Socioepidemiological screening of serologically ineligible blood donors due to Chagas disease for the definition of inconclusive cases

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Epidemiological screening combined with serological tests has become an important tool at blood banks for the characterization of donors with or without Trypanosoma cruzi *infection. Thus, the objective of the present study was to describe the sociodemographic and epidemiological characteristics of blood donors with non-negative serology for* T. cruzi *to determine possible risk factors associated with serological ineligibility. Sociodemographic and epidemiological data were collected by analysis of patient histories and interviews. The data were analyzed descriptively using absolute and relative frequencies and odds ratio (OR) evaluation. The frequency of serological ineligibility was 0.28%, with a predominance of inconclusive reactions (52%) and seropositivity among first-time donors (OR = 607), donors older than 30 years (OR = 3.7), females (OR = 1.9), donors from risk areas (OR = 4) and subjects living in rural areas (OR = 1.7). The risk of seropositivity was higher among donors who had contact with the triatomine vector (OR = 11.7) and those with a family history of Chagas disease (OR = 4.8). The results demonstrate the value of detailed clinical-epidemiological screening as an auxiliary tool for serological definition that, together with more specific and more sensitive laboratory methods, will guarantee a higher efficacy in the selection of donors at blood centres.*

Key words: blood donors - Chagas disease - serological ineligibility - inconclusive serology - socioepidemiological screening

Chagas disease continues to be a severe endemic disease that affects about eight million people in Latin America (OPS 2006). Most infected individuals are from rural areas and more than 60% of them have migrated to urban centres over the past six decades because of social and urbanization policies (Dias et al. 2002).

Since the 1980s when vector control programs in Brazil became effective and achieved broad coverage, vector transmission of Chagas disease has decreased, with a consequent gradual decline in the number of acute cases in the country (Dias 1993). A similar situation has been observed throughout Latin America, where the rural-urban migration, as well as effective combat against triatomine bugs, has led to the emergence of secondary mechanisms of Chagas disease transmission, including blood transfusion, organ transplantation and congenital transmission (Dias 2009).

On the other hand, the obligatory requirement for serological screening of blood donors and the accelerated increase in the coverage of serological testing (due to government initiatives or programs led by the Pan-American Health Organization, such as the Southern Cone Initiatives in 1991, the Central American countries in 1997 and the Andean countries in 1999) permitted a rapid and progressive reduction in the prevalence of chagasic infection among blood donors in Brazil and Latin America. In Uberaba, Chagas disease alone excluded 16.6% of blood donors at the end of the 1960s, a rate that fell to 0.31% at the beginning of the millennium (Moraes-Souza et al. 2006).

In contrast to these low rates, the greatest challenge currently encountered by blood banks is the frequent occurrence of inconclusive reactions, most of them observed among non-chagasic donors, a finding characterizing failure in the specificity of the tests used (Salles et al. 1996, Melo et al. 2009). Several investigators have proposed the inclusion of a clinical-epidemiological chart to assist in the investigation and definition of the true serological profiles of ineligible donors, especially those presenting inconclusive reactions (Salles et al. 1996, Reesink 2005, Furuchó et al. 2008). This chart includes questions regarding the place and type of residence, a family history of Chagas disease, a history of contact with the vector and a history of blood transfusion and surgery. This procedure has been proven effective in differentiating between donors with or without *Trypanosoma cruzi* infection when used simultaneously with serological and/or molecular biology tests (Salles et al. 1996, Furuchó et al. 2008) and will certainly provide an important tool for the selection and exclusion of blood donors at blood centres in countries or geographic regions where Chagas disease is not endemic.

In view of these considerations, the objective of the present study was to analyze the sociodemographic and epidemiological characteristics of blood donors with positive and inconclusive *T. cruzi* serology in an attempt to determine possible risk factors for Chagas disease and their association with serological ineligibility.

