Prevalence of Trypanosoma Cruzi Infection in Blood Donors

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Abstract

Background: Chagas disease (CD) is considered a public health problem in Latin America. The northeastern region, especially the state of Ceará, still represents a major concern in terms of the risk of transmission of CD.

Objective: To estimate the prevalence of T. cruzi in blood donors from the state of Ceará.

Methods: This is a retrospective descriptive study that was performed in the period from 2010 to 2015 from data recorded in the computerized system of the Hematology and Hemotherapy Center of Ceará (HEMOCE in Portuguese).

Results: Of the 763,731 potential blood donors, 14,159 were serologically ineligible; 1,982 (0.33%) were serologically ineligible due to positive / inconclusive diagnosis for CD. A total of 425 individuals came to the HEMOCE to repeat the test, with 28.2% (120/425) declared ineligible for donations due to CD.

Conclusion: No significant reduction of positive / inconclusive serology was observed in the period between 2010 and 2015, but a reduction was observed when compared to 1996/1997 in the state. The determination of the prevalence of CD in blood banks may be relevant as an indicator of the risk of CD transmission through blood transfusions in a given region. New serological tests for triage with better accuracy in screening are needed, in an attempt to reduce the unnecessary disposal of blood bags, reduce costs for the Brazilian Unified Health System, and diminish insecurity for the patient and family members. (Arq Bras Cardiol. 2020; 115(6):1082-1091)

Keywords: Chagas Disease/complications; Chagas Disease/epidemiology; Trypanosoma Cruzi; Blood Banks; Sorologic Tests.

Introduction

Chagas disease (CD) is considered to be a public health problem in Latin America. This disease used to be exclusive to the Americas, but, in recent decades, it has spread to other continents, due to the internalization of the disease caused by the migration of people from endemic regions to these locations. In Brazil, it is estimated that there are some two to three million infected people.2

In the northeast region of Brazil, the state of Ceará is one of the states in which there is still a great concern in terms of the transmission of CD. This concern is due to three main factors: the region is still, from a social point of view, quite needy. It also has the highest indices of homes conducive to the colonization of triatominae bugs, along with the low operational level of Chagas Disease Control Program (CDCP) throughout Brazil.3,4

As regards CD transmission, the two most important epidemiological forms are the vectorial and blood transfusions.2,6 As of 1940, the practice of blood transfusions became widespread throughout Latin America, which contributed of the risk of CD by blood transfusion.7-12 In this light, blood transfusions began to take on epidemiological importance as of 1944 and became technically approved in 1951. In 1969, the obligation of serological triage for CD was instituted in blood banks in Brazil. This action was established by the Brazilian Health Ministry in an attempt to control and increase safety against related diseases.13

Governmental decree number 158, ratified on February 04, 2016, by the Health Ministry, considers an ineligible donor to be those who have had home contact with the triatominae in an endemic area, and those with a clinical or laboratorial diagnosis for CD. In addition, in Article 130 of the same governmental decree, the conducting of serological tests of high sensitivity for CD became mandatory in every blood donation.14

Therefore, the present study sought to estimate the prevalence of T. cruzi in blood bank donors in the state of Ceará, given that the region has scarcely been explored. The results of the present study will serve as a warning to the epidemiological surveillance agency teams, so that they may establish measures for the prevention, treatment, and follow-up of individuals infected by Trypanosoma cruzi as well as for individuals who live in high-risk areas.
Metodology

Study design, location, and sample

This work is a retrospective descriptive study conducted through the collection of data recorded in the information technology (IT) system of the Hematology and Hemotherapy Center of the state of Ceará (HEMECE in Portuguese) of all of the potential blood donors of the state’s Public Blood Bank Network from 2010 to 2015 (Figure 1). The donors’ personal data were preserved, and these were identified by their registration number to guarantee confidentiality.

