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Irregular red blood cell antibodies, abnormal hemoglobin and dangerous universal blood donor insights from a public blood center in a Brazilian metropolitan area

Laiane da Silva Santos [a,b], Sérgio Eduardo Soares Fernandes [b,c], Anna Luiza Oliveira Sant'Anna [d], Flávio Ferreira Pontes Amorim [d], Felipe Ferreira Pontes Amorim [e], Fábio Ferreira Amorim [a,b,c,*]

[a] Graduation Program in Health Sciences of School Health Sciences, Escola Superior de Ciências da Saúde (ESCS), Brasília, Federal District, Brazil
[b] Brasília Blood Center Foundation, Federal District, Brazil
[c] Medical School, Escola Superior de Ciências da Saúde (ESCS), Brasília, Federal District, Brazil
[d] Medical School, Universidade Católica de Brasília (UCB), Brasília, Federal District, Brazil
[e] Medical School, (ESCS), Centro Universitário do Planalto Central Apparecido dos Santos (UNICEPLAC), Brasília, Federal District, Brazil

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ABSTRACT

Background: Immunohematology tests are crucial in transfusion safety. This study aimed to assess irregular red blood cell (RBC) antibodies, abnormal hemoglobin and dangerous universal blood donors at a public blood center in a Brazilian metropolitan area.

Methods: A cross-sectional study included all consecutive blood donors from January 2018 to December 2021 at the Brasília Blood Center Foundation, Federal District (FD), Brazil.

Results: Among 205,965 blood donations, irregular RBC antibodies were found in 743 (0.4 %). Abnormal hemoglobin was observed in 5396 (2.6 %): 3959 (1.9 %) with Hb AS, 1344 (0.7 %) with Hb AC, and 93 (< 0,1 %) with other hemoglobin variants. Of O group donors, 12.5 % (9646) had hemolysins: 12.5 % (2410) both anti-A and anti-B, 8.7 % (9646) only anti-A, and 1.6 % (1763) only anti-B hemolysins. Female sex ($p < 0.001$) and increasing age ($p < 0.001$) were associated with irregular RBC antibodies. O and/or Rh(D)-positive blood groups had a lower prevalence of irregular RBC antibodies compared to other ABO and/or Rh(D)-negative groups. Age ($p < 0.001$) and female sex ($p < 0.001$) were associated with anti-A/anti-B hemolysins, while FD residency was associated with reduced incidence ($p < 0.001$).

Conclusion: Anti-A/anti-B hemolysins in O group donors, abnormal hemoglobin and irregular RBC antibodies pose risks to transfusion practice and should not be overlooked. Advancing age, female sex, ABO blood group other than O, or Rh(D)- negative are independently associated with the presence of irregular RBC antibodies. Dangerous universal blood donors were associated with advanced age, female gender, Rh(D)-positive blood type, and individuals residing in a Brazilian state other than where the blood center was located.

1. Introduction

Blood transfusion is an integral aspect of patient care and serves as a potentially life-saving intervention [1,2]. Approximately 118.5 million blood donations are gathered annually, catering to diverse clinical needs across various medical conditions [3]. However, it is crucial to recognize that blood cells and plasma proteins, being foreign components, can elicit an immune response in the recipient. Notably, plasma, antibodies, and various immune mediators may engage recipient cells, introducing the inherent risks of immunologic reactions associated with blood transfusions. To address this, multiple layers of safety measures are imperative, striving toward the aspirational goal of achieving zero risk for recipients of blood transfusions [3–5].

In addition to screening for transfusion-transmitted infections (TTIs), the process of blood transfusion therapy involves multiple steps to select safe blood components for transfusion, ensuring complete ABO

* Correspondence to: Coordenação de Pesquisa e Comunicação Científica - Escola Superior de Ciências da Saúde, SMHN Quadra 03, conjunto A, Bloco 1 - Edifício FEPECS, CEP: 70710-907 Brasília, DF, Brazil.
E-mail address: ffamorim@gmail.com (F.F. Amorim).

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compatibility and the absence of any clinically significant irregular red blood cell (RBC) antibodies, including alloantibodies and autoantibodies [5–7]. Transfusions from donors carrying abnormal hemoglobin, such as Hemoglobin C trait (Hb AC) and sickle cell trait (Hb AS), can be harmful to specific patients, especially those with hemoglobin disorders, severe acidosis, newborns, or those undergoing exchange transfusions, and intrauterine transfusions [8–10]. Additionally, assessment of anti-A and anti-B hemolysins in O blood group donors is recommended to prevent transfusion reactions [11].