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SUBJECTS, MATERIALS AND METHODS

Screening tests used at the Uberaba Regional Blood Center (URBC)/Hemominas Foundation - At the URBC, blood donors are screened for Chagas disease using three different techniques: enzyme-linked immunosorbent assay (ELISA cruzi®, bioMérieux Diagnóstica SA, Rio de Janeiro, Brazil), indirect immunofluorescence (IFI Imunocruzi®, Biolab-Merieux Diagnóstica SA, Rio de Janeiro, Brazil) and indirect haemagglutination (Chagatest HAI®, Wiener Labs, Rosario, Argentina).

Such techniques were used by the Hemominas Foundation due to high sensitivity (95-100%) and specificity (99% and 100%) found by the manufacturers and in several other studies (Caballero et al. 2007).

In this study, we considered all 269 serologically ineligible blood donors (i.e., blood donors with positive and inconclusive serology) at URBC. Serology was considered positive when all three tests were repeatedly positive and inconclusive when discordant results were obtained with the techniques, i.e., positive results in one or two tests and negative results in the other one or two tests or ELISA absorbance values within the indeterminate zone, which in blood banks is considered to be ± 20% of the cut-off. The serology laboratory of URBC performed quality control for each batch of the three kits used for anti-*T. cruzi* serology. For this purpose, internal panels of positive and negative control sera were used, beyond those provided by the manufacturers, thus confirming the good performance of the tests and quality of the kits. External Quality Control was performed every six months using the serological panels from the Biomanguinhos-Fiocruz laboratory, Brazil.

Study population and data collection - The target population of this study was stratified based on a retrospective study of data from the database of the URBC conducted between January 2000-December 2005. The following data were obtained from the records of these donors: type of donor (first-time or repeat donor), gender, age (≤ 30 years and > 30 years), skin color, origin (born and/or lived in risk areas - rural area with a high rate of active infection in the past - or in urban areas with low or no incidence of the vector) and place of residence (rural or urban area).

Subsequently, ineligible donors were divided in five age groups, ≤ 20, 21-30, 31-40, 41-50 and > 50 years old, to assess a possible tendency for the percentage of positive using Pearson's correlation.

For clinical and epidemiological evaluation, a sample of 60 donors was selected from the 269 donors serologically ineligible due to Chagas disease (30 positive and 30 inconclusive) and a second sample of 30 donors was selected from donors with proven negative serology. This number was fixed in view of difficult access to ineligible donors, especially those ineligible before 2003, which led to sample loss. The main reasons for this loss were change of address and telephone number (30.4%), living more than 120 km from Uberaba (26.4%), refusal to participate in the study (7%) and death (2%). This group received detailed information about the objectives of the study and was invited to return to the Blood Center.

The control group (negative donors) was randomly selected among blood donors with more than five donations at URBC in the last six years (study period). When they appeared for donation, they were informed about the purpose of the study and invited to participate.

After signing a free informed consent form, the subjects answered a socioepidemiological questionnaire for the collection of sociodemographic data and specific information regarding risk factors for Chagas disease, such as residence in a risk area, a family history of Chagas disease and episodes of contact with the triatomine vector.

TABLE I

Total number of blood donations with negative serology and ineligible donors (positive and inconclusive serology) due to Chagas disease at the Uberaba Regional Blood Center according to sociodemographic characteristics

	Negative donations			Positive serology				Inconclusive serology			
Variables	n	%	OR	n	%	OR	CI (95%)	n	%	OR	CI (95%)
Donor type											
First-time	16,658	17.40	1	128	99.22	607[a]	84.9-4348.3	73	52.14	5.2[a]	3.7-7.2
Repeat	79,063	82.60	1	1	0.78	0.002[a]	0.0002-0.012	67	47.86	0.19[a]	0.14-0.27
Age											
≤ 30 years	45,339	47.37	1	25	19	0.27[a]	0.2-0.4	59	42	0.8	0.6-1.1
> 30 years	50,382	52.63	1	104	81	3.7[a]	(2.4-5.8)	81	58	1.20	0.9-1.7
Gender											
Male	69,541	72.65	1	76	59	0.5[a]	0.4-0.8	108	77	1.3	0.9-1.9
Female	26,180	27.35	1	53	41	1.9[a]	1.3-2.6	32	23	0.80	0.5-1.2
Skin color											
White	64,323	67.20	1	85	65.9	0.9	0.7-1.4	90	64.30	0.9	0.6-1.2
Non-white	31,398	32.80	1	44	34.1	1.1	0.7-1.5	50	35.70	1.10	0.8-1.6

a: significant; CI: confidence interval; OR: odds ratio.

