Original article

The bioelectrical impedance phase angle as an indicator of undernutrition and adverse clinical outcome in cardiac surgical patients

Marlieke Visser\textsuperscript{a,b}, Lenny M.W. van Venrooij\textsuperscript{a,c,*}, Dominique C.M. Wanders\textsuperscript{d}, Rien de Vos\textsuperscript{e}, Willem Wisselink\textsuperscript{b}, Paul A.M. van Leeuwen\textsuperscript{b}, Bas A.J.M. de Mol\textsuperscript{a}

\textsuperscript{a}Department of Cardiothoracic Surgery, Academic Medical Center University of Amsterdam, The Netherlands
\textsuperscript{b}Department of Surgery, VU University Medical Center, Amsterdam, The Netherlands
\textsuperscript{c}Department of Dietetics, Academic Medical Center University of Amsterdam, Amsterdam, The Netherlands
\textsuperscript{d}Institute of Health Sciences, Faculty Earth and Life Sciences, VU University, Amsterdam, The Netherlands
\textsuperscript{e}Clinical Epidemiology and Biostatistics, Academic Medical Center University of Amsterdam, Amsterdam, The Netherlands

\textbf{Article history:}
Received 11 January 2012
Accepted 3 May 2012

\textbf{Keywords:}
Phase angle
Undernutrition
Clinical outcome
Cardiac surgery
Bioelectrical impedance spectroscopy
Fat free mass

\textbf{SUMMARY}

\textbf{Background & aims:} In cardiac surgical patients, undernutrition increases the risk of adverse clinical outcome. We investigated whether the bioelectrical impedance phase angle is an indicator of undernutrition and clinical outcome in cardiac surgery.

\textbf{Methods:} In 325 cardiac surgical patients, we prospectively analyzed the associations between a preoperative low phase angle, measured by bioelectrical impedance spectroscopy, and well-established indicators of undernutrition such as body mass index (kg/m\textsuperscript{2}), unintended weight loss, and fat free mass index (kg/m\textsuperscript{2}) and muscle strength (handgrip strength (kg)), immune function (C-reactive protein and albumin), and adverse clinical outcomes.

\textbf{Results:} A low phase angle (<5.38\textdegree) was present in 29.8\% (n = 96) of the patients, and was associated with low body mass index (p < 0.001), low fat free mass index (p < 0.001), and less handgrip strength (p = 0.063), but not with unintended weight loss or immune function. Furthermore, a preoperative low phase angle was associated with a prolonged intensive care unit and hospital stay (adj. hazard ratio: 0.68; 95\%CI: 0.49–0.94; p = 0.020 and adj. hazard ratio: 0.74; 95\%CI: 0.55–0.99; p = 0.048, respectively).

\textbf{Conclusions:} A preoperative low bioelectrical impedance phase angle is associated with undernutrition, and increases the risk of adverse clinical outcome after cardiac surgery. The phase angle might help to identify undernourished cardiac surgical patients.

\textcopyright{} 2012 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

1. Introduction

Disease-related undernutrition is common in patients undergoing cardiac surgery: 10–25\% of these patients appeared to be undernourished.\textsuperscript{1–3} Preoperative undernutrition increases the risk of postoperative infectious and non-infectious complications, a prolonged stay at the ICU and hospital, and impaired wound healing.\textsuperscript{1–3} Therefore, undernutrition should be identified and treated preoperatively.

Recent research showed that half of cardiac surgery patients with a low fat free mass index (FFMI; kg/m\textsuperscript{2}), were misclassified and scored as well-nourished when identified by BMI (kg/m\textsuperscript{2}) and unintended weight loss (UWL) only.\textsuperscript{4} In this study,\textsuperscript{4} FFMI was determined by bioelectrical impedance spectroscopy (BIS) which calculates fat free mass (FFM; kg) from measurements of body water.\textsuperscript{5} Most likely, a low FFMI reflects less muscle mass. Muscle mass functions as an important source of amino acids for protein synthesis and gluconeogenesis in times of stress and starvation.\textsuperscript{5} Therefore, cardiac surgical patients with a low FFMI have too little reserves to respond adequately to operative stress resulting in worse muscle function, an increased risk of infectious and non-infectious complications, and a longer convalescence period after surgery.\textsuperscript{5,6}
Another variable derived from BIS, the phase angle (PA; °) might offer a clinically applicable technique to identify undernutrition. The PA reflects the ratio between reactance (the resistive effect produced by cell membranes, i.e., cell membrane capacity = and resistance = the restriction to the flow of an electric current through the body, primarily related to the amount of water present in tissue). In agreement with FFMI, the PA includes the assessment of total body water, and therefore reflects body cell mass. In contrast to FFMI, the PA also measures cell membrane capacity and therefore also reflects body cell quality. For that reason, the PA has been regarded as a marker of undernutrition and of cellular health. Hence, the PA might offer possibilities to improve identification of undernourished patients in addition to FFMI.

