-wall strain was an independent prognostic marker of mortality without the analysis of other parameters such as TAPSE and S' of the tricuspid annulus.[63] In addition to RV strain, RV dilatation was a prognostic marker of patients with the severe form of the disease.[64] In conclusion, echocardiography plays a key role in this scenario, not only the comprehensive but also the problem-focused echocardiogram (point of care), as recently addressed by the position statement of the Department of Cardiovascular Imaging of the Brazilian Society of Cardiology (DIC/SBC).[65]

2.4. Recommendations

1 - Bedside echocardiography is a mandatory test for all hemodynamically unstable patients with clinically suspected PE.[39,43,44] (grade of recommendation: I/level of evidence: C).

2 - Echocardiography is not mandatory in the diagnostic routine for hemodynamically stable patients with suspected PE in diagnostic algorithms.[1,35-37] However, it represents an important tool for discriminating and defining differential diagnoses.[38-41] (grade of recommendation: IIa/level of evidence: B).

3. Intravascular Ultrasound in Deep Vein Thrombosis Diagnosis

3.1. Ultrasound Signs of Vein Thrombosis: B-mode and Doppler

If not properly diagnosed and treated, deep vein thrombosis (DVT) can lead to pulmonary embolism (PE), a potentially fatal condition.[66] Thrombus formation often occurs in regions of slow flow within the venous system, such as the cusp of a valve, spreading along the vein lumen, which is completely or partially filled by the thrombus. During the acute phase, the thrombi induce an inflammatory response on the adjacent venous wall. This is known as thrombophlebitis, and symptoms include cramp and local pain.[67] Many factors can trigger venous thrombosis. In the case of venous catheters, wall trauma induces the inflammatory response that leads to thrombosis within the vein lumen.[68,69] However, clinical signs of DVT can often be nonspecific; therefore, medical history, physical examination, and awareness of the main factors associated with the thrombotic process are essential.[16,70]

Risk factors for venous thrombosis can be grouped in clinical prediction models. The Wells score is a well-established method used to assess the clinical probability of a DVT diagnosis. Considering that only 50% of clinical DVT diagnoses may be correct, supplementary imaging is required to confirm or rule out diagnosis.[70] The evaluation of patients with suspected DVT usually involves stratification, clinical score, D-dimer measurement, and intravascular ultrasound (VUS).[6]

3.1.1. Imaging Methodology – Technical Aspects

VUS is the method of choice for suspected DVT, providing information on venous anatomy and function. In addition, it is noninvasive, does not use nephrotoxic contrast, and is reproducible and inexpensive, although it is operator- and device-dependent.[71] Therefore, real-time B-mode ultrasound (US) images of the vein wall and lumen are obtained with transducer compression maneuvers and color flow Doppler (CFD), pulsed Doppler, or power Doppler.[72]

As recently stated by the Department of Cardiovascular Imaging,[73] the imaging technique to study DVT includes the following items:

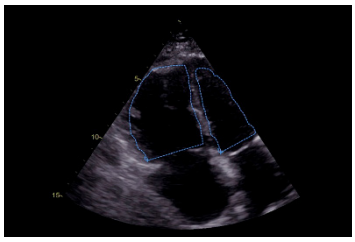
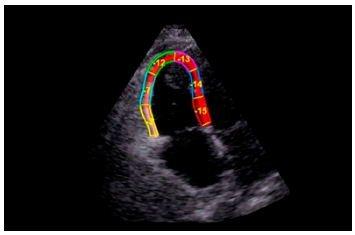
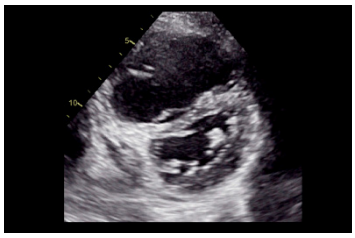
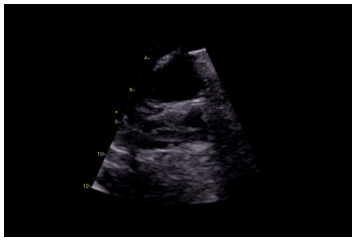
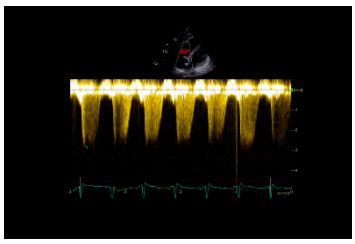
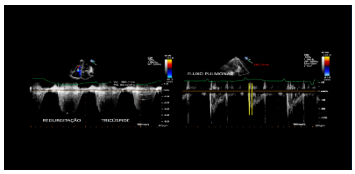
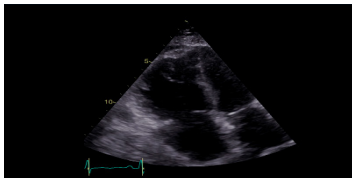
a) Patient's position:

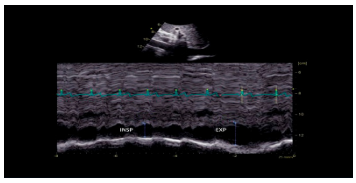
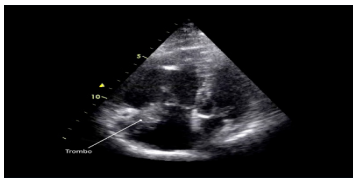
For imaging of the lower extremities, the patient should be placed in a comfortable supine position with the torso and head elevated up to 30°, lying close to the edge of the bed, on the same side of the operator, with the hip slightly abducted and the knee slightly flexed. For the common and superficial femoral veins and the posterior tibial vein, the patient should keep the leg externally rotated and the knee slightly flexed. For the popliteal vein and peroneal veins, the patient should be placed in a prone or lateral decubitus position, resting on the contralateral limb with the knee slightly flexed. In case of any clinical condition, the patient may remain seated at the edge of the bed with the legs hanging down.[74] For the peroneal, muscular, and posterior tibial veins of the calf, the patient may sometimes be placed in the supine position, with the leg bent and the foot resting on the bed. Conversely, imaging study of iliac veins and the vena cava is conducted with the patient in the supine position. Prior preparation is required to reduce intestinal gases. In case of suspected post-thrombotic syndrome, if possible, the patient should also be examined in the upright position. For imaging of the upper extremities, the patient should be placed in the supine position, with the limb stretched alongside and slightly away from the body.

b) US equipment adjustment

Devices that produce high-quality images in B-mode and that are equipped with spectral Doppler and color Doppler should be used. The choice of transducer is primarily based on the relationship between the transducer's frequency and the location of the object to be studied. High-frequency transducers produce high-quality images but have low tissue

Chart 8 – Summary of echocardiographic changes in acute pulmonary embolism (PE)

PARAMETERS	ECHOCARDIOGRAPHIC CHANGE	IMAGE
Dimensions of the right ventricular cavity	2DE: basal linear diameter > 4.2 cm and longitudinal, > 8.1 cm. 3DE: end-diastolic volume > 87 mL/m2 in men and 74 mL/m2 in women; end-systolic volume > 44 mL/m2 in men and 36 mL/m2 in women. RV/LV dimension ratio > 1.0	
RV systolic function	Free-wall hypokinesia or akinesia. FAC < 35%. TAPSE < 17 mm. Tissue Doppler: S' wave < 9.5 cm/s. Free-wall longitudinal strain above -20% (<20% in absolute values). 3DE: ejection fraction < 45%.	
Interventricular septum	Straightening or leftward shift due to RV pressure overload.	
Pulmonary trunk diameter	Dilatation (above 3.0 cm diameter).	
Tricuspid regurgitation	Increased tricuspid regurgitation due to increased pulmonary artery systolic pressure.	
"60/60 sign"	Pulmonary ejection acceleration time < 60 ms with mid-systolic notch + tricuspid valve peak systolic gradient < 60 mm Hg.	
"McConnell sign"	Hypokinesia or akinesia of basal and mid segments of the RV free wall and preserved apical contractility.	

Inferior vena cava	"Collapsibility index" <50% during inspiration.	
Intracavitary thrombus	Single pathognomonic finding (present in right cavities and/or pulmonary trunk or branch arteries in only 4% of cases).	

penetration. On the other hand, low-frequency transducers penetrate deeper, but produce lower quality images. Linear transducers with frequencies between 4 and 10 MHz are often used to investigate upper- or lower-extremity deep veins, whereas linear transducers with frequencies between 7 and 13 MHz are used to investigate upper- or lower-extremity superficial veins. For evaluation of swollen lower limbs and patients with obesity (ie, with greatly increased diameters), iliac veins, and the inferior vena cava, a multi-frequency convex transducer (between 1 and 5 MHz) may be necessary, as it has lower wave frequencies and deeper US wave penetration. Presets should be properly calibrated for imaging of the deep and superficial venous systems, with adjusted focal zone and gray scale, so that the lumen is dark in the absence of stasis or thrombosis. Spectral or color Doppler gain should be adjusted to a low velocity scale suitable for evaluating venous flow. For deep vein evaluation, VUS devices must be adjusted with proper focus and line density for two-dimensional imaging. The structures are evaluated in cross-sectional and longitudinal views.[73] In general, veins are easily compressible by the transducer.[71]

c) Imaging sequence:

B-mode: the transducer is initially positioned below the inguinal ligament, allowing visualization of the saphenofemoral junction. Longitudinal and cross-sectional scanning of the entire lower-extremity venous system is then performed. In the cross-sectional view, transducer compression must be applied especially along the common femoral, femoral, and popliteal veins with a distance of approximately 2 to 3 cm between each compression. The use ofVUS as a diagnostic technique for DVT was first described by Talbot in 1982, and the method has been improved over the years.[75] A normal vein without thrombosis shows complete lumen collapse during transducer compression of the walls (Figures 7 and 8).

The figures show maximum common femoral vein compressibility in B-mode (top) and color flow Doppler (bottom). A = artery, V = vein.

Doppler: This technique is intended for recording venous flow velocities, which are low under normal pulsed Doppler conditions. In the presence of venous thrombosis, velocities may be further reduced, requiring scale adjustment. Lower-extremity venous flow is spontaneous and phasic in response to breathing and variations in intra-abdominal pressure (Figure 9). During inspiration, velocities decrease due to increases in intra-abdominal pressure; conversely, distal compression maneuver (eg, calf compression) shows a flow peak in the patent interrogated vein. In some cases, proper flow detection is only possible by power Doppler, which can detect very low velocity flows despite not showing flow direction.

d) Changes in B-mode, spectral Doppler, and color Doppler in the diagnosis of recent/acute DVT:

US diagnosis of recent/acute DVT is based on total or partial changes in the affected vein under transducer compression, whereas vein dilation is based on the presence of intraluminal thrombotic material. Changes in vein compressibility, even if a very recent episode of DVT has occurred, may be the most important finding for venous thrombosis diagnosis. The presence of a thrombus can only be ruled out if the vein lumen is obliterated after compression. Otherwise, the lumen may be partially filled with thrombi. During the compression procedure, the adjacent artery should be observed.

In most cases, recently thrombosed veins are distended and have larger diameters than the adjacent arteries. This distension is also important in the differentiation between acute and chronic thrombi. Occlusive thrombus is characterized by vein dilation and absence of detectable flow by pulsed Doppler, CFD, and power Doppler (Figure 10 A and B). Vein dilation has been described as an accurate parameter for identifying acute DVT.[76] An acute thrombus is composed primarily of a dense fibrin mesh that persists for approximately 5 to 7 days. During this phase, the thrombus is more echolucent or has intermediate echogenicity, appearing less bright than adjacent tissues. Diagnosing acute/fresh thrombus is important, given that anticoagulation and fibrinolytic therapies are more effective during this phase. Venous thrombosis is generally considered acute within the first 2 weeks of onset.[77] A fresh clot often forms at the end of an acute thrombus and floats freely inside the vein. Caution is needed during manipulation to prevent it from moving (Figure 10 A-C). Besides venous compressibility, venous wall appearance (DVT is often characterized by thin and smooth walls), vein lumen size, valve functionality, and the presence of collateral circulation should also be evaluated.

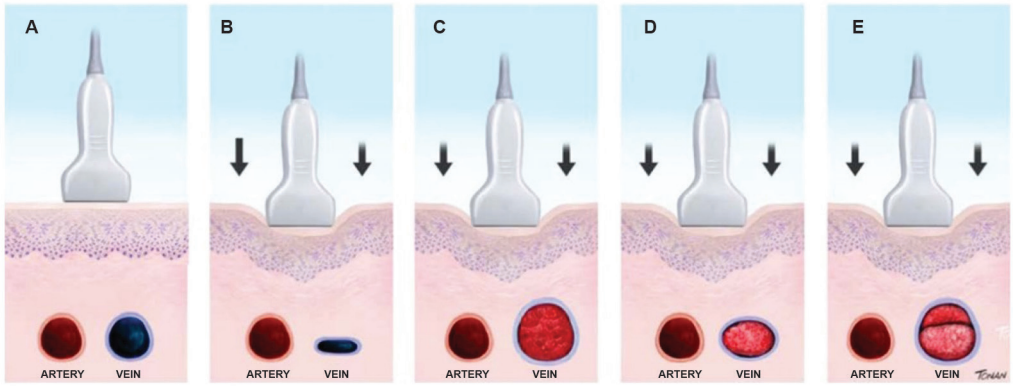


Figure 7 – *A-E: Venous compressibility evaluation by the ultrasound transducer in the investigation of venous thrombosis. A: artery and vein without transducer compression. B: normal vein with maximum compression. C: dilated and noncompressible vein with acute/fresh thrombus. D: normal- to small-caliber vein, slightly compressible by transducer maneuvers and consistent with findings of chronic/old venous thrombosis. E: recurrent thrombosis.*

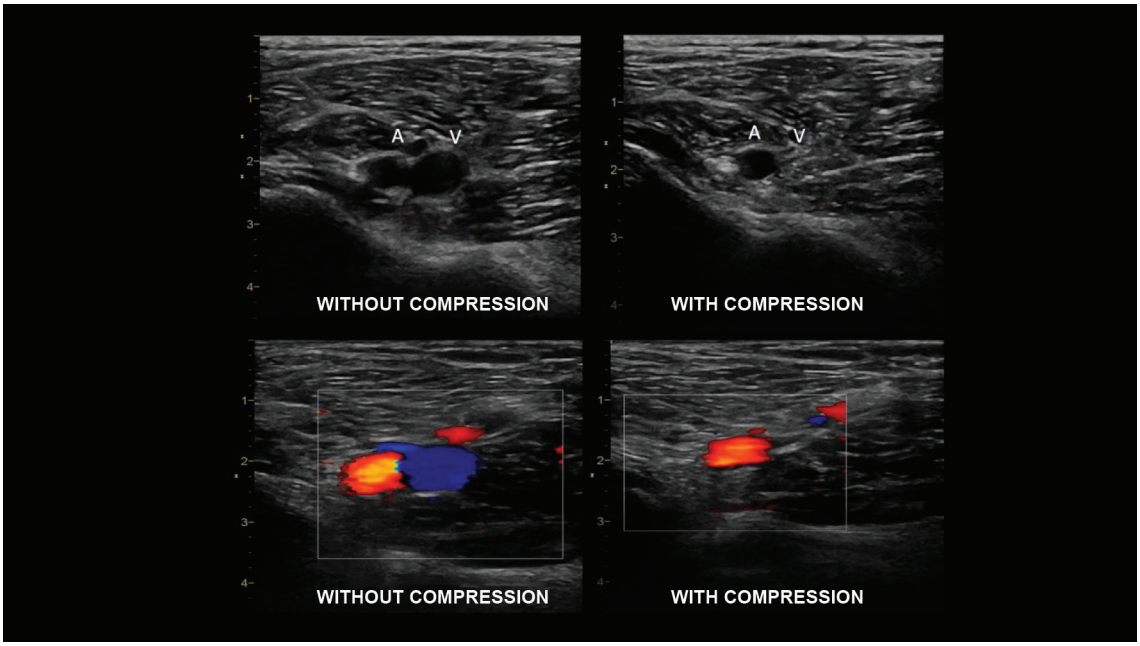


Figure 8 – *Cross-sectional view of the common femoral artery and vein for venous compressibility evaluation.*

Regarding flow, it may be reduced or absent depending on the degree of partial or complete obstruction of the lumen, with absence of respiratory variation. However, a partially obstructed lumen may not cause changes in Doppler's flow signs.

e) Changes in B-mode and color Doppler in the diagnosis of clinically subacute and chronic/old DVT[11]:

In general, clinical evaluation of DVT considers the thrombus to be acute within the first 2 weeks of onset, subacute between 2 weeks and 6 months, and chronic after 6 months of onset.[72,78] Although the thrombus becomes more echogenic as DVT progresses, this change is variable and, most often, the age of the thrombus cannot be accurately estimated solely by echogenicity. For this reason, recently formed hypoechoic or anechoic thrombi are consistent with a diagnosis of acute thrombosis. Isoechoic or hyperechoic intraluminal images, however, make it more difficult to accurately estimate the age of the thrombus. During this period, retraction or thrombolysis may occur with a less distended vein; hence the importance

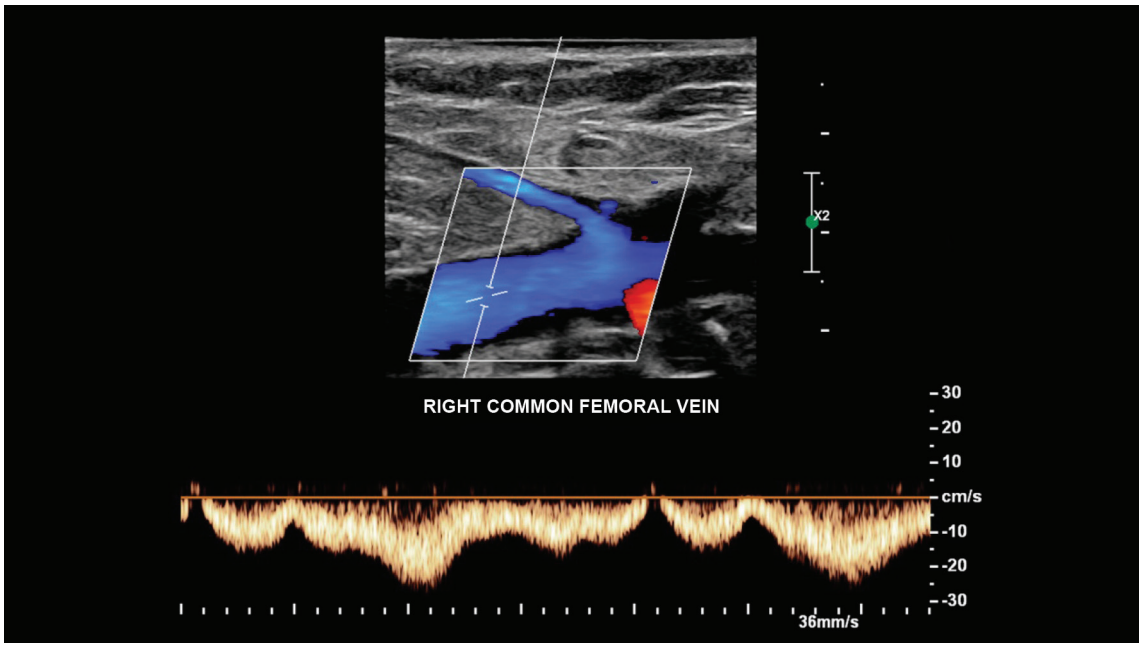


Figure 9 – *Pulsed Doppler of the common femoral vein showing normal phasic flow in response to breathing movements, consistent with the absence of occlusive thrombosis.*

