Revista Española de Nutrición Humana y Dietética

Spanish Journal of Human Nutrition and Dietetics

RESEARCH – post-print version

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Phase Angle for Nutritional Risk Screening in Cardiac Critically Ill Patients

Ángulo de fase para el tamizaje del riesgo nutricional en pacientes cardiacos críticos

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Received: 09/02/2022; Accepted: 12/19/2022; Published: 02/01/2023

Editor: Edna J. Nava-González, Universidad Autónoma de Nuevo León, México.

HIGHLIGHTS

1) The phase angle has been listed as a nutritional marker and its useful role in critically ill patients, but its role as a patient-to-patient tool has not yet been tested.

2) The PhA had good results of sensitivity and specificity but should be used with caution to determine the nutritional risk in critically ill cardiac patients, as it did not detect the risk in approximately 30% of the individuals.

3) It is suggested, therefore, that PhA should not be used in isolation as a tool for screening nutritional risk and carrying out other studies to reach more definitive conclusions.

ABSTRACT

Introduction: The phase angle has been listed as a nutritional marker and its useful role in critically ill patients, but its role as a patient-to-patient tool has not yet been tested. This study sought to evaluate the phase angle as a proposal to determine nutritional risk in critically ill patients hospitalized in cardiac intensive care units.

Methods: Transversal study, coupled to a prospective analysis variable (hospitalization outcome) and involving adult and elderly male and female patients in cardiac intensive care units. The nutritional risk was determined by using the NUTRIC score, the phase angle was obtained through bioelectrical impedance analysis and other data, through the clinical record. A significance level of p <0.05 was used for all statistical analysis.

Results: 79 patients were included and resulted in homogeneous distribution among the sexes and an average age of 67.2 ± 13.7 years. Most of the sample had malnutrition according to the body mass index (BMI) (46.7%; CI: 36.0-57.8) and due to the adequacy of the arm circumference (40.8%; CI: 34.0-52.0). According to the NUTRIC score, 59.5% (CI: 48.5-69.3) had a high nutritional risk, and 68.4% (CI: 57.4-77.6) had a low phase angle (≤5.5°). Correlation between the phase angle and age (p = 0.010) and BMI (p = 0.023) was verified. A good sensitivity (72%; CI: 55.6-81.9) and specificity (68%; CI: 42.5-77.5) of the low phase angle were obtained to detect nutritional risk by NUTRIC.
Conclusions: The PhA had good results of sensitivity and specificity but should be used with caution to determine the nutritional risk in critically ill cardiac patients.

Keywords: Nutritional screening; Risk factors; Bioelectrical Impedance; Critical care; Intensive care units; Mortality.

RESUMEN

Introducción: El ángulo de fase se ha catalogado como un marcador nutricional y su papel útil en pacientes críticos, pero aún no se ha probado su papel como herramienta de paciente a paciente. Este estudio buscó evaluar el ángulo de fase como propuesta para determinar el riesgo nutricional en pacientes críticos hospitalizados en unidades de cuidados intensivos cardíacos.

Metodología: Estudio transversal, acoplado a una variable de análisis prospectivo (resultado de hospitalización); participaron pacientes adultos y adultos mayores del sexo masculino y femenino en unidades de cuidados intensivos cardíacos. El riesgo nutricional se determinó mediante el NUTRICscore, el ángulo de fase se obtuvo a través del análisis de impedancia bioeléctrica y otros datos, a través de la historia clínica. Se utilizó un nivel de significación de p <0,05 para todos los análisis estadísticos.

Resultados: Se incluyeron 79 pacientes y resultó una distribución homogénea entre los sexos y una edad promedio de 67,2±13,7 años. La mayor parte de la muestra presentaba desnutrición según índice de masa corporal (IMC) (46.7%; CI: 36.0-57.8) y por adecuación del perímetro braquial (40,8%; CI: 34.0-52.0). De acuerdo con el puntaje NUTRIC, el 59,5% (CI: 48.5-69.3) tenía un riesgo nutricional alto y el 68,4% (CI: 57.4-77.6) tenía un ángulo de fase bajo (≤ 5,5°). Se verificó correlación entre el ángulo de fase y la edad (p = 0,010) y el IMC (p = 0,023). Se obtuvo una buena sensibilidad (72%; CI: 55.6-81.9) y especificidad (68%; CI: 42.5-77.5) del ángulo de fase bajo para detectar riesgo nutricional por NUTRIC.