The indicative value of the PA for undernutrition and its relation to clinical outcome has been investigated in several patient populations. A low PA was associated with disease severity in heart failure, and with undernutrition and prognosis in patients with cancer, cirrhosis, HIV, in patients undergoing gastrointestinal surgery, and in haemodialysis patients. Moreover, the PA increased after protein supplementation in undernourished gastrointestinal patients. In cardiac surgical patients, the indicative value of the PA for undernutrition and its association with clinical outcome was studied. Therefore, the PA was assessed. In this study we investigated the association of the PA with other indicators of undernutrition, muscle and immune function, and adverse clinical outcome after cardiac surgery.

2. Materials and methods

2.1. Study population

This prospective cohort study used data from a study that was performed between February 2008 and December 2009 at the Department of Cardiothoracic Surgery at the Academic Medical Center of the University of Amsterdam (AMC). Selected patients were admitted at this department for elective coronary artery bypass grafting (CABG) and/or heart valve surgery, both with extracorporeal circulation. Exclusion criteria were not willing or able to give written informed consent, pacemaker, congenital heart abnormality, or open-heart surgery within the preceding three months. The process for obtaining informed consent was approved by the Medical Ethical Committee of the AMC.

2.2. Patient and operation characteristics

Patient characteristics including age, gender, operative risk attained by the European System for Cardiac Operation Risk Evaluation score (EuroSCORE), a validated risk stratification system to determine the risk profile for mortality of cardiothoracic surgery patients, inflammatory activity (as CRP), presence of hypalbuminaemia, and heart failure (defined by NT-proBNP), type of operative procedure (CABG and/or heart valve surgery), and operation time were extracted from medical files and the standard electronic database of the cardiothoracic surgery department. Age was modelled as dichotomised variable, >65 years vs. ≤65 years. Operative risk was dichotomised as EuroSCORE ≥6 (high risk) vs. EuroSCORE <6 (low risk). The levels of albumin, CRP and NT-proBNP were examined in accordance with routine hospital procedures on the day of admission or, if not carried out, from the last time the patient had visited the preoperative outpatient clinic. Heart failure was defined as NT-proBNP >600 ng/L. Operative procedure was dichotomised as isolated CABG vs. isolated heart valve surgery or both. Operation time was divided into cardiopulmonary bypass (CPB) time and aortic cross-clamp (ACC) time. CPB and ACC time were dichotomised as ≥130 min vs. <130 min and ≥95 min vs. <95 min respectively.

2.3. Measurement of the PA, body composition and muscle strength

On the day of admission at the cardiothoracic surgery ward, PA, body height and weight, and body composition were measured while patients were barefoot and in underwear. Body height was measured to the nearest 0.5 cm using a stadiometer (Seca, Germany) and body weight was measured using an electronic beam scale with digital read-out to the nearest 0.1 kg (Inventum Scalas PW200). The PA and FFMI were determined by BIS (BodyScout Fresenius Kabi, Germany). A BIS measurement was done while patients were lying supine with legs apart and arms abducted. The measurement was performed on the right side of the body using four electrodes (3 M Red Dot, Health Care, Borken, Germany) of which two electrodes were placed on the dorsum of the hand and two on the dorsum of the foot. The electrodes introduced a 5–1000 kHz electrical current into the body. At low frequency, the electrical signal travels predominantly through the extracellular space, whereas high-frequency signals travel through both extracellular and intracellular space. Thereby, the multiple frequencies enable a difference between intracellular fluid and extracellular fluid to be measured. Subsequently, FFMI was calculated. Simultaneously, reactance (Xc) and resistance (R) were measured. The PA at 50 kHz was calculated by using the following equation: PA (°) = arc tan (Xc/R) × (180/π). Handgrip strength (HGS) was measured three times by using a portable dynamometer (Jamar handgrip dynamometer, Sammons Preston Rolyan, IL, USA) in the non-dominant hand after which mean HGS was calculated. To adjust for differences in body height, FFMI was calculated by dividing FFMI (kg) by squared body height (m²).