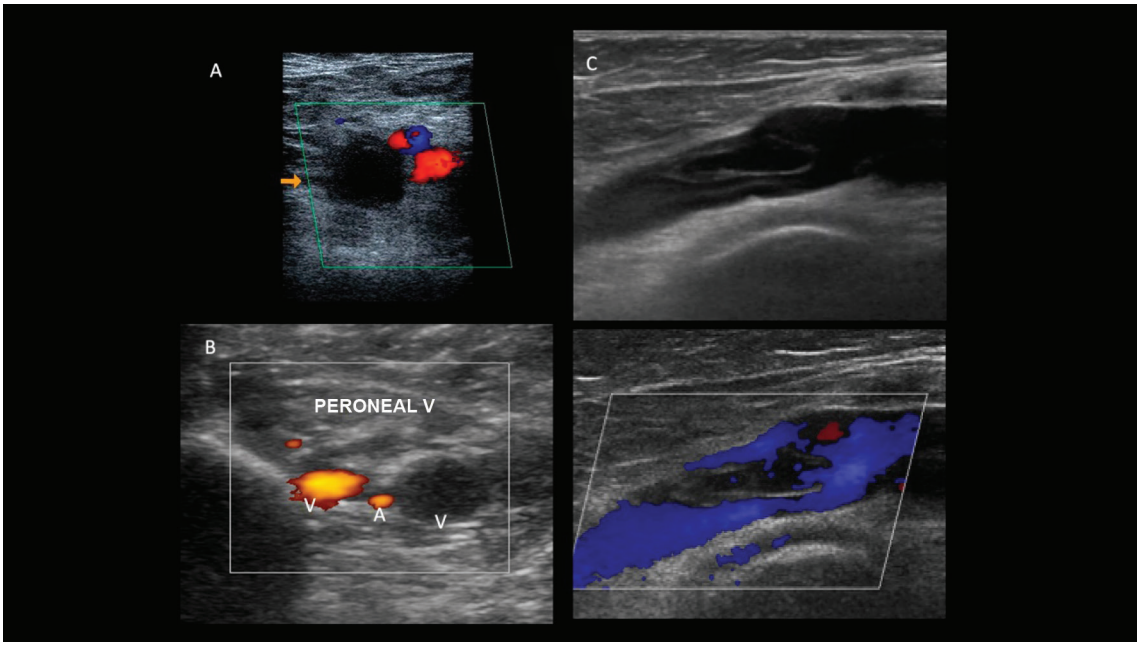


Figura 10 – *Trombose oclusiva e não oclusiva de aspecto agudo ao ultrassom. A: corte transversal da veia femoral comum; B: veias fibulares evidenciando dilatação e incompressibilidade sem evidências de fluxo ao mapeamento de fluxo em cores e ao Power Doppler; C: corte longitudinal da veia femoral comum com e sem mapeamento de fluxo em cores evidenciando trombo flutuante de aspecto recente e não oclusivo.*

of serial imaging. After retraction or thrombolysis, flow may not be necessarily restored to normal, and the vein walls become thick (Figure 11).[71,72,79] Thus, VUS cannot always determine the age of a thrombus solely by its appearance or echogenicity.[71,72,79]

If the thrombus does not heal completely after acute thrombosis, it will become infiltrated with fibroblasts, organize, and reendothelialize. Fibrosis produces scarring, wall thickening, and synechiae that can cause partial vessel obstruction and persist for years. This residual material is no longer called thrombus. Thickening of the venous wall is a common finding, the caliber may be reduced and, depending on the venous caliber, the flow can be altered. In some cases, post-thrombotic fibrotic scars appear as the condition progresses. They are echogenic and sometimes have calcifications that produce acoustic shadows, causing plaque-like images along the venous wall. The Society of Radiologists in Ultrasound recommends that the term "chronic post-thrombotic changes" should be used to describe the changes that persist on US after acute DVT. Therefore, US abnormalities associated with DVT should be classified into "acute venous thrombosis, chronic post-thrombotic changes, or indeterminate changes." The last one should be used when findings are uncertain and the age of the thrombus cannot be determined only by VUS, as with clinically subacute DVT. According to these recommendations, exceptionally, some cases may be classified as subacute thrombosis on the US report if:

1. There is a previous US showing acute DVT;

2. There is a new study showing that the appearance of the thrombus has changed.[80]

These classifications are supported by a recent SBACV publication.[81] However, in an effort to prevent the term "indeterminate changes" from creating confusion as to whether clinicians should start treatment or not, the publication recommends that the vascular sonographer should report image features in detail, analyze the clinical information regarding time of occurrence, and preferably refer to these changes as "predominance of acute or recent changes" or "predominance of chronic or old changes." Thus, the term "indeterminate changes" would only be used when the time of the event cannot be determined.

DVT can affect several venous segments alone or together, as in plantar vein thrombosis, an uncommon diagnosis. It should be suspected in the presence of acute pain and/or edema of the foot. Recently observed US features of plantar DVT are venous dilation and luminal thrombus with reduced vein compressibility (Figure 12).

US features that may be present in acute/recent and chronic/old DVT are shown in Chart 9, adapted from Gornik HL and Sharma AM.[72] In some cases, there is overlap of features, which should be reported as of "indeterminate age."

Impaired respiratory phasicity and reduced flow velocity in a venous vessel of normal appearance, with a present and compressible flow, may be associated with the thrombotic involvement of a venous segment proximal to the vessel being evaluated (Figure 13).

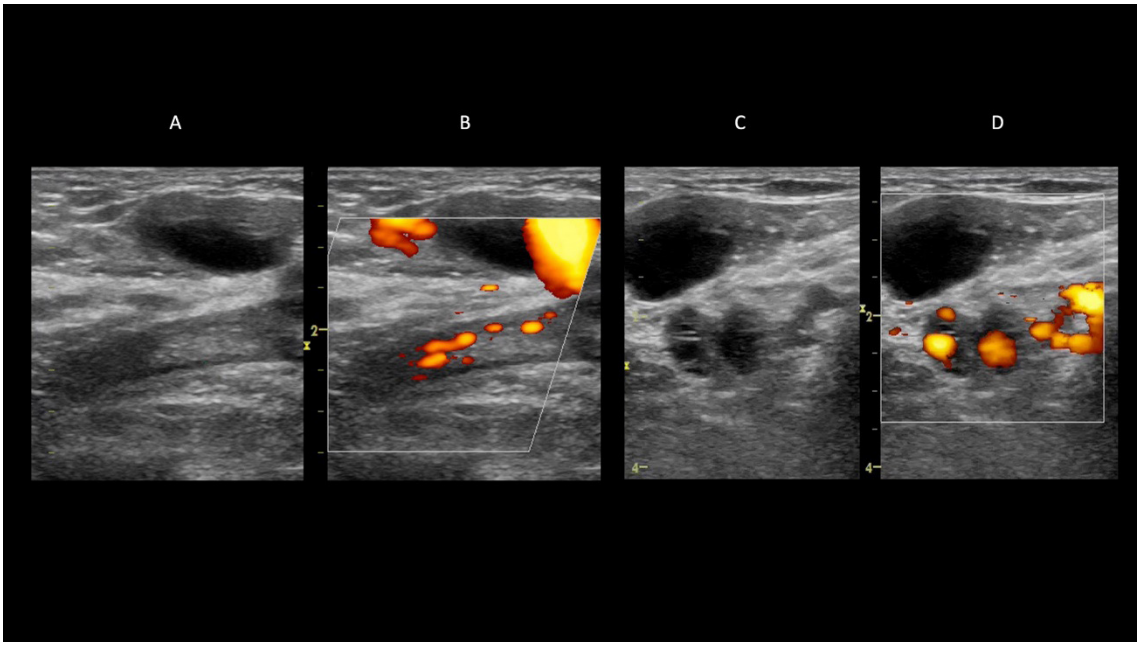


Figure 11 – *Chronic post-thrombotic changes. A: B-mode image showing no dilation of the femoral vein. Echogenic luminal image consistent with chronic post-thrombotic changes; B: power Doppler image showing reduced luminal flow; C: B-mode image showing posterior tibial veins with thick walls; D: power Doppler image showing partial luminal narrowing of posterior tibial veins.*

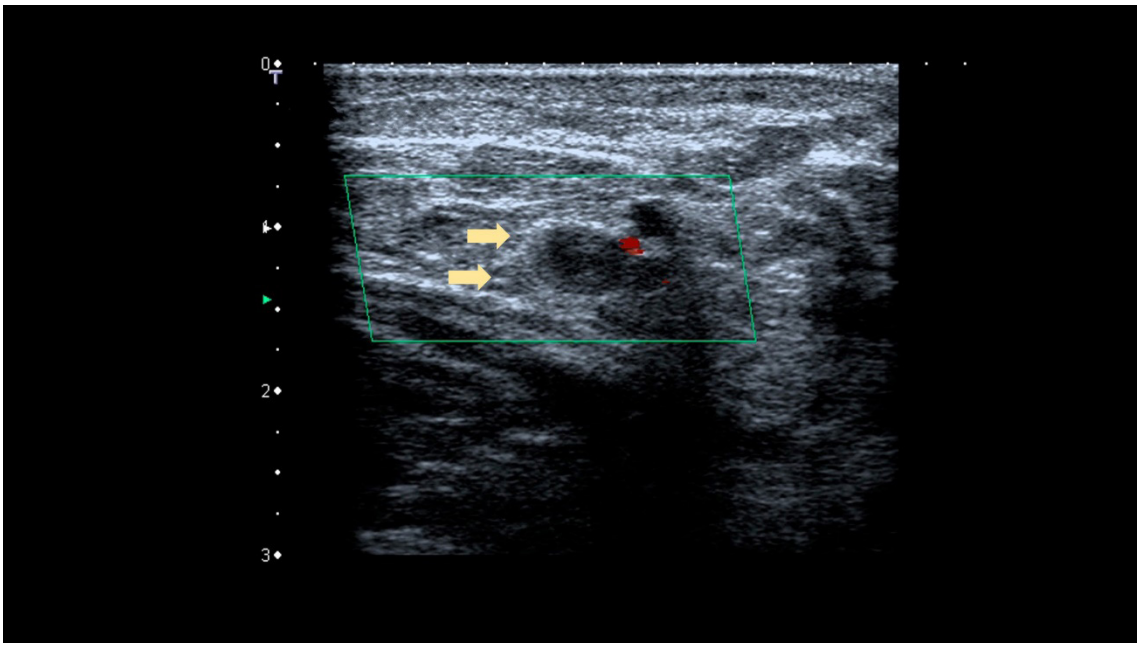


Figure 12 – *Color flow Doppler image showing venous thrombosis of noncompressible plantar veins without flow.*

Chart 9 – Ultrasound features of deep vein thrombosis

Feature	Acute/recent	Predominance of acute thrombus or chronic thrombus	Chronic/old post-thrombotic changes
Thrombus echogenicity	Hypoechoic or isoechoic	Variable (more echoic than acute DVT)	Hypoechoic
Presence of mobile thrombus	May be present	Generally absent	Absent
Attachment of thrombus to vein wall	Loosely attached	Firmly attached	Hyperechoic fibrous tissue attached to the vein wall or lumen
Venous wall appearance	Variable	Variable	Venous wall thickening and scarring Calcium deposits may be seen
Vein lumen	Dilated	Retracting to normal size	Smaller than normal size
Compressibility	Slightly compressible or deformable	More compressible than acute DVT	Partially compressible
Compressibility	Generally absent	May be present	May be present
Venous valve function	Generally competent	May be incompetent	Generally incompetent

3.2. IVUS Protocols in DVT

IVUS has been used since the 1960s for the diagnosis of venous thrombosis, and the method has been improved over the years by the results of imaging studies of venous blood flow measurement and venous compressibility. As previously stated, venous compressibility evaluation combined with color and spectral Doppler of flow is the recommended approach for the detection of venous clots. This is partly due to availability, ease of implementation in a variety of clinical settings, and accuracy.

IVUS has been the reference method for diagnosis since the first accuracy studies of symptomatic and asymptomatic patients with suspected above- and below-knee DVT.[82] Lower extremity venous compression US has largely replaced venography in DVT diagnosis, as it has a sensitivity above 90% and a specificity of approximately 95% for the diagnosis of proximal thrombosis. For distal thrombosis, the specificity is the same (above 90%), but the sensitivity is reduced to approximately 65%. Conversely, duplex US (a combination of B-mode and Doppler of all veins) is the first-line imaging test for DVT diagnosis, with a sensitivity of 96% and a specificity of 98% to 100%. However, duplex US is time-consuming, requires patient transport to a diagnostic imaging center, and the immediate availability of an imaging specialist.[38,83,84] In

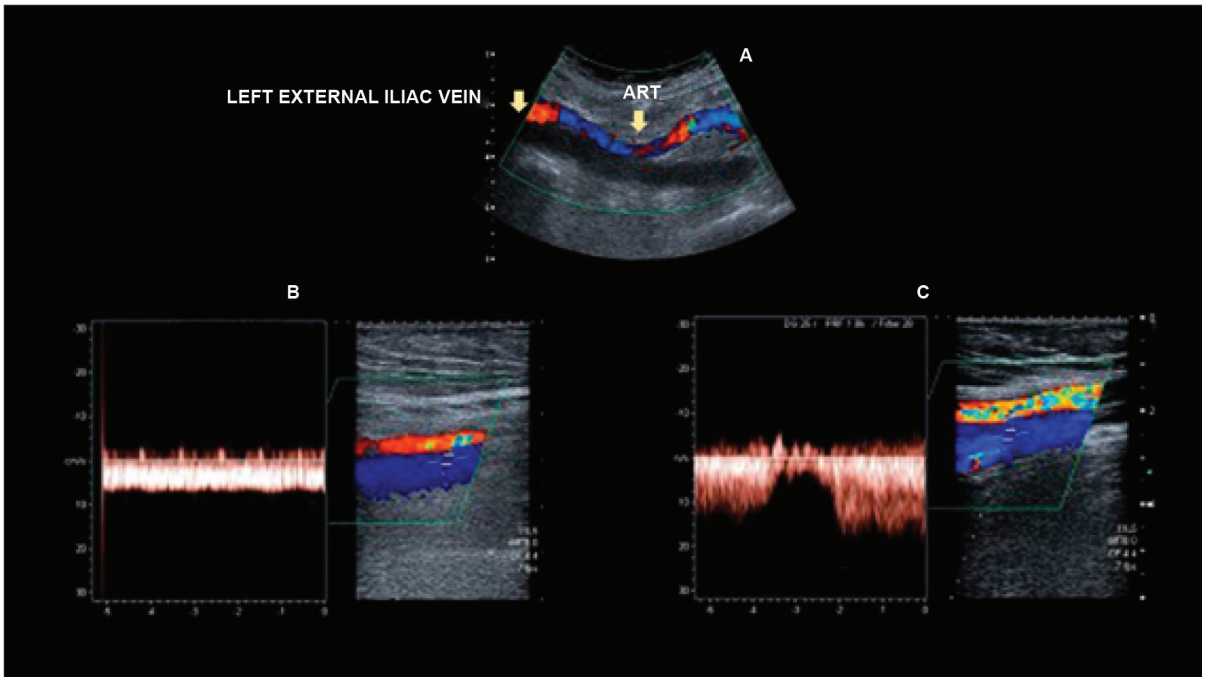


Figure 13 – *Left external iliac vein thrombosis: flow comparison between the left and right common femoral veins. A: color Doppler image showing no flow in the dilated left external iliac vein, with lumen filled with a thrombus of recent appearance; B: spectral Doppler image showing continuous flow in the common left femoral vein secondary to thrombosis of the ipsilateral external iliac vein; C: normal phasic flow in the contralateral common femoral vein.*

addition, to obtain an accurate diagnosis, the operator must be familiar with the method's indications and limitations. Only 20% to 30% of imaging tests of suspected DVT show abnormal findings, and 90% of patients with fatal PE are asymptomatic for DVT.[16,18] Test limitations and, therefore, accuracy are associated with obesity, mobility restrictions, major edema, and/or presence of wounds, orthopedic devices, bandages/plasters, etc. Relevant clinical information and focused physical examination are essential to assist in the interpretation of IVUS results during DVT investigation, and adequate protocol training is imperative. Despite these limitations, the easy handling, harmless nature, and wide availability of portable devices make this tool extremely applicable in daily practice. In addition, advances in electronic miniaturization have resulted in the creation of pocket-sized US scanning devices equipped with linear array transducers, further disseminating the use of compression VUS for DVT diagnosis.[85]

Regardless of the test protocol of choice, the examination room should be comfortably warm to prevent venospasm. To evaluate the inferior vena cava (IVC), the patient should be placed in a reverse Trendelenburg position.[71] The Society for Vascular Ultrasound recommends an examination time of approximately 75 minutes for a complete bilateral examination: 40 to 60 minutes for the actual imaging test and 15 minutes for clinical data analysis, consultation of previous imaging tests, and patient and room preparation.[86]

3.3. Test Protocols

With increased availability of VUS devices, which usually have excellent resolution, the training of nonexperts has become very frequent and gained increased acceptance, especially in the emergency and urgent care departments and in distant locations. Thus, imaging tests for suspected DVT performed by critical care and emergency physicians and last-year radiology/US/VUS residents is a current reality. The accuracy of emergency physician-performed US was assessed in a systematic review and meta-analysis of 16 studies with 2,379 patients. The prevalence of DVT was 23% (7.4%-47.3%), with a mean sensitivity of 96.1% (95%CI: 90.6%-98.5%) and a specificity of 96.8% (95%CI: 94.6%-98.1%). However, the studies included were very heterogeneous, the training process of emergency physicians was poorly described, and most of the studies had short follow-up periods. These data were not reproduced in a prospective study published in same year with a much smaller sample size, indicating the need for strict training protocols for these professionals, given that only the first three tests performed by nonexperts were supervised.[84,87] Many of the protocols performed by nonexperts have been developed for settings where experts are not available or to reduce the number of VUS performed after normal laboratory working hours. Thus, a single simple imaging protocol is not enough to definitively rule out DVT. In addition to proper supervised training, pretest DVT probability analysis is essential, that is, the use of clinical PE/DVT probability scores and awareness of the lower

accuracy of VUS in the diagnosis of asymptomatic below-knee DVT. Studies on the topic did not report any major disadvantage for patients, and well-structured protocols with Wells' clinical probability criteria helped improve test accuracy and proper referral of patients without easy access to imaging experts.[82,88-90]

During VUS investigation of venous thrombosis, the parameters previously described should be analyzed, ie, the combination of grayscale components (B-mode) with compression transducer maneuvers and CFD and spectral Doppler evaluation. Imaging test protocols use the following parameters to reach diagnosis: vein compressibility (the most important), vein caliber (diameter), US features of vein wall and lumen, and CFD and spectral Doppler evaluation.

There are some well-established complete and focused (point-of-care) DVT imaging protocols. They are chosen according to device availability at the facility where the patient is being examined, time of patient arrival, and whether there is an expert available to perform VUS.

DVT imaging protocols (Figures 14 and 15):

1. Whole-leg VUS – Figure 14A-C.

2. Complete lower extremity venous compression US – Figure 15A.

3. Three-point extended compression US – Figure 15B.

4. Two-point extended compression US – Figure 15C.

3.3.1. Complete DuplexVUS

The Society of Radiologists in Ultrasound and the Brazilian Society of Angiology and Vascular Surgery currently recommend (grade of recommendation: I; level of evidence: A) complete duplex US for the diagnosis of acute DVT. In addition to being a more complete patient approach, examining the infrapopliteal segment allows the diagnosis of other musculoskeletal diseases that are part of the differential diagnosis of DVT, such as Baker's cyst, hematomas, and muscle diseases.[80,81] The complete protocol involves using all US resources to diagnose thrombosis: evaluation of compressibility, caliber, vein wall and lumen appearance on US, and color and spectral Doppler of all veins from below the inguinal ligament to ankle in all segments (femoral, popliteal, posterior tibial, and peroneal veins) (Figure 14A-C). In general, the investigation of venous thrombosis in the anterior tibial veins is not included in this protocol due to the rarity of the condition. It is only investigated when there are signs/symptoms. Evaluation of the superficial and muscular venous systems should be conducted especially in symptomatic regions.

Duplex ultrasound includes compression at 2-cm intervals, CFD analysis of vessel filling (using necessary scale adjustments), observation of flow phasicity, and spectral Doppler analysis of femoral and popliteal veins morphology. If complete testing is performed only in the symptomatic limb, spectral Doppler evaluation should be performed in both common femoral veins to assess symmetry. In case of asymmetric flow pattern, the intra-abdominal veins should be investigated (grade of recommendation: I; level of evidence: A).[91-94]

3.3.2. Complete Lower Extremity Venous Compression US

In 1982, Steve Talbot described the compression technique that would become the standard diagnostic method for DVT.[75] This protocol involves only the evaluation of compressibility in all veins below the inguinal segment to the ankle in all segments (Figure 15A). As previously stated, loss of vein compressibility is the most reliable indicator of thrombus.

