Enhanced classification of Chagas serologic results and epidemiologic characteristics of seropositive donors at three large blood centers in Brazil

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BACKGROUND: A major problem in Chagas disease donor screening is the high frequency of samples with inconclusive results. The objective of this study was to describe patterns of serologic results among donors to the three Brazilian REDS-II blood centers and correlate with epidemiologic characteristics.

STUDY DESIGN AND METHODS: The centers screened donor samples with one *Trypanosoma cruzi* lysate enzyme immunoassay (EIA). EIA-reactive samples were tested with a second lysate EIA, a recombinant-antigen based EIA, and an immunfluorescence assay. Based on the serologic results, samples were classified as confirmed positive (CP), probable positive (PP), possible other parasitic infection (POPI), and false positive (FP).

RESULTS: In 2007 to 2008, a total of 877 of 615,433 donations were discarded due to Chagas assay reactivity. The prevalences (95% confidence intervals [CIs]) among first-time donors for CP, PP, POPI, and FP patterns were 114 (99-129), 26 (19-34), 10 (5-14), and 96 (82-110) per 100,000 donations, respectively. CP and PP had similar patterns of prevalence when analyzed by age, sex, education, and location, suggesting that PP cases represent true *T. cruzi* infections; in contrast the demographics of donors with POPI were distinct and likely unrelated to Chagas disease. No CP cases were detected among 218,514 repeat donors followed for a total of 718,187 person-years.

CONCLUSION: We have proposed a classification algorithm that may have practical importance for donor counseling and epidemiologic analyses of *T. cruzi*–seroreactive donors. The absence of incident *T. cruzi* infections is reassuring with respect to risk of window phase infections within Brazil and travel-related infections in nonendemic countries such as the United States.

Chagas disease is a parasitic infection caused by *Trypanosoma cruzi*, which is transmitted by hematophagous triatomine insects. The parasite can also be transmitted vertically (through the placenta or peripartum) and by transfusions of blood products and organ transplantations.[1,2] Screening for Chagas disease has been mandatory in Brazil since 1969. Parallel testing with two different *T. cruzi* antibody assays was mandatory in Brazil until 2004; since then screening was performed using a single high-sensitivity enzyme immunoassay (EIA).

Laboratory diagnosis of either acute or chronic *T. cruzi* infection is challenging.[3,4] Diagnosis is generally based on serologic assays because the direct detection of parasites is difficult even with modern molecular techniques such as polymerase chain reaction (PCR), due to

ABBREVIATIONS: CP = confirmed positive; FP = false positive; IFA = immunofluorescence assay; POPI = possible other parasitic infection; PP = probable positive.

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very low levels or even absence of parasitemia.[5-7] Most of the commercially available antibody assays use crude parasite extracts or subcellular fractions of cultured parasites as antigen preparations.[4] More recently assays using immunodominant recombinant *T. cruzi* antigens have been developed.[8,9] Recombinant antigens for *T. cruzi* are more specific than parasite extracts that cross-react with sera from patients with other diseases such as leishmaniasis[10-12] and *Trypanosoma rangeli* infection.[13]

Samples with low-level reactivity and inconclusive *T. cruzi* antibody results are frequently found in large-scale screening, especially when parallel testing with two or more assays is performed.[4,14,15] Such samples present challenges not only for donor counseling but also when evaluating the performance of new tests or estimating prevalence or incidence rates for national or regional epidemiologic surveillance. These samples could represent cases of cross-reactivity with other parasitic infections, self-limited (resolved) infections with waning antibodies, or active Chagas infection with low antibody responses or with antibody not detected by currently employed commercial assays. In the latter cases, failure of robust detection of infected donors would imply that the blood supply could still be a route for *T. cruzi* transmission.

