### Acuracy and Concordance of Anthropometric Indicators and Body Composition in Heart Failure

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### Abstract

**Background:** Anabolic / catabolic disorder in heart failure (HF) favors cardiac cachexia, implying a reduction in HF survival.

**Objectives:** To assess the accuracy and concordance of the diagnosis of protein malnutrition and excess fat among the anthropometric and body composition methods in individuals with HF.

**Method:** A study of accuracy that included 60 individuals with HF. Body mass index (BMI), arm circumference (AC), triceps skinfold thickness (TST), adductor pollicis muscle thickness (APMT), arm muscle circumference (AMC) and corrected arm muscle area (cAMA). Fat free mass index (FFMI) and body fat percentage (BF%), obtained by electrical bioimpedance (EBI), were used to compare the diagnosis of protein malnutrition and excess fat. Accuracy was assessed by calculating sensitivity, specificity, positive and negative predictive value. The concordance of the EBI diagnosis and other methods was performed by the chi-square test and kappa (k) statistic, where p<0.05 was considered significant.

**Results:** Higher frequencies of protein malnutrition were identified by cAMA and AMC, and excess fat by BF%. BMI presented low sensitivity (43%) and accuracy (38.5%), with moderate concordance (0.50). AMC sensitivity was 86%, accuracy 66.4%, and acceptable concordance (0.36) compared to FFMI. Similar percentages of moderate sensitivity and low accuracy were observed for TST and BMI.

**Conclusion:** AMC may be useful to identify protein malnutrition and TST has not been adequate to diagnose adiposity. BMI was not sensitive to assess muscle and adipose reserve. EBI was more accurate. (Int J Cardiovasc Sci. 2019;32(2)143-151)

Keywords: Heart Failure; Nutrition Assessment, Anthropometry; Body Composition; Cachexia.

### Introduction

Heart failure (HF) is a clinical syndrome characterized by the heart's inability to supply the body's metabolic demands or to do so at the expense of high filling pressures as a result of structural or functional abnormalities of the heart.<sup>1</sup> It is the common final pathway of a number of cardiac diseases that determine activation of the neurohormonal and inflammatory axis and energetic metabolism, resulting in cardiac cachexia, worsening the prognosis and greater morbidity and mortality of individuals with HF.<sup>2</sup> On the other hand, overweight protects individuals with HF, which is called the "obesity paradox".<sup>3</sup>

In this context, methods of anthropometric assessment and body composition are proposed, but without consensus.<sup>4</sup> Although the body mass index (BMI) does not clearly reflect body composition, low values for this index have been associated with worse prognosis in individuals with HF, whereas increased values act as a protective factor.<sup>56</sup> The evaluation of triceps skinfold thickness (TST) is a way of identifying adiposity in these

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individuals and is related to the prognosis of the disease.<sup>7</sup> Other parameters of indirect musculature assessment, such as the arm muscle circumference (AMC) and corrected arm muscle area (cAMA) are widely accepted, but there are few studies that consolidate its use in HF.<sup>8</sup> Another parameter used as an indicator of musculature reserve is the adductor pollicis muscle thickness (APMT).<sup>9</sup>

More accurate methods for assessing body composition, such as computed tomography and magnetic resonance imaging, are costly and difficult to perform.<sup>10</sup> As an alternative, electrical bioimpedance (EBI) has been suggested and can be used to evaluate the prognosis and follow-up of individuals with HF.<sup>11</sup> However, the higher cost of acquiring the equipment makes anthropometry the most feasible method for outpatient evaluation and follow-up, despite its still questionable sensitivity and accuracy.<sup>12</sup>

Given the controversies between the methods of anthropometric evaluation and body composition in individuals with HF, and the scarcity of specific indicators in the literature, the objective of this study was to evaluate the accuracy and concordance of the diagnosis of protein malnutrition and excess fat among anthropometric and body composition methods.

#### **Methods**

### Selection of participants and study design

The accuracy study was developed at *Hospital Universitário Onofre Lopes* (HUOL) – UFRN/Natal, in *Ambulatório Interprofissional de Insuficiência Cardíaca* (AMIIC). This study was approved by the Ethics and Research Committee of Onofre Lopes University Hospital / UFRN (N°: CAAE 59827516.2.0.0.0.0.5292). All individuals signed an Informed Consent Form.