TABLE II

Total number of blood donations with negative serology and ineligible donors (positive and inconclusive serology) due to Chagas disease at the Uberaba Regional Blood Center, according to risk area and local of residence

	Negative donations			Positive serology				Inconclusive serology			
Risk factors	n	%	OR	n	%	OR	CI (95%)	n	%	OR	CI (95%)
Risk area											
Yes	82,571	86.26	1	124	96.12	4[a]	1.6-9.7	119	85	1	0.6-1.4
No	13,150	13.74	1	5	3.88	0.3[a]	0.1-0.6	21	15	1.1	0.7-1.8
Residence											
Rural	11,967	14.49	1	28	22.58	1.7[a]	1.1-2.6	13	10.92	0.7	0.4-1.3
Urban	70,604	85.51	1	96	77.42	0.6[a]	0.4-0.9	106	89.08	1.4	0.8-2.5

the type of residence was stratified based on the total number of donors in the risk areas. *a*: significant; CI: confidence interval; OR: odds ratio.

Statistical analysis - The data were first analyzed descriptively using absolute and relative frequencies. The Chi-square test, Fisher's exact test and odds ratios (OR) were then applied to describe associations and risks of interest, with the level of significance set at 5%. Statistical analysis was performed using the GraphPad InStat® program, version 3.06 (GraphPad Software, San Diego CA, USA).

RESULTS

A total of 95,990 blood donations were performed at the URBC between 2000-2005. Of these, 269 donations (0.28%) were from donors with non-negative serology for Chagas disease, consisting of 129 (48%) with positive serology and 140 (52%) with inconclusive results.

Comparison of the groups with positive and negative serology showed that a higher frequency of donations with positive serology was significantly associated with donations from first-time donors (OR = 607; 84.9-4348.3), donors older than 30 years old (OR = 3.7; 2.4-5.8) and female donors (OR = 1.9; 1.3-2.6) (Table I). The prevalence of donors living in risk areas and in rural areas was also significantly higher among donors with positive serology compared to those with negative serology (OR = 4; 1.6-4.7 and OR = 1.7; 1.1-2.6, respectively) (Table II). No difference was observed with respect to skin color.

Comparison of the groups with negative and inconclusive serology showed a higher frequency of donations with inconclusive serology among first-time donors (OR = 5.2; 3.7-7.2). No significant difference in sociodemographic or epidemiological characteristics was observed between the two groups (Table II).

The distribution of ineligible donors in five age groups showed a strong linear correlation and was directly proportional to the percentage of individuals serologically positive for Chagas disease with increasing age (r = 0.93) (Fig. 1). Thus, the labeling index, which in the range of 18-20 years old was only 1.5% of positive individuals, came to represent 18%, 23%, 27% and 31%, respectively, in the ranges of 21-30, 31-40, 41-50 and greater than 50 years old. For indeterminate serology, there was a weak linear correlation with age (r = 0.22) (Fig. 2A), but if we eliminated 18-20-year-old donors from the analysis, there was a strong downward trend in the percentage in relation to higher ages (r = -0.97) (Fig. 2B).

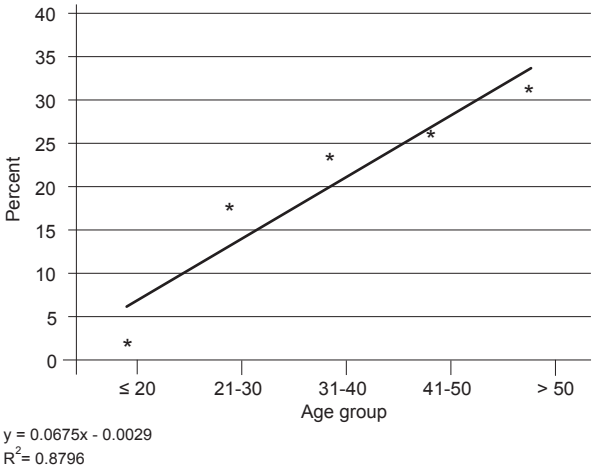

Fig. 1: correlation between seropositivity for Chagas disease and age groups for the 129 Uberaba Regional Blood Center blood donors. Strong linear correlation and directly proportional between age group and percentage of serologically positive donors. Linear Pearson correlation: 0.93.