Recently, a recombinant *T. cruzi* antigen–based EIA was developed and commercialized that has eliminated cross-reactivity with *Leishmania*-seropositive samples while retaining sensitivity to *T. cruzi* antibodies similar to other lysate-based EIAs and radioimmunoprecipitation assay and other confirmatory test results.[4,14] We hypothesized that if a subset of the low-level *T. cruzi* EIA-reactive donor samples are due to cross-reactivity to other parasitic infections, the recombinant EIA could provide a useful tool to employ in a supplemental testing algorithm to differentiate these groups.

We describe here the Chagas serologic patterns obtained by testing at two large blood centers during 2007 and 2008 as part of the REDS-II international study in Brazil.[16] Based on our findings we propose a new algorithm for classification of *T. cruzi*–seroreactive donor specimens. We also report the epidemiologic characteristics of *T. cruzi* confirmed-seropositive Brazilian donors and demonstrate virtual absence of seroconversions attributable to incident infections in these regions in Brazil.

MATERIALS AND METHODS

Blood screening and supplemental testing

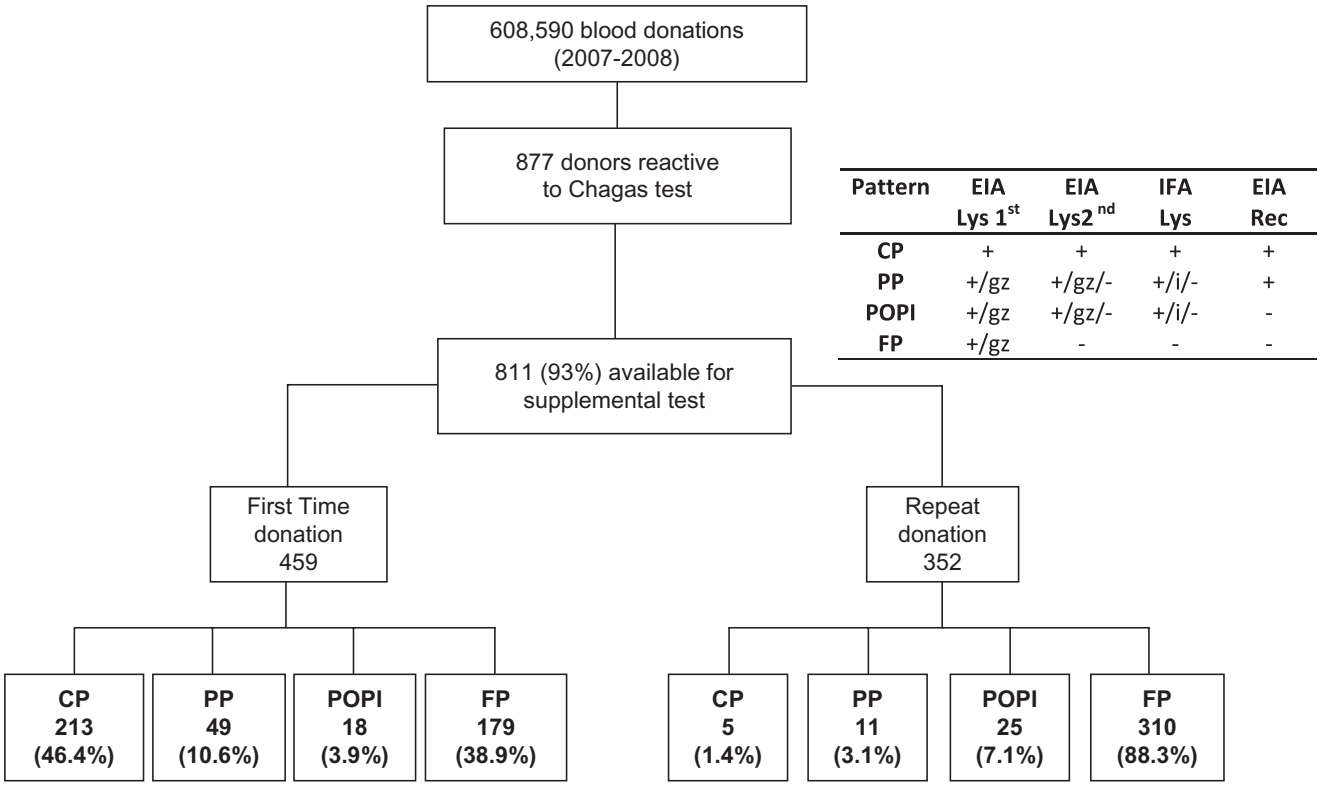
The three blood centers included in this study are as follows: Hemope, Recife, Pernambuco; Hemominas, Belo Horizonte, Minas Gerais; and Fundacao Pro-Sangue, Sao Paulo. All centers screened for *T. cruzi* antibodies using one EIA based on crude parasite extracts. Table 1 lists the manufacturers of the screening EIAs employed at each blood center during the 2-year analysis period (January 2007-December 2008). Independent of the kit used, the centers classified as "gray zone" all samples with signal-to-cutoff ratios of 0.8 to 1.2. All positive or gray zone units were discarded, and the samples were sent to a central laboratory in Sao Paulo and tested with a second lysate EIA (Chagatek, Biomérieux, Argentina; or Elisa cruzi, Biomérieux, Rio de Janeiro, Brazil), a recombinant *T. cruzi* antigen EIA that does not react with *Leishmania* samples (Chagatest rec. V.3.0, Wiener Lab, Rosario, Buenos Aires, Argentina), and a *T. cruzi* immunfluorescence assay (IFA; Biolab Merieux, Rio de Janeiro, Brazil). In the central laboratory we used the manufactures' criteria to consider samples positive, gray zone, or negative: the Wiener and Chagatek kits classify samples as positive or negative, while Elisa cruzi classifies samples with signal-to-cutoff between 0.8 and 1 as gray zone. IFA testing was initially performed on 1/20 dilutions of EIA-reactive donor sera and if reactive further dilutions were tested to determine IFA titers. Samples were considered IFA positive when the titer was higher than 1/20 and indeterminate when the titer was equal to 1/20 (see Fig. 1).