Ensuring a safe and sufficient blood supply is an integral part of each country's national healthcare policy and infrastructure, constituting a vital component in the pursuit of universal health coverage. In this respect, national blood systems should rely on a consistent and secure supply of safe blood, primarily sourced from regular, voluntary, and unpaid blood donors [3,4]. In Brazil, the scenario of blood donations was predominantly characterized by remuneration and engagement with private blood centers until the early 1980s, leading to significant challenges in blood banking policies and practices [12,13]. With the onset of the acquired immunodeficiency syndrome (AIDS) pandemic, the Brazilian Ministry of Health ensured blood safety as a national security concern. A comprehensive national program was enacted to improve transfusion safety and enhance the quality standards of blood and its components, including prohibiting remunerated blood donations. Public blood centers have also been established across all Brazilian states [12–14]. Annually, over 3 million blood donations are gathered in Brazil, with 70 % originating from the public healthcare system [13,15]. Brazilian public blood centers routinely conduct immunohematology tests encompassing ABO and Rh(D) blood typing, abnormal hemoglobin screening, and irregular RBC antibody testing. In addition to these, donors with the O blood group undergo testing for anti-A and anti-B hemolysin [16,17].

The primary objective of this study was to evaluate the frequency of irregular RBC antibodies, abnormal hemoglobin, and dangerous universal donors at a public blood center in a Brazilian metropolitan area. The secondary purpose was to evaluate factors associated with irregular RBC antibodies and dangerous universal donors.

2. Methods

2.1. Study design

A cross-sectional study including all consecutive blood donors from January 2018 to December 2021 at the Brasília Blood Center Foundation (BBCF), Federal District (FD), Brazil. Data were retrospectively obtained from the electronic medical record and the BBCF database of blood donations (SistHemo).

2.2. Settings and participants

The study included all blood donors at the BBCF from January 2018 to December 2021. No exclusion criteria were applied. The DF encompasses a metropolitan area with a population of 2,469,489, encompassing Brasilia, the capital of Brazil. The BBC serves as the unique blood center responsible for collecting, testing, and processing all blood donations within the Federal District (FD) public healthcare system, including 16 public hospitals. According to the BBCF standard blood donation requirements, all blood donors are volunteers aged 16 or over and weigh more than 50 kg. Candidates for blood donation were submitted to a rapid and pre-donation interview and physical examination. Prospective blood donors underwent a pre-donation interview and physical examination. All blood units were screened for irregular RBC antibodies and abnormal hemoglobin. Additionally, O-group donated blood was tested for anti-A and anti-B hemagglutinins.

2.3. Data collection

The variables collected from the electronic medical record and the SistHemo were age, sex, city of residence, blood donation date, ABO and Rh(D) blood type, presence of abnormal hemoglobin, and irregular RBC antibodies. For O-group blood donors, anti-A and anti-B hemolysins tests were also collected.

For the determination of ABO and Rh(D) blood type, the microplate hemagglutination technique was employed utilizing an automated instrument, the Galileo NEO (Immucor, Rödemark, Germany). Additionally, reverse grouping (serum grouping) methods were used.

Irregular RBC antibodies were screened using the Capture-R Ready Screen (pooled Cells) test (Immucor Inc., Atlanta, USA) on an automated instrument, Galileo NEO (Immucor Inc., Rödemark, Germany).

For anti-A and anti-B hemolysin tests in O-group donated blood, 60 μL of blood sera were incubated in microplates with a 20 μL aliquot of a 3 % commercial suspension of RBCs (Revercel MAG A1 and B - Fresenius Kabi, São Paulo, Brazil) for one hour at 37 °C. Anti-A and/or anti-B hemolysins were considered positive if hemolysis in A and/or B red blood cells was observed after homogenization.

For abnormal hemoglobin screening, ion exchange-high performance liquid chromatography (HPLC) was performed on Variant II Turbo (Rio-Rad, São Paulo, Brazil).

2.4. Statistical analysis

The analysis of continuous variables distribution and normality was conducted through the Shapiro–Wilk test. Quantitative data are presented as either mean ± standard deviation (SD) or median and interquartile range (IQR 25–75 %), while categorical variables are represented as numbers and percentages (%).