When considering the sample of 90 donors, positive serology was significantly associated with donors who had contact with the triatomine vector (OR = 11.7; 3.4-40.2) and those with a family history of Chagas disease (OR = 4.8; 1.6-14.2), whereas negative and inconclusive serology was associated with donors who had no contact with the vector (OR = 0.1; 0.02-0.3) and those without a family history of Chagas disease (OR = 0.21; 0.07-0.63). No difference in the sociodemographic variables was observed between donors with inconclusive and negative serology (Table III).

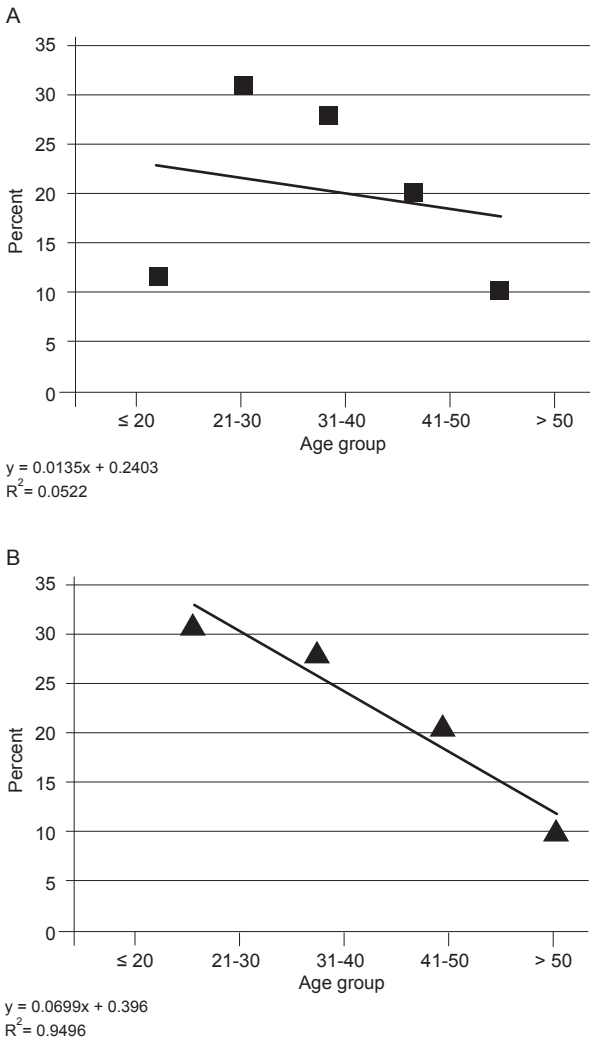

Fig. 2: correlation between percentage of inconclusive serology for Chagas disease and age groups for the 140 Uberaba Regional Blood Center blood donors. A: distribution in five age groups (≤ 20 years until > 50 years). Linear Pearson correlation: 0.22, showed a weak linear correlation; B: distribution in four age groups (from the 21 years until > 50 years). Linear Pearson correlation: 0.97, showed a strong downward trend in the percentage in relation to higher ages.

DISCUSSION

Analysis of the serological profiles of the 269 donors with non-negative serology for Chagas disease, who corresponded to 0.28% of all serologically ineligible donors seen at the URBC between 2000-2005, showed a discrete predominance of inconclusive reactions (52%) over repeatedly positive results (48%). Various studies have demonstrated that inconclusive reactions frequently account for more than 50% of cases of serological ineligibility due to Chagas disease at blood banks, with this rate exceeding 70% at some services (AABB 2007, Bern et al. 2008).