Conclusiones: El PhA tuvo buenos resultados de sensibilidad y especificidad, pero debe ser utilizado con precaución para determinar el riesgo nutricional en pacientes cardíacos críticos.

Palabras clave: Tamizaje nutricional; Factores de riesgo; impedancia bioeléctrica; cuidado crítico; Unidades de cuidados intensivos; Mortalidad.
INTRODUCTION

Critically ill patient is an individual who is at constant risk of death, loss of organ or system function in the human body, as well as a fragile clinical condition resulting from trauma or other conditions that require intensive and immediate care, both clinical and surgical, or mental health. In this context, many metabolic and hormonal changes occur in these patients in an attempt to maintain the organism in due homeostasis.

Thus, they develop an imminent catabolic state, with a picture of complications due to increased morbidity, organ dysfunction, prolonged hospitalization and significant mortality rate. And linked to the catabolic state, critically ill patients can still suffer from hypermetabolism condition, induced by signs of stress hormones, inflammatory cytokines and other mediators, resulting in malnutrition.

Malnutrition in critically ill patients can occur mainly due to persistent inflammation, chronic organ failure, persistent protein catabolism and inadequate nutrition, in addition to being associated with problems in wound healing and immunosuppression, with greater susceptibility to secondary infections and low levels of survival in long term. Consequently, the correct and early identification of nutritional risk in critically ill patients is essential, since recent studies have shown that not everyone benefits from aggressive nutritional therapy in the initial phase of critical illness, and the exception is patients with higher nutritional risk.

Nutrition Risk in the Critically ill (NUTRIC score) is the first screening tool developed for critically ill patients. This instrument uses risk factors that can be modified by nutritional therapy in the intensive care unit, considering that not all patients are at the same risk of suffering adverse events with repercussions on nutritional status. Therefore, the variables contained in this score were incorporated because they are significantly associated with mortality and are easily collected in the routine of intensive care units. They are: age, the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, the Sequential Organ Failure Assessment (SOFA) score, comorbidities, days in the hospital before admission to the intensive care unit, and levels of interleukin 6 (IL-6), the latter being available or not, without interference in the final classification of low or high risk.

On the other hand, the phase angle (PhA) is a parameter derived from bioelectrical impedance analysis that is calculated directly from the resistance and reactance. Resistance is the body's opposition to the flow of an alternating electric current and reactance, refers to the properties...
of capacitance of the cell membrane\textsuperscript{9}. This emerged as a sensitive indicator of cell health, with higher values reflecting the integrity of the cell membrane or the vitality of the living tissue, due to the fact that healthy cell membranes behave as good capacitors, which store the current and consequently cause a delay in the flow. The phase difference between voltage and current, caused by the delay in the current that penetrates cell membranes and tissue interfaces, is expressed as phase angle (PhA)\textsuperscript{10}.

PhA measurement does not require pre-parameters, body weight and laboratory tests, being calculated directly as $\text{PhA} = \tan^{-1}(\frac{\text{Xc}}{\text{R}}) \times \frac{180}{\pi}$ (expressed in radians) and can be considered a prognostic indicator in several clinical situations, such as malnutrition, cancer and HIV infection to predict clinical results, including survival and mortality\textsuperscript{9,11}.

Few studies in patients with heart disease have evaluated PhA and its relation with adverse outcomes. An investigation that associated PhA with nutritional status in critically ill heart disease patients evidenced that this could be a marker of malnutrition and a predictor of poor prognosis\textsuperscript{12}. Another recent study in surgical cardiac patients demonstrated that PhA was a clinically useful prognostic biomarker\textsuperscript{13}. Despite these findings, PhA has not been tested as a nutritional screening marker in critically ill heart disease patients yet.

In this context, the objective of this study was to evaluate PhA as a tool to determine nutritional risk in critically ill patients hospitalized at Cardiac Intensive Care Unit.

**METHODS**

This transversal study coupled to a prospective analysis variable (hospitalization outcome), occurred in the cardiac intensive care units of an university cardiology hospital in Brazil involving all hospitalized patients from May to November 2019 with those who filled the eligibility criteria.