Inclusion criteria were adult and elderly individuals of both sexes, with a diagnosis of HF, according to the Boston point system and the Framingham criteria, confirmed by the Doppler echocardiogram. The study did not include adolescents, pregnant women, patients with cognitive deficit, with renal dysfunction and under dialysis, and consumptive diseases such as cancer. Individuals with pacemaker, metal valve, orthopedic prostheses/metal implants on the left or right side, as well as those with abnormal limb or trunk limitations, amputation and/or dystrophies were not included.<sup>13</sup>

We considered a convenience sample composed of individuals with HF followed up on an outpatient basis. Of 112 individuals enrolled from January to November 2017, 60 individuals were included in the study. The most frequent causes of sample losses included refusals to participate and the presence of exclusion criteria.

The participants came for a routine outpatient visit and were examined by the medical staff for their cardiac condition, including clinical history and clinical examination, to determine functional capacity and classify HF based on disease progression, according to the American Heart Association criteria, investigation of the etiology and type of HF and left ventricular ejection fraction. The clinical data were collected by going through the participants' online records. For anthropometric and body composition assessment, the participants were instructed to do a food and liquid fasting and to exercise for at least 8 hours before the evaluation.<sup>13</sup>

#### Anthropometric evaluation and body composition

Anthropometric and body composition assessment was performed by a single well trained anthropometrist with calibrated equipment. The intraevaluator technical error of measurements (TEM).<sup>14</sup> The score assigned according to the TEM calculations was of TST (1.03), for the APMT (0.77) and perimeter of the arm circumference (AC) (0.31), characterizing the evaluator as capable of taking the measurements. The evaluation was performed twice and a third measurement was taken in case of discrepancy to obtain the mean.

To measure body mass, a digital scale with capacity of 150 kg and 0.1 kg precision (Balmak Premium<sup>®</sup>) was used. Stature was measured using a stadiometer. BMI was calculated and classified according to cut-off points proposed by the Food and Nutrition Surveillance System.<sup>15</sup>

AC and TST measurements were taken as defined in standardized protocols. TST was evaluated using a Lange Skinfold Caliper<sup>®</sup> and classified according to Frisancho.<sup>16,17</sup> AC and TST measurements were used to calculate the anthropometric indicators of muscle reserve, arm muscle circumference (AMC) and corrected arm muscle area (cAMA), according to Frisancho.<sup>16</sup> The APMT measurement was assessed according to previously published guidelines, considering the classification proposed by Lameu.<sup>9</sup>

Body composition was assessed using a tetrapolar EBI (Biodynamics 450<sup>®</sup>) to analyze the body compartments of fat free mass, fat mass and water. The measurement was taken with the individual lying down with the limbs apart and the emitter electrodes placed on the surface of the hand and

right foot at the midpoint between the distal prominences of the radius and ulna of the right wrist and between the medial and lateral malleolus of the right ankle.<sup>13</sup>

For the diagnosis of protein malnutrition, the fat free mass index (FFMI) was used, corresponding to the ratio between the amount in kilograms of fat free mass (FFM) obtained by electrical bioimpedance and the squared height (FFM/height<sup>2</sup>), considering the following cutoff points:  $15 \text{ kg/m}^2$  for women and  $17 \text{ kg/m}^2$  for men.<sup>18</sup> Excess fat was classified according to sex, age and fat percentage evaluated by the EBI, using the following reference values: for women aged 20-39 years ( $\geq$  33%); 40-59 years ( $\geq$  34%); 60-79 years ( $\geq$  36%), for men aged 20-39 years ( $\geq$  25%).<sup>1920</sup>

### Statistical analyses

In the descriptive analysis of the continuous variables, mean and standard deviation were used. Absolute and percentage frequencies were calculated for the binary and categorical variables. To demonstrate the frequencies of anthropometric variables, absolute frequencies, percentages and their respective 95% confidence intervals were considered.