The state’s Public Blood Bank Network consists of a Blood Bank Coordinator, with its headquarters located in the city of Fortaleza; four Regional Blood Banks, located in the municipalities of Sobral, Quixadá, Crato, and Iguatu; one Blood Bank Nucleus, in Juazeiro do Norte; one Blood Bank Collection Clinic at the Dr. Jose Frota Institute (IJF in Portuguese); and 64 Blood Transfusion Agencies, located in the hospitals attended to by the Blood Bank Network of Fortaleza and the municipalities throughout the countryside of Ceará. Each blood bank is responsible for receiving blood donors and patients in the coverage area, with jurisdiction to conduct all of the blood cycle steps, with the exception of serology, which, for than a decade, has been centralized in the Blood Bank of Fortaleza, where the data referent to the blood donors was collected.

The serological triage for CD during the study period was performed by the chemiluminescence technique, which consists of the interaction of the antibodies present in the serum of the infected patients with antigenic epitopes present in the plaque, which, after incubation of biotinylated anti-gamma globin and streptavidin conjugated with the enzyme, in the presence of a luminal reagent, are capable of emitting light.\(^\text{15,16}\)

If the result is positive or inconclusive, the test is repeated in duplicate in the same sample. If the result remains positive or inconclusive, the patient is called to the blood bank service for a second blood collection. After the collection, the triage test (chemiluminescence) is again performed, and, if the result is positive or inconclusive in the repetition of the test, a confirmatory test will be performed and the donor is already definitively refused, even if the confirmatory test is negative. The confirmatory test must be a highly specific test, with the HEMOCE using indirect immunofluorescence (IIF) or western blot,\(^\text{15}\) depending on the bidding processes, since this is a governmental agency.

The IIF consists of a detection of antibodies in the infected serum, which, when incubated upon a slide with fixed \(T. cruzi\) antigens, connect themselves, forming the antigen-antibody compound, and which are revealed by a human anti-gamma globulin marked with fluorescein isothiocyanate. The western blotting test consists of a method in molecular biology and biochemistry to detect proteins in a homogenate or a biological tissue extract. This technique uses a gel electrophoresis to separate the denatured proteins by mass. The proteins are then transferred from the gel to a nitrocellulose membrane, where they are used as specific protein antibody probes.\(^\text{15}\)

If the result is positive or inconclusive in the confirmatory test, the patient is referred to the medical clinic of the Walter Cantidio University Hospital or to Messejana Hospital, which are reference units for the clinical follow-up of individuals with chronic CD in the state of Ceará (Figure 2).

Data analysis

The occurrence of ineligible donors due to CD was calculated, and a descriptive statistical analysis was conducted from the absolute and relative frequencies. For the epidemiological characterization of individuals who are blocked from donating blood due to a confirmed diagnosis of CD, the following parameters were considered: gender (male and female), age range (18 – 29 years of age and over 30 years of age), profession, hometown, and city from which the individual came (Fortaleza or other municipality). The descriptive analysis was performed using Microsoft Excel, version 2013.
Ethical aspects

The present study was approved by the Research Ethics Committee of the Federal University of Ceará (COMEPE-UFC in Portuguese), logged under protocol number 1.482.674, CAAE: 53833816.5.0000.5054, which was judged according to the guidelines for research with human beings from the National Health Council (Resolution CNS 466/12).

Results

During the period of the study (January 2010 to December 2015), the state’s Public Blood Bank Network of the state of Ceará received a total of 763,731 potential blood donors. Of these, 155,378 (20.3%) were excluded in the clinical triage, and among the reasons for exclusion were: anemia, hypertension, alcoholism, drug use, high-risk behavior for sexually transmitted diseases, hepatitis, malaria, CD, among other causes. This study rejected 38 (0.02%) potential donors, of which 21 (55.2%) were men and 17 (44.8%) were women. Hence, the research on antibodies against T. cruzi (Serological triage) was conducted with 608,353 (79.6%) individuals.