Students' t-test or the Mann–Whitney test was employed to compare quantitative variables, depending on appropriateness. Categorical variables were analyzed using contingency tables, and the statistical tests employed were Pearson's chi-square test ($\chi 2$) or Fisher's exact test, depending on appropriateness.

To evaluate independent factors associated with irregular RBC antibodies and O blood group anti-A and/or anti-B hemolysins, non-collinear variables associated with the outcome with a p-value < 0.05 in the univariate analysis and the confounding factors according to previous knowledge with a p-value < 0.20 in the univariate analysis were assessed using enter method binary logistic regression analysis. Acceptance of non-collinearity was based on a tolerance level higher than 0.10 and a variance inflation factor (VIF) lower than 10.0.

Statistical analyses were performed using IBM Statistical Package for the Social Sciences version 20.0 for Mac (SPSS 20.0 Mac, SPSS Inc., Chicago, Illinois, USA) and Jamovi 2.3.24 (https://www.jamovi.org). The level of statistical significance was defined as a two-sided P-value ≤ 0.05.

The Ethics Committee of the Education and Research Foundation of Health Sciences (FEPECS), Brasília, Federal District, Brazil, approved the study that was conducted following the Declaration of Helsinki.

3. Results

A total of 205,965 blood donations were included. The mean age of blood donors was 33.2 ± 10.8 years, and 181,409 (48.4 %) were females. The assessment of the ABO blood group system observed that the O blood group was the most prevalent (110,450/205,965, 53.6 %), succeeded by the A blood group (67,430/205,965, 32.7 %), B blood group (21,574/205,965, 10.5 %), and AB blood group (6511/205,965, 3.2 %). Most blood donors were Rh(D)-positive (177,120/205,965, 86.0 %). Irregular RBC antibodies were detected in 743 blood donations (0.4 %). Abnormal hemoglobin was observed in 5396 (2.6 %) blood donations: 3959 (1.9 %) with Hb AS, 1344 (0.7 %) with Hb AC, and 93 (< 0,1 %) with other hemoglobin variants, Table 1.

Table 1
Demographic characteristics, ABO and Rh(D) blood type, abnormal hemoglobin and irregular red blood cell (RBC) antibodies among blood donors from January 2018 to December 2021 (n = 205965).

Variable	Value
Age, years,	
mean (SD)	33.2 (10.8)
median (IQ 25–75 %)	32.0 (24.0-40.0)
Female, n (%)	84,409 (48.4)
Residence in the Federal District, n (%)	181,029 (87.9)
ABO blood group, n (%)	
O	110,450 (53.6)
A	67,430 (32.7)
B	21,574 (10.5)
AB	6511 (3.2)
Rh(D) Factor, n (%)	
Rh(D) positive[a]	177,120 (86.0)
Rh(D) negative	28,845 (14.0)
Blood type, n (%)	
O positive	94,198 (45.7)
A positive	58,957 (28.6)
B positive	18,466 (9.0)
O negative	16,252 (7.9)
A negative	8473 (4.1)
AB positive	5499 (2.7)
B negative	3108 (1.5)
AB negative	1012 (0.5)
Irregular RBC antibodies, n (%)	753 (0.4)
Abnormal hemoglobin, n (%)	5396 (2.6)
Sickle cell trait (Hb AS)	3959 (1.9)
Hemoglobin C trait (Hb AC)	1344 (0.7)
Hemoglobin D trait (Hb AD)	1 (< 0.1)
Hemoglobin SC (Hb SC)	1 (< 0.1)
Others	91 (< 0.1)

IQ25–75 %: interquartile range 25–75; SD: standard deviation.
[a] One blood donor had a serological weak D phenotype.

Among O blood group donors, 9646 (12.5 %) had hemolysins in their sera: 2410 (12.5 %) both anti-A and anti-B hemolysins, 9646 (8.7 %) only anti-A hemagglutinin, and 1763 (1.6 %) only anti-B hemolysins, Table 2.

Table 3 presents the univariate and multivariate analysis of factors associated with irregular RBC antibodies. Female sex (OR: 3.260, 95 % CI: 2.780–3.816, $p < 0.001$), and increasing age (OR: 1.020, 95 % CI: 1.010–1.026, $p < 0.001$) were independently associated with increased likelihood of irregular RBC antibodies in the donated blood. Blood donors with O and/or Rh(D)-positive blood groups had a lower prevalence of irregular RBC antibodies in the donated blood than donors with other ABO and/or Rh(D)-negative blood groups.