The sample was non-probabilistic, attended by convenience, including adult and elderly male and female patients diagnosed with myocardial infarction, heart failure, aortic aneurysm and acute pulmonary edema. Those with amputation, pacemaker or mechanical valve prosthesis were excluded. The sample size was determined considering the correlation ($p$) between the phase angle and the NUTRIC score of 0.5 obtained in a pilot study, a variability ($d$) of 0.17, an $\alpha$ error of 5% a $\beta$ error of 20%. The minimum sample size of 68 individuals was obtained and an increase of 15% was added to cover eventual losses, totaling 79 patients.
Bioelectrical impedance analysis (BIA) was performed within 72 hours of admission with Biodynamics model 310 portable equipment, which applies a current of 800 µA, with a simple frequency of 50 kHz. The patient remained supine, on a non-conductive surface, with hands and legs parallel to the body. An electrode, brand heart beat and model for bioelectrical impedance, was placed on the hand, at the middle level of the finger, and one on the wrist joint, both on the right side. Another pair of electrodes was placed on the foot, at the middle level of the toes, and on the ankle joint, also on the right side. At first, the patients' skin was cleaned with 70% alcohol, where the electrodes were fixed.

The phase angle was determined by the relationship between different resistance (R) and reactance (Xc) measurements provided in the BIA, (PhA = tangent arc Xc / R). To convert the result from radian to degrees (º), the result obtained was multiplied by 180º / π and the cutoff point to establish low PhA was ≤ 5.5º.

The nutritional risk screening tool, modified NUTRIC score, was also applied within 72 hours of admission, obtained with support of the sector's multiprofessional team, being calculated according to the variables that compose it, such as: age, the evaluation of acute physiology and chronic health II (APACHE II), assessment of sequential organ failure (SOFA), comorbidities and days in the hospital before admission to the intensive care unit.

Anthropometric data (body mass index, weight, height, arm circumference and knee height) were obtained from their report, measured at the bed, through the medical records, or even through an estimate, considering predictive equations. Sociodemographic data (age, gender), clinical data (definitive or provisional clinical diagnoses, the presence of comorbidities and the value of SOFA, APACHE II) and mortality during hospitalization were obtained from the medical record.

Data analysis was performed using the SPSS version 13.0 statistical package (SPSS Inc., Chicago, IL, USA). Continuous variables were tested according to normal distribution using the Kolmogorov-Smirnov test and as they presented normal distribution, they were expressed as means and standard deviations. The Student's t test was applied to compare the mean age and clinical and nutritional variables as a function of mortality. Pearson's linear correlation was used to test the correlation between PhA with continuous covariates. A significance level <0.05 was adopted for all statistical analysis. The agreement between the PhA and the modified NUTRIC score was expressed by the percentages of sensitivity and specificity.
The study followed the ethical standards for research involving human beings, contained in resolution 466/12 of the National Health Council, being approved by the Ethics and Research Committee under protocol number CAAE 09989319.6.0000.5207.

In addition, the participants and / or legal guardians of the research participants were previously informed about the research objectives, as well as the methods that were adopted. Upon their consent, they signed the Free and Informed Consent Form.

RESULTS

The sample consisted of 79 critical patients admitted to the Cardiac Intensive Care Unit, with an average age of 67.2 ± 13.7 years and homogeneous distribution between the male and woman. There was a predominance of elderly individuals (77.2%), with ages ranging from 22 to 89 years.

The main clinical diagnoses of hospitalization were acute myocardial infarction and congestive heart failure. The prevalence of hypertension and type 2 diabetes mellitus was 79.7% (CI: 69.6-87.1) and 38.5% (CI: 28.4-39.6), respectively. Chronic kidney disease was identified in 26.6% (CI: 18.1-37.2) of patients, and of these, 11% (CI: 6.1-20.2) were on renal replacement therapy (hemodialysis). Mortality during hospital internment was observed in 53.2% (CI: 42.3-63.8) of patients.

The average body mass index (BMI) was 26.4 ± 4.8 kg / m², with a high percentage of malnutrition (46.7%; CI: 36.0-57.8). According to the adequacy of the arm circumference, a similar percentage of malnutrition was found (40.8%; CI: 34.0-52.0) (Table 1).