During the period of the study, 14,159 blood bags were discarded, because they were believed to be inappropriate for donation due to positive serology for some type of disease traced at the blood bank, representing 2.32% of the total number of blood bags collected during the period. Of the 608,353 blood donor candidates approved in the clinical triage, 1,982 (0.32%) were considered ineligible for blood donation due to positive/inconclusive serology due to CD by means of the chemiluminescence test. Of these, 602 (30.37%) were positive and 1,380 (69.62%) were inconclusive (Table 1). The inconclusive results corresponded to 9.75% (1,380) of the discarded bags.

The results showed an increase of 16.35% in the number of potential donors, when compared to 2010 to 2015. As regards the prevalence per year, a reduction of 50% (0.34% in 2010, 0.17% in 2015) was observed.

To verify if CD is still present in historically endemic areas, the prevalence was analyzed in the blood banks of Sobral, Quixadá, and Iguatu, considering positive and inconclusive results. The blood bank of Sobral presented the highest prevalence for CD in 2010 at 0.57% (78/13,674). In 2011, blood bank of Quixadá also presented 0.55% (29/5312), and in 2012 this same blood bank presented 0.71% (43/6,075), and 0.56% (33/5,914) in 2013 (Figure 3).

The blood banks of Quixadá, Sobral, and Iguatu presented the highest indices of serum prevalence for infection by T. cruzi in the period studied – 0.51% (171/33,413), 0.42% (356/85,150), and 0.40% (140/35,286), respectively.

The years of 2012 and 2013 presented the highest percentages of blood donors declared ineligible due to CD, 0.47% and 0.49%, respectively, with a subsequent reduction in
the following years of 2014 and 2015, with the lowest values recorded of 0.19% and 0.17% (Table 1). Of the ineligible donors due to CD, 847 (42.73%) had already donated blood some time before the period of this study.

Of the 1,982 donors selected to repeat the test (positive or inconclusive diagnosis in the serological triage due to CD), 757 (38.19%) individuals did not return to the Blood Bank to repeat the test. Hence, 1,225 (61.8%) individuals repeated the test.

In the repetition of the chemiluminescent triage test, 92 individuals presented inconclusive results, while and 333 presented positive results. These sample reagents were submitted to the confirmatory test (IIF or Western blot). Of these, 305 individuals presented a negative confirmatory test, 48 an inconclusive result, and 72 a positive result (Table 2). Thus, 120 (28.23%) individuals were declared ineligible for blood donation due to CD.

Of these ineligible individuals due to CD (n=120), 78 (65%) were male, 100 were over 30 years of age, 100 (83.3%) were first-time donors, and 20 (16.7%) had already donated blood before. Forty-five (37.5%) of these individuals had completed high school and 32 (26.7%) had not completed elementary school, while 88 (73.3%) came from municipalities in the countryside of the state of Ceará and 62 (51.7%) came from the capital city of Fortaleza (Table 3).

From the point of view of blood banks, the disease continues to be the object of continuous surveillance, given that the population of donors encompasses a considerable number of individuals who have already lived in sanitary and environmental conditions that were favorable to the transmission of the disease, and some of these individuals still live in these conditions. Thus, epidemiological studies in hemotherapy services becomes important, not only because of the relevance in blood transfusions, but also as an option to evaluate the transmission of the disease in specific municipalities.

In the present study, the prevalence of CD was of 0.33% in the serological triage of the Public Blood Bank Network of the state of Ceará. Similar results were also found in other regions of the country, such as in the study conducted at the Blood Bank of the city of Ituiutaba, in the Triângulo Mineiro region, which detected that 0.23% of the candidates presented a positive serology for CD, while 0.27% presented inconclusive serological results in the period of 2011 to 2011.19 In the period from 1995 to 2009, a study was conducted in the Blood Bank of the Center Region of Uberaba (HRU in Portuguese) and a serum prevalence of 0.2% of ineligible donors due to CD was found.20

A bibliographic review conducted by Costa et al.21 analyzed the ineligibility of candidates for blood donation due to seropositivity for CD in different regions of Brazil (northeast, southeast, south). In this sense, the greater prevalence of the ineligible donors due to seropositivity for CD occurred in the Northeast, specifically in the city of Iguatu, CE, while in the southeast region, in the city of Patos de Minas, MG, the recorded percentages were of 1.90% and 1.20%, respectively. In the southern region of the country, the percentages of prevalence for ineligible donors due to seropositivity for CD varied between 0.40% and 0.47% in Porto Alegre and Pelotas, respectively.