Table 4 presents the univariate and multivariate analysis of factors associated with anti-A and/or anti-B hemolysins among O blood group donors. Age (OR: 1.022, 95 % CI: 1.020–1.024, $p < 0.001$) and female (OR: 1.301, 95 % CI: 1.255–1.349, $p < 0.001$) were independently associated with increased occurrence of anti-A and/or anti-B hemolysins, while residence in the FD with reduced incidence (OR: 0.899, 95 % CI: 0.852–0.945, $p < 0.001$).

4. Discussion

Our study, which included a large sample of non-remunerated blood donors at a public blood center in Brazil, showed a prevalence of 0.4 % for irregular RBC antibodies, consistent with the range reported in previous studies [5,7,18–21]. Additionally, our findings revealed a 2.6 % prevalence of abnormal hemoglobin. Notably, more than one in ten O blood group donors exhibited anti-A and/or anti-B hemolysins, a finding that mirrors the prevalence of dangerous universal donors observed in studies conducted in the Brazilian state of São Paulo [11,22].

Table 2
Prevalence of anti-A and anti-B hemolysins among O blood group donors from January 2018 to December 2021 (n = 110,450).

Hemolysin type, n (%)	
Total	13,819 (12.5)
Anti-A	9646 (8.7)
Anti-B	1763 (1.6)
Anti-A and B	2410 (2.2)

Although RBC antibodies may be involved in acute and delayed hemolytic transfusion reactions, few large studies have assessed the prevalence of these antibodies in healthy blood donors [23]. In our study, we found a prevalence of 0.4 % for irregular RBC antibodies. Noteworthy previous studies reported a prevalence of irregular RBC antibodies from 0.05 % to 0.51 % in donated blood [5,7,18–21]. A study including blood donors from four US blood centers reported that 0.51 % of donated blood exhibited positivity in the irregular RBC antibody screen [23]. Studies in India observed a prevalence of irregular RBC antibodies between 0.05 % and 0.36 % [5,7,18–21].

In our study, we observed an association between increased irregular RBC antibodies and advancing age, female sex, Rh(D)-negative, and ABO blood group other than O. This finding is an expected observation since prior transfusion, which increases with aging, and antigenic exposure during pregnancies are risk factors for RBC alloimmunization [5,19–21,24–27]. A study including blood donors from four US blood centers also reported a high incidence of irregular RBC antibodies linked to advancing age, Rh(D)-negative, and being female [23]. Interestingly, a study in India showed a higher alloimmunization among males than females. However, this finding might be influenced by the low frequency of donations made by women in the study [20].

Our study observed a 2.6 % prevalence of abnormal hemoglobin among the blood donations, with Hb AS being the most prevalent (1.9 %). Although transfusions from donors carrying abnormal hemoglobin can potentially harm specific patients, notably those with hemoglobin disorders, severe acidosis, newborns, exchange transfusions, and intrauterine transfusions, there is no global standard practice to screen donated blood for abnormal hemoglobin systematically. In the United States, there is a lack of nationwide directives advocating the routine screening of donated blood for hemoglobinopathies, leading to policy variations across institutions [5–7,10]. Conversely, in Brazil, the Hb AS has a prevalence of 4 % in the general population, and a study has estimated that one in every six newborns has abnormal hemoglobin [10,28]. In this respect, the country implemented a national abnormal hemoglobin screening in donated blood in 2005 [10]. Previous Brazilian studies reported a varying prevalence of abnormal hemoglobin, ranging from 1.4 % to 3.4 % among donated blood [29–31]. A study including first-time blood donors in Brazil exhibited a prevalence of Hb AS of 1,8 %, similar to the prevalence of Hb AS observed in our study [10]. It is crucial to emphasize that the frequency of abnormal hemoglobin in blood donations displays significant regional variability across Brazil, influenced by the demographic characteristics and ancestry of the population. For instance, an analysis of 101,000 blood samples from 65 cities spanning all Brazilian regions revealed that the prevalence of Hb AS was higher in the northern region (4.5 %) and gradually decreased towards the south: northeast (4.0 %), midwest (3.1 %), southeast (1.9 %), and south (1.9 %) [31].