In the present study, 27% of all blood donations were from women. A similar rate of 26% has been reported by ANVISA (2002) in Brazil. Although the frequency of non-negative serology was found to be similar among genders (0.32% for women and 0.26% for men, data not shown), the frequency of seropositivity was significantly higher among women ($p < 0.0005$). The significant proportion of women infected with *T. cruzi* observed in the present series is in contrast to literature data showing no association between Chagas disease and gender (Guariento et al. 1996), a fact impairing the interpretation of our finding. However, pertinent data, although not scientifically confirmed, suggest that the habit of farm women to sleep close to the wall of their mud wall houses increases their exposure to the blood-sucking vectors.

The highest rate of inconclusive serology was observed among first-time donors, with this rate being 5.2-fold higher than that among repeat donors. This finding might be explained by the fact that inconclusive serology is already detected during the first donation and these serologically ineligible individuals are therefore excluded from the group of repeat donors (Salles et al. 1996). However, although low, the occurrence of inconclusive results among repeat donors indicates the need for more detailed laboratory investigation at blood banks by using more accurate tests that are capable of detecting possible cross-reactions with other pathogens or autoimmune diseases. The finding of 80% negative results by immunoblotting (TESA blot) among samples previously defined as inconclusive by ELISA (Umezawa et al. 2004, Furuchó et al. 2008) strongly suggests that, in most cases, inconclusive reactions do not indicate the presence of *T. cruzi* infection.

The correlation study showed an increase of seropositivity until the age of 50 years old, with a slight decrease in donors older than this age. The low prevalence of serological ineligibility for Chagas disease in younger individuals, i.e., up to 30 years old, corresponds to the period of the introduction of control measures of vector transmission in our region and during the last 20 years, due to recruitment, selection and loyalty programs, there has been a clear change in the profile of URBC donors.

With respect to origin, we observed no significant difference in the proportion of donors living in a risk area among donors with positive and inconclusive serology when compared to the negative serology group. The lack of statistical significance might be explained by the fact that Chagas disease is endemic in the study area, where a high percentage of seronegative individuals (73.3%) are also from risk areas, as well as by small sample size. However, the chance of positive serology was 5.1-fold higher among donors living in a risk area compared to those from non-risk areas.

The significantly higher prevalence of positive donors compared to those with inconclusive serology ($p = 0.015$) who are living in rural areas may be another good epidemiological indicator that an inconclusive reaction is poorly correlated with Chagas disease. However, the high proportion of serologically positive donors living in cities (77.42%) demonstrates the urbanization of Chagas disease in this geographic region, a fact also observed in other countries (Briceño-León 2009).

Until the 1980s, individuals living in rural areas presented a high probability of contracting Chagas disease because they generally lived in clay or mud wall houses covered with plant material and located close to forests and domestic ani-

TABLE III
Distribution of the 90 selected donors according to risk factors and serology for Chagas disease

	Negative donations			Positive serology				Inconclusive serology			
Risk factors	n	%	OR	n	%	OR	CI (95%)	n	%	OR	CI (95%)
Risk area											
No (n = 17)	8	26.67	1	2	6.67	0.2	0.04-1.01	7	23.33	0.8	0.3-2.7
Yes (n = 73)	22	73.33	1	28	93.33	5.1	0.98-26.4	23	76.67	1.20	0.4-3.9
Contact with the vector											
No (n = 52)	25	83.33	1	9	30	0.1[a]	0.02-0.3	18	60	0.3	0.1-1.01
Yes (n = 38)	5	16.67	1	21	70	11.7[a]	3.4-40.2	12	40	3.30	0.98-11.14
Family history											
No (n = 42)	19	63.33	1	8	26.67	0.21[a]	0.07-0.63	15	50	0.6	0.2-1.6
Yes (n = 48)	11	3.67	1	22	73.33	4.8[a]	1.6-14.2	15	50	1.70	0.6-4.8

a: significant; CI: confidence interval; OR: odds ratio.

mals, factors that favor the entry and installation of the vector in the dwelling (Briceño-León 2009). The current vector control of *Triatoma infestans* established in the Southern Cone and improved housing conditions indicate that patients with Chagas disease who continued in rural areas were infected three or four decades ago, with the prevalence of seropositivity being higher among these age groups.