Accuracy assessment was performed by calculating the sensitivity, specificity, positive predictive value, negative predictive value and the proportion of correct anthropometric measurements compared to FFMI for the diagnosis of malnutrition (muscular depletion) and the EBI %FM for the diagnosis of overweight. The formula A=(a+d)/(a+b+c+d) was used. Diagnoses of protein malnutrition and overweight with accuracy and sensitivity above 60% were considered acceptable.<sup>20</sup>

For the concordance analysis, the observed concordance and the expected concordance for the Kappa statistic calculation were calculated at first. The Kappa index values were determined by the concordance classification according to Landis and Koch,<sup>21</sup> which establishes the following classifications: poor (Kappa equal to 0), light (Kappa between 0-0,20), acceptable (Kappa between 0.21-0.40), moderate (Kappa 0.41 to 0.60), substantial (Kappa 0.61 to 0.80) and excellent (Kappa 0.81 to 1.0). Statistical analyses were performed using the software IBM<sup>®</sup> SPSS<sup>®</sup> 13.0 and Epi InfoTM 7.2.2.2.

### Results

The mean age of the individuals was 55.3 (13) years, predominantly males (70%), and diagnosis of HF with

non-ischemic etiology (55%). Significant percentages of individuals with hypertension (70%) were found. Most individuals were in functional class I (76.7%), despite the diagnosis of 60% of the individuals with reduced ejection fraction heart failure (HFrEF). The BMI revealed that the study population presented average values indicative of overweight and total body water (TBW) percentage within normality (Table 1).

It was found that protein malnutrition, when evaluated by cAMA (43.9%) and AMC (40%), was more frequent when compared to FFMI (13.2%), BMI (11.7%) and APMT (5.1). There was similarity in the diagnoses of excess fat indicators, where BF was the one that identified a higher frequency of excess fat (63.5) (Table 2).

Analyzing the indicators of accuracy of anthropometric measures related to protein malnutrition, it was found that BMI presented low sensitivity (43%) and accuracy (38.5%), but moderate concordance (0.50). On the other hand, AMC presented greater sensitivity (86%) and accuracy (66.4%) with acceptable concordance (0.36) compared to FFMI, being considered acceptable for the diagnosis of protein malnutrition. It was not possible to calculate the accuracy and concordance for the cAMA and APMT indicators because one of the categories presented a null value (Table 3).

Regarding overweight, the anthropometric measures related to BF% revealed similarity between the percentage of sensitivity of the TST (60%) and BMI (67%) indicators, as well as low accuracy (20.3% and 22.2%, respectively). Considerable concordance values were found for both indicators, compared to BF% (Table 4).

### Discussion

This pioneering study certifies the complexity of anthropometric assessment and body composition in individuals with HF. The results showed that there was a higher frequency of excess fat/weight than of protein malnutrition in our population diagnosed with HF. Among the methods of anthropometric evaluation reviewed, AMC was considered more sensitive for the diagnosis of protein malnutrition, despite the lower concordance with EBI. Indicators of excess fat/adiposity presented similar results and low sensitivity.

Comorbidities diagnosed in individuals with HF may coexist or even be the cause of the disease, having a negative impact on the quality of life, increasing the frequency of hospitalization and mortality.<sup>22</sup> In this

# Table 1 - Biodemographic, clinical, anthropometric and body composition characteristics of patients with HF

	n = 60		
Variables	n (%)		
Sex			
Male	42 (70)		
Female	18 (30)		
Comorbidities			
Arterial hypertension	43 (71.7)		
Diabetes mellitus	18 (30)		
Chronic kidney disease under conservative treatment	11 (18.3)		
Dyslipidemia			
Hypercholesterolemia	3 (5)		
Hypertriglyceridemia	18 (30)		
Mixed hypertriglyceridemia	2 (3.3)		
Low HDL-c	52 (86.7)		
Smoking			
Smoker	5 (8.3)		
Non-smoker	31 (51.7)		
Former smoker	24 (40)		
Medications			
ARA/ACEI	56 (93.3)		
Diuretics	49 (81.7)		
Beta-blocker	57 (95)		
Vasodilator	14 (23.3)		
Digoxin	14 (23.3)		
Antiplatelet agent	29 (48.3)		
Functional class			
Ι	46 (76.7)		
П	10 (16.7)		
III and IV	4 (6.6)		
LVEF			
HFrEF	36 (60)		
HFiEF	12 (20)		
HFpEF	12 (20)		