The mean PhA was 4.9 ± 1.9° and the modified NUTRIC Score was 5.1 ± 2.2. It was observed that 59.5% (CI: 48.5-69.3) had a high nutritional risk by NUTRIC and 68.4% (CI: 57.4-77.6) had low PhA (≤5.5 °).

Table 2 shows the correlation of PhA with demographic, clinical and nutritional parameters, in which an inverse correlation with age was verified (r = -0.289; p = 0.010) and a direct correlation with body mass index (r = 0.259; p = 0.023).
TABLE 1. Demographic and clinical characteristics of critically ill patients admitted to the Cardiac Intensive Care Unit (n = 79).

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients (n = 79)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (mean, SD)</strong></td>
<td>67.2 ± 13.7</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>40 (50.6); CI: 39.8-61.4</td>
</tr>
<tr>
<td>Female</td>
<td>39 (49.4); CI: 38.6-60.2</td>
</tr>
<tr>
<td><strong>Co-morbidities</strong></td>
<td></td>
</tr>
<tr>
<td>Hypertension (n, %)</td>
<td>63 (79.7); CI: 69.6-87.1</td>
</tr>
<tr>
<td>Diabetes (n, %)</td>
<td>30 (38.5); CI: 28.4-39.6</td>
</tr>
<tr>
<td>Renal disease (n, %)</td>
<td>21 (26.6); CI: 18.1-37.2</td>
</tr>
<tr>
<td>Death (n, %)</td>
<td>42 (53.2); CI: 42.3-63.8</td>
</tr>
<tr>
<td><strong>BMI (mean, SD)</strong></td>
<td>26.4 (4.8)</td>
</tr>
<tr>
<td><strong>Nutritional status (BMI) (n, %)</strong></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>36 (46.7); CI: 36.0-57.8</td>
</tr>
<tr>
<td>Eutrophy</td>
<td>28 (36.3); CI: 26.5-47.5</td>
</tr>
<tr>
<td>Overweight</td>
<td>13 (16.9); CI: 10.1-26.8</td>
</tr>
<tr>
<td><strong>Nutritional status (MAC) (n, %)</strong></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>31 (40.8); CI: 34.0-52.0</td>
</tr>
<tr>
<td>Eutrophy</td>
<td>31 (40.8); CI: 34.0-52.0</td>
</tr>
<tr>
<td>Overweight</td>
<td>14 (18.4); CI: 11.3-28.6</td>
</tr>
<tr>
<td><strong>Phaseangle (mean, SD)</strong></td>
<td>4.9 (1.9)</td>
</tr>
<tr>
<td>Phaseangle ≤ 5.5°</td>
<td>54 (68.4); CI: 57.4-77.6</td>
</tr>
<tr>
<td>Phaseangle &gt; 5.5°</td>
<td>25 (31.6); CI: 22.4-42.5</td>
</tr>
<tr>
<td><strong>NUTRIC (mean, SD)</strong></td>
<td>5.1 (2.2)</td>
</tr>
<tr>
<td><strong>NUTRIC</strong></td>
<td></td>
</tr>
<tr>
<td>Lowrisk</td>
<td>32 (40.5); CI: 30.4-51.5</td>
</tr>
<tr>
<td>High risk</td>
<td>47 (59.5); CI: 30.4-51.5</td>
</tr>
<tr>
<td><strong>SOFA (mean, SD)</strong></td>
<td>8.0 (4.9)</td>
</tr>
<tr>
<td><strong>APACHE II (mean, SD)</strong></td>
<td>17.4 (7.9)</td>
</tr>
</tbody>
</table>

Ci: Confidence Interval; BMI- Body mass index; MAC – Mid-arm circumference; NUTRIC - Nutrition Risk in the Critically Ill; SOFA – Sequential Organ Failure Assessment; APACHE II – Acute Physiology and Chronic Health Evaluation. Values are mean (standard deviation) or n (%).
Table 2. Correlation of the phase angle, nutritional and clinical parameters of critically ill patients admitted to a Cardiac Intensive Care Unit.