Classification criteria

Samples were classified as confirmed positive (CP) when reactive by all four assays as probable positive (PP) when reactive on the screening EIA and recombinant antigen EIA, but inconclusive or negative by the supplemental lysate EIA or IFA as possible other parasitic infection

TABLE 1. Description of the kit used in each site during the study period

Center	Period	Screening test kit	Number of screened units	Percentage of screened units	FP	Percentage FP
Hemope (Recife)	1. Jan 1, 2007-Jul 21, 2007	C: Bioschile	56,132	9.2	10	0.02
	2. Jul 22, 2007-Dec 31, 2008	B: Elisa Cruzi-Biomerieux	145,363	23.9	64	0.04
Hemominas	1. Jan 1, 2007-Feb 11, 2007	C: Bioschile	9,056	1.5	0	0.00
(Belo Horizonte)	2. Feb 12, 2007-Sep 26, 2007	D: Gold Chagas REM	42,589	7.0	221	0.52
	3. Sep 27, 2007-Jan 16, 2008	B: Elisa Cruzi-Biomerieux	20,653	3.4	4	0.02
	4. Jan 17, 2008-Dec 31, 2008	D: Gold Chagas REM	64,589	10.6	25	0.04
FPS/HSP	1. Jan 1, 2007-Feb 22, 2007	B: Elisa Cruzi-Biomerieux	20,352	3.3	7	0.03
(São Paulo)	2. Feb 23, 2007-Feb 27, 2007	A:Chagateck-Biomerieux	1,666	0.3	1	0.06
	3. Feb 28, 2007-Dec 31, 2008	B: Elisa Cruzi-Biomerieux	248,190	40.8	157	0.06



Pattern	EIA Lys 1st	EIA Lys2 nd	IFA Lys	EIA Rec
CP	+	+	+	+
PP	+/gz	+/gz/-	+/i/-	+
POPI	+/gz	+/gz/-	+/i/-	-
FP	+/gz	-	-	-

Fig. 1. Distribution of the samples according to serologic algorithm (see Materials and Methods). EIA Lys 1st = EIA based on parasite lysate used for routine screening; EIA Lys 2nd = EIA based on parasite lysate used as a supplemental test; EIA Rec = EIA based on parasite recombinant DNA expressed proteins; gz = gray zone; i = indeterminate by IFA (titer, 20).