HF etiology	
Ischemic	27 (45)
Non-ischemic	33 (55)
Hypertensive	10 (30)
Chagas' disease	3 (9)
Alcoholic	4 (12)
Rheumatic	1 (3)
Peripartum	2 (6)
To be clarified	13 (39)
Anthropometric indicators	Md (SD)
Height (m)	1.63 (0.9)
Weight (kg)	71.1 (15)
BMI (kg/m²)	26.7 (4.7)
AMC (cm)	23.8 (3.6)
cAMA (cm <sup>2</sup> )	38.2 (13.4)
APMT (mm)	20.3 (5)
TST (mm)	20.4 (8.6)
Body composition	Md (SD)
FM (kg)	20.8 (7.2)
FFM (kg)	52 (11.9)
%TBW	52.6 (5.5)
TBW (L)	38.4 (9.7)
ICW (L)	20.5 (5.6)
ECW (L)	17.9 (3.5)

ARA/ACEI: Angiotensin receptor antagonist/angiotensinogen converting enzyme inhibitor; LVEF: Ventricular ejection fraction; HFrEF: Heart failure with reduced ejection fraction; HFiEF: Heart failure of intermediate ejection fraction; HFpEF: Heart failure with preserved ejection fraction; BMI: Body mass index; AMC: Arm muscle circumference; cAMA: Corrected arm muscle area; APMT: Adductor pollicis muscle thickness; TST: Triceps skinfold thickness; FM: Fat mass; FFM: Fat free mass; TBW: Total body water/Weight; ICW: Intracellular water; ECW: Extracellular water.

study, a higher frequency of arterial hypertension was observed, as found in other studies that also identified higher frequencies of this comorbidity, in addition to diabetes mellitus.<sup>23</sup>

One fact that caught our attention was that more than half of the participants had reduced ventricular ejection fraction (HFrEF), but from a clinical point of view, they

Table 2 - Frequency of muscle depletion and excess         adiposity/body fat in patients with HF						
Variables/categories	Ν	%	95%CI*			
Indicators of protein malnutritic	m					
FFMI						
Presence of malnutrition	7	13.2	5.5 - 26.3			
Eutrophy	46	86.5	74.7 - 94.5			
BMI						
Presence of leanness	7	11.7	4.8 - 22.6			
Eutrophy	53	88.3	77.4 - 95.2			
AMC						
Muscle deficit	24	40	27.6 - 53.5			
Eutrophy	36	60	45.5 - 72.4			
cAMA						
Reduced musculature	25	43.9	30.7 - 57.6			
Eutrophy	32	56.1	42.4 - 69.3			
APMT						
Reduced musculature	3	5.1	1.1 - 14.2			
Eutrophy	56	94.9	85.8 - 98.9			
Indicators of excess adiposity/b	ody fat					
BF%						
Excess body fat	33	63.5	48.9 - 74.4			
Eutrophy	19	36.5	23.6 - 51			
BMI						
Excess weight	31	51.7	38.4 - 64.8			
Eutrophy	29	48.3	35.2 - 61.6			
TST						
Excess adiposity	28	46.7	33,7 - 60			
Eutrophy	32	53.3	40 - 66.3			

\*CI: 95% confidence interval. FFMI: Fat free mass index; BMI: Body mass index; AMC: Arm muscle circumference; cAMA: Corrected arm muscle circumference; AMPT: Adductor pollicis muscle thickness; BF%: Body fat percentage; TST: Triceps skinfold thickness.

were classified as functional class I. This is opposed to the findings reported by Garlet. et al.,<sup>24</sup> whose data revealed that individuals with HF evaluated in functional class I presented higher LVEF than those in functional class II, III or IV. At this point, it can be said that the inconsistent

data can be due to the fact that the individuals with HF in our study are followed up, in an outpatient setting, by a well-structured interprofessional and multidisciplinary team, reinforcing that high-quality multidisciplinary care can benefit individuals with HF in the prevention or early detection of acute decompensation.<sup>25,26</sup>

It is important to know the state of clinical compensation of individuals with HF upon anthropometric and body composition evaluation, as it may have repercussions on inaccuracies due to the inability to follow the evaluation protocols in the presence of symptoms. In our study, it was found that the participants had body water volume within the acceptable ranges. It has been found that the total body water volume of individuals with compensated HF is reported to be equal to that of normal individuals.<sup>23</sup> Studies evaluating total, intercellular and extracellular body water volumes in individuals with HF, either followed up in an outpatient setting or not, but who were compensated at the time of the evaluation, found results similar to those of this study.<sup>27-29</sup>