2.4. Definition of low PA, undernutrition, and worse immune and muscle function

A low PA was defined based on the cut-off value with the highest indicative accuracy (details are described in the Statistical analysis). In advance of the results this cut-off value was 5.38. Indicators of undernutrition assessed in this study were low BMI, UWL and low FFMI. A low BMI was defined as a BMI <21.0 kg/m², UWL as ≥10% UWL in the preceding six months, and low FFMI was set at FFMI ≤14.6 kg/m² for women, and ≤16.7 kg/m² for men conform previous research in cardiac surgery. Muscle function was described by HGS. In accordance with literature, a low HGS was defined as an HGS <85% of its age–sex standardized HGS. Immune function was described by CRP and albumin; inflammatory activity was defined as CRP ≥5 mg/L and hypoalbuminaemia as ≤39 g/L.

2.5. Adverse clinical outcome

Data about clinical outcome were collected from medical files and from the standard electronic database of the cardiothoracic surgery department at the AMC. The main adverse clinical outcome parameters were: 1) the occurrence of postoperative infection, 2) death, 3) prolonged time of mechanical ventilation, 4) prolonged length of ICU stay, and 5) prolonged length of hospital stay, all during the period of admission at the hospital for surgery (i.e. AMC) and the period of admission at the referring hospital where patients were transferred back to from the AMC. Postoperative infection was defined as the composite of sepsicaemia, respiratory tract infection, mediastinitis, deep sternal wound and leg wound infection which were defined in accordance with the definitions of the Society of Thoracic Surgeons. Postoperative death was defined as death.
during hospital admission. Prolonged time of mechanical ventilation was defined as ≥12 h (re-intubation hours included). Prolonged ICU stay was defined as ≥48 h (re-admission hours included, patients who died were excluded) and prolonged hospital stay was defined as postoperative hospital stay ≥10 days (patients who died were excluded).

2.6. Statistical analysis

Before analyzing the associations between a low PA and other indicators of undernutrition, muscle and immune function, we determined the optimal cut-off value for a low PA in our cardiac surgery population. Receiver operating characteristic (ROC) curves were drawn for different cut-offs of the PA and its association with the five adverse clinical outcome parameters mentioned above. First, the 10th, 20th, 30th, 40th and 50th percentiles of the PA were used as cut-off values. Secondly, a cut-off value was created for each patient based on the 5th percentile of a sex-, age- and BMI-stratified reference value. Finally, a cut-off value was created by standardizing the PA of each patient according to the equation: standardized PA = (observed PA – mean PA)/SD of the PA), from which the mean and SD were from sex-, age- and BMI-stratified reference values. A low PA was indicated by a standardized PA < -1. For all PA cut-off values area under the curves (AUC) were calculated to determine the accuracy of the cut-off value as indicator of adverse clinical outcome. The optimal cut-off value for a low PA in our cardiac surgery population was determined based on the highest AUC which was demonstrated by the PA cut-off with the majority of significant associations with the five adverse clinical outcome parameters.

After the optimal cut-off value for a low PA was determined, the associations between a low PA and indicators of undernutrition, muscle strength, and immune function were assessed with the Chi-square test, or if necessary, with the Fisher’s exact test. To assess the association between a preoperative low PA and the occurrence of postoperative infection, death, and prolonged time of mechanical ventilation, univariate logistic regression analyses were executed. Odds ratios (OR) with a 95% CI were calculated, with postoperative infection, death, or prolonged time of mechanical ventilation as dependent variable and PA as independent variable. Then, if p-value < 0.10 in the univariate regression model was present, multivariate regression analyses were performed. The multivariate models adjusted for age, gender, operative risk, CRP, albumin, NT-proBNP, operative procedure, CPB and ACC. In order to take into account time-related information Cox regression analyses were used to assess the association between a preoperative low PA and length of ICU and hospital stay. Discharge at any point during the admission period was coded as event and death or loss to follow up as censored data. The proportional hazard assumptions of Cox regression analysis were met. Adjusted hazard ratios (HR) and their 95% CI were calculated. A p-value ≤ 0.05 was considered statistically significant. All statistical analyses were performed with SPSS (version 18.0; SPSS Inc., Chicago, IL, USA), statistical software for WINDOWS.