Contact with the triatomine vector (inside or close to the dwelling) was reported by 70% of positive donors vs. 40% of inconclusive and 16.7% of negative donors. In addition, the risk of seropositivity was 11.7-fold higher among those who had contact with the vector when compared with those who had no contact. However, there was no significant difference between the inconclusive and negative groups. The fact that most positive donors reported frequent contact with the vector in their houses during some phase of their life agrees with the location of this region that, until a few decades ago, was considered highly endemic for Chagas disease. Prolonged contact with triatomines is known to be essential for vector transmission of chagasic infection since the chance of contamination through a single contact with the infected vector is only 1:1000 or 0.1% (Prata 2001). In this study, despite the small size of the sample, a strong correlation between a history of vector contact and positive serology was evident.

Furthermore, a family history (especially siblings) of Chagas disease is an important factor since it is a good indicator of previous co-existence with the triatomine vector. The possibility of congenital transmission should be considered in cases where the mothers of the donors are carriers of Chagas disease. A family history of Chagas disease was found to be a good indicator for seropositivity as demonstrated by the finding of a significantly higher frequency among positive donors when compared to the negative serology group (p = 0.01).

With respect to epidemiological characteristics, the group of inconclusive donors in general more closely resembled the group of seronegative donors, as shown in Tables I-III, for age, gender, risk area, residence in rural or urban areas and contact with the vector.

In a study by Ferreira-Silva (2006), a parasitological reassessment of 30 inconclusive donors showed that 100% tested negative by blood culture, whereas among seropositive donors, blood culture was positive in 40% of donors. In addition, only 24% of inconclusive donors presented more than two risk factors for Chagas disease vs. 61% of seropositive donors, a finding further indicating the absence of the disease among inconclusive donors (Ferreira-Silva 2006).

In agreement with other studies conducted on blood bank donors, the present investigation demonstrated the importance of epidemiological data for differentiation between donors with or without *T. cruzi* infection. Although it is not the only parameter used in endemic countries, epidemiological screening has been recommended as the first criterion at blood banks in non-endemic countries (Castro 2009), especially North America (O'Brien et al. 2009) and Europe (Garraud et al. 2007) where an expressive flow of immigrants from endemic countries of Latin American is observed (Schmunis 2007).

Cases of transfusion-associated Chagas disease and vertical transmission have been documented in non-endemic countries such as the United States (O'Brien et al. 2009), Canada (Comeau 2007), Spain (Piron et al. 2008) and France (Garraud et al. 2007) and were the subject of a workshop sponsored by the World Health Organization in Geneva in July 2007.

These facts demonstrate the importance of detailed and systematic clinical-epidemiological screening of blood donors, even in endemic countries with a low prevalence of Chagas disease among donors such as Brazil. In addition to being an auxiliary method to exclude risk donors, this procedure may help with the evaluation and establishment of the serological profiles of inconclusive donors. In non-endemic countries such as Europe and North America, clinical-epidemiological screening is one of the tools used to exclude donors presenting risk factors for Chagas disease infection, such as emigration from a country where the disease is endemic. In addition, the occurrence of Chagas disease transmission through blood transfusions supports the need for in-

tensified studies evaluating tests that, in addition to high sensitivity, also present high specificity.

In repeated studies, the TESA blot has shown sensitivity and specificity of 100% (Umezawa et al. 2004, Furuchó et al. 2008), including the absence of cross-reactions and has been proposed as a possible test for the confirmation of positive, negative or inconclusive results in the serology of Chagas disease.

In conclusion, in addition to increasing the safety of blood transfusion, the combination of clinical and epidemiological data with results of high performance diagnostic tests will permit a more accurate view of the serological profiles of blood donors, which will contribute significantly to reducing the unnecessary disposal of blood bags and the number of donors mistakenly classified as suffering from *T. cruzi* infection.

Additionally, we believe that, trusting the involvement of sanitary and health authorities of the country and the permanent monitoring and action of the scientific community and in agreement with the words of Dr João Carlos Pinto Dias (Dias 2007), transfusion-transmitted Chagas disease will become only a sad memory within the next 10-15 years.

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