Analyzing the diagnosis of indicators of protein malnutrition/muscle depletion, we found discrepancies between anthropometric and body composition methods. Although reduced sensitivity presents moderate concordance, it suggests that BMI assessment compared to other methods is not a good predictor of protein malnutrition. Authors reaffirm this finding, pointing out that BMI may not provide a good overview of the nutritional status of patients with HF, especially when used for the diagnosis of malnutrition, as this index does not distinguish between fat and lean body mass.<sup>30</sup>

However, the literature is controversial when it recommends the use of BMI as a marker of prognosis in individuals with HF. A retrospective study comparing the levels of N-terminal pro-brain natriuretic peptide (NT-proBNP), which is a strong risk factor for mortality, for the increase in the BMI of individuals with decompensated HF, suggests that overweight is not associated with mortality in this population, reporting that BMI is inversely proportional to the increase of NT-proBNP.<sup>31</sup> However, Cescau et al.,<sup>32</sup> found that although BMI is not an independent predictor of mortality, it is significantly related to greater reverse myocardial remodeling, promoting a protective effect of overweight in the course of HF. In a meta-analysis that assessed the association between BMI and all causes of death in individuals with HF, it was found that both BMI corresponding to malnutrition and morbid obesity are associated with higher mortality in this population.<sup>33</sup>

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Variables/categories	Protein malnutrition (FFMI kg/m²)		S	Е	PPV	NPV	Accuracy	Kappa
	Yes	No						
Low weight (BMI)								
Yes	3	1	4201	0.9//	750/	0201	20 50/	0 50
No	4	45	43%	98%	15%	92%	38.5%	0.50
Mass deficit lean (AMC)								
Yes	6	12	9601	7407	220/	0701	66 101	0.26
No	1	34	00/0	7470	3370	91/0	00.4/0	0.30
Low musculature (cAMA)								
Yes	5	15	00/					
No	0	31	070	-	-	-	-	-
Musculature reduced (APMT)								
Yes	0	1	0%	_	-	-	-	-
No	6	45	070	-				

# Table 3 - Accuracy and concordance between anthropometric methods and fat free mass index in the evaluation of protein malnutrition in patients with HF

S: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; BMI: body mass index; AMC: arm muscle circumference; cAMA: corrected arm muscle circumference; AMPT: adductor pollicis muscle thickness.

## Table 4 - Accuracy and concordance between anthropometric methods and BF% indicator in the evaluation of excess fat and adiposity in patients with HF

Variables/categories	Excess fat (BF%)		6	-			•	T.
	Yes	No	- Sp	E	PPV	NPV	Accuracy	карра
Excess adiposity (TST)								
Yes	20	6	60%	68%	77%	50%	20.3%	0.27
No	13	13						
Overweight (BMI)								
Yes	22	8	67%	58%	73%	50%	22.2%	0.24
No	11	11						

S: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; BF%: body fat percentage; TST: triceps skinfold thickness; BMI: body mass index.

On the other hand, for the evaluation of muscle depletion, AMC and cAMA identified higher percentages of individuals with muscle depletion, with AMC being the most sensitive one, with acceptable concordance with the FFMI parameter. In view of these findings, it is assumed that the AMC and cAMA anthropometric indicators may overestimate the cases of muscle depletion, which may be associated to the evaluation method, which presupposes equations that require the value of two anthropometric measures, as well as classification in tables of percentiles.

However, considering the importance of monitoring the skeletal muscles of individuals with HF, since

cachexia and sarcopenia are associated with worse prognosis and higher mortality,<sup>34</sup> it is preferable to use the AMC values even in a potential overestimation of malnutrition due to the impossibility of acquiring and maintaining more sensitive equipment such as EBI.

For the APMT, a lower frequency of muscle depletion was found, compared to FFMI, suggesting that this measure has a reduced diagnostic capacity to evaluate compensated individuals followed up in an outpatient setting as in this study. However, its use in an intensive care unit has proven to be a good method of assessing nutritional risk.<sup>35</sup>

One of the most striking aspects of our study was the high percentage of individuals who had excessive adiposity/fat. This fact may have a positive impact on the course of the disease, since there is an inverse association between overweight or obesity and mortality in individuals with HFrEF, as found in our population.<sup>36</sup> In our study, there was a uniformity of diagnoses considering the indicators used for this purpose. However, both BMI and TST showed low sensitivity, accuracy and concordance with the percentage of fat evaluated by EBI.