(POPI) when positive or gray zone reactive by the lysate assays (EIAs and IFA) but negative in the recombinant antigen EIA, and as false positive (FP) when reactive only by the screening assay.

Defining seroconversion status

The REDS-II donation data set classifies donors as first time versus repeat and provides the date of previous donation for repeat donors. Since this computerized data set does not include *T. cruzi* antibody test results, we determined if seroconversion occurred in a positive repeat donor by manually verifying that a previous donation was *T. cruzi* antibody negative. This step was performed to confirm that donors were not misclassified as seroconverters. We also reviewed if the donor had returned subsequent to a positive antibody test to provide samples for follow-up testing.

We computed the incidence rate of Chagas infection by dividing the number of new events by the number of person-years of follow-up. The follow-up time was measured in years, from the first available donation date to the last available donation date or date of confirmed or probable Chagas testing. The pre–REDS-II donation dates were used when available, and those dates could go back to 1988 for Minas, 1994 for Sao Paulo, and 1998 for Recife. Donors could be followed up to 2008 in all centers.

Statistical analysis

We computed 2-year prevalence rates (2007 and 2008) per 100,000 donors and corresponding 95% confidence intervals (CIs). The 95% CI was calculated using the normal approximation. Chi-square tests were used to assess differences in proportions according to the classification of infection types in first time donors. A p value of less than 0.05 based on two-tailed alternatives was considered to be significant. All statistical tests were performed using statistical software (SAS 9.1, 2004, SAS Institute, Cary, NC).[17]

RESULTS

Study population

The population for this analysis consisted of 615,428 donations by 410,457 donors during 2007 and 2008; Chagas screening testing was not conducted on 6775 (1.1%) donations and these donations were excluded from the analysis. Additionally, for 66 specimens (22 from Recife and 44 from Belo Horizonte) with reactive Chagas

screening test results no residual samples were available for supplemental testing at the central laboratory in Sao Paolo; these cases were excluded from the analysis of interpretive algorithms Our primary analysis data set therefore consisted of 608,590 donations, including donations from 186,970 first-time donors and 421,620 donations from repeat donors.

With regard to demographic characteristics of the donor population, the majority were male (69.8%), the mean age was 33.3 years old (SD, 10.38 years), and 67.3% of donations were given by community donors and 32.7% by replacement donors. With respect to education status, 13% of donations were by donors who did not finish high school, 16.5% by donors who had a high school degree, 54.5% by donors who had completed at least 3 years of college, and 16% by donors who were college graduates. More donations were made in Sao Paulo (44%), followed by Recife (33%) and Minas (23%).

Reactivity patterns in all donors

During the 2-year study period 877 samples were discarded due to reactive or gray zone primary *T. cruzi* EIA reactivity, with 811 (93%) available for confirmatory testing. Figure 1 summarizes the samples classification according to first-time and repeat donation status. The number of reactive donations and prevalence rates per 100,000 donations for each serologic pattern are shown in Table 2, sorted by blood center. Of the 811 primary EIA-reactive samples evaluated by the three supplemental assays, 322 (40%) were reactive by at least one of these assays while 489 (60%) were negative by all three supplemental assays and were classified as FP. The FP rates were very different depending on the screening test used (Table 1), with one kit lot responsible for almost half of the FP cases. The overall prevalence of CP donations was 36 per 100,000 (95% CI, 31-41/100,000). Sao Paulo had the highest prevalence of CP donations followed by Belo Horizonte and Recife (Table 2). The distribution of reactive donations with the PP pattern was similar to that of the CP cases in terms of relative rates by blood center.