3.3.3. Extended Compression US (Three-point)

This protocol involves only the evaluation of compressibility from the common femoral vein to the popliteal vein, where all leg veins converge. It is also called the three-point protocol, as it assesses the compressibility of all proximal veins of the investigated lower extremity (Figure 15B). The sensitivity found in this protocol was significantly higher than that in the two-point compression protocol (90.57% vs. 82.76%), although specificity was the same (98.52%).[95] A diagnostic accuracy study of the three-point protocol performed by emergency physicians found very similar results (91.7% accuracy; 95% CI, 85%-95.6%).[96] A recent meta-analysis of 17 studies from 16 original articles compared three-point vs. two-point point-of-care US (POCUS) protocols. Overall, the two-point POCUS had similar pooled sensitivity (0.91; 95% CI, 0.68-0.98; P = 0.86) and specificity (0.98; 95% CI, 0.96-0.99; P = 0,60) to the three-point POCUS (sensitivity 0.90; 95% CI, 0.83-0.95 and specificity 0.95; 95% CI, 0.83-0.99). The false-negative rates of two-point (4.0%) and three-point POCUS (4.1%) were almost similar. Meta-regression analysis showed that high sensitivity and specificity tended to be associated with the initial POCUS performer and separate POCUS training for DVT[97] (grade of recommendation: II; level of evidence: A).

3.3.4. Two-point Compression US

In this protocol, compressibility is evaluated at only two sites: the common femoral vein, 1-2 cm above and below the saphenofemoral junction (groin); and the popliteal vein, up to where the leg veins converge (Figure 15C).

In general, emergency physicians are able to perform this protocol after training, with good reproducibility. There are several methods for performing this imaging protocol in the emergency setting. Most training programs include theoretical lectures on imaging technique, mock training, and observation of a number of different tests. However, some studies mention training programs that last only 10 minutes or less than 2 hours. This significant difference between protocols hinders comparisons between studies and, consequently, affects accuracy.[95-100] Emergency physicians can achieve a level of competence equivalent to that of experts, but substantial training and practice are required to achieve and maintain this performance. To reach this level of excellence, they need regular training in US imaging.

A study comparing complete two-point procedures performed by experienced emergency physicians showed that 362 of 2,451 patients had DVT. Two-point US would not have been able to diagnose 23 patients with proximal DVT (6.2%).[100] A randomized controlled trial with more than 2,000 patients comparing whole-leg vs. serial two-point US

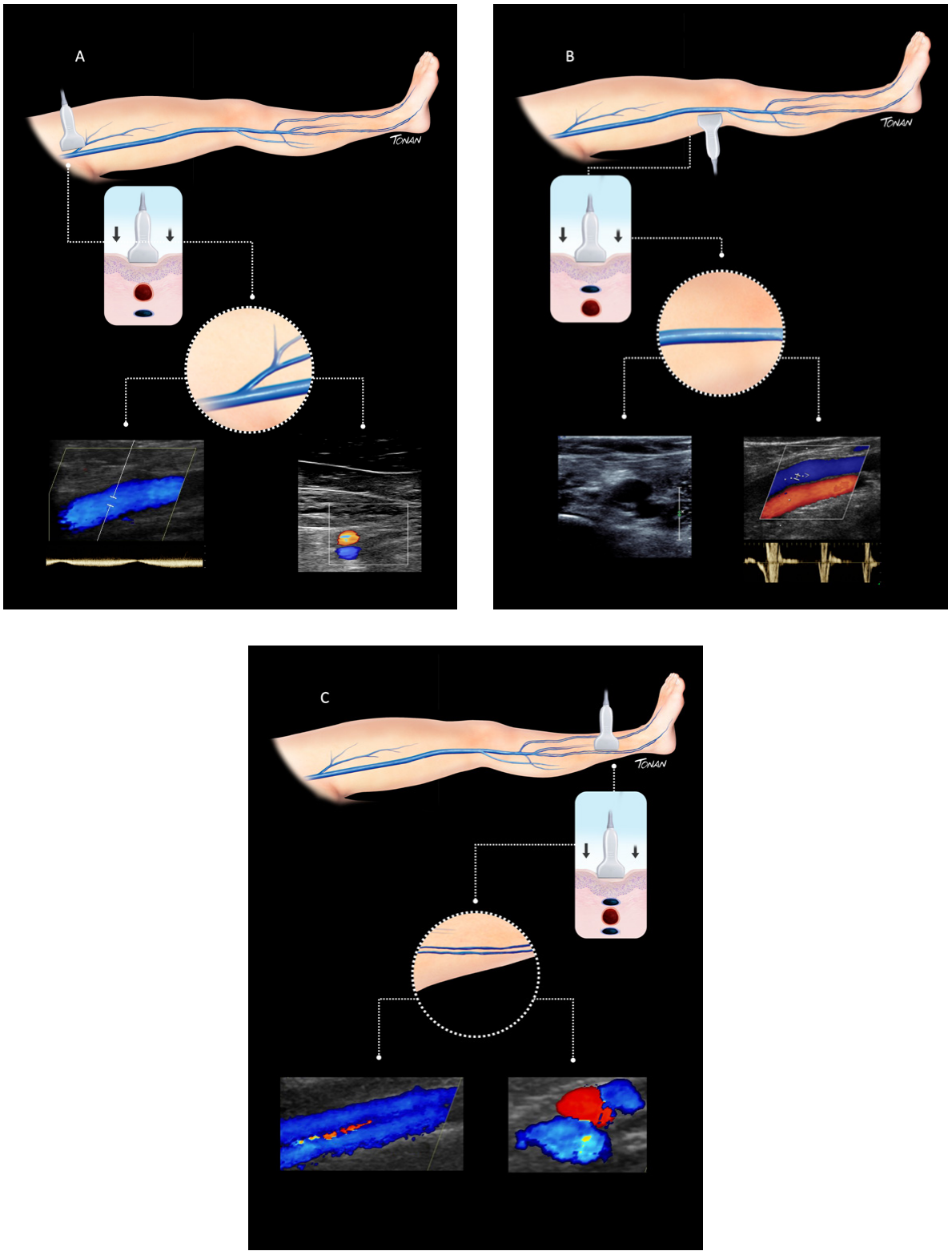


Figure 14 – *Whole-leg vascular ultrasound protocol for deep vein thrombosis diagnosis. A: schematic representation of compression points throughout the above-knee venous system and color flow Doppler and spectral Doppler of the femoral segment; B: schematic representation of compression and color flow Doppler and pulsed Doppler of the popliteal segment; C: schematic representation of compression and color flow Doppler of the distal segment.*

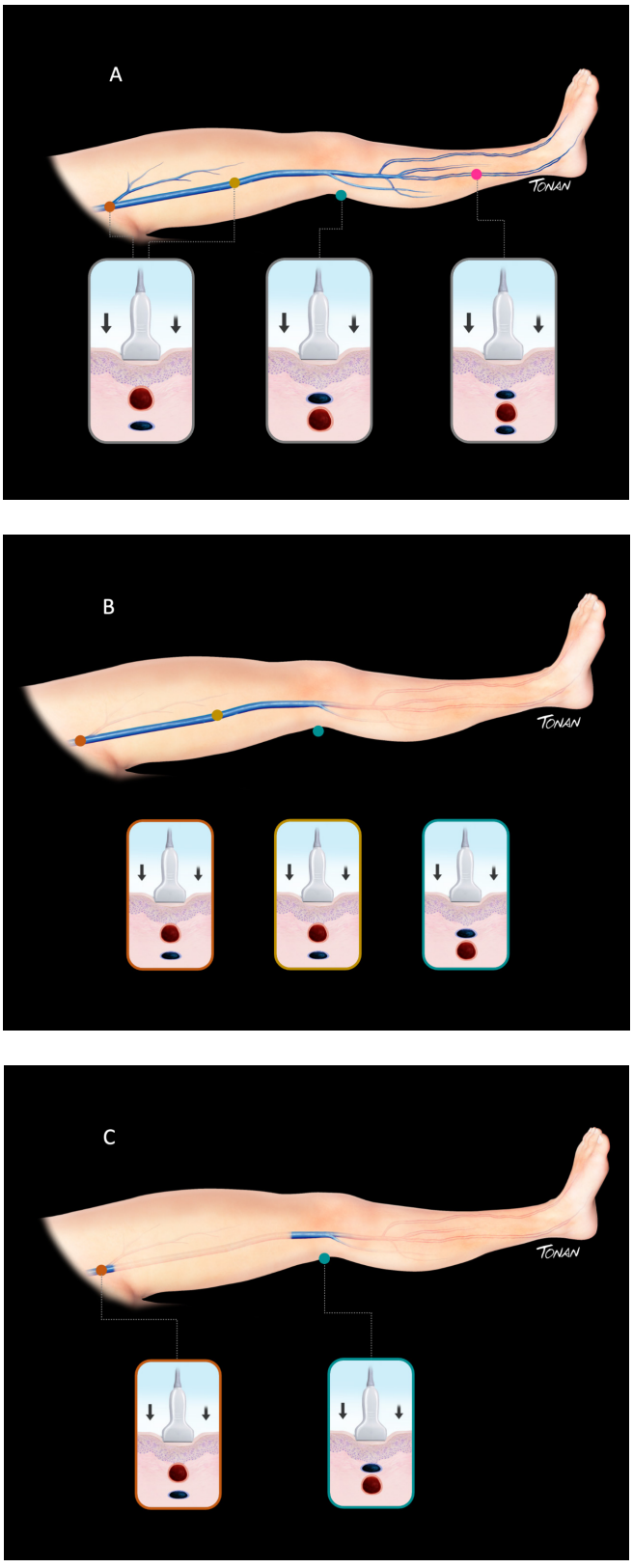


Figure 15 – *A: complete lower extremity venous compression ultrasound protocol; B: three-point extended compression ultrasound protocol; C: two-point extended compression ultrasound protocol.*

plus D-dimer measurement showed that the two strategies are equivalent for the management of symptomatic outpatients with DVT. However, in the latter, the physicians were VUS experts, which indicates that this testing protocol should be performed by well-trained physicians and follow clinical probability scores to be accurate. In most cases, if symptoms persist or if D-dimer is elevated, compression US must be repeated.[101]

Finally, the recommended complete imaging protocol is the one intended for DVT investigation because it scans the entire deep vein leg system, and it should be performed by experts. In addition, it requires dedicated high-grade devices and patient transport from the emergency department/ward to the imaging center.

Conversely, two- and three-point compression US protocols are simple, fast, do not require experts or sophisticated devices, and can be performed at the bedside. These protocols are suitable for emergency departments and hospital wards, especially after working hours and on weekends. Together with pretest probability evaluation, they should be able to provide an initial assessment of patients with suspected DVT.

Two-point compression US protocols are an extremely important tool for critically ill patients in certain settings, such as the current coronavirus disease 2019 (COVID-19) pandemic. There is a relationship between COVID-19 and a prothrombotic state, which favors the occurrence of thromboembolic phenomena. Thus, in case of a clinical possibility of DVT, performing a quick, focused US of the suspected limb should be considered when the diagnosis has an impact on patient management.[102]

3.4. Differential Diagnosis

The symptoms triggered by a thrombotic event are common to several other acute or chronic conditions, such as ruptured synovial cysts, chronic venous insufficiency, hematomas, and muscle tears.[103] In other cases, venous thrombosis may occur due to extrinsic compression by vascular abnormalities, such as aneurysms and pseudoaneurysms, or extravascular conditions, such as hematomas, abscesses, synovial cysts, lymphadenomegaly, and neural and hematological tumors.[104] Complete US imaging of the limb provides the ability to identify associated clinical conditions and differentiate them from nonthrombotic abnormalities. Conversely, a swollen limb associated with stasis or decreased respiratory variability in the femoral vein may indicate extrinsic compression in the pelvic region. Lymphadenomegaly, uterine fibroids, retroperitoneal tumors, and May-Thurner syndrome are among possible causes.[105] In this case, the sonographer should draw attention to these findings and suggest them as possible causes on the report.

Lymphadenomegaly, especially in the inguinal region due to lymphoproliferative disease, may lead to clinical manifestations of marked limb edema due to a possible compressive venous component or insufficient regional lymphatic drainage. Although easy to identify, occasionally the tumor mass can be so large that it hides the vein, making it difficult to characterize the flow or even the presence or absence of thrombus.[106]

Pseudoaneurysms, despite having well-defined color VUS features, can develop variable echogenicity in B-mode when thrombosed, depending on the volume and time of occlusion. When massive, they can also lead to venous compression and be associated with other conditions, such as blunt trauma or bone tumors, hindering vein identification.[107]

A common differential diagnosis of DVT on US is muscle hematoma, especially when located in the calf muscles. Clinical presentation is very similar to venous occlusion and is not always clear. Trauma or sudden pain are often mentioned in medical history. Su et al.[108] have described features suggestive of hematoma: mixed echogenicity, absence of anechoic regions, perilesional hyperechogenicity (especially enhancement in the underlying tissue), and a relationship between the longitudinal and transverse axis of lesions > 2.

One of the most common additional nonvascular findings on venous imaging is the popliteal synovial cyst, or Baker's cyst. It is most commonly located in the posteromedial surface of the popliteal fossa and most often does not trigger symptoms in the vascular system. However, in rare conditions, an intact cyst can present posterolaterally, with varying degrees of venous, arterial, and nervous compression, thus triggering neurovascular symptoms in the affected limb. In case of rupture towards the calf, Baker's cyst may mimic the main features of acute venous thrombosis. Despite clinical similarity, differentiation between articular and vascular cysts must be made because each condition requires a completely different treatment approach. Some cyst features may help differentiate it from thrombotic events. Baker's cyst communicates with the joint cavity close to the medial head of the gastrocnemius muscle, which is a key diagnostic feature (Figure 16). Despite its liquid content (therefore anechoic), it may have clots inside. Baker's cysts are usually single and multilocular.[109]

Soft tissue tumors, benign tumors (schwannomas, fibrous histiocytoma, neuromas), or malignancies (sarcomas, osteosarcoma) may mimic the clinical features of venous thrombosis or present as a compressive condition (Figure 17).[110]

VUS is a very accessible and widely available tool for the initial investigation of venous thrombosis; however, nonthrombotic conditions should be considered in the differential diagnosis. In experienced hands, IVUS allows alternative diagnoses of conditions that often occur in cases of suspected venous obstruction.

3.5. Recommendations

1. Complete duplex US should be used for the diagnosis of acute DVT (grade of recommendation: I; level of evidence: A).

2. Spectral Doppler of both common femoral veins should be performed to evaluate symmetry. In case of asymmetric flow pattern, the intra-abdominal veins should be investigated[91-94] (grade of recommendation: I; level of evidence: A).

3. Based on the consensus, the following terminology should be used to describe US abnormalities associated with DVT:

1) Acute/recent;

2) Chronic/old;

3) Predominance of acute changes;

4) Predominance of chronic changes.

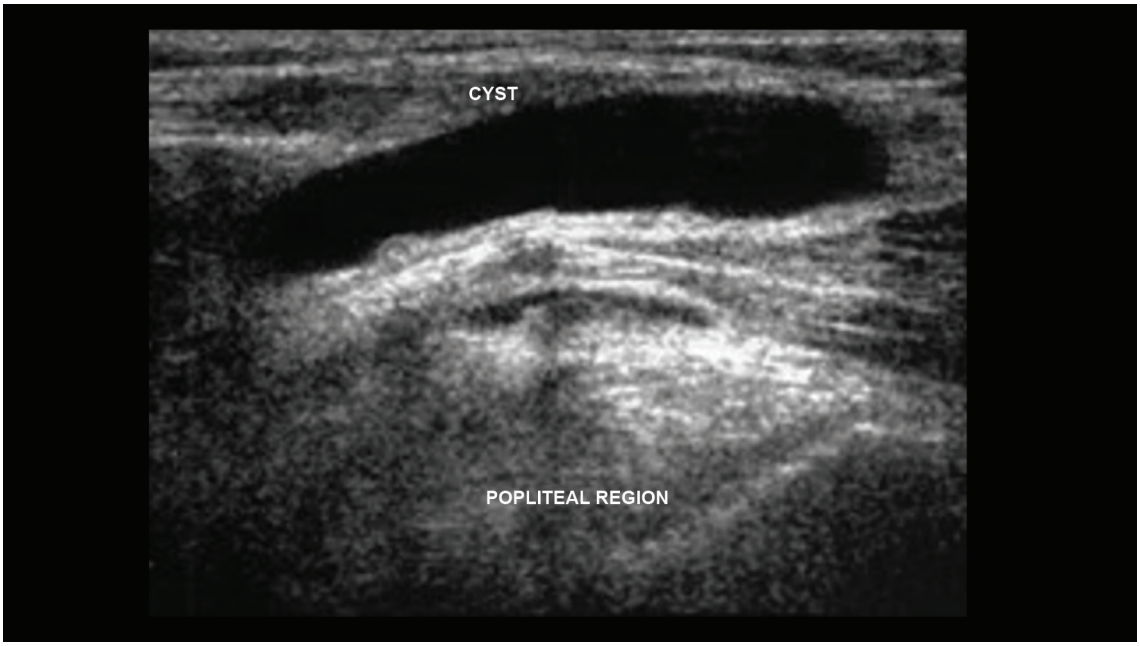


Figure 16 – *Baker's cyst in the posteromedial region of the knee (differential diagnosis of deep vein thrombosis).*

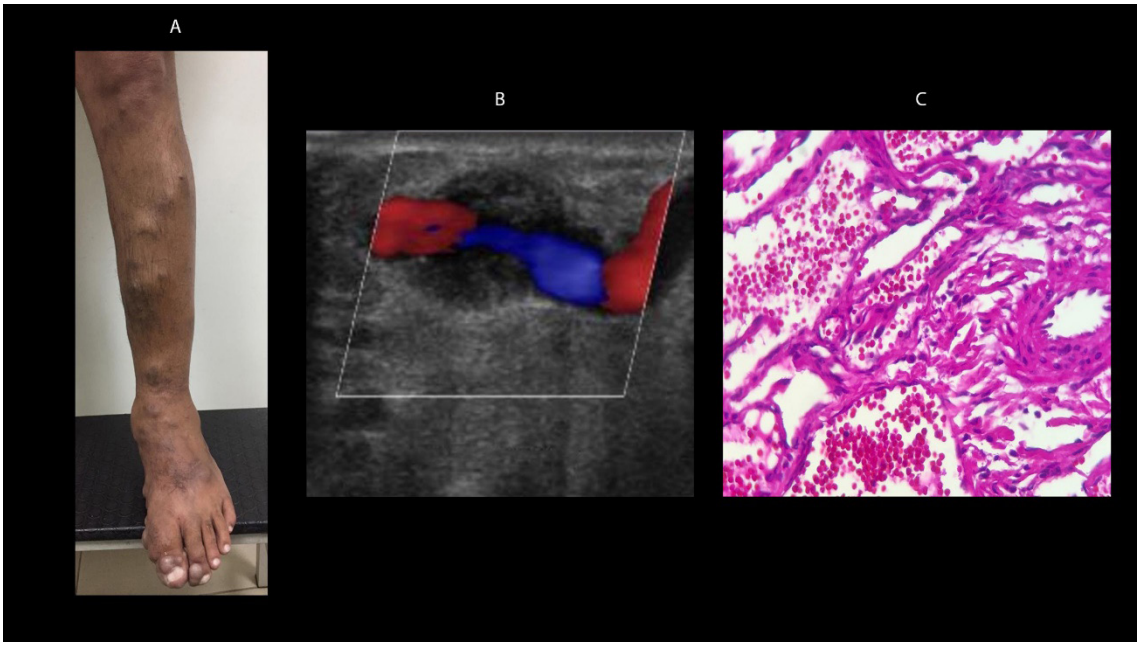


Figure 17 – *Venous hemangioendothelioma. A: clinical appearance; B: hypoechoic image surrounding a superficial vein on color flow Doppler; C: histopathologic examination.*

The term "indeterminate" might be used only in the few occasions that the time of the event cannot be determined.

3.6. How to Write Imaging Test Reports

Findings: first, it is fundamental to describe technical quality, the conditions in which the test was performed (if urgent or not), whether the patient was able to stay in the proper position, and if any major edema or other factors that could prevent proper technical quality were detected.