<table>
<thead>
<tr>
<th>Variables</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.289</td>
<td>0.010*</td>
</tr>
<tr>
<td>BMI</td>
<td>0.259</td>
<td>0.023*</td>
</tr>
<tr>
<td>MAC</td>
<td>0.194</td>
<td>0.093</td>
</tr>
<tr>
<td>NUTRIC</td>
<td>-0.197</td>
<td>0.085</td>
</tr>
<tr>
<td>SOFA</td>
<td>-0.116</td>
<td>0.308</td>
</tr>
<tr>
<td>APACHE II</td>
<td>-0.033</td>
<td>0.783</td>
</tr>
</tbody>
</table>

BMI- Body mass index; MAC – Mid arm circumference; NUTRIC - Nutrition Risk in the Critically Ill; SOFA - Sequential Organ Failure Assessment; APACHE II - Acute Physiology and Chronic Health Evaluation. *Value of Pearson correlation.

Variables were compared in relation to the occurrence of death during hospitalization (table 3) and was observed that the patients that evolved to death were older (p = 0.036), with a higher score on the modified NUTRIC score (p <0.001), on the Sofa (p <0.001) and Apache II (p = 0.003). A good sensitivity (72%; CI: 55.6-81.9) and specificity (68%; CI: 42.5-77.5) of low PhA was found to detect nutritional risk by Nutric (data not shown in tables).

Table 3. Comparison between age, nutritional and clinical parameters according to mortality in hospitalization of critically ill patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mortality</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n = 37)</td>
<td>Yes (n= 42)</td>
</tr>
<tr>
<td>Age</td>
<td>63.8 ± 14.7</td>
<td>70.2 ± 12.0</td>
</tr>
<tr>
<td>BMI</td>
<td>27.0 ± 5.1</td>
<td>25.8 ± 4.5</td>
</tr>
<tr>
<td>MAC</td>
<td>30.6 ± 5.1</td>
<td>29.4 ± 4.2</td>
</tr>
<tr>
<td>Phaseangle</td>
<td>5.2 ± 1.9</td>
<td>4.7 ± 1.9</td>
</tr>
<tr>
<td>NUTRIC</td>
<td>4.0 ± 1.7</td>
<td>6.0 ± 2.1</td>
</tr>
<tr>
<td>SOFA</td>
<td>5.7 ± 4.7</td>
<td>10.1 ± 4.1</td>
</tr>
<tr>
<td>APACHE II</td>
<td>14.3 ± 6.3</td>
<td>19.8 ± 8.2</td>
</tr>
</tbody>
</table>

BMI- Body mass index; MAC – Mid arm circumference; NUTRIC - Nutrition Risk in the Critically Ill; SOFA - Sequential Organ Failure Assessment; APACHE II - Acute Physiology and Chronic Health Evaluation. *p-value refers to T Student test of means comparison. Results with mean and standard deviation.
DISCUSSIONS

The present study evaluated critical cardiac patients admitted to the Cardiac Intensive Care Unit in order to assess the applicability of the phase angle as a tool to determine the nutritional risk in these patients.

The high prevalence of malnutrition, both due to the body mass index (46.7%), as well as the adequacy of the arm circumference (40.8%), is a fact that corroborates the results reported by Santos and Araújo\textsuperscript{15}, who described 44% of malnutrition with the adequacy of the arm circumference.

Silva et al.\textsuperscript{16} in their study with 110 patients, found 38.2% of the study population with a nutritional diagnosis of malnutrition due to the adequacy of the arm circumference. The research findings are approximate, probably because the populations of the studies are similar such as, both with mostly elderly patients, hospitalized in a cardiac intensive care unit, with the same clinical diagnoses and comorbidities. However, although the percentages of malnutrition were similar, the percentage of patients considered to have low PhA (42.7%) does not corroborate with the findings of the present study (68.4%).

PhA has been identified as a good indicator of nutritional status\textsuperscript{17}, nevertheless there is no universally established or recommended cut off point for the diagnosis of malnutrition and it is emphasized that the value of the classification must be different in several chronic conditions\textsuperscript{18}. Thus, care should be taken when comparing results that have adopted different cuts.

Contrary to the outcomes of this study, which found no correlation between PhA and the modified NUTRIC score, Razzera et al.\textsuperscript{14}, in a prospective cohort with similar objective and population characteristics, reported that a PhA<5.5\textdegree{} in the prediction of a high nutritional risk according to the NUTRIC Score had an accuracy of 79% (95% CI 0.59-0.83) by analyzing the ROC curve. The sensitivity (62.3%) and specificity (65%) values reported were similar to current study results. No other studies were found testing the performance of PhA as a marker of nutritional risk, using the NUTRIC Score tool.