In our study, the prevalence of anti-A and/or anti-B hemolysins (12.5 %) mirrors the frequency of dangerous universal donors observed in studies conducted in the state of São Paulo, Brazil (12.4 %, 13.1 %, and 13.6 %) [32–34]. These frequencies are lower than reported in other studies [11,22,35–37]. For instance, a study conducted in Minas Gerais, another Brazilian state, observed a significantly higher prevalence, with 30.5 % of O blood group individuals classified as dangerous universal donors [11]. Additional studies in diverse regions have reported varying prevalence rates [22,37–41]. In South Texas, USA, a study of 3274 male Group O donors found that 13.0 % had high-titer antibodies [40]. In Boston, a study evaluating platelet apheresis O blood group donors revealed that 25.0 % of the components were classified as high titers

Table 3
Univariate and multivariate analysis of factors associated with a presence of irregular red blood cell (RBC) antibodies (n = 205,965).

Variable	Present (753)	Absent (205,212)	p-value	Multivariate OR (95 % CI)	Multivariate p-value
Age, mean (SD)	34.6 (10.5)	33.2 (10.8)	< 0.001	1.012 (1.010–1.025)	< 0.001
Female, n (%)	530 (70.4)	89,948 (43.8)	< 0.001	3.250 (2.774–3.807)	< 0.001
Residence in the FD, n (%)	675 (89.6)	180,356 (87.9)	0.141	-	-
ABO blood group, n (%)			< 0.001		
A	263 (34.9)	67,167 (32.7)		1.559 (1.254–1.939)[a]	< 0.001
B	106 (14.1)	21,468 (10.5)		1.281 (1.091–1.504)[a]	0.003
AB	35 (4.6)	6476 (3.2)		1.701 (1.200–2.411)[a]	0.003
O	349 (46.3)	110,101 (53.7)		-	-
Rh(D)-positive, n (%)	562 (74.6)	176,558 (86.0)	< 0.001	0.448 (0.4136–0.576)	< 0.001
Abnormal hemoglobin, n (%)	24 (3.2)	5372 (2.6)	0.329	-	-

95 % IC: 95 % confidence interval; FD: Federal District; OR: odds ratio; SD: standard deviation.
[a] The reference group is O blood type donors.

Table 4
Univariate and multivariate analysis of factors associated with anti-A and/or anti-B hemolysins among O blood group donors from January 2018 to December 2021 (n = 110,450).

Variable	Present (13,819)	Absent (96,631)	Univariate p-value	Multivariate OR (95 % CI)	Multivariate p-value
Age, mean (SD)	35.3 (10.8)	32.9 (11.0)	< 0.001	1.022 (1.020–1.024)	< 0.001
Female, n (%)	6685 (48.4)	42,263 (43.7)	< 0.001	1.301 (1.255–1.349)	< 0.001
Residence in the FD, n (%)	12,040 (87.1)	85,082 (88.0)	0.002	0.899 (0.852–0.945)	< 0.001
Rh(D)-positive	11,904 (86.1)	82,294 (85.2)	0.002	1.118 (1.061–1177)	< 0.001
Abnormal hemoglobin, n (%)	356 (2.6)	2579 (2.7)	0.526	-	-

95 % IC: 95 % confidence interval; FD: Federal District; OR: odds ratio; SD: standard deviation.

[38]. In Nigeria, a study reported notably elevated prevalence (55.4 %) of anti-A and/or anti-B hemagglutinins in O blood group donors, primarily consisting of both anti-A and anti-B hemagglutinins (32.5 %) [22]. In a Cameroon study, hemolysin prevalence was reported as 52.1 %, with significant hemolysin titers detected in 18.5 % of cases [37]. A study in Thailand found a frequency of 75.7 % of dangerous universal blood donors due to IgM anti-A antibodies, 93.0 % due to IgG anti-A, 80.0 % due to IgM anti-B antibodies, and 95.3 % due to IgG anti-B antibodies [41]. In Malaysia, a study showed that 76.5 % of O blood donors had high anti-A and/or anti-B antibody titers [39]. Notably, the prevalence of anti-A and anti-B hemagglutinins largely depends on the ethnic background and environmental factors associated with the studied population [22,35]. In Japan, titers of anti-A and anti-B antibodies exhibited a consistent decrease between 1986 and 2001, aligning with dietary patterns [42].