Vein evaluation should be described separately, especially if there is venous thrombosis. All imaging features must be described: B-mode and Doppler (which can lead to the conclusion on the presence or absence of thrombosis), time of clinical onset, and whether the thrombus is acute, indeterminate, or has chronic post-thrombotic changes.

1. Vein features to be included in the findings:

2. Vein caliber (compare with the contralateral vein and/or with the diameter of the adjacent artery) and wall appearance in all evaluated segments;

3. Total or partial compressibility and specification of the affected segment;

4. Presence or absence of intraluminal material. If present, evaluate echogenicity, complete or partial luminal obstruction, and presence or absence of a mobile component;

5. Presence or absence of collaterals;

Regarding flow:

- Color Doppler: describe if present or absent and if it causes partial or complete luminal obstruction;

- Spectral imaging: flow may be absent. If present, describe whether the flow is continuous or phasic in response to breathing and whether it increases (or not) distal compression and/or associated reflux.

6. If applicable, include possible differential diagnoses: Baker's cyst, tumors, signs suggestive of lymphedema, and images suggestive of hematoma.

Conclusions: Report if there are signs of thrombosis or not. If so, specify whether it is totally or partially occlusive and whether the vein(s) and venous segments are affected. Specify whether it is acute/recent or there are chronic/old post-thrombotic changes. In cases of clinically subacute thrombosis (between 2 weeks and 6 months), the following terminology should be used: predominant signs of acute thrombosis or predominant signs of chronic thrombosis. In cases of nonocclusive thrombosis, specify whether there is associated flow or not. Features that should be reported to support the conclusions are summarized in Chart 10.

4. Vascular ultrasound in the diagnosis of recurrent deep vein thrombosis and post-thrombotic syndrome

4.1. Recurrent venous thrombosis

This refers to a new episode of venous thromboembolism (VTE) in an individual with a previous history of this type of event. In cases of lower-extremity thrombosis, recurrence may manifest as a thrombus in a new location (propagation into a segment proximal or distal to the initial event, different segment of the same extremity, different extremity) or in a previously affected segment.[111] In current medical practice, the recommendation is that a patient diagnosed with a first episode of DVT be treated with an anticoagulant for 3 to 6 months,[112] which most often results in resolution of symptoms due to recanalization of the vein or emergence of collateral circulation.

Recurrent thrombosis is a common event. After anticoagulant discontinuation, the recurrence rate is estimated to progressively increase over time, reaching 40% among all patients within 10 years (cumulative incidence: 7.2% within 6 months, 11% within 1 year, 19.6% within 3 years, 29.1% within 5 years, 34.3% within 8 years, and 39.9% within 10 years).[113]

The most important risk factor for recurrence is the cause of the first episode of DVT. In patients with DVT caused by a transient risk factor (eg, after major surgery), the annual risk is only 1%, and a 3-month treatment may be considered depending on other risk factors.[114] When there is no identifiable main causal factor, or when there is only a transient minimal cause ("unprovoked"/idiopathic thrombosis), which occurs in approximately half of DVT cases, the prevalence of recurrence increases to 5%-10% at 1 year and up to 30% at 5 years,[115] which warrants treatment continuation with vitamin K antagonists (VKAs) or non-vitamin K oral anticoagulants (NOACS) for 6 months or over, as these significantly reduce the risk of recurrence by 80%-90%.[116]

Chart 10 – Ultrasound features to be reported that support the conclusions

ULTRASOUND SIGNS	TIME OF CLINICAL ONSET	DEGREE OF COMPRESSIBILITY	THROMBUS ECHOGENICITY	VEIN DIAMETER	TYPE OF FLOW
Acute/recent thrombosis	Less than 15 days	Total or partial noncompressibility	Hypoechoic or echolucent	Increased	Absent/continuous or reduced phasicity
Chronic/old thrombosis	More than 6 months	Total or partial noncompressibility	Hyperechoic/residual fibrotic trabeculations	Reduced or normal	Continuous/absent/with residual changes
Predominant signs of acute thrombosis, predominant signs of chronic thrombosis or, as an exception, indeterminate	Clinically subacute: Between 2 weeks and 6 months	Total or partial noncompressibility	Isoechoic/mixed echogenicity/mostly hypoechoic/mostly hyperechoic	Normal or slightly changed	Reduced/continuous/absent phasicity

Recurrence after stopping anticoagulation primarily depends on two factors: first, whether acute DVT has been effectively treated, including minimum duration of anticoagulant treatment; and second, the presence of intrinsic risk factors that increase recurrence, notably malignant disease and thrombophilias.[117] Optimal duration of anticoagulant treatment remains under discussion and is based on the extent of thrombosis (proximal or distal veins) and on the cause of the thrombotic event, when related to a main predisposing factor. We follow the recommendations of the American College of Chest Physicians (ACCP).[112]

In addition to the precipitating cause of DVT, other factors should be considered to determine the risk of recurrence. The sonographer should be attentive to clinical factors that are associated with recurrent thrombosis, such as: unprovoked DVT (RR, 2.3; 95% CI, 1.8-2.9), obesity (RR, 1.6; 95% CI, 1.1-2.4), male sex (RR, 2.8; 95% CI, 1.4-5.7), positive D-dimer test (RR, 2.6; 95% CI, 1.9-3.5), residual thrombosis (RR, 1.5; 95% CI, 1.1-2.0), hereditary thrombophilia (RR, 1.5; 95% CI, 1.1-1.9), inflammatory bowel disease (RR, 2.5; 95% CI, 1.4-4.2), antiphospholipid antibody (RR, 2.4; 95% CI, 1.3-4.1), and malignancy.[117]

Malignant disease is a major risk factor for both thrombosis and recurrent DVT. The risk for recurrent DVT is 2- to 4-fold higher in patients with cancer, reaching 4.2-fold in those undergoing chemotherapy.[118] This treatment affects the vascular endothelium and the coagulation cascade, thereby releasing prothrombotic substances during tumor lysis.

Knowing the natural history of DVT is important to choose the appropriate therapy and, in the case of vascular ultrasound, to conduct patient follow-up. Recanalization is a dynamic process that depends on lytic or procoagulant forces; it starts in the first week after an episode of DVT and continues for months, with most of the thrombotic mass being reduced in the first 3 months.[119] Complete resolution has been demonstrated in 56% of patients undergoing ultrasound scanning during follow-up,[120] and this rate is different for proximal and distal thromboses. In distal thrombosis, complete recanalization is more frequent and there are no sequelae such as significant reflux or obstruction. Conversely, the prevalence of reflux or obstruction in proximal thrombosis or thrombosis involving the two segments is much more significant.[121]

The recanalization rate is related to the prevalence of reflux in the venous system. The faster the recanalization, the greater the association with competent valves.[120,122] Reflux, associated or not with venous obstruction due to incomplete recanalization, may cause persistent venous hypertension, leading to the appearance of signs of chronic venous hypertension. These are known as post-thrombotic syndrome (PTS).

Although several studies have highlighted the importance of residual venous thrombosis, which is usually diagnosed by VUS examinations in the follow-up of patients with DVT,[123-125] a recent meta-analysis of 14 studies showed only a mild increase in the risk of recurrence (HR, 1.5; 95% CI, 1.1-2.0) in patients with residual venous thrombosis.[126]

4.2. Ultrasonic Diagnosis of Recurrent Thrombosis

There are no isolated VUS parameters that characterize recurrent thrombosis, or validated clinical models that allow its diagnosis. The diagnosis is based on reappearance of symptoms and clinical findings of DVT, considering the probability associated with risk factors and the presence of ultrasound findings suggestive of recurrent thrombosis.

Comparative findings of an examination of currently suspected recurrence versus a baseline examination at the end of treatment are the only validated method for the diagnosis of recurrent DVT. Therefore, the existence of previous examinations that clearly and accurately describe the extent of involvement and the degree of recanalization of the previous thrombosis is key.

Ultrasound findings are similar to those described for the diagnosis of the first thrombosis, and the presence of an old thrombotic mass may hinder the diagnosis of recurrence. Such findings are listed in Chart 9 of this document.

The diagnosis may be easier when recurrent thrombosis occurs in the contralateral extremity or in a new segment that was clearly not involved in the previous venous thrombosis. When it occurs in a previously involved segment, an increase in vein diameter by 2 mm under compression of the previously thrombosed segment has been reported as a criterion for the diagnosis of recurrent DVT.[127,128] Because an increase of 2 mm at maximum compression has a low positive predictive value, other authors have proposed the use of an increase in vein diameter ≥4 mm for the diagnosis of recurrent DVT.[129] A recent study that reviewed 36 published articles demonstrated that a new noncompressible vein or an increase in the diameter of a previously thrombosed vein segment ≥4 mm is sufficient to confirm the diagnosis of recurrent DVT. An increase in vein diameter of less than 2 mm allows recurrence to be ruled out, and an increase between 2 and 4 mm is considered unclear.[130]

Thrombus echogenicity, even when correlated with in vitro thrombus organization, makes the in vivo evaluation subjective. Importantly, even acute thrombi may have different stages of organization.[131]

La Gal et al.[132] demonstrated that a diagnostic strategy comparing an examination of currently suspected recurrence with a baseline examination at the end of treatment can be safely used to rule out recurrent DVT.

Such considerations reinforce the need for a thorough examination at the end of treatment to be used for comparison with subsequent procedures. All findings should be described, including residual diameter after maximum compression and site of measurement (it should be performed at the site of greatest residual thrombotic mass).

4.3. Post-thrombotic Syndrome

Post-thrombotic syndrome (PTS) is a chronic condition characterized by signs and symptoms that develop as a consequence of previous DVT. The most common complication appears in the long term and affects 20%-40% of cases of lower-extremity DVT, even in the presence of adequate anticoagulation.[133]

PTS has a significant impact on the quality of life of patients, compromising and limiting their daily activities and productivity. This impact can be compared to that of cancer and heart disease, in addition to causing a significant cost increase to the health care system. Data from Swedish studies showed costs about 75% higher than those of primary DVT.[133]

4.3.1. Pathophysiology

The pathophysiology of PTS is a combination of obstructive phenomena and venous reflux due to valve insufficiency, which result in venous hypertension. Increased venous pressure in subcutaneous capillaries and microcirculation culminates in valve incompetence in perforating veins. In most cases, the obstructive process is characterized by recanalization occurring within 6-12 months of the acute event, which leads to a combination of partial obstruction and varying degrees of reflux in the affected segments. All changes ultimately result in destruction of venous valves, development of collateral valves in sites where a greater degree of obstruction persists, and a greater tendency to recurrence of acute episodes.[133,134]

A system of competent saphenous veins may act favorably in venous drainage when there is a significant obstructive component. Conversely, when there is insufficiency of preexisting saphenous veins, venous return will be even more compromised, and the clinical status of these patients may deteriorate more quickly.

The inflammatory process is the main factor in PTS, being present in the resolution of thrombosis. Fibrinolysis, thrombus organization, and neovascularization involve interleukin-6 and intercellular adhesion molecule-1, which causes valve damage within the first months of the acute phase of DVT. Prandoni and Kahn suggest that the absence of recanalization in this period is an important predictor of PTS.[133,135,136]

4.3.2. Clinical Manifestations

PTS includes a wide spectrum of manifestations ranging from mild clinical signs to severe symptoms such as chronic pain in the affected limb (limiting daily activities), intractable edema, and ulcers. Other signs may appear, such as hyperemia, hyperpigmentation, venous dilations, and lipodermatosclerosis, in addition to symptoms such as pain, heaviness, paresthesia, pruritus, and cramps. In more advanced cases, cutaneous, subcutaneous, and lymphatic infections may occur.

The degree of residual luminal obstruction is directly related to the severity of repercussion of these clinical manifestations, leading to severe pain and venous claudication.

The following risk factors may contribute to the development of PTS:

- Obesity;
- Varicose veins;
- Proximal thrombosis and recurrence;
- Inadequate use of oral anticoagulant;
- Time: the slower the resolution of DVT, the greater the probability of PTS;
- Age and gender (there are contradictory studies);
- Other potential but not yet proven factors: malignancy, immobilization, surgery, pregnancy, and thrombophilia.

4.3.3. Diagnosis

This condition should be diagnosed after the end of treatment of the DVT episode, ie, PTS cannot be characterized before the first three months.[133]

The diagnostic strategy for PTS should consist of clinical examination, laboratory tests, imaging studies, and air plethysmography. There is no reference laboratory, imaging, or functional test for the diagnosis of PTS. However, the presence of venous obstruction and reflux must be recorded by an imaging test, and an attempt should be made to inform which of the two pathophysiological components predominates, even if nonquantitatively.

The clinical, etiological, anatomical, and pathological (CEAP) classification and the venous clinical severity score (VCSS) used for chronic venous insufficiency (CVI) can be appropriately applied to quantify PTS with different measures.[133,137-139]

The Brandjes scale, the Ginsberg measure, and the Villalta scale were specifically developed to assist in the diagnosis of PTS, but unfortunately they have not been adopted comparatively in other studies. At the 2008 meeting of the International Society on Thrombosis and Haemostasis, held in Vienna, the Villalta scale was recommended for defining the presence of PTS, with a score >5 indicating the diagnosis. The presence of ulcer makes the score even more severe (Chart 11).[140,141]

VUS is the imaging method of choice because of its low cost, availability in small centers, and high accuracy. In patients with a clinical presentation suggestive of PTS but no history of DVT, ultrasound is indicated to screen for evidence of previous DVT.

VUS provides the location, the measurement of vein diameters after the compression maneuver (Figure 18), and, more subjectively, the degree of luminal obstruction in the affected veins, valve damage, and reflux characterization (Figures 19 and 20). Although rarely used and infrequently available, air plethysmography is effective in quantifying venous reflux and is useful for the control of recanalization and diagnosis of rethrombosis.

In addition to defining the diagnosis,VUS can be used for therapeutic planning. The test is important in the evaluation of the involvement of the iliocaval segment for endovascular approach and restoration of patency as well as in post-treatment follow-up, having a key role in the diagnosis of recurrent thrombosis.[137,142]

When there are barriers to the use of VUS, such as the presence of severe edema and lipodermatosclerosis, which often includes focal fibrocalcific nodules, some strategies should be adopted for improving image acquisition.

Modification of transducer position and angle of insonation of the ultrasound beam, reduction in transducer frequency, and use of trapezoid mode with linear transducers are recommended; if necessary, the transducer should be replaced with lower-frequency devices that enable greater penetration, such as convex, sector, and endocavitary transducers.

Chart 11 – Villalta scale for post-thrombotic syndrome[141]

SYMPTOMS	NONE	MILD	MODERATE	SEVERE
Pain	0	1	2	3
Cramps	0	1	2	3
Heaviness	0	1	2	3
Paresthesia	0	1	2	3
Pruritus	0	1	2	3
Signs	0	1	2	3
Pretibial hematoma	0	1	2	3
Skin induration	0	1	2	3
Hyperpigmentation	0	1	2	3
Redness	0	1	2	3
Venous ectasia	0	1	2	3
Pain on calf compresssion	0	1	2	3
Venous ulcer	0	1	2	3

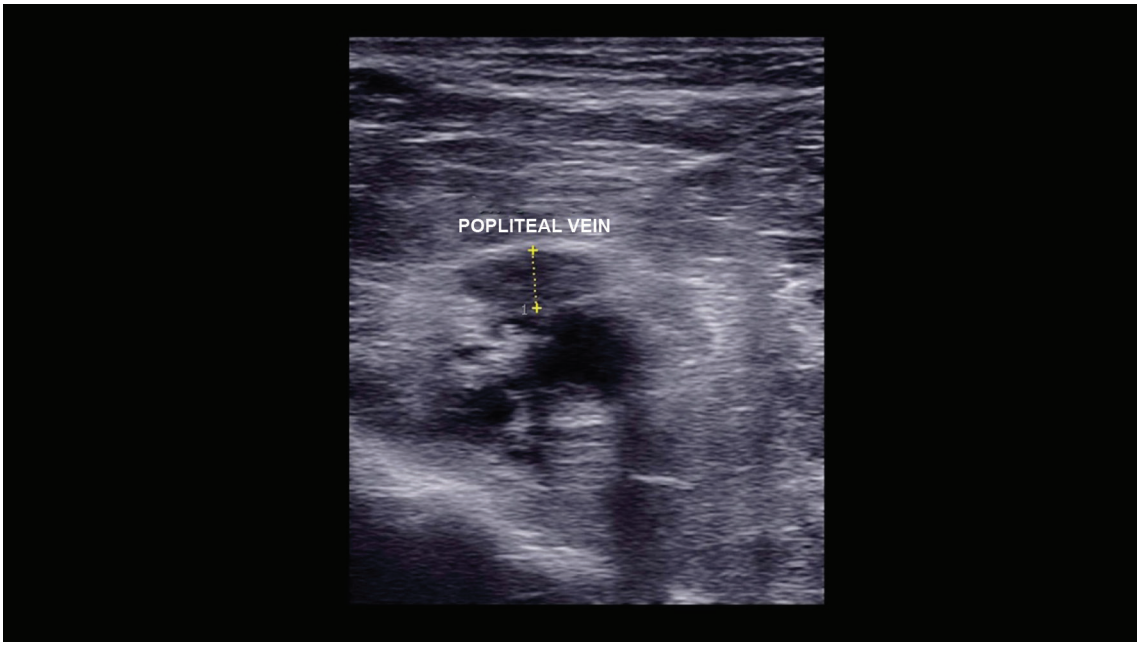


Figure 18 – *Two-dimensional ultrasound image with measurement of popliteal vein diameter after a compression maneuver.*

Patients with PTS should be examined in the supine and upright positions, which allows a better visualization of luminal changes, such as thickening and trabeculae, and promotes a more effective investigation of reflux (Figures 19A-C and 20). Below-knee veins can also be evaluated with the patient seated, feet resting lightly on a platform, and muscles more relaxed, which facilitates the insonation of deeper veins.

In the iliocaval segment, not only should the physician assess patency but also look for signs of extrinsic compression, especially compression of the left common iliac vein between the direct common iliac artery and the adjacent vertebral body, usually L5.