It was observed in the study by Paes et al.\textsuperscript{19} that PhA presented satisfactory performance in identifying patients with high nutritional risk, when studying 31 seriously ill patients with cancer, suggesting that the use of PhA may be a viable tool for this population.
Al-Kalaldehet. Al20 in a study in Jordan with 411 critical patients found weak congruence between NUTRIC and electrical bioimpedance measurements, including the phase angle, as seen in this research and when assessing the risk of malnutrition, found a limited contribution from NUTRIC in estimating malnutrition.

PhA reflects the amount and types of tissues, such as muscle and fat mass, including hydration status, being the main biological factors that affect it, age, sex and body mass index21. The hypothesis is that PhA may also reflect nutritional status, as it is believed that metabolic changes, such as those of cell membranes, are primarily affected by malnutrition in its initial stage21, which is why it has been so explored in recent research as a nutritional assessment strategy.

It was identified a negative correlation between PhA and age, which corroborates Cioffi et. Al22, who evaluated patients diagnosed with acute and chronic Crohn's disease. However, these authors found a positive correlation between PhA and fat-free mass but not correlation between PhA and body mass index. It is believed that the different clinical diagnoses can influence body reserves and, consequently, alter PhA, which may be an explanation for the variations in results found in different pathologies. It should be considered that an adjusted analysis would be important to identify possible confounding factors.

Low PhA can reflect a reduction in muscle mass, cellular dysfunction and has been correlated with a worse prognosis in oncology, amyotrophic lateral sclerosis, geriatrics, renal dialysis in chronic renal failure, liver cirrhosis, acquired immunodeficiency syndrome and gastrointestinal surgical patients11. PhA values may vary depending on the underlying disease. Therefore, it is important to consider that, as this study included patients with heart disease and some with chronic kidney disease (CKD) in the same analysis, these PhA values may be affected, especially in more advanced stages of the disease.

Mortality is documented in the literature in association with several factors, including increasing age17,23-25. Costa26 stated 117 critical patients with sepsis an increase in mortality in older age groups and in patients with a higher SOFA score at admission. The study by Bector et al.27 with retrospective data from Canadian patients admitted to intensive care units and coronary care units, found a high score on APACHE II, associated with an increase in mortality. And as in the present study, Ozbilgin et al.28 obtained a positive correlation between mortality and parameters such as age, APACHE II, SOFA and NUTRIC score.
There was no difference in the mean PhA with the occurrence of death, although this is strongly suggested as a prognostic, health, functional and nutritional indicator\textsuperscript{21}. Other studies that obtained results different from the present study were found\textsuperscript{17,19,29,30}, among them, Garlini et al.\textsuperscript{31}, a systematic review that evaluated 48 studies and found an association between PhA and mortality in 42 of these articles, including in these findings research with cardiac patients and also critical patients.

Some aspects should be considered when interpreting the results, such as the relatively small sample size and the unicentric investigation, thus limiting the generalization of the results to other populations. Furthermore, we did not excluded patients with CKD from the study and it is known that chronic kidney disease (CKD) has, in itself, a deleterious effect on cell membranes. One should also consider the variability of the results obtained by the BIA depending on the model of the equipment used and the applied frequency (multifrequency, unifrequency, low or high frequency). Finally, considering that this study included a sample of critically ill patients, who may have PA values affected by the inflammatory process\textsuperscript{32}.

**CONCLUSIONS**

The PhA had good results of sensitivity and specificity, but should be used with caution to determine the nutritional risk in critically ill cardiac patients, as it did not detect the risk in approximately 30% of the individuals. Although it is important to consider that patients with CKD on hemodialysis were included in the study.

It is suggested, therefore, that PhA should not be used in isolation as a tool for screening nutritional risk and carrying out other studies to reach more definitive conclusions.

**AUTHORS’ CONTRIBUTIONS**

DT.D.M., I.G.R. and C.P.S.P. contributed to design conception of the study; DT.D.M., I.G.R. and J.G.M.M.J. developed data collection; C.P.S.P. interpretative analysis of the results; DT.D.M. writing the manuscript. All authors review of the manuscript.

**FUNDING**

This project did not receive any financial support.
CONFLICTS OF INTEREST

The authors state that there are no conflicts of interest in preparing the manuscript.

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