The present study observed that the blood bank of Iguatu presented one of the highest prevalence of the studied period (0.41%); however, when compared to the findings from Costa et al.21 a reduction was observed in the prevalence of ineligible donors due to seropositivity for CD in Iguatu, CE, between 2010 and 2015.
The blood bank of Sobral presented one of the highest prevalence of the studied period when compared to the other blood banks of the state (Figure 3). These data can be explained by the fact that the city of Sobral is considered a high-risk region for vectorial CD. This municipality presents a unique eco-epidemiological scenario, characterized by foci of triatominae in informal urban settlements with low-quality housing. Species of native triatominae, such as *Triatoma brasiliensis* and *Triatoma pseudomaculata* can often be seen inside of houses, and are often infected with *T. cruzi*. These foci have the potential to develop acute cases of CD, illustrating that this region still represents a real concern for the population.

Santana et al. analyzed the positivity for CD among blood donors in Piauí between 2004 and 2013. The prevalence of the reagent serology for CD in the triage of blood donors was greater than that observed in the present study, at approximately 1%. Only 34.5% of the positive samples in the triage were referred for complementary tests. In the confirmatory tests, 84.4% presented negative results.

In the state of Ceará, Silva et al. studied the seroprevalence for CD in public blood banks from 1996 to 1997, where 34,943 donors were observed, of which 377 (1.1%) presented seropositivity for CD. When compared to the present study, it could be observed that there was a reduction in the ineligibility of donors due to CD in the sorological triage that, until 2015,
The present study verified that many donors did not return for the repetition of the test, and this fact represents a limitation of the present study, as many potential donors did not perform the confirmatory tests. The non-return of the potential donors represents a loss of information, such as the more precise prevalence of CD in blood banks.

The blood bank network should formulate new strategies to recruit these individuals so as to confirm the diagnosis and, in the case of a negative result, allow them to become blood donors once again.\textsuperscript{11}

This loss of information is worrisome and can result from a combination of factors, among which is the place of origin of many donors. In addition to the difficulty of the donors travel from his/her city of origin to the capital, the letter of summons is an instrument that scares people, as it indicates that the result of the exam was not normal, and many end up not wanting to know the true result.\textsuperscript{15}

The present study observed that the majority of ineligible donors due to CD (positive/inconclusive) was of the male sex, over 30 years of age, from the capital city of Fortaleza, but born in the municipalities in the countryside of the state of Ceará. Although other studies also show this predominance of males in individuals diagnosed with CD, there is no positive correlation between the sex of the donor and the reagent serology for the disease, as it indistinctly affects both men and women, which can be justified by the fact that many men work in rural areas, with greater chances of coming into contact with the triatominae, as well as due to cultural differences in the practice of blood donations, which commonly attribute to the best donor role to the men.\textsuperscript{28,32-34}

As regards the place of origin, it is well-known that many knowingly infected individuals live or have lived in countryside regions that constitute the natural ecotopes of the insect vector and, later, many end up migrating to large urban centers, such as Fortaleza, fleeing from droughts and in search of jobs and opportunities.\textsuperscript{15} Despite the certificate of the eradication of CD by Triatoma infestans, it is important to clarify that this species was never found in the state of Ceará; therefore the risk of transmission of CD in Ceará is due to the most prevalent species of triatominae, the Triatoma brasiliensis and the Triatoma pseudomaculata. Thus, the state of Ceará still presents a high risk for the transmission of the disease.\textsuperscript{3,35-37}

### Table 2 – Blood donors considered ineligible in the serology triage and recruited to repreat the test and take the confirmatory test from 2010 to 2015 (n: 1982)