The main ultrasound findings are reduced vein diameters and chronic luminal changes, such as parietal thickening and hyperechoic trabeculae, in addition to valve insufficiency. A less frequent finding is the presence of flow with an arteriovenous fistula pattern in focal points of venous segments affected by thrombosis, without repercussions in adjacent axial veins and arteries and of unknown clinical significance.[142]

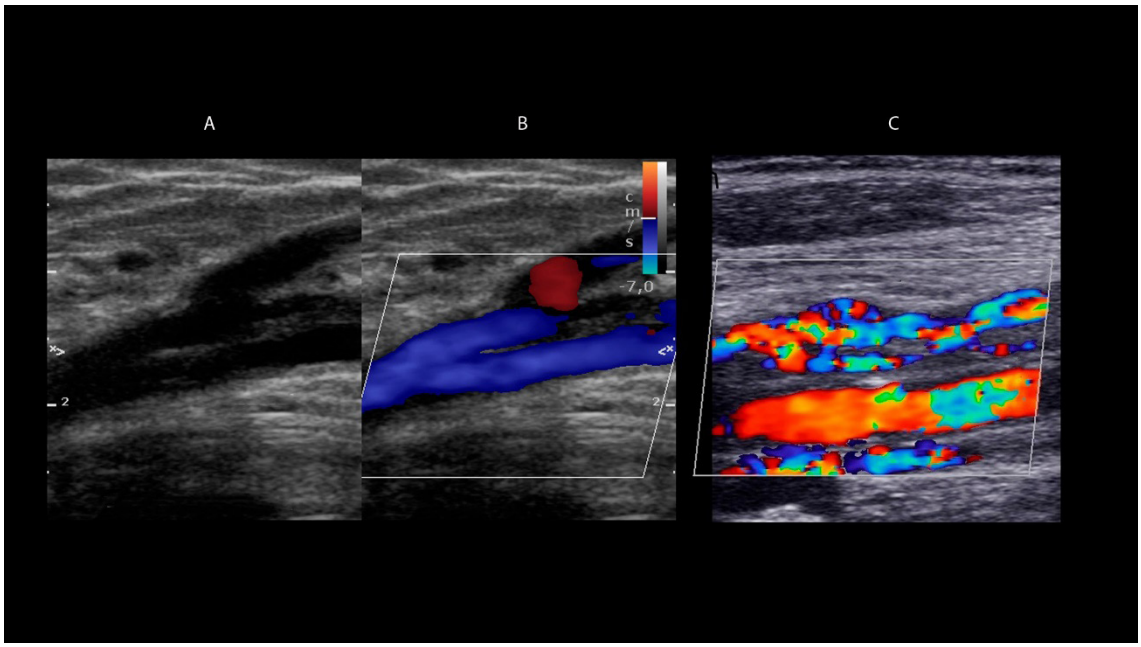


Figure 19 – *A: two-dimensional longitudinal image of the common femoral vein with parietal thickening and trabeculations/fibrous tissue inside the vessel. B: color flow Doppler showing flow around the trabeculation. C: similar findings in the popliteal vein.*

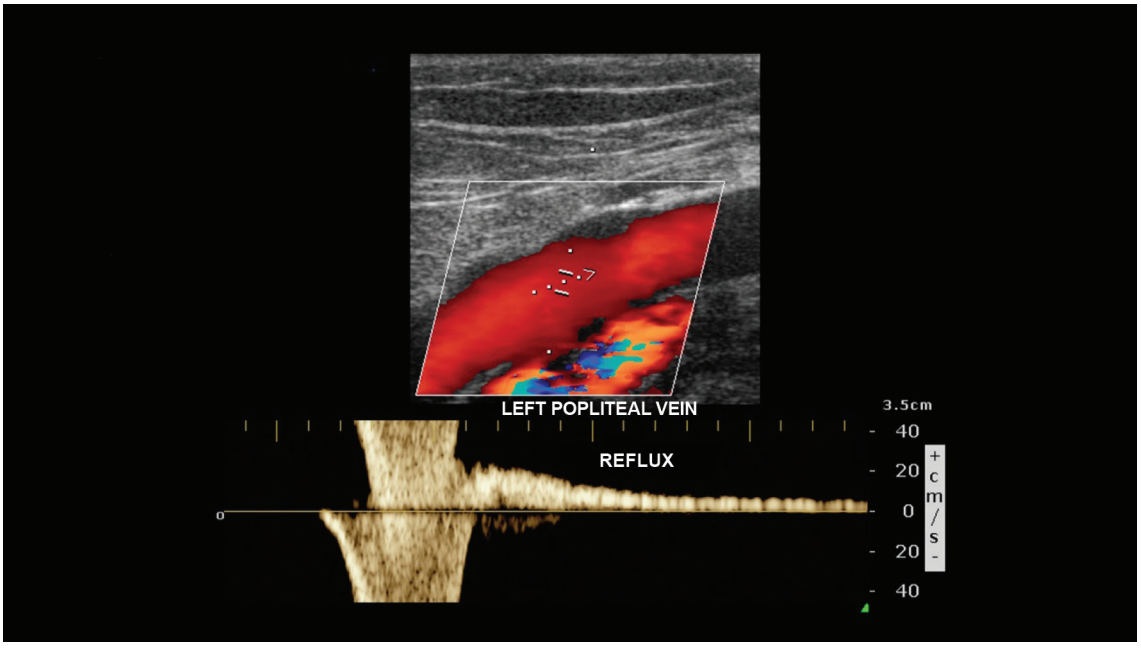


Figure 20 – *Reflux in the popliteal vein on color flow Doppler (red) and pulsed Doppler.*

5. Follow-up Protocols with Intravascular Ultrasound after Deep Vein Thrombosis

5.1. Introduction

DVT is a dynamic process with periods of recanalization, progression, and recurrence, and VUS is an important tool for the diagnosis and follow-up of DVT. Ultrasound findings play an important role in the outcome of this disease and are useful at different stages, which will be described below.

5.2. Recanalization

Recanalization of venous thrombosis is complex and involves mechanisms of intrinsic and extrinsic fibrinolysis, peripheral fragmentation, neovascularization, and thrombus retraction.[134,143,144] Such data have been confirmed by studies using VUS, which demonstrated thrombus regression within the first three months and complete recanalization in approximately half of patients within 6 to 9 months of the episode of DVT.[8,145] Partial recanalization and persistently occluded segments occur in approximately 20% and 5% of cases, respectively[146] (Figure 21A).

Images of small tortuous vessels inside the thrombus and adjacent to the venous wall, detected on color flow Doppler and pulsed wave Doppler, suggest the presence of neovascularization in the complex process of recanalization[134,143] (Figure 21B).

5.3. Recurrent Thrombosis

Although recanalization is an important step in the process, recurrent thrombotic events are not uncommon. The recurrence rate of symptomatic thromboembolism ranges from 5% to 13%, depending on the duration of patient follow-up with VUS. However, VUS-detected thrombotic events without clinical manifestations tend to be greater.[8]

The diagnosis of recurrent thrombosis is highly dependent on the ultrasound examination performed during the first episode. The following criteria are suggestive of recurrence:

1) Identification of new sites of thrombosis;

2) Occlusion of partially recanalized venous segments recorded in a previous examination;

3) An increase greater than 4 mm in the diameter of partially recanalized venous segments recorded in previous examinations[111,130] (Figure 22).

5.4. Valve Insufficiency

Reflux secondary to DVT, resulting from valve damage, is described in approximately 33% to 59% of the affected venous segments (Figure 22B). The popliteal vein is known to be the most frequently affected vein, followed by the femoral vein.[146] Reflux evaluation with distal compression maneuvers is considered abnormal when time is greater than 1000 ms in the common femoral, femoral, and popliteal veins, and equal to or greater than 500 ms in other veins.[147]

A: longitudinal view of the femoral artery (red) and femoral vein (blue). Presence of partial recanalization of the venous segment surrounding residual fibrosis. B: longitudinal view of the femoral vein. Normal ascending venous flow is detected in blue by color flow Doppler, and reflux is shown in red after distal compression maneuver. C: cross-sectional view of the calf muscle vein, with increased diameters and reduced compressibility. In 1, an echogenic image refers to a previous DVT, and in 2, a hypoechoic image refers to a current recurrence.

Some factors associated with persistent post-thrombotic changes, such as the presence of residual thrombi and/or post-phlebitic valve insufficiency, were considered predictors of PTS. However, whether such findings can be used to change a therapeutic plan remains a controversial topic in the literature.[148,149]

5.5. Discussion

5.5.1. Initial Negative VUS

The US Society of Radiologists in Ultrasound has recently recommended full compliance with the VUS protocol for diagnosing DVT.[80] They also recommend that a comprehensive ultrasound examination be performed within 5 days to 1 week of an initial negative comprehensive or point-of-care ultrasound in the following situations:

a) Persistent or worsening symptoms;

b) Patients at high risk for DVT whose etiology of symptoms has not been elucidated;

c) Technically compromised previous examinations.

5.5.2. Proximal vs. Distal Thrombosis

There has been a debate regarding follow-up protocols with VUS for patients undergoing treatment for proximal DVT, ie, in the iliac, femoral, and popliteal territories. It is not clear whether patients on adequate anticoagulant medication would benefit from VUS during treatment, or whether the conclusions of a repeated examination would change the established treatment strategy. However, VUS performed at the end of treatment has been shown to be an important basis for future evaluations, being crucial for detecting which veins were recanalized and which veins still have post-thrombotic changes.[80,123,150-152]

Regarding isolated leg vein thrombosis, classified as axial when it involves the tibial and/or peroneal veins, and as muscular when it involves the muscular veins only, some centers do not include its study in the investigation of DVT with VUS. With this initial approach, patients should undergo a second examination on the seventh day after the onset of symptoms. The second procedure is indicated for assessing the possibility of involvement of the proximal segment, if there has been propagation of distal DVT not diagnosed in the acute phase. When the results of the second examination are normal, the possibility of thrombosis is ruled out and the patient is not anticoagulated. In such centers, the estimated risk of pulmonary embolism (PE) at the end of 3 months is 0.5% (95% CI, 0.4%-0.9%).[18,153,154]

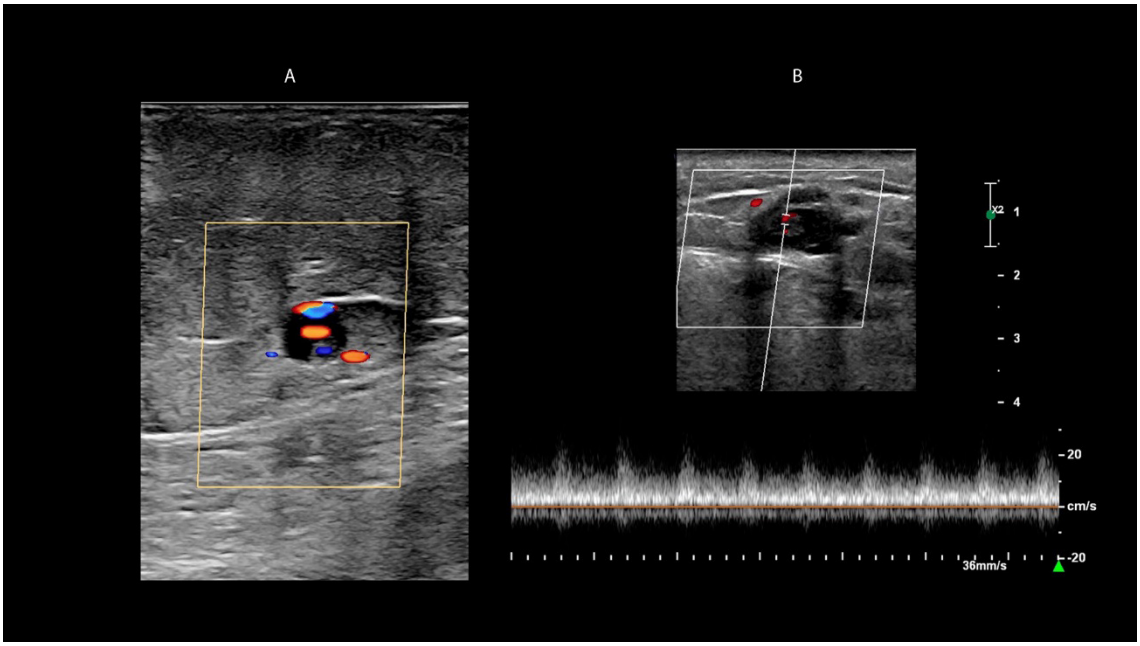


Figure 21 – *Process of neovascularization in recanalized thrombosis. A: cross-sectional view of a vein with echogenic images corresponding to the thrombus inside the vessel. Color Doppler demonstrates the presence of flow within the thrombus. B: Pulsed wave Doppler revealing the presence of flow within the thrombus.*

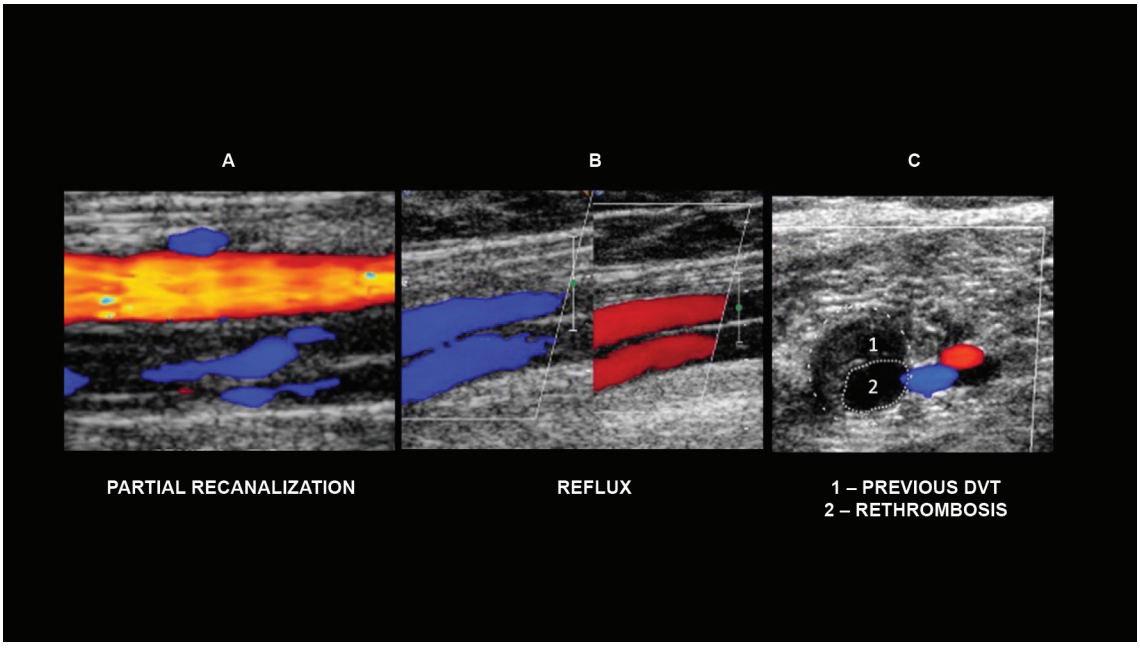


Figure 22 – *Ultrasound changes that may be found in the follow-up of patients with deep vein thrombosis. A: longitudinal view of the femoral artery (red) and femoral vein (blue). Presence of partial recanalization of the venous segment surrounding residual fibrosis. B: longitudinal view of the femoral vein. Normal ascending venous flow is detected in blue by color flow Doppler, and reflux is shown in red after distal compression maneuver. C: cross-sectional view of the calf muscle vein, with increased diameters and reduced compressibility. In 1, an echogenic image refers to a previous DVT, and in 2, a hypoechoic image refers to a current recurrence.*

A serial follow-up protocol with VUS is recommended given the low rate of proximal propagation of popliteal vein thrombosis, which ranges from 3 to 15%,[155-158] with limited benefits from anticoagulation. A meta-analysis conducted by Masuda et al.[159] reinforced the low rate of propagation. In a randomized study, Schwartz et al.[160] demonstrated no statistically significant differences between patients treated with anticoagulation for 10 days and individuals without treatment (3.7% vs. 3.8%). The propagation rate described by the authors for the popliteal vein was 1.9%.

Few studies have addressed recurrence in distal DVT. Masuda et al.[159] found no robust publications to compare patients treated with anticoagulation or followed-up with VUS. Lagerstedt et al.[161] studied patients with symptomatic distal DVT and treated with warfarin for 5 days. Patients were then allocated to warfarin maintenance for 3 months or treatment discontinuation. After 3 months, the recurrence rate of DVT was 29% and 0% in the "treated" versus "untreated" groups, respectively.

Anticoagulation treatment is chosen for cases of symptomatic distal DVT. Most authors suggest a short duration, up to 6 weeks, in low-risk patients.[160,162] In high-risk cases (cancer, previous DVT, involvement of two or more veins, age over 50 years, and thrombophilia), they recommend a 12-week treatment.[163]

Centers using a comprehensive VUS protocol choose to treat patients regardless of venous thrombosis being proximal or distal. The estimated risk of PE with this approach, at the end of 3 months, is also low (0.6%; 95% CI, 0.3%-0.9%).[101,164]

Evidence regarding post-thrombotic changes used as a parameter to guide the duration of anticoagulation is not robust. Additional research is needed in this field.[123,165]

A study on patients with proximal DVT treated with rivaroxaban evaluated ultrasound changes found at 1, 3, 6, and 12 months as well as recanalization rates in the affected veins.[166] Other authors[167] have highlighted the link between the presence of reflux in the popliteal vein, the permanence of fibrotic tissue, and the development of PTS. Sartori et al.[168] followed-up 172 patients with isolated leg vein thrombosis treated with enoxaparin for 6 weeks and assessed the degree of recanalization with IVUS. The examination was performed during diagnosis and at the end of treatment. Approximately half of patients (49.5%) had recanalized veins, and there was no statistical significance between recanalization and thrombus size or anatomical site involved (axial vs. muscular veins).

5.5.3. Recurrent Deep Vein Thrombosis

After an initial episode of DVT, recurrence may occur in approximately 25% of patients within 5 years and account for an increased risk of PE and PTS.[169] The diagnosis of recurrent DVT is more complex because, different from the approach to the initial episode of DVT, which includes validated algorithms of pretest probability combined with the use of D-dimer and VUS,[19] such criteria are not validated for recurrence.[170] An VUS finding of non-compressibility of a previously unaffected segment can be considered diagnostic.[152]

At times, an initial thrombus will not resolve completely, which results in chronic post-thrombotic changes and may hamper the diagnosis. In this case, the diagnosis of recurrent DVT in a previously affected venous segment can be made by detecting an increase in thrombus diameter of at least 4 mm compared with a previous study. However, the accuracy of this finding is controversial,[171] and the diagnosis depends on a previous good-quality examination with an adequate number of images for comparison, together with caliber measurements in the different affected venous segments; however, this is not observed in daily clinical practice. Also, this criterion is not used in previous episodes of calf vein thrombosis, which makes patient approach even more difficult.

The follow-up of patients with recurrent DVT should follow the criteria used during the initial episode, as there is a lack of studies addressing the follow-up of this specific group. We also highlight the importance of a comprehensive study at the end of treatment, or a follow-up examination after 6 months in cases of prolonged anticoagulation, including the investigation of chronic post-thrombotic changes with an assessment of venous insufficiency with the patient in the upright position.

5.4. Recommendations

Based on medical literature data and guideline expert discussions, we suggest the following:

1) Negative initial VUS: perform a comprehensive ultrasound examination within 5 days to 1 week of an initial negative comprehensive or point-of-care ultrasound in the following situations: (a) persistent or worsening symptoms, (b) patients at high risk for DVT whose etiology of symptoms has not been elucidated, and (c) technically compromised previous examinations.

2) Initial VUS with diagnosis of proximal DVT (iliac, femoral, and popliteal territories): repeat comprehensive ultrasound examination towards the end of treatment to assess the degree of recanalization and presence of post-thrombotic changes. Or, at any time the patient presents with symptoms during treatment, for evaluation of recurrence or extent of the initial thrombosis.

3) Initial VUS with diagnosis of distal DVT (tibial, peroneal, or muscular veins): repeat ultrasound examination within 6 weeks or 12 weeks of the onset of clinical manifestations, according to the established therapeutic plan.

4) Patients with recurrent DVT: repeat ultrasound examination at the end of treatment, or a follow-up examination after 6 months in cases of prolonged anticoagulation, or at any time in the presence of symptoms.

6. Diagnosis of Pulmonary Embolism by CT Angiography, MR Angiography, and Pulmonary Angiography

6.1. CT angiography

The PIOPED II study[172] evaluated the role of CT pulmonary angiography combined with lower-extremity CT venography in the diagnosis of PE. Patients were clinically classified

according to the probability of PE using the Wells score,[33] and pretest results were compared with posttest results. The authors demonstrated that CT angiography combined with CT venography has a positive predictive value (PPV) of 96% in patients with high clinical probability of PE, and a negative predictive value (NPV) of 97% in patients with low clinical probability. In patients with moderate clinical probability, both NPV and PPV were 92%, demonstrating an excellent case-solving capacity.

CT angiography also allows the assessment of the aorta, lung parenchyma, chest wall, and pleural space, with excellent spatial resolution, and the making of alternative diagnoses in cases of suspected PE, which may occur in up to 2/3, such as aortic dissection, pneumothorax, pneumonia, and lung cancer. Thus, the treatment for each of these conditions can be properly initiated.[173]

CT angiography is currently the method of choice for evaluating patients with suspected PE after clinical risk stratification. A negative CT angiogram in patients with low clinical probability is sufficient to rule out this diagnosis. A positive CT angiogram in patients with high clinical probability confirms the diagnosis of PE.

As CT angiography is the gold standard for the diagnosis of PE, studies that attempt to characterize prognostic factors using CT for evaluation of the RV have emerged.[174-177]

In a retrospective study, Schoepf et al.[177] demonstrated that an increased RV, assessed by CT angiography using the ratio between the dimensions of the RV and the LV (abnormal when > 0.9), is an independent mortality factor (OR = 5.17; 95% CI),[1,16,35,63] which confirms the potential use of this tool in the risk stratification of patients with acute PE.

Araoz et al.[175] selected three types of CT angiography findings (ventricular septal bowing, embolic burden, and RV/LV ratio) to assess the risk of short-term death, defined as in-hospital death or death within 30 days of CT angiography due to PE (Figure 23). They concluded that ventricular septal bowing may have some clinical value as a predictor of short-term death, with high specificity (87%-88%) and low sensitivity (18%-21%). The other factors did not increase the risk of short-term death in the study sample.