3. Results

3.1. Patient characteristics

A total of 396 patients were asked to participate. Of these patients 17.9% (n = 71) refused to take part. These patients had a higher operation risk and were older than those patients who gave informed consent (p < 0.005 and p = 0.01, respectively). The remaining data of 325 patients were used in the analyses. Their characteristics and adverse clinical outcome data are summarized in Tables 1 and 2 respectively. No patients were admitted at the ICU after transferred back to the referring hospital.

3.2. Optimal low PA cut-off value

The ROC curves revealed that the 30th percentile of the PA determined from our cardiac surgery population (i.e. PA of 5.38) was the cut-off that provided the highest accuracy to indicate adverse clinical outcome (infections: AUC: 0.54, 95%CI: 0.44–0.65, p = 0.434; mortality: AUC: 0.70, 95%CI: 0.52–0.88, p = 0.043; prolonged time of mechanical ventilation: AUC: 0.57, 95%CI: 0.50–0.63, p = 0.037; prolonged ICU length of stay (LOS): AUC: 0.58, 95%CI: 0.51–0.65, p = 0.020; and prolonged hospital LOS: AUC: 0.58, 95%CI: 0.51–0.65, p = 0.021) compared to the 10th, 20th, 40th and 50th percentiles of the PA, the cut-off value based on the 5th percentile of a sex-, age- and BMI-stratified reference values, and compared to the standardized PA (data not shown). The presence of this low PA of <5.38° was higher in women compared to men (50.0% vs. 22.2%), in elderly patients compared to the younger cohort (44.3% vs. 10.8%), in high operative risk compared to low operative risk patients (49.2% vs. 17.7%), in patients undergoing heart valve surgery (with or without CABG) compared to those undergoing CABG solely (37.0% vs. 23.2%), and in patients suffering from severe heart failure compared to those who did not suffer from severe heart failure (44.2% vs. 24.0%), (p ≤ 0.05). No associations were found with CPB or ACC time.

3.3. A low PA in relation with indicators of undernutrition, muscle strength, and immune function

From those patients with a low PA, 22.9% had a low FFMI while in Tables 1 and 2 respectively. No patients were admitted at the ICU after transferred back to the referring hospital.

Table 1 Patient and operation characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>% or mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (female)</td>
<td>90</td>
<td>27.7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>325</td>
<td>66.2 ± 9.8</td>
</tr>
<tr>
<td>Nutritional status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA (g/L)</td>
<td>322</td>
<td>5.9 ± 1.0</td>
</tr>
<tr>
<td>UWL</td>
<td>17</td>
<td>5.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>325</td>
<td>27.2 ± 4.1</td>
</tr>
<tr>
<td>FFMI (kg/m²)</td>
<td>325</td>
<td>18.9 ± 2.5</td>
</tr>
<tr>
<td>HGS (kg)</td>
<td>320</td>
<td>36.7 ± 11.2</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>323</td>
<td>45.1 ± 3.1</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>325</td>
<td>3.9 ± 8.0</td>
</tr>
<tr>
<td>Operative risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EuroSCORE ≥ 6</td>
<td>127</td>
<td>39.1</td>
</tr>
<tr>
<td>Heart failure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-proBNP &gt;600 ng/L</td>
<td>107</td>
<td>34.9</td>
</tr>
<tr>
<td>Operative procedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CABG</td>
<td>168</td>
<td>51.7</td>
</tr>
<tr>
<td>Heart valve surgery</td>
<td>105</td>
<td>32.2</td>
</tr>
<tr>
<td>CABG combined with heart valve surgery</td>
<td>52</td>
<td>16.0</td>
</tr>
<tr>
<td>Duration of extracorporeal circulation</td>
<td>106</td>
<td>32.7</td>
</tr>
<tr>
<td>Cardiopulmonary bypass time &gt;130 min</td>
<td>96</td>
<td>29.6</td>
</tr>
</tbody>
</table>

Table 2 Prevalence of adverse clinical outcome parameters.