Caution is needed in this interpretation, especially when BMI is used for assessing body fat. A prospective study with patients with HF found results similar to ours for the diagnosis of BMI. The authors raise questions that individuals with protein malnutrition may possibly be classified as overweight by BMI, underestimating cases of malnutrition and increasing the risk of mortality.<sup>37</sup>

According to a study that investigated the association between body composition assessed by dual energy x-ray absorptiometry (DEXA), an imaging test that promotes the identification of body extracts (gold standard) and mortality of patients with HF, it was observed that the BMI misclassified 41% of patients for body fat,<sup>5</sup> similarly to the one found in our study, where we identified 50% of negative predictive value for BMI.

Although our results did not confirm good indicators of accuracy and concordance of TST in the evaluation of excess adiposity, other authors point out the use of TST as a predictor of mortality, further reinforcing the obesity paradox, since excess TST presented an inverse relation with mortality.<sup>7</sup> It is suggested to evaluate subcutaneous fat from the association between multiple skinfolds, used in formulas to evaluate adiposity, as well as was evaluated by Gastelurrutia et al.,<sup>37</sup> associated with other methods to identify the nutritional status of individuals with HF, aiming to identify adipose reserve more accurately when there is no evaluation of fat percentage by EBI.

Our study is not free of limitations: (1) the low frequency of anthropometric disorders may be biased in the statistical analysis; (2) a small number of participants may have caused lower values of sensitivity and concordance, but the results found were relevant to signal the need to consider several methods of assessing body composition in this population.

The results reveal important information about the use of anthropometric indicators in the evaluation of individuals with HF, reinforcing that, for a more accurate evaluation, EBI assessment may be better indicated. EBI measurements should be standardized to obtain reproducible results and the use of body composition prediction equations must meet the specifications of each population studied. It should be noted that when it is not feasible to use equations already defined in specific studies, it is possible to use data from the EBI provided by the equipment at the time of measurement, such as resistance (R) and reactance (Xc) data, resulting in the formation of data such as phase angle, which is used in the diagnosis of malnutrition and clinical prognosis. Additionally, another potential data for the diagnosis and prognosis of body mass reserves is the analysis of bioelectrical impedance vectors.38,39

### Conclusion

In conclusion, it is suggested that although AMC overestimates muscle depletion, it can be used as an indicator of protein malnutrition. In addition, BMI was not sensitive in the evaluation of muscle and body fat components, therefore caution is recommended in the use of this indicator in patients with compensated HF followed up in an outpatient setting. TST did not present good sensitivity for the evaluation of adiposity and body fat, reinforcing the importance of the evaluation considering multiple folds. The anthropometric parameters should be incorporated into the clinical practice for their low cost and practicality, but for greater accuracy in the evaluation of body composition, more accurate methods, such as EBI, should be considered.

### Author contributions

Conception and design of the research: Rocha DO, Diniz RVZ, Lira NRD, Sena-Evangelista KCM.

Acquisition of data:Rocha DO, Dantas RCS, Andrade FL, Avelino RRS, Diniz RVZ, Lira NRD. Analysis and interpretation of the data: Rocha DO, Dantas RCS, Andrade FL, Avelino RRS, Lyra CO, Diniz RVZ, Lira NRD, Sena-Evangelista KCM. Statistical analysis: Rocha DO, Lyra CO, Sena-Evangelista KCM. Obtaining financing: Sena-Evangelista KCM. Writing of the manuscript: Rocha DO, Dantas RCS, Andrade FL, Avelino RRS, Lyra CO, Diniz RVZ, Lira NRD, Sena-Evangelista KCM. Critical revision of the manuscript for intellectual content: Rocha DO, Dantas RCS, Andrade FL, Avelino RRS, Lyra CO, Diniz RVZ, Lira NRD, Sena-Evangelista KCM.

### **Potential Conflict of Interest**

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

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### **Study Association**

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### Ethics approval and consent to participate

This study was approved by the Ethics Committee of the *Hospital Universitário Onofre Lopes* under the protocol number CAAE 59827516.2.0.0.0.0.5292. 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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