We calculated ratios of PP/CP and of POPI/CP donations to gain insights into the possible relationships of these patterns to confirmed *T. cruzi* infection rates in each center. For all centers the PP/CP ratio was approximately 0.30, suggesting that this PP pattern is probably related to Chagas disease exposure. In contrast, the POPI/CP ratio was significantly different, being higher in Recife followed by Belo Horizonte and Sao Paulo ($p < 0.001$), implying that POPI reactivity is unrelated to *T. cruzi* exposure.

Prevalence in first-time donors

Of the 811 reactive samples, 459 were from first-time donors and 352 were from repeat donors. The prevalence per 100,000 donations for each serologic pattern among

TABLE 2. Serologic pattern by location among all donors

	Blood center region							
	Recife		Belo Horizonte		Sao Paulo		Total	
	(total donations n = 204,124)		(total donations n = 139,429)		(total donations n = 271,880)		(total donations n = 615,433)	
Classification	Number positive	Prevalence/100,000 donations (95% CI)	Number positive	Prevalence/100,000 donations (95% CI)	Number positive	Prevalence/100,000 donations (95% CI)	Number positive	Prevalence/100,000 donations (95% CI)	p value*
CP	31	15 (10-21)	46	34 (24-43	141	52 (44-61)	218	36 (31-41)	<0.001
PP	10	5 (2-8)	13	9 (4-15)	37	14 (9-18)	60	10 (7-12)	0.011
POPI	19	9 (5-14)	12	9 (4-14)	13	5 (2-7)	44	7 (5-9)	0.136
FP	74	37 (28-45)	250	183 (160-205)	165	61 (52-70)	489	80 (73-87)	<0.001
Total	134	NA	321	NA	356	NA	811	NA	NA
PP/CP, ratio	0.32		0.28		0.26		0.28		0.876
POPI/CP, ratio	0.61		0.26		0.09		0.20		<0.001

* p value for chi-square statistics comparing the Recife, Belo Horizonte, and Sao Paulo blood centers.
NA = not applicable.

the first-time donors is shown in Table 3. The prevalence of CP donations was 114 per 100,000 (95% CI, 99-129/100,000). Significantly different rates of CP infections were found by donor age, education status, and blood center location variables. As expected there was a marked relationship between donor age and CP results, ranging from a prevalence of 15 per 100,000 (95% CI, 6-24) in the less than 25 years old group to 756 per 100,000 (95% CI, 499-1013) in the 55 years and older group ($p < 0.001$). On the other hand, an inverse relationship was observed for education with CP prevalence higher among those who did not graduate high school versus those with a college degree or higher level educations ($p < 0.001$). With respect to blood center location, we found a higher prevalence in Sao Paulo, followed by Belo Horizonte, and Recife ($p < 0.001$). CP prevalence rates did not significantly differ by sex or donor type (replacement vs. community donors).

For the PP cases, the overall prevalence in FT donors was 26 per 100,000 donations (95% CI, 19-34/100,000). As with the overall donor analysis, we found similar patterns of PP and CP prevalence rates when first-time donors were analyzed by age, sex, education, donation history, and location.

The prevalence of POPI was not significantly different in the three regions, but the demographic correlates of POPI were different than CP and PP. For example, the prevalence of POPI was higher among males and demonstrated an opposite trend to that noted for PP and CP rates with respect to center location, with the highest rate of POPI in Recife and lowest rate in Sao Paolo (Table 3).

Assuming that PP and CP cases both represent true *T. cruzi* seropositivity, these groups were combined and we calculated a total seroprevalence rate among FT donors of 140 (123-157)/100,000. Demographic correlates of the combined CP and PP groups are summarized in Table 4.