<table>
<thead>
<tr>
<th>Event</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections</td>
<td>35 (12.3)</td>
</tr>
<tr>
<td>Mortality</td>
<td>320 (9.3)</td>
</tr>
<tr>
<td>Mechanical ventilation ≥12 h</td>
<td>152 (47.1)</td>
</tr>
<tr>
<td>ICU LOS &gt;48 h</td>
<td>116 (36.4)</td>
</tr>
<tr>
<td>Hospital LOS &gt;10 days</td>
<td>178 (62.7)</td>
</tr>
</tbody>
</table>
Furthermore, patients with a low PA had more often a low BMI (10.4% vs. 0.9%, p < 0.001) and probably a low HGS (58.5% vs. 47.1%, p = 0.063).

A low PA was not associated with UWL, inflammatory activity or hypoalbuminemia.

### 3.4. A low PA in relation to adverse clinical outcome

Univariate logistic regression analyses showed that a preoperative low PA was associated with postoperative mortality (7.6% of patients with a low PA died vs. 1.5% of patients with a high PA, OR: 5.43; 95% CI: 1.42–22.26; p = 0.019), prolonged time of mechanical ventilation (57.9% vs. 41.8%, OR: 1.92; 95% CI: 1.18–3.12; p = 0.009), and prolonged stay at ICU and hospital (HR: 0.63; 95% CI: 0.49–0.80; p < 0.001 and HR: 0.60; 95% CI: 0.45–0.79; p < 0.001 respectively) (Table 4). After multivariate analyses adjusting for age, gender, operative risk, CRP, albumin, NT-proBNP, operative procedure, CPB and ACC, the associations between a preoperative low PA and prolonged length of stay at the ICU and at the hospital did persist (adj. HR: 0.74; 95% CI: 0.55–0.99; p = 0.048 and adj. HR: 0.68; 95% CI: 0.49–0.94; p = 0.020 respectively), while the others did not (Table 4).

### 4. Discussion

This prospective cohort study shows that a low bioelectrical impedance PA is associated with well-established indicators of undernutrition and probably muscle strength. Furthermore, a preoperative low PA is associated with an increase in the risk of prolonged postoperative ICU and hospital LOS in patients undergoing cardiac surgery independently from other risk factors such as operative risk and severity of heart failure. A cut-off value for a low PA of 5.38 determined from our cardiac surgery population had the highest indicative accuracy and was present in 29.8% of the patients. Furthermore, it was observed that a low PA was largely present in women, elderly, patients with severe heart failure, high operation risk and heart valve surgery.

Our findings are in-line with results from several other studies that found the PA to be a prognostic marker for impaired outcome in different medical conditions. In HIV patients, a low PA was an independent prognostic marker of adverse clinical progression and survival. In patients with liver cirrhosis and cancer, a low PA was associated with increased mortality rates. In our study, there was no association between a low PA and mortality. Probably, the prevalence of mortality in our population (n = 9 (3.2%)) was too low and there was a lack of power to find an association.

While our and previous studies clearly show an association between a low PA and adverse clinical outcome, the biological meaning of the PA is difficult to interpret. The PA has been considered a global marker of health and of cellular vitality. The PA reflects the ratio between cellular membrane capacity (reactance) and the amount of water present in tissue (resistance). Undernutrition affects the PA by loss of metabolic active cell mass which is reflected by reduced reactance and increased resistance, both resulting in a lower PA. However, it should be stressed that undernutrition might also result in an increase of extracellular water which influences resistance, and thereby possibly also the PA, in an opposite direction.

In our study, the associations between the PA and BMI and low FFMI suggest that the PA is indeed related to undernutrition. On the other hand, this also implies that the associations of the PA with adverse clinical outcomes might depend on abnormalities of these other indicators of undernutrition. However, in contrast to the PA, none of the well-established indicators of undernutrition were associated with hospital LOS in this cardiac surgery population. Therefore, measurement of the PA might be of additional benefit to identify undernourished patients and those who have an increased risk of adverse clinical outcome after cardiac surgery.