6.1.1. Imaging Technique and Protocols

In CT pulmonary angiography, the aim is to achieve maximum pulmonary arterial opacification while keeping venous contamination and motion artifacts at a minimum, in addition to reducing radiation and contrast doses.

The main parameters to be observed are:

a) Imaging protocols: depend on available equipment and information such as patient's height and weight. Ideally, they should include:

- Acquisition performed with the patient holding the breath (apnea) without exercising a Valsalva maneuver or respiratory pause;

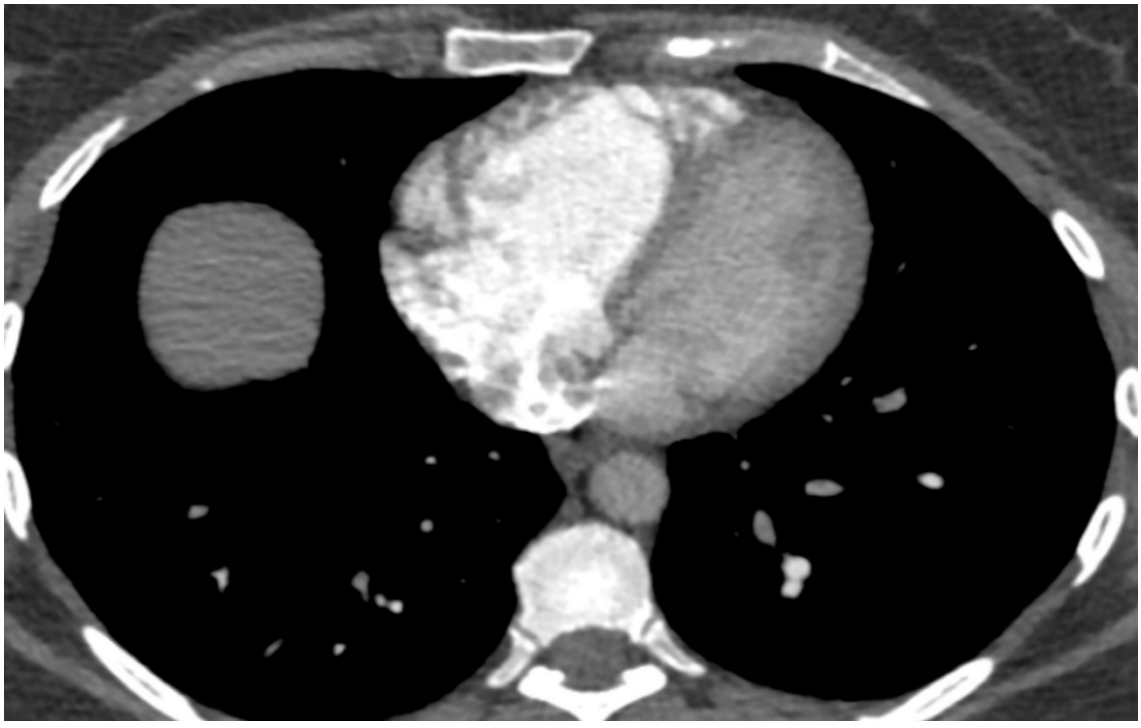


Figure 23 – *Positive CT angiography for acute PE. Signs of RV dysfunction; note increased RV dimensions (RV/LV ratio >1) and ventricular septum bowing leftward.*

- Use of test bolus or bolus tracking with an ROI on the pulmonary trunk to monitor the arrival of the contrast material in the pulmonary arterial circulation and to perform the examination at the appropriate time;

- Imaging from the lung base to the apex, which minimizes motion artifacts in the most important regions and reduces the artifact resulting from concentrated contrast in the superior vena cava;

- Acquisition and reconstruction of images with thin slice thickness (1 mm or less) and without gaps between images to allow multiplanar reconstructions with no loss of spatial resolution;

- Comprehensive study of all thoracic structures, which allows the assessment of possible differential diagnoses for the patient's complaint;

- Use of lower kV, which significantly attenuates iodine and substantially reduces the radiation dose. This reduction causes an increase in image noise: the use of 100 kV instead of traditional 120 kV should be preferred, as long as the signal-to-noise ratio is kept at acceptable levels. Obese patients may need higher kV;

- Use of iterative reconstruction, which reduces radiation doses and keeps image noise at acceptable levels.

b) Contrast material injection protocols: depend on the available type of contrast material and venous access. Ideally, they should include:

- Large-bore antecubital vein access that allows the injection of contrast material with an injection pump at a minimum speed of 4 mL/s;

- Use of more concentrated contrast materials, such as 350 mg I/mL.

6.2. Dual-energy computed tomography

Dual-energy CT is able to acquire images simultaneously (or nearly) at two different energy levels.[178] The main advantages of this method for evaluation of PE are the following (Figure 24):

a) It generates an iodine-enhanced map as a by-product, which reflects at that time the distribution of contrast material across the lung parenchyma. A joint evaluation of the iodine-enhanced map with angiographic images can sensitize the detection of small thrombi in distal pulmonary branch arteries,[179,180] allowing monoenergetic reconstructions.

b) Conversely, monoenergetic reconstructions at low energy levels (eg, 50 keV) increase iodine attenuation, but they also increase image noise. This type of reconstruction improves angiographic images in cases of suboptimal vascular opacification or allows reductions in contrast dose, eg, in patients with renal dysfunction.[179,180]

6.3. Older Generations of CT Scanners

Performing CT pulmonary angiography using older scanners can be a major challenge. However, after adjustments to imaging parameters and optimization of contrast use, using larger volumes or adjusting the injection rate may be required to compensate for a slower acquisition and to obtain good-quality diagnostic images.

6.4. Diagnostic Criteria

The diagnosis of acute PE with CT angiography is based on the identification of occlusive or nonocclusive thrombi within the pulmonary branch arteries. The diagnostic criteria for acute PE on CT angiography are[181]:

a) Occlusive filling defect causing an increase in caliber of the affected vessel (Figure 25A);

b) Contrast-edged, central, nonocclusive filling defect (Figure 25B);

c) Nonocclusive filling defect adhering to the vessel wall and forming an acute angle with it (Figure 25C).

Acute PE is the main cause of filling defects in pulmonary branch arteries. Possible pitfalls and differential diagnoses include:

- Flow and/or motion artifacts;

- Incorrect anatomical interpretation;

- Reflex vasoconstriction;

- Chronic PE;

- Nonthrombotic emboli;

- Congenital anomalies (pulmonary artery agenesis and/or hypoplasia);

- Inflammatory diseases (vasculitis, fibrosing mediastinitis);

- Neoplasms (thrombi and/or tumor invasion or primary neoplasms of the pulmonary arteries).

An indirect sign of acute PE on CT is pulmonary infarction (Figure 26), which can be identified even on noncontrast studies. In this situation, the study should be complemented with an angiogram to confirm the diagnosis. The typical image of pulmonary infarction is a peripheral opacity with a pleural base and central foci of attenuation that are better characterized with mediastinal window settings (vessel sign) and without air bronchograms.[12,182]

6.5. Prognostic Criteria

The changes with prognostic value that can be characterized on CT angiography are related to an acute overload of the right heart chambers. Although dilations in the superior vena cava and azygos veins and in the pulmonary trunk as well as objective measurements of RV function have been cited as prognostic markers, the most important sign of RV dysfunction is an increase in RV dimensions with an RV/LV ratio greater than 1.0 in an axial CT plane (Figure 27).[181]

6.6. Contraindications and Special Situations

As CT angiography is an important tool in the diagnosis of acute PE, the only absolute contraindication is a history of severe allergy to iodinated contrast material. In such cases, alternative diagnostic methods include V/Q scintigraphy, MR angiography, gadolinium-enhanced CT angiography, or serial Doppler ultrasound of the lower extremities. A history of mild-to-moderate allergic reactions can be approached with the use of premedication for desensitization.

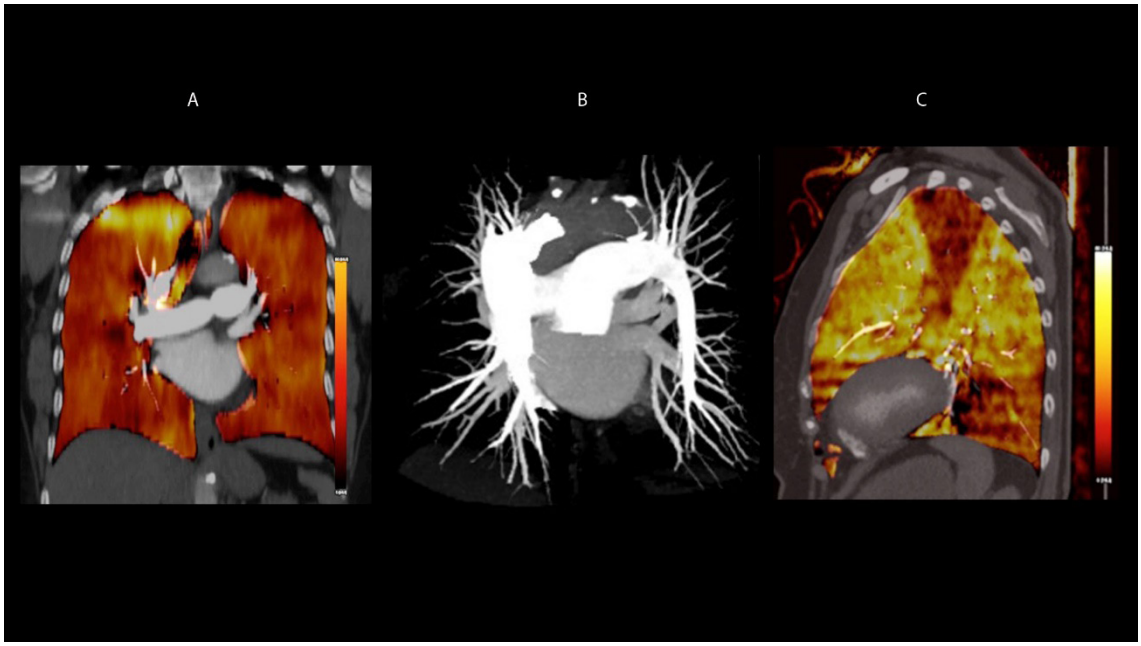


Figure 24 – *Dual-energy computed tomography pulmonary angiography. A: iodine-enhanced map reflecting the homogeneous distribution of contrast material within the lung parenchyma; B: MIP reconstruction in CT angiography. Dual-energy CT combines functional information with anatomical angiographic images. C: iodine-enhanced map of dual-energy CT in another patient, showing wedge-shaped perfusion defects in the left upper and lower lobes, compatible with pulmonary infarctions. Angiographic images (not shown) confirmed the diagnosis of acute PE.*

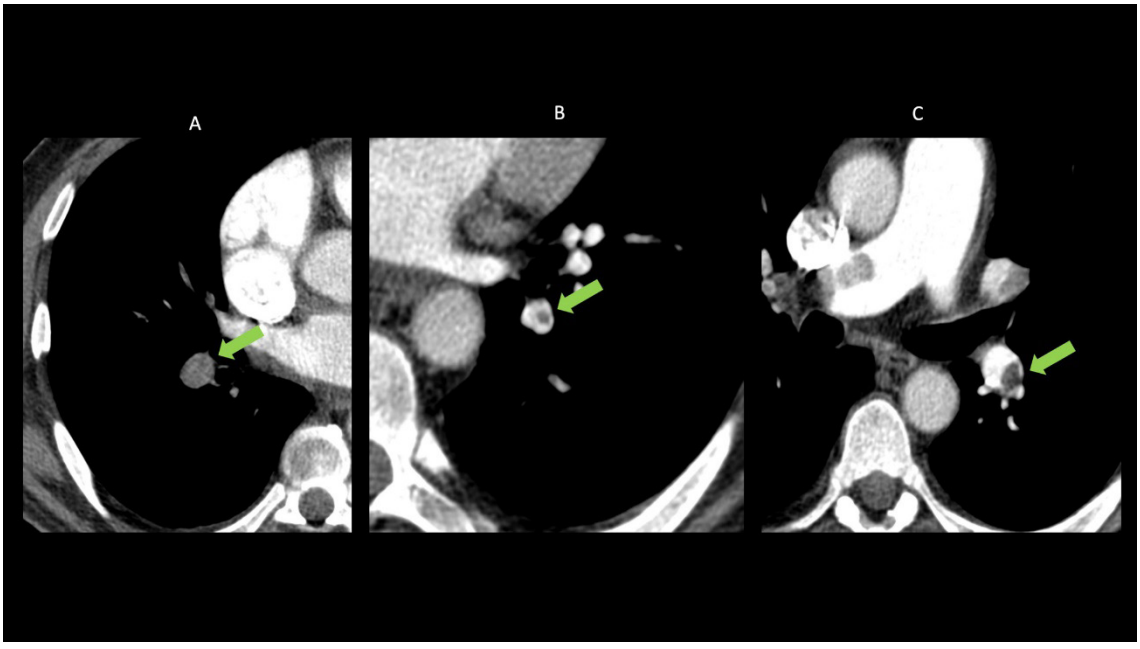


Figure 25 – *A: occlusive filling defect causing an increase in vessel caliber; B: contrast-edged, central filling defect; C: filling defect adhering to the vessel wall and forming an acute angle with it.*

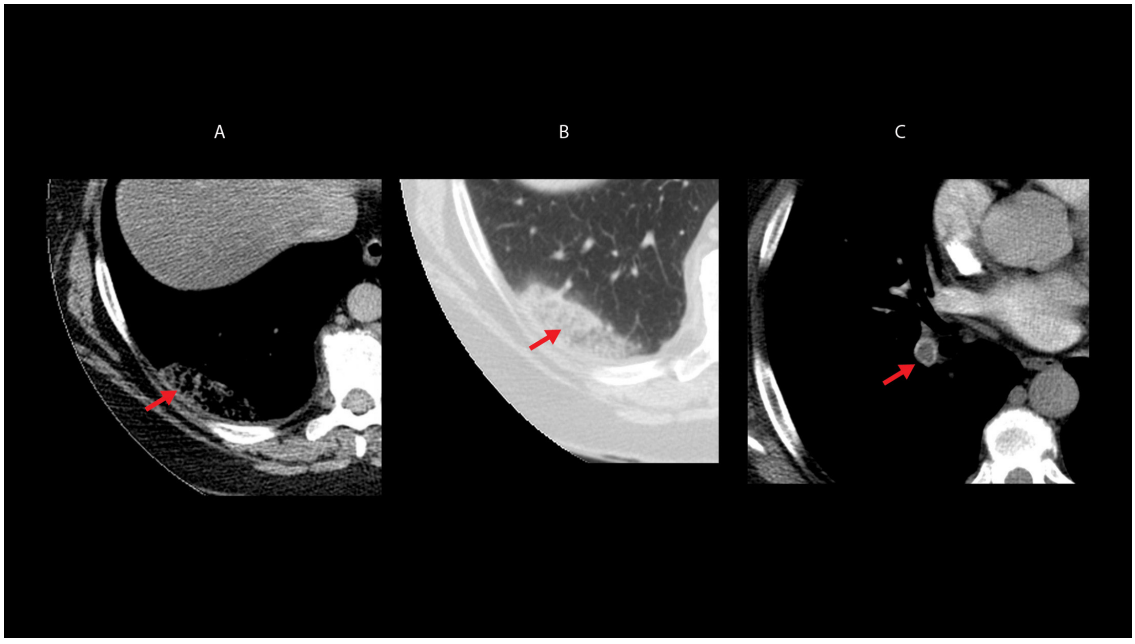


Figure 26 – *Pulmonary infarction. A and B: peripheral opacity with central foci of attenuation and no air bronchograms. C: acute thrombus in the basal segmental pulmonary artery to the right lower lobe.*

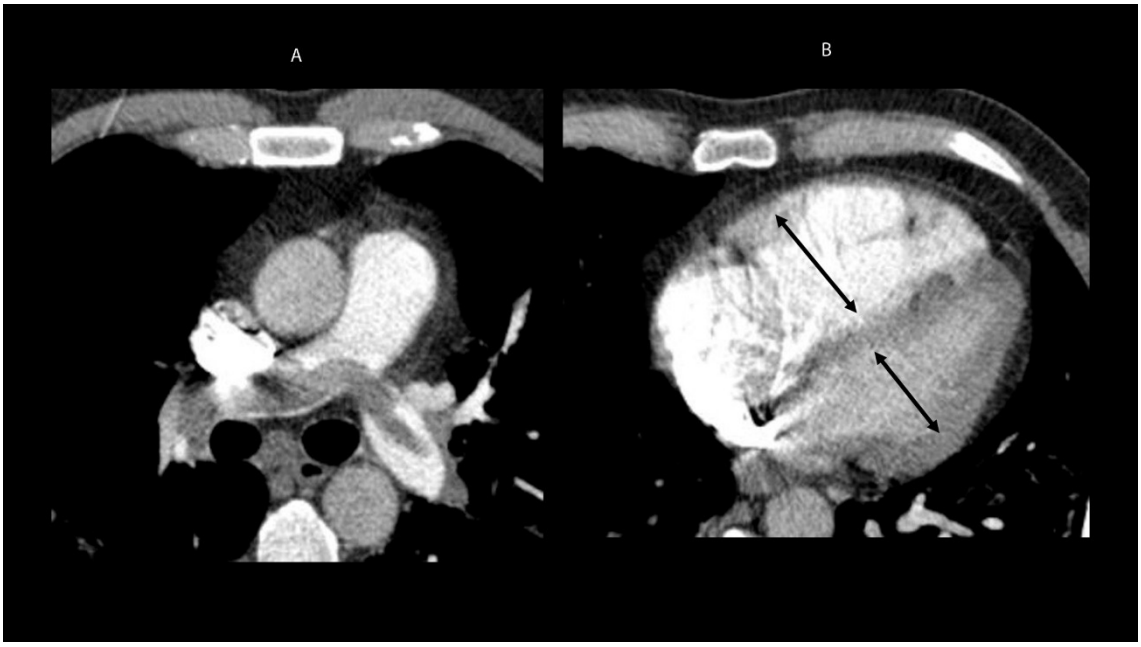


Figure 27 – *CT pulmonary angiography. A: typical signs of acute PE; B: signs of overload of the right heart chambers. Note RV/LV ratio >1.0 and straightening of the interventricular septum.*

There are other special situations that may require adaptation of the protocol or replacement of CT angiography with other diagnostic methods:

Situation 1: young women of reproductive age or pregnant women

To avoid exposing the breast tissue to ionizing radiation, especially in patients with a normal chest radiograph, V/Q scintigraphy (or the perfusion-only study) or serial Doppler ultrasound of the lower extremities can be alternatives.

When CT angiography is used, the protocol should be optimized by ensuring adequate venous access and injection rates to achieve high diagnostic quality and avoid repetitions.

Situation 2: chronic renal insufficiency under conservative treatment

This is not an absolute contraindication. The patient should preferably be hydrated before the examination, but cautiously to avoid congestion. In urgent cases, CT angiography can be performed even without prior preparation. V/Q scintigraphy and serial Doppler ultrasound of the lower extremities remain as alternatives.

If available, dual-energy CT scanners and monoenergetic reconstructions can be used to reduce the dose of iodinated contrast material and still ensure adequate vascular opacification.

Situation 3: ICU patients who cannot be transported

This case requires examinations that can be performed at the bedside, such as echocardiography and Doppler ultrasound of the lower extremities.

6.7. MR Angiography

MR angiography is an alternative to CT angiography, allowing a direct study of the pulmonary arteries.[174,183] This diagnostic method was evaluated in the PIOPED III study,[183] which combined MR pulmonary angiography with lower-extremity MR venography for diagnosis.

MR angiography has some advantages over CT angiography. The main benefits are the absence of radiation and the employment of a contrast material (gadolinium) that can be used in patients with iodinated contrast allergy.[183] Also, MR angiography allows the performance of other techniques, such as lung perfusion, great vessel flow quantification, and cardiac function assessment.[174,183]

Currently, the indication of MR imaging for patients with renal insufficiency, especially those receiving dialysis, has been revised because of the occurrence of progressive systemic fibrosis related to the use of gadolinium.[174]

The main disadvantages of MR angiography are lower spatial resolution, higher cost, greater complexity, longer examination, reduced availability, and difficulty monitoring critically ill patients inside the machine due to the high magnetic field.[174,183]

The results of the PIOPED III study(183) showed that MR angiography was technically inadequate in 25% of patients. With these technically inadequate tests included, the method diagnosed PE in 57% of patients. When the technically adequate tests were analyzed separately, MR angiography had a sensitivity of 78% and a specificity of 99% for the diagnosis of PE. When MR angiography was combined with MR venography, the combination showed a sensitivity of 92%, superior to MR angiography alone, and a specificity of 96% for the diagnosis. However, the tests were technically inadequate in 52% of patients, which caused a major problem.