<table>
<thead>
<tr>
<th>YEAR</th>
<th>DID NOT RETURN</th>
<th>REPETITION NEG</th>
<th>REPETITION IND</th>
<th>REPETITION POS</th>
<th>TOTAL CONF. NEG</th>
<th>CONF. IND</th>
<th>CONF. POS</th>
<th>TOTAL</th>
</tr>
</thead>
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<td>2010</td>
<td>103</td>
<td>142</td>
<td>11</td>
<td>26</td>
<td>262</td>
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<td>13</td>
<td>47</td>
<td>310</td>
<td>49</td>
<td>11</td>
<td>11</td>
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<tr>
<td>2012</td>
<td>145</td>
<td>131</td>
<td>21</td>
<td>101</td>
<td>398</td>
<td>102</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>2013</td>
<td>160</td>
<td>235</td>
<td>18</td>
<td>54</td>
<td>467</td>
<td>41</td>
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<td>6</td>
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<tr>
<td>2014</td>
<td>105</td>
<td>80</td>
<td>20</td>
<td>39</td>
<td>244</td>
<td>45</td>
<td>7</td>
<td>19</td>
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<tr>
<td>2015</td>
<td>120</td>
<td>86</td>
<td>9</td>
<td>66</td>
<td>261</td>
<td>47</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>TOTAL</td>
<td>757</td>
<td>800</td>
<td>92</td>
<td>333</td>
<td>1,982</td>
<td>305</td>
<td>48</td>
<td>72</td>
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</table>

In addition to the vectorial form, the form of transmission has been especially attacking the northern and northeastern regions of the country. In the case of men, this transmission can occur in a sporadic manner, through the ingestion of food contaminated with the parasite or its feces.38

As regards the age range, during the period of study, a reduction in the number of younger infected individuals could be observed, which can be evaluated as a reflection of the vectorial control measures implemented in the state of Ceará. With the overall reduction of the incidence of the disease, the entrance of individuals diagnosed with CD became less frequent in the age group of people who donate blood, parallel to the progressive exit of infected individuals from the list of donors by age or by morbidity due to CD. However, it is important to highlight that the

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### Table 3 – Sociodemographic characteristics of the donors considered ineligible with confirmation of CD at the Ceará Public Blood Bank from 2010 to 2015

<table>
<thead>
<tr>
<th>Variables</th>
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<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
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<td>59.7</td>
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<td>73</td>
<td>78</td>
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<td>100</td>
<td>48</td>
<td>100</td>
<td>120</td>
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<td>≤ 30 years</td>
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<td>12</td>
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<td>75</td>
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<td>83.3</td>
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<td>100</td>
<td>120</td>
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<td>100</td>
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<td>100</td>
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<tr>
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<td>48</td>
<td>100</td>
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<tr>
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<td>100</td>
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<td>100</td>
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<td>100</td>
<td>120</td>
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</tr>
</tbody>
</table>

increase in the launching of blood donation campaigns generates a greater demand for people of highly varied age groups, making it difficult to identify a greater difference between the analyzed age ranges.19,40

Parallel to the reduction in blood donors diagnosed with CD in Ceará, what calls attention is the high proportion of inconclusive reactions. During the period of the study (2010-2015), we observed that, in the serological triage, 70.9% (1,380) of the results of ineligible donors due to CD were inconclusive, representing 5.99% of the total number of bags discarded during the period. Many studies demonstrate that the inconclusive reactions often represent more than 50% of the cases of donor eligibility due to seropositivity for CD in blood banks, with this rate being higher than 70% in some services.41,42

In Brazil, it is estimated that 60% of the occurrences of inconclusive reactions in the three million annual blood donations, of which 0.6% of the collected blood bags are discarded due to a positive serology for T. cruzi, that is, 10,800 bags are being discarded due to inconclusive serology.43 The significant number of inconclusive reactions is worrisome, especially since, in addition to the high costs caused by the disposal of blood bags, there is also the consequence that this brings to the donor, who is labeled as an individual diagnosed with a severe disease such as CD.