Although also other studies have demonstrated significant associations between the PA and indicators of undernutrition, there was no agreement about the accuracy of the PA as an indicator of undernutrition. In patients with advanced colorectal cancer and in haemodialysis patients, the PA was associated with the risk of undernutrition (as assessed by subjective global assessment) respectively. In cancer patients, the PA was an independent predictor of impaired nutritional and functional status (as measured by SGA and HGS respectively). Furthermore, Barbosa-Silva et al. concluded that the PA independently predicted prognosis in patients undergoing gastrointestinal surgery while other nutritional variables (weight loss, SGA, and extracellular mass/body cell mass ratio) lost their association with postoperative complications. It is clear that a low PA is associated with undernutrition and adverse clinical outcome in several populations and in patients undergoing cardiac surgery. However, it remains unclear whether this association is causal.

A clinical trial randomizing nutritional intervention vs. control in (cardiac surgical) patients with a low PA on adverse clinical outcome should clarify this.
In a previous study in our population, the indicator low FFMI was superior to low BMI and/or UWL in predicting adverse clinical outcome. Furthermore, this study demonstrated that a low FFMI was independently associated with the occurrence of infections and tended to be associated with ICU LOS. In this current study, the PA was not only independently associated with ICU LOS but also with hospital LOS. This suggests that a low PA might be of additional benefit to identify undernutrition in cardiac surgical patients besides low FFMI. Measuring both indicators is easy since BIS measures both PA and FFMI.

An advantage of the PA compared to BIS-assessed FFMI might be that the PA is a raw BIS measurement and therefore requires fewer assumptions such as tissue hydration. Criteria which might not be fulfilled by all cardiac surgical patients. Furthermore, the PA does not require recalculation into an index as FFM and body weight (FFMI; BMI).

The limitations of our study need to be addressed. First, the complication rates and the magnitude of the effect sizes might be underestimated as those patients who were not willing to participate were older and had a higher operative risk. Secondly, the results might be context specific. Therefore, studies are needed to validate the PA cut-off value of 5.38° in other cardiac surgical patient populations. Finally, in cardiac surgery, impedance measurements, or more specifically the PA, should be interpreted with caution. Extracellular fluid imbalances are expected because of underlying cardiac disease, and impedance measurements are sensitive to hydration abnormalities. It was observed in this specific population that compensatory cords accompanied by extra fluid, was present in 8%. This may have resulted in some bias and overestimation of the PA and thereby in underestimation of undernutrition (Extra fluid results in a lower resistance and thus higher PA). No further bias was expected in the stable chronic heart failure patients because others have observed similar extracellular water/intracellular water ratios compared to healthy controls (0.73 vs. 0.75). Furthermore, it should be realized that a low PA might be initiated by a low reactance, a high resistance or both. It is unknown which component of the ratio causes a low PA without knowing their values. In undernourished (low muscle mass) patients most likely both components change, i.e., reactance decreases and, to a larger extent, resistance increases. In patients with fluid overload, the decrease in reactance and especially resistance might result in a falsely more adequate nutritional status because of a higher PA.

Our results imply that the bioelectrical impedance PA can help to identify undernutrition in patients admitted for cardiac surgery, in order to start nutritional interventions and to reduce the risk of adverse clinical outcome. Now, further research is needed to investigate the effect of nutritional supplementation on the PA and clinical outcome in cardiac surgical patients with a low PA.

Conflict of interest
There are no conflicts of interest.

Author agreement
All authors have made substantial contributions and final approval of the conceptions, drafting, and final version.

Acknowledgements
The authors’ responsibilities were as follows. MV: performed the data analysis, writing of the manuscript, and was responsible for the design and protocol; LMWV: was responsible for the patient selection, data collection and analysis, and the writing of the manuscript; DCMW: participated in the data analysis and writing of the manuscript; RdV: participated at the methodological development of the study protocol, reviewed the analysis, and co-authored the manuscript; WW and PAML reviewed the manuscript and were responsible for the final content; BAJMdB: participated in the development of the protocol, supervised the study, and reviewed the manuscript.

References