The PIOPED III study[183] concluded that MR angiography should only be used for the diagnosis of PE at centers of excellence in MR imaging and when other methods are contraindicated. Currently, the main indication for MR imaging is as an alternative method to CT scanning in patients with iodinated contrast allergy.[174,183]

6.8. Digital Subtraction Angiography

For a long time, digital angiography was considered the gold standard for the diagnosis of PE. Invasive nature, greater exposure to ionizing radiation, and complications of up to 0.5% of mortality, 1% of severe complications, and 5% of minor complications,[184] together with the advances of CT angiography (similar accuracy with a less invasive procedure), are determining factors for digital subtraction angiography to have virtually fallen into disuse for the diagnosis of acute PE. However, the method remains an important diagnostic tool in the evaluation of chronic PE, especially in candidates for balloon angioplasty.

6.9. Recommendations

a) The indication of imaging methods for the diagnosis of acute PE should be based on hemodynamic status and clinical assessment of pretest probability, with the application of validated rules (Wells score, Geneva score, PERC criteria) combined with D-dimer. The aim is to avoid unnecessary use of imaging methods (grade of recommendation: I; level of evidence: A);

b) CT angiography is the imaging method of choice for diagnosing acute PE. It can be replaced with V/Q scintigraphy in cases of severe iodine allergy or in special situations such as pregnant women or patients with severe renal insufficiency. MR angiography is reserved for centers of excellence in the method or for when CT angiography and V/Q scintigraphy are inappropriate or unavailable;

c) The diagnosis of acute PE should be rejected in patients with low or moderate probability and negative CT angiography (grade of recommendation: I; level of evidence: A);

d) The diagnosis of acute PE should be accepted in patients with moderate or high probability and CT angiography demonstrating thrombi in segmental or proximal branches (grade of recommendation: I; level of evidence: B);

e) Lower-extremity CT venography is not recommended because of high exposure to ionizing radiation and should be replaced with IVUS for the evaluation of DVT (grade of recommendation: III; level of evidence: B);

f) In pregnant patients, both CT angiography and V/Q scintigraphy can be used. With modern techniques, both

tests cause little maternal and fetal exposure to ionizing radiation.[78,185] In normal radiograph cases, both CT angiography and V/Q scintigraphy can be used; however, in abnormal radiograph cases, the recommendation is to proceed with CT angiography (grade of recommendation: IIa; level of evidence: C);

g) Increased dimensions of the right heart chambers, with RV/LV ratio > 1.0, are linked to 5-fold higher mortality rates due to acute PE.[186] This should be considered a tomographic criterion of RV dysfunction and a marker of poor prognosis, even in patients at clinically low risk (grade of recommendation: IIa; level of evidence: B).

Diagnostic flowcharts in hemodynamically stable and unstable patients, according to the clinical probability of PE, are shown in Charts 5 and 6. Chart 12 shows the advantages and disadvantages of using CT pulmonary angiography in the diagnosis of PE.

7. Lung scintigraphy

7.1. Evidence

7.1.1. Introduction

For practical purposes, "lung scintigraphy" is defined in this section as a single test containing different combinations of lung ventilation/perfusion scans, or even a perfusion-only study. Based on international guidelines proposed by the US Society of Nuclear Medicine and Molecular Imaging (SNMMI), the European Association of Nuclear Medicine (EANM), and the European Society of Cardiology (ESC), the main indications for lung scintigraphy are listed and discussed below.[22,187,190]

Primary indication: determine the probability of PE.[22,187,196]

Secondary indications:

a) Record the degree of PE resolution in the follow-up of chronic PE;

b) Quantify differential lung function before surgical resection of lung cancer;[197-199]

c) Assess lung transplants;[200,201]

d) Assess congenital heart or lung disease, or conditions such as cardiac shunting, pulmonary artery stenosis, and arteriovenous fistula, including their treatment.[202]

e) Confirm the presence of a bronchopleural fistula.[203-205]

f) Assess chronic parenchymal lung disorders such as cystic fibrosis;[206,207]

g) Assess PE as a cause of pulmonary hypertension.[208]

The following findings are considered positive for PE:[189]

• Disagreement between ventilation and perfusion studies in at least one segment or two subsegments, with hypouptake present only or more extensively in perfusion compared with normal ventilation (known as a mismatch), conforming to the pulmonary vascular anatomy (wedge-shaped hypoperfusion areas with the base projecting to the lung periphery).

The following findings are considered negative for PE:

• Normal perfusion pattern conforming to the anatomic boundaries of the lungs;

• Matched pattern between ventilation and perfusion studies (defects of any size, shape, or number);

• Mismatch between ventilation and perfusion that does not follow a lobar, segmental, or subsegmental pattern.

The following findings are considered nondiagnostic for PE:

• Widespread ventilation/perfusion abnormalities not typical of specific diseases.

7.1.2 Evidence Regarding Imaging Interpretation Criteria

In scintigraphic evaluation for the detection of PE, there is sufficient scientific evidence for the use of well-defined interpretive criteria, as proposed by the Prospective Investigation of Pulmonary Embolism Diagnosis (PIOPED), PIOPED II, and PISAPED studies, as well as for interpretation based on the clinical experience of the nuclear medicine or imaging physician (clinical Gestalt).

The revised PIOPED criteria proved to be more accurate than the original PIOPED criteria,[209,210] while the PIOPED II (which uses fewer scan categories) and PISAPED criteria resulted in a smaller number of indeterminate or inconclusive studies.[211,212] Both PIOPED II and PISAPED criteria had equivalent performances in terms of interpretation when chest radiography was combined with lung ventilation scintigraphy.[213] There are, however, insufficient studies replicating these results, with no double-blind multicenter comparisons between the two strategies, which jeopardizes the finding that the methods have equivalent accuracy.

Therefore, the combination of ventilation studies is recommended whenever possible, mainly because of the increased specificity of the combined study compared with

Chart 12 – Advantages and disadvantages of CT angiography for the diagnosis of PE

CT PULMONARY ANGIOGRAPHY	
ADVANTAGES	**DISADVANTAGES**
Widespread availability	Ionizing radiation exposure
Excellent accuracy	Need for iodinated contrast material
Small number of inconclusive studies	Tendency to indiscriminate use because of easy access
Quick procedural time	Overdiagnosis – able to identify subsegmental thrombi, whose clinical significance is uncertain
Possibility of investigating alternative diagnoses	

radiography alone. In older adults, up to 60% of false-positive tests may result from absent ventilation, given the high prevalence of respiratory diseases.[214]

An analysis based on the experience of the nuclear medicine physician (clinical Gestalt) may provide a more accurate interpretation than studies using well-defined criteria alone. For the interpretation to be the best possible, the physician should provide an opinion based on detailed knowledge of the different objective criteria listed above.[209]

With regard to the imaging method of single-photon emission tomography (SPECT), there is a considerable body of literature supporting its use, including evidence regarding SPECT studies combined with low-dose CT, which seems to increase diagnostic accuracy.[215] The most recent EANM consensus (2019) suggests that tomographic imaging (SPECT or SPECT/CT) should always be performed routinely, leaving planar imaging for when the former is no longer possible.[188]

Although there are no robust multicenter studies comparing SPECT with planar scintigraphic imaging or even CT angiography,[216] or robust prospective studies evaluating patient outcomes with SPECT versus planar scintigraphy and CT angiography,[190] some reports have showed an increased diagnostic accuracy of SPECT and SPECT/CT. There remains some uncertainties about the interpretation of SPECT and SPECT/CT with the PIOPED and similar criteria, the best SPECT protocol for ventilation imaging, and the optimal imaging protocol (ventilation/perfusion SPECT, SPECT/CT with or without contrast enhancement, etc.).[213,216] However, findings of a segmental ventilation/perfusion mismatch should be regarded as positive. Findings of a single subsegmental mismatch should not be regarded as suspects for PE.[166]

Additionally, it is worth noting that, regardless of the criteria adopted for interpretation, there is a consensus that patients with low probability of PE on lung scintigraphy have a favorable prognosis[191-193] and that a normal perfusion study virtually excludes PE.[194-196]

7.1.3. Diagnostic Accuracy

There is a marked difference in the diagnostic accuracy of lung scintigraphy between older studies, performed in the 1980s and 1990s, and more recent studies using updated machines and tomographic methods.[217] Older studies had a significantly lower accuracy, in addition to a rate of nondiagnostic studies nearing 65% in the original PIOPED study. The rate currently ranges from 1% to 4%, which is significantly lower than that of other imaging methods.[189,209,217]

Overall, the diagnostic accuracy of more recent studies, especially those of SPECT, is equal to or greater than those of CT angiography; in some studies, such as the PIOPED II, scintigraphy had a greater capacity to detect subsegmental PE. Therefore, there are no robust scientific data to support the superiority of CT angiography over scintigraphy.[218]

Lung scintigraphy performed as SPECT is the imaging test with the lowest rate of inconclusive results (<3%).[189,190] However, in practice, CT angiography remains the method of choice, mainly because of its quick procedural time and greater availability (24 hours) compared with nuclear medicine tests.[176]

The main differences in diagnostic accuracy between planar and SPECT studies are due to the greater detection of changes in subsegments and in medial segments close to the mediastinum; in the latter, SPECT can detect up to 53% more areas suggestive of PE than planar studies.[219] There was an important variability in sensitivity (Se) and specificity (Sp) values in the initial studies: Se = 67% for planar imaging and Se = 93% for SPECT in an animal model (pigs);[220] Se = 80% and Sp = 78% for planar imaging; Se = 80% and Sp = 96% for SPECT;[221] and Se = 76% and Sp = 85% for planar imaging vs. Se = 97% and Sp = 91% for SPECT.[222] Therefore, diagnostic accuracy in the initial studies generally rose from 70%-80% with planar imaging to more than 90% with SPECT.

More recent studies of over 5,000 SPECT tests have shown a negative predictive value = 97%-99%, Se = 96%-99%, and Sp = 96%-98%, which provides wide support for its use instead of planar imaging.[222-229] Combining SPECT with low-dose CT improves the method even further by increasing its specificity and adding the capacity of low-dose CT. This is useful in the differential diagnosis for identifying emphysema, pneumonia, and/or other parenchymal changes that cause vascular compression and may therefore explain perfusion defects.[215,230] (Figure 28)

More recent articles have compared SPECT with CT angiography and provided new evidence, but there remains some controversy about which method performs better.[231] Apparently, SPECT and SPECT/CT provide a more accurate diagnosis of PE in cases of pulmonary diseases with comorbidities that may hamper the diagnosis by CT angiography.[223,232-234] In a systematic review with meta-analysis, SPECT performed better than CT angiography,[231] and direct comparisons showed that SPECT is superior to CT angiography in a ROC curve analysis.[235] Importantly, SPECT has greater sensitivity in patients with chronic PE, thus being considered the reference test.[236]

In conclusion, both tests have advantages and disadvantages, with scintigraphy being superior to CT angiography in cases of concomitant diseases, chronic PE, and indeterminate CT angiography results.[223,231-234] Thus, when both modalities are available, lung scintigraphy performed as SPECT (or SPECT/CT, preferably) should be recommended, as it has no contraindications, a lower rate of indeterminate test results, and reduced exposure of the female breast to radiation (see below).[237-241]

Comparisons of perfusion-only scintigraphy with CT pulmonary angiography as the gold standard demonstrated a sensitivity of 86% and a specificity of 93% for perfusion imaging. When the PISAPED criteria were applied to the PIOPED II study population, perfusion-only scintigraphy had a weighted sensitivity of 82% and a weighted specificity of 96%.[213] Perfusion-only studies are recommended in patients suspected of massive PE and pregnant patients, as discussed below. Using low-dose CT instead of a ventilation study increases sensitivity compared with perfusion-only studies; however, this strategy leads to a higher rate of false-positives[215,23,242] and increased radiation exposure in pregnant women.

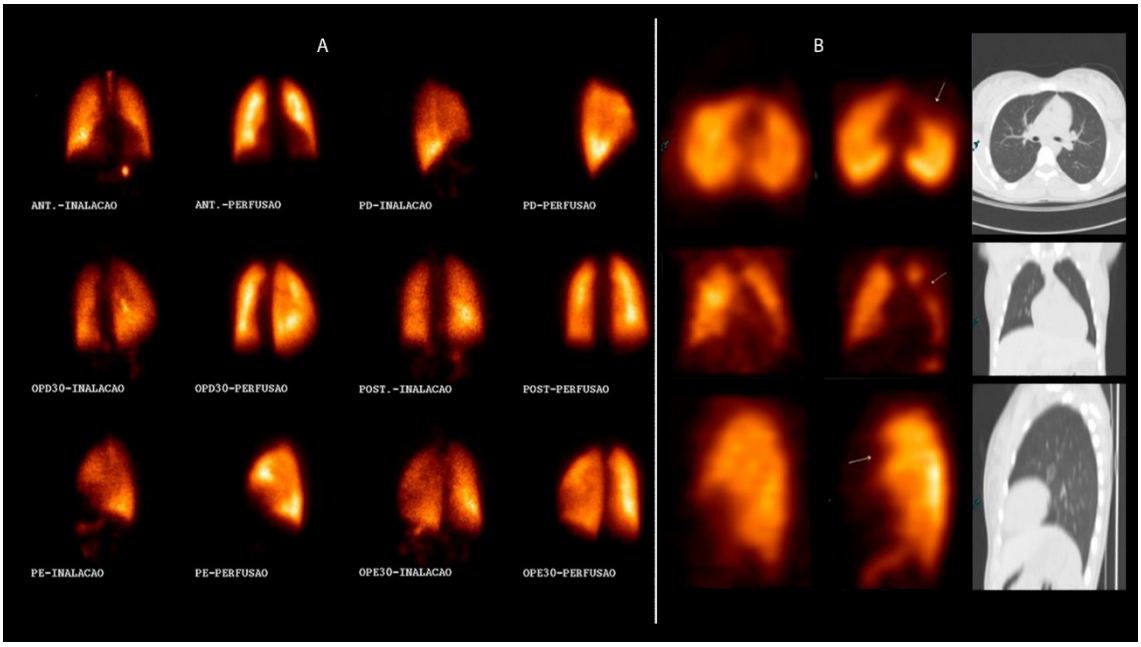


Figure 28 – *A: series of images of planar V/Q scintigraphy with 99mTc-labeled DTPA/MAA, with the anterior, posterior, lateral, and oblique views showing normal ventilation and perfusion. B: series of images of V/Q SPECT combined with low-dose CT with 99mTc-labeled DTPA/MAA, from left to right: first column, ventilation SPECT; second column, perfusion SPECT; and third column, chest CT. The ventilation image is normal, while the perfusion image shows a perfusion defect in the lower segment of the left upper lobe (arrow) and normal CT, which characterizes PE.*

7.1.4. Indications for Lung Scintigraphy Due to Limitations of CT Pulmonary Angiography

In addition to the previously discussed items, lung scintigraphy has been the preferred choice over other imaging methods in pregnant women because of reduced breast exposure to radiation and in some cases of limitation of contrast use on CT angiography. Such indications will be detailed below.

a) Exposure to radiation and use during pregnancy:

Since ventilation studies and chest radiographs are commonly normal in young individuals, a usual recommendation is skipping this portion of the study in those known to be at risk for the effects of radiation, such as women in the first trimester of pregnancy, because of the likely greater vulnerability of the fetus to the effects of radiation.[189,190]

In pregnant women, a protocol including a perfusion-only study and reduced radioactivity is initially recommended (see section on imaging techniques). A normal perfusion study excludes PE, and the ventilation study should only be performed in exceptional cases or cases of uncertainty about a positive result the day after the perfusion scan.[243] This ensures reduced radiation exposure compared with CT angiography and no exposure to iodinated contrast material.[189]

The critical maternal organ to be protected in this case is the breast, which absorbs radiation doses between 8.6 to 44 mSv on CT angiography[237,238,244] and less than 1 mSv on perfusion-only scintigraphy when not combined with tomography or ventilation studies.[237] Fetal doses seem to be low and similar in both methods.

b) Other clinical limitations of CT angiography:

Because of the intravenous use of iodinated contrast material, CT angiography might not be performed in a large number of critically ill patients, such as those with renal insufficiency, those who have recently had a myocardial infarction, those requiring ventilatory support, and those with a history of iodinated contrast allergy/anaphylaxis. In such cases (in addition to those mentioned in item A and in the section on diagnostic accuracy) and in rare cases of inability to perform the test, lung scintigraphy should always be indicated, as it has no contraindications, no related complications, and a low rate of inconclusive test results.[189,190,223]

7.1.5. Protocol

1. Ventilation study

a. Radiopharmaceuticals:

i. Inert gases:

1. ^{133}Xe:

a. This is historically the agent used in ventilation studies.[187,245,246]

b. It has a half-life of 5 days and a (low) energy of 81 keV.[187,209]

c. In the PIOPED I study, the single-breath technique was the most used.[187,209]

d. ^{133}Xe is inhaled during the first 20 seconds, and one image should be obtained from the posterior view.[187,247]

e. This gas is not available in Brazil.

2. ^{81m}Kr:

a. This is produced from a high-cost generator of rubidium (^{81}Ru).[187,248]

b. It has the ideal gamma energy (193 keV) and a half-life of 13 seconds.[187,249] When a patient is breathing air with ^{81m}Kr at a normal respiratory rate, the regional alveolar concentration of this gas is at steady state, proportional to regional ventilation.[187] During steady-state ^{81m}Kr inhalation, multiple planar imaging or SPECT imaging are feasible. Very recently, the combination of SPECT with low-dose CT has been described.[187,215]

c. ^{81m}Kr is a true gas that does not cause artifacts due to central airway deposition. An advantage is that ventilation and perfusion can be imaged simultaneously, as the gamma energy of ^{81m}Kr is greater than that of ^{99m}Tc (used as a perfusion marker).[187]

d. Low radiation exposure makes ^{81m}Kr the best choice for children.

e. This gas is not available in Brazil.

ii. Radiolabeled aerosols:

1. Given the unavailability of ^{133}Xe and ^{81m}Kr in Brazil for ventilation studies, radioaerosols are generally used.

2. An aerosol is a relatively time-stable, two-phase system consisting of particles suspended in air. The radiolabeled particles may be liquid, solid, or a combination of the two. The percentage of particles remaining in the lung after inhalation (deposition fraction) depends on the aerodynamic properties of the particles, particularly on their size. The deposition fraction is up to 50%, with ultrafine nanoparticles (diameter, 0.02), which are deposited predominantly in the alveolar region by diffusion.[187,250]

3. The most commonly used radiolabeled aerosol is ^{99m}Tc-DTPA, which is cleared from the alveolar region by transepithelial diffusion. It has a biological half-life of 80 minutes in healthy nonsmokers, 45 minutes in healthy passive smokers, and 24 minutes in healthy active smokers.[251]

4. Another radiolabeled aerosol available is Technegas, which consists of extremely small ^{99m}Tc-labeled solid graphite particles generated at high temperatures.[252,253] These particles have a diameter of about 0.005 to 0.2 micrometers,[254] are hydrophobic, and tend to aggregate, which is why they should be used within 10 minutes of being generated. They are suspended in argon gas (therefore having the property of gas). Studies using ^{99m}Tc-Technegas and ^{81m}Kr have provided comparable results.[255-259] However, the availability of Technegas equipment in Brazil is low, especially because of its high cost.

b. Imaging protocols:

i. As PE is a life-threatening condition, the recommendation is that the diagnosis be made as soon as possible, usually with 1-day ventilation/perfusion protocols. Lung ventilation studies, which will be used for comparison with the corresponding perfusion study, should be performed before perfusion imaging, requiring very low radioactivity. Sequential perfusion imaging with low-to-moderate radioactivity produces high-quality comparative images, with the diagnosis being made in a short time.

ii. Whenever possible, tomographic imaging should be performed as follows:

1. Ventilation with the appropriate radiolabeled aerosol, patient in the supine position, using a 1 to 4 dose ratio between ventilation and perfusion, respectively (usually 25 to 30 MBq for ventilation studies).