Discrepancies in the results of the serological tests occur quite often, sometimes questionable in a given text and positive or negative in another. The present study verified that 847 individuals had previously donated blood and such discrepancies become even more evident and conflicting in repeat donors, when more than a dozen repeatedly negative serological reactions in previous donations now present an inconclusive or eventually positive serology in a subsequent donation.44-45

The present study verified that of the 1,982 ineligible donors due to CD, 1,225 (61.8%) returned to repeat the test, and of these, only 72 (5.8%) were positive in the confirmatory test (IIF or western blot) and 48 (3.9%) were inconclusive. The occurrence of inconclusive reactions and false-positive reactions in the tests of serological triage show flaws in the specificity of the serological tests, as there may be many individuals co-infectected with other diseases, and therefore sensitized with other antigens, which can entail crossed reaction in the serological tests.

Consequently, many healthy individuals end up being labeled as CD carriers, when in fact they are not actually infected with the disease, leading to psychological, social, and economic issues for the donor who was excluded for wrongly being considered infected with CD, in addition to promoting the unnecessary disposal of blood bags in blood banks and important financial losses for the Brazilian Unified Health System.46

The data obtained in this study reflect the difficulties in the approach and guidance of donors with inconclusive serological reactions, who are almost all non-chagasic, as well as the indispensability of the implementation of measures that allow one to minimize, or even eliminate, the questionable or false-positive serological results for CD in serological triage tests.

The confirmatory tests present good sensitivity and specificity; however, the triage tests, such as chemiluminescence, present a high sensitivity, and it is well-known that tests with a high sensitivity have their specificities compromised, generating false-positive results. Due to this factor, it is important to implement more specific tests in the serological triage of blood donors.26,31,33

The determination of the prevalence of the disease in blood banks can be relevant as an indicator of the risk for CD in blood transfusions and of the level of transmission of the disease in a determined region, as well as allow one to evaluate the vectorial control indirectly, providing up-to-date information about the disease in the state.

In this sense, it is possible to trace local strategies that involve the efforts of all of the sectors related to the area, such as sanitation agencies, blood banks, and laboratories, unified in the effort to eliminate the transmission through blood transfusions and improve the quality of the transfused blood.

In addition, the results from this study warn of the need in the blood banks to introduce a complementary serological method that is more specific, in an attempt to minimize the unnecessary disposal of blood bags and consequently indicate the real values of the disease in blood donors. Hence, there is a need for studies that propose new measures for the improvement in the accuracy of the serological tests, which could consequently reduce the unnecessary disposal of blood bags, thus diminishing the costs for the Brazilian Unified Health System.

Conclusions

Of the potential donors in the studied period, 1,982 were considered ineligible donors due to a positive/inconclusive serology for CD. Confirmed as ineligible (positive or inconclusive) were 28.2% (120/425) due to CD. No significant reduction in positive/inconclusive serology was observed in the period between 2010 and 2015; however, a reduction was observed in relation to 1996/1997 in the state. The determination of the prevalence of CD in blood banks can be relevant as an indicator of risk for transmission through blood transfusion in a given region. New serology tests for triage, with a better accuracy, are necessary in order to reduce the unnecessary disposal of blood bags, reduce the costs for the Brazilian Unified Health System, and diminish the insecurity for patients and family members.

Author contributions

Conception and design of the research, Obtaining financing and Writing of the manuscript: Costa AC, Oliveira MF; Acquisition of data: Costa AC, Silva Filho JD, Fidalgo ASOBV, Comes VBAF, Oliveira MF; Analysis and interpretation of the data: Costa AC, Rocha EA, Silva Filho JD, Oliveira MF; Statistical analysis: Costa AC, Rocha EA, Silva Filho JD, Viana CEM, Oliveira MF; Critical revision of the manuscript for intellectual content: Costa AC, Rocha EA, Fidalgo ASOBV, Nunes FMM, Viana CEM, Oliveira MF.

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