Among O blood group donors, an increased prevalence of anti-A and/or anti-B hemagglutinins was independently associated with advancing age, being female, Rh(D)-positive, and residence in another Brazilian state than FD in our study. Parallel findings were observed in other studies, noting an association between a high frequency of dangerous universal blood donors and being female [11,34]. In Malaysia, a study also reported a high titer of anti-A and anti-B hemagglutinins among women. However, in contrast to our results, some studies found that young ages were associated with high antibody titers [39]. Other studies conducted in different countries did not observe associations between anti-A and anti-B hemagglutinins and age [22,36,37], sex [22,36,37], and Rh(D) blood group [35,37]. It is worth noting that most of these studies did not apply multivariate analysis to adjust their results for confounding factors, and the discrepancy in findings may be explained by differences in diet, lifestyle, socioeconomic status, ethnicity, and environmental factors [43,44].

The present study has some limitations. First, the retrospective design of the study. Second, the study included patients from only one center, with intrinsic limitations for generalizing the results to another setting. Third, we cannot rule out that the difference in outcomes among the groups may have been affected by factors not evaluated in our study. Despite these limitations, our study demonstrated the importance of immunohematology tests in donated blood, which are straightforward examinations that add a layer of safety to transfusion practices. New technologies, programs, and quality systems adopted by blood transfusion services have significantly reduced the risk of infectious and noninfectious complications, greatly enhancing transfusion safety in recent times. However, inherent risks of immunologic reactions associated with blood transfusions persist [11,23,35]. Furthermore, it is essential to highlight the significance of screening for hemoglobinopathies in first-time blood donors, as many recipients may also have hemoglobin disorders or other conditions that increase the risk of transfusing blood containing abnormal hemoglobin [8]. Given the variability in prevalence rates of anti-A and anti-B hemolysins among O group donors, as well as irregular RBC antibodies and abnormal hemoglobin across different regions and populations, blood transfusion services should consider implementing enhanced screening protocols, especially in regions with higher prevalence rates [8,35,42]. They should also engage in continuous monitoring and evaluation of blood donations to track changes in prevalence over time and identify emerging trends that may necessitate adjustments to screening protocols [38]. For example, regions with a higher prevalence of irregular RBC antibodies, abnormal hemoglobin, and potentially dangerous universal blood donors may require more frequent and comprehensive screening. Additionally, a unified computerized registry across national blood systems may allow the identification of blood donors who have had irregular RBC antibodies and abnormal hemoglobin and O group donors with anti-A and/or anti-B hemolysins. By implementing these recommendations, blood transfusion services can enhance the safety and efficacy of transfusions for patients in need. Besides, the screening for conditions, such as hemoglobinopathies, allows for informing donors about their health status [29].

5. Conclusion

Anti-A and/or anti-B hemolysins in O blood group donors, irregular RBC antibodies, and abnormal hemoglobin pose risks to transfusion practice, and their occurrence should not be neglected. Abnormal hemoglobin was observed in 2.6 % of blood donations, while irregular RBC

antibodies were detected in 0.4 %. Individuals with advanced age, female sex, ABO blood group other than O, or Rh(D)-negative blood type had an increased risk of irregular RBC antibodies in donated blood. More than one in ten O blood group donors (12.5 %) had anti-A and/or anti-B hemolysins, and this finding was associated with advancing age, female sex, Rh(D)-positive blood type, and residence in a Brazilian state other than where the blood center. Immunohematology tests in donated blood are straightforward examinations that add a layer of safety to transfusion practice, mitigating the potential risk of adverse events during transfusions.

Ethics Approval

This study was approved by the Ethics Committee of the Fundação de Ensino e Pesquisa em Ciências da Saúde (FEPECS), Brasília, Federal District, Brazil, and was conducted following the Declaration of Helsinki.

Data sharing

Relevant data can be available after a reasonable request to the corresponding author.

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CRediT authorship contribution statement

Fabio Ferreira Amorim: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Felipe Ferreira Pontes Amorim:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation. **Flavio Ferreira Pontes Amorim:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation. **Anna Luiza Oliveira Sant'Anna:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation. **Sérgio Eduardo Soares Fernandes:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laiane da Silva Santos:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of interests

The authors report no conflicts of interest in this work.

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