2. A large field-of-view, dual- or triple-head gamma camera and a general-purpose collimator should be used. At least 128 images will be obtained in a 64 x 64 matrix and 10 seconds per image.

3. Perfusion images should be obtained immediately after ventilation images. To avoid patient movement, peripheral venous access for perfusion imaging should be placed before the start of the ventilation study.

2. Perfusion study

a. Radiopharmaceutical:

i. ^{99m}Tc-labeled macroaggregated albumin (^{99m}Tc-MAA) is used in perfusion studies.

b. Imaging protocols:

i. Patient should be in the supine position, and venous access should have been previously obtained (prior to ventilation imaging, so that the patient does not move);

ii. Venous access is used to administer activity between 100 and 120 MBq ^{99m}Tc-MAA (number of MAA particles is approximately 200,000 to 700,000 in specific clinical situations, such as pulmonary hypertension and right-to-left shunting. The number of particles should be reduced to 100,000 to 150,000).

iii. In the same gamma camera used for ventilation imaging, without any patient movement, perfusion images should be obtained as follows:

iv. 128 tomographic images (SPECT) in a 64 x 64 matrix and 5 seconds per image.[260]

3. Perfusion-only study: in pregnant patients and those suspected of massive PE, a perfusion-only study is indicated.

7.1.6. Image Reconstruction

Images should be iteratively reconstructed with ordered-subsets expectation maximization (OSEM), according to a suggested protocol with 8 subsets and 2 iterations.[219,260,261]

7.1.7. Interpretation

a) In V/Q SPECT and planar V/Q scintigraphy, images should be interpreted based on:

i. Basic criteria for evaluating imaging studies;

ii. Knowledge and experience of the interpreting physician (clinical Gestalt);

iii. Pretest probability according to holistic interpretation principles.

b) For them to be clinically relevant, lung scintigrams should be described as positive or negative for the presence of PE (PE: YES or NO), not based on probability categories.[84]

c) Basic criteria for interpreting V/Q scintigrams:

i. Reports of absence of PE are based on:

i.1. Normal lung perfusion;

i.2. Matched or reversed-mismatched V/Q defects (presence of ventilation change, no perfusion superposition) of any size, shape, or number, in the absence of true mismatch;

i.3. Mismatch with no lobar, segmental, or subsegmental pattern.

ii. Reports of presence of PE are based on:

ii.1. V/P mismatch of at least 1 segment or 1 subsegment conforming to the lung perfusion anatomy.

iii. A study is nondiagnostic when:

iii.1. Multiple V/P abnormalities not typical of specific diseases are observed.

7.1.8. Chronic Pulmonary Embolism

Chronic PE is a medical condition different from acute PE. It is an insidious, progressive disease that has no treatment and a poor prognosis.[262,263] Mortality results from pulmonary hypertension, right heart failure, and arrhythmias. V/Q scintigraphy is generally used as an auxiliary tool in the diagnosis of chronic thromboembolic pulmonary hypertension,[208,264] with greater sensitivity and specificity than multidetector CT.[265]

7.1.9. Additional Diagnostic Findings

Ventilation and perfusion scintigraphy with tomographic imaging (V/Q SPECT) has been able to demonstrate the presence of clinical situations other than PE, such as COPD, left heart failure, and pneumonia. In 39% of patients who undergo the diagnostic procedure and do not have PE, and in 22% of those with PE, some of the following additional findings will be present.[223]

a) COPD: characterized by matched ventilation/perfusion defects. Ventilation defects are frequently more pronounced than perfusion defects, a phenomenon known as reverse mismatch.[266,267] A significant correlation between the degree of ventilation abnormalities detected on scintigraphic studies and the results of pulmonary function tests has been described.[268] PE is frequent in patients with COPD,[269] accounting for about 10% of deaths in those with stable COPD.[270]

b) Left heart failure: perfusion is redistributed to the upper lung regions.[271,272] Ventilation redistribution is less extensive than perfusion redistribution. A mismatch can be observed in the dorsal regions, with a diffuse nonsegmental pattern, and should not be interpreted as PE.

c) Pneumonia: matched ventilation/perfusion defects are observed, and the former are usually larger, causing a reverse mismatch.[273,274] A finding suggestive of pneumonia is the stripe sign, which refers to the maintenance of perfusion in the pleural surface adjacent to a central area of matched defect.[275] The stripe sign pattern is best detected on tomographic studies.

7.1.10. Pitfalls in the Interpretation of V/Q Studies

As is the case with any diagnostic method, V/Q scintigraphy requires the nuclear medicine physician reporting the study to be aware of possible errors inherent to the test, such as the following:

a) Technical artifacts may arise from preinjection handling of ^{99m}Tc-MAA. Drawing blood from a patient into a syringe containing the ^{99m}Tc-MAA solution may cause aggregation of particles, creating the so-called hot spots. A similar defect may arise from failure to resuspend ^{99m}Tc-MAA particles prior to intravenous administration.

b) Planar imaging may underestimate the presence and/or extent of perfusion abnormalities due to superposition of normally perfused areas. This artifact is eliminated by scintigraphic imaging with tomographic views (SPECT).

c) Whenever possible, the use of Technegas is preferred over liquid aerosols in patients with COPD. In rare patients with emphysema, Technegas particles are trapped in bullae, leading to a pattern that may be mistaken for a mismatch.[222]

d) Mismatched perfusion defects with no clear segmental pattern may be seen in older patients in cases of partially resolved PE, but not in cases of acute PE. Also, mismatched perfusion defects are observed in other lung conditions, such as lung cancer, mediastinal lymphadenopathy, actinic sequelae, pneumonitis, fibrosis, and heart failure.

e) Ventilation and perfusion scintigraphy may fail to detect thromboembolism that causes only partial vascular obstruction with few hemodynamic effects. This problem has low clinical significance. A possible explanation is that nonocclusive emboli are usually associated with other signs of PE in other regions, leading to a correct diagnosis. Nonetheless, when the presence of partial occlusion is identified (segmental perfusion defect found in the presence of normal ventilation), the study should be reported as positive for PE.

f) Overall, the complete absence of unilateral perfusion in the presence of normal ipsilateral ventilation and without any other mismatch in the contralateral lung is not due to PE.[276,277] In such cases, chest CT scans usually reveal the presence of other conditions, such as tumors or other mediastinal processes, congenital pulmonary vascular abnormalities, or aortic aneurysm.

7.1.11. The future of Nuclear Medicine Evaluation of PE

In the 1970s, ^{68}Ga, one of the first isotopes used in lung perfusion imaging, was introduced as the future of nuclear medicine pulmonary evaluation. As it is a positron-emitting isotope, ^{68}Ga requires a PET system and is used as ^{68}Ga-labeled microspheres to assess perfusion. Ventilation, in turn, is assessed with a similar technique to that of Technegas, using ^{68}Ga-labeled particles called Galligas[278] (Figure 29).

The advantages of transitioning from V/Q SPECT to PET/CT include superior imaging characteristics, such as higher sensitivity of the ring detector (covering 360 degrees simultaneously), better count statistics, higher spatial and temporal resolution, and reduced acquisition time (approximately 10 min, decreasing to 5 min with new

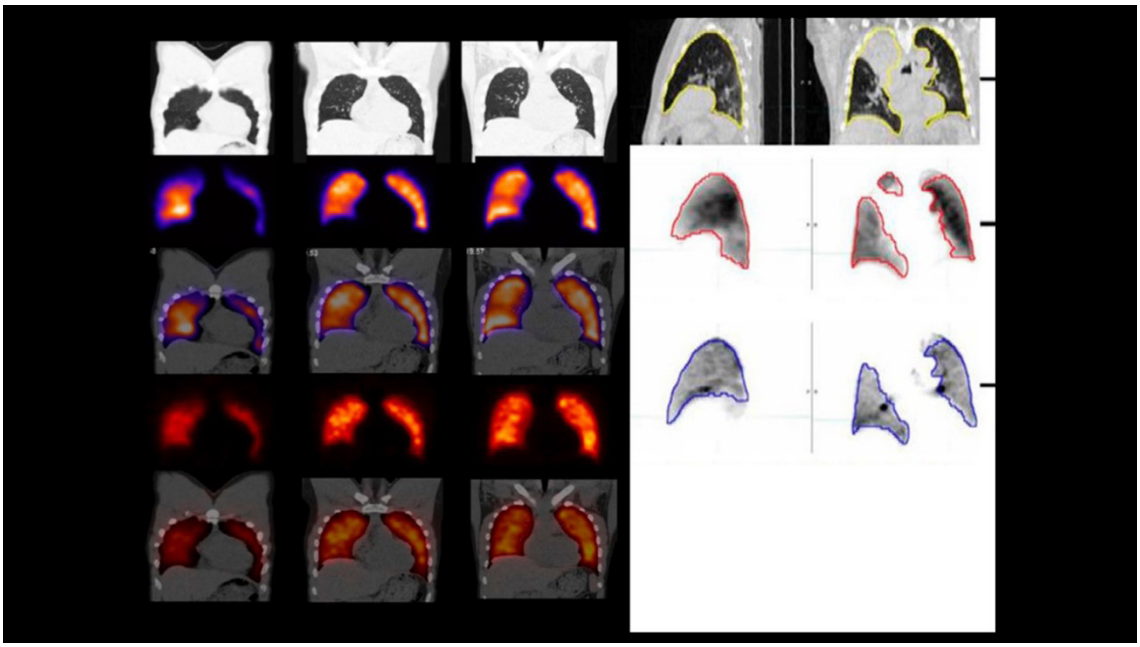


Figure 29 – *On the left, a series of images showing a normal V/Q SPECT/CT scintigram with ^{99m}Tc-labeled Technegas/MAA; from top to bottom: low-dose CT; ventilation SPECT; fused ventilation/CT; perfusion SPECT; and fused perfusion/CT. On the right, a series of images showing a normal V/Q PET/CT scan with ^{68}Ga-labeled Galligas/MAA; from top to bottom: sagittal views of fused ventilation/CT (left) and perfusion/CT (right). Adapted from Le Roux PY et al.[279] and Bailey DL et al.[280]*

digital PET/CT scans). Other advantages are the commercial availability of respiratory-gated acquisition systems that reduce artifacts, especially at lung bases; the possibility of multiple ventilation studies with or without bronchodilator on the same day owing to the short half-life of ^{68}Ga; and important characteristics such as being a simple and noninvasive test without contraindications or side effects related to the use of contrast material (eg, allergy and renal dysfunction). At the technical level, the transition does not imply major difficulties or investments because the radiopharmacy requirements remain relatively simple with the use of existing Technegas equipment. The only difference is the modified MAA synthesis and its consequent labeling with ^{68}Ga (whose generators are increasingly available, enabling widespread adoption) instead of ^{99m}Tc, with a radiation dose very similar to that of conventional V/Q SPECT/CT scans[108] (Figure 30).

In 2018, Le Roux et al.[281] proposed a protocol for systematic review and meta-analysis of diagnostic accuracy and clinical outcome of patients evaluated with V/Q SPECT, whose results are still to be published and will certainly provide more evidence for the adoption of this technique.

7.1.12. Clinical Algorithm for the Investigation of Patients with Suspected PE

Assessing the clinical probability of PE helps physicians choose the most appropriate objective test to diagnose or exclude the diagnosis (Chart 6). Measurement of D-dimer (a degradation product of cross-linked fibrin clot) is widely used in the investigation of patients with suspected venous thromboembolism (VTE). The quantitative D-dimer assay, based on a rapid ELISA test, has high sensitivity (close to 95%) for VTE.[22,23] However, the assay has low specificity (40%) because D-dimer levels may be increased in several conditions other than VTE, such as in acute myocardial infarction, stroke, inflammation, active cancer, and pregnancy. Specificity also decreases with age, and it can be as low as 10% in older adults.[120,214] Consequently, a negative quantitative D-dimer test has a high negative predictive value for VTE. The results of the studies reveal that the risk of development of PE in patients with low clinical probability who are not treated after a negative D-dimer test is <1% within three months of the initial evaluation.[282] Conversely, because of its low predictive value, a positive quantitative D-dimer test does not modify the pretest (clinical) probability and is therefore clinically useless. Recent evidence, however, suggests that very high D-dimer levels are associated with a 4-fold increase in the probability of PE,[30] which is important for assessing the burden of thromboembolic disease and may have prognostic significance.[27,29]

Diagnostic strategies will depend on the patient's hemodynamic stability, as follows:

a) Stable patients:

Based on these considerations, when the clinical probability of PE is low and the quantitative D-dimer test is negative, the diagnosis is unlikely and additional investigations are not needed (Chart 5). When the clinical probability of PE is

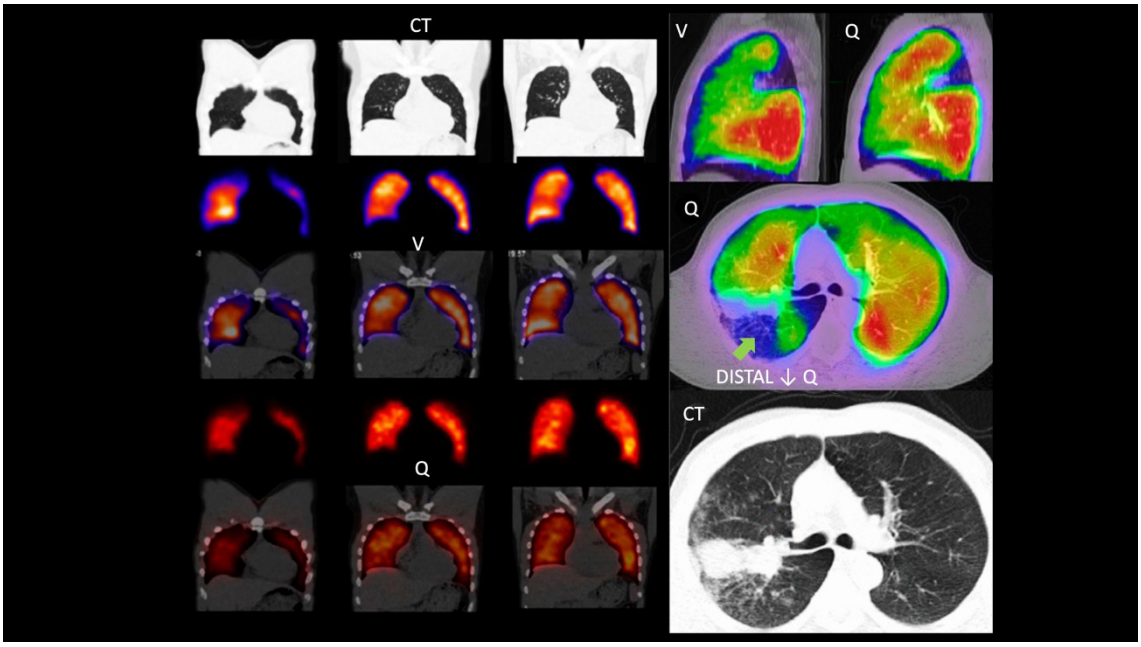


Figure 30 – *On the left, a series of images showing a normal V/Q SPECT/CT scintigram with ^{99m}Tc-labeled Technegas/MAA; from top to bottom: low-dose CT; ventilation SPECT; fused ventilation/CT; perfusion SPECT; and fused perfusion/CT. On the right, a series of images showing a V/Q PET/CT scan with ^{68}Ga-labeled Galligas/MAA; from top to bottom: sagittal views of fused ventilation/CT (left) and perfusion/CT (right); matched defect, fused PET/CT perfusion image showing a perfusion defect (arrow); and a CT image showing a tumor obstructing the bronchus, corresponding to the ventilation/perfusion defect on PET imaging. Adapted from Le Roux PY et al.[279] and Bailey DL et al.[280]*

low and the quantitative D-dimer test is positive, additional investigations may be needed for a range of diagnoses, including PE, especially when D-dimer levels are markedly high. When the clinical probability is other than low, it seems more reasonable to skip the D-dimer test and refer the patient directly to the appropriate imaging technique (Chart 5). It can be V/Q SPECT or CT angiography, depending on local availability, medical knowledge, and the patient's clinical status. V/Q SPECT has virtually no contraindications and produces a substantially lower radiation burden than CT angiography.[22]

b) Hemodynamically unstable patients:

When the patient presents with severe hypotension or cardiogenic shock (Chart 6), transthoracic echocardiography remains the first-line imaging technique, as it detects right heart dilatation and hypokinesia. Embolism is rarely seen within the right heart chambers or the main pulmonary artery. Lung perfusion scintigraphy is an alternative to CT angiography, as it quickly shows several segmental or lobar perfusion defects typical of acute PE.[120] When acute thoracic aortic dissection is suspected due to chest pain, CT angiography allows this differential diagnosis to be evaluated.

Given the need for expediting the diagnosis and treatment of these patients, the strategy employed in a specific health facility should be adapted to each clinical situation and local context. When the initial examination suggests a massive PE, other actions should be adapted to the clinical situation, and antithrombotic therapy may be administered.

7.1.13. Diagnostic Algorithm

When suspected, PE should be confirmed or refuted to avoid the risk of over- and undertreatment, which requires imaging tests. Only the optimal techniques, ie, CT angiography and V/Q SPECT, are recommended. The choice of imaging modality will depend on availability.[120] Although CT angiography is more readily available, it is contraindicated in a substantial number of patients, as shown in the PIOPED II study.[217] Currently, V/Q SPECT is rarely available 24 hours a day, 7 days a week. Consequently, the two tests should be ready for use at least in tertiary care centers, as both are crucial for appropriate diagnostic algorithms of PE. In each center, the diagnostic algorithm used for PE should be based on local conditions and particularly on the availability of V/Q SPECT and CT angiography.

V/Q SPECT, when available, has considerable advantages over other imaging techniques for the diagnosis of PE, such as high sensitivity and specificity, lower predictable radiation burden, and suitability to the follow-up of patients with PE and its natural history.

Diagnostic flowcharts for hemodynamically stable and unstable patients, according to the clinical probability of PE, are shown in Charts 5 and 6. Chart 13 shows the advantages and disadvantages of lung scintigraphy for the diagnosis of PE.

Chart 13 – Advantages and disadvantages of lung scintigraphy for the diagnosis of PE

LUNG SCINTIGRAPHY	
ADVANTAGES	**DISADVANTAGES**
Low radiation exposure	Limited availability
Can be used in pregnant women	Does not detect the occluded artery with absolute certainty
Radiopharmaceutical does not impair renal function	
High accuracy	
Procedure covered by SUS and by the ANS health procedure list	
Allows the physician to assess chronic PE	
Allows the physician to assess resolution of acute PE	

7.1.14. Conclusions

a) V/Q scintigraphy is strongly recommended for the diagnosis of PE, with high accuracy even in the presence of COPD and pneumonia;

b) Technegas is superior to DTPA in patients with COPD;

c) When available, ^{81m}Kr is advantageous;

d) The radiation dose should be reduced as much as possible, not affecting image quality (ALARA principle). Overall, 30 MBq ^{99m}Tc-labeled aerosol should be used for ventilation and 100-120 MBq ^{99m}Tc-labeled MAA for perfusion;

e) In pregnant patients, the perfusion-only study is recommended;

f) The interpretation should be holistic; the probabilistic interpretation is obsolete;

g) A key criterion for the diagnosis of PE is the presence of mismatch in more than 1 subsegment.

Erratum

In the "Joint Guideline on Venous Thromboembolism - 2022", with doi number: https://doi.org/10.36660/abc.20220213, published in the journal Arquivos Brasileiros de Cardiologia, 118(4): 797-857, the following corrections were made:

Included the institution Hospital DF Star, Rede D'Or, Brasília, DF Brazil, for the author Simone Nascimento dos Santos.

At Angiolab Vitória, Laboratório Vascular, corrected the location "Rio de Janeiro, RJ" for "Vitória, ES".

On page 804, Chart 2, an arrow from "Positive" to "VUS" was inserted.

Only in the Portuguese version, page 806, Chart 5, right column, the position of "Positivo" and "Negativo" was changed.

Only in the Portuguese version, page 822, Chart 9, line 7, corrected the spelling of " Compressibilidade".

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