Incidence in repeat donors

Of 426,166 repeat donations collected in 2007 through 2008, there were 352 *T. cruzi* EIA repeat-reactive samples at screening. Of those 310 (88.3%) did not react to any of the supplemental assays and were considered FP, while 42 (11.7%) samples were reactive by at least one supplemental assay with five classified as CP, 11 as PP, and 26 as POPI. However, after reviewing the test result history of these donors (Table 5) using all available blood center records, we established that none of the five CP cases were true seroconverters: four were *T. cruzi* antibody repeat reactive on their previous donation and donated again by mistake (i.e., due to failure of the notification and deferral process), and one tested *T. cruzi* antibody negative by EIA on follow-up samples and hence the CP result probably resulted from a specimen mix-up or contamination problem at the time of donation or initial sample processing. Of the 11 PP cases, 10 were negative on previous donations, seven of whom returned for a follow-up counseling and repeat sampling and testing; five of the follow-up samples tested negative and two were gray zone. Our expectation was that if cases of true seroconversion were observed, most of them should be classified CP cases because the interdonation interval among possible seroconverters was large (the median, 15.13 months; range, 1.17-145.67 months). The window phase for Chagas is expected to be less than 60 days. It would not be reasonable to think that all seroconverters would come to donate soon after getting infected and during the transient window phase when the serologic pattern could be inconclusive based on differential detection by the different assays. The fact that at follow-up many of these cases did not fully seroconverted corroborate our conclusion that these were not true incident infections. Hence, although not all PP cases could be investigated, there were no observed cases of confirmed *T. cruzi* antibody seroconversion among repeat donors with preliminary CP or PP results. Overall 218,514 repeat donors were followed for a total of 718,187 person-years. The median follow-up time per donor was 2.32 years (range, 0.03 to 20.57 years). Thus, no incident case of confirmed *T. cruzi* infection was identified during our follow-up period which yield an upper 95% CI for possible *T. cruzi* incidence of approximately 1:240,000 person-years.

Of the 26 possible POPI cases in repeat donors, 19 were previously negative on all donations, and of these 14 returned for a follow-up sample: eight were *T. cruzi* EIA negative, three had borderline reactivity on EIA and were classified as inconclusive, and three were again EIA reactive with similar levels of reactivity on plasma from the index donation and at follow-up (probable POPI).

Sensitivity of assays and classification algorithm

Figure 2 summarizes the test results of the samples classified as POPI or PP. Of the 60 PP samples, only seven were positive or indeterminate by IFA, so if we assume that all CP and PP cases represent true *T. cruzi* exposure, the sensitivity of IFA for confirmation was 81.1% (218 + 7/218 + 60). Of the 60 PP cases, 18 were negative by the second EIA based on crude antigen (and by IFA), so those 18 cases would have been missed if the second lysate-based EIA were it to have been used as the primary screening assay. Of the 44 POPI samples, only six were reactive to the second EIA based on crude antigen and to IFA, 30 samples were only reactive to EIA and eight only to IFA. Hence, detection of donors with POPI reactivity was inconsistent among the supplemental assays, presumably reflecting variable cross-reactivity to antibodies directed against *Leishmania* or other parasites endemic to Brazil.

TABLE 3. Serologic pattern by demographic characteristics among first-time donors

		Serologic pattern							
		CP Chagas		PP Chagas		POPI		FP	
Characteristics	Number of donations	Number positive	Prevalence/100,000 donations (95% CI)	Number positive	Prevalence/100,000 donations (95% CI)	Number positive	Prevalence/100,000 donations (95% CI)	Number positive	Prevalence/100,000 donations (95% CI)
Overall	186,970	213	114 (99-129)	49	26 (19-34)	18	10 (5-14)	179	96 (82-110)
Age (years)									
<25	73,596	11	15 (6-24)*	6	8 (2-15)*	4	5 (0-11)	64	87 (66-108)
≥25-<35	61,857	45	73 (52-94)	15	24 (12-37)	8	13 (4-22)	53	86 (63-109)
≥35-<45	31,329	67	214 (163-265)	15	48 (24-72)	3	10 (0-20)	38	121 (83-160)
≥45-<55	15,761	57	362 (268-455)	10	63 (24-103)	2	13 (0-30)	19	121 (66-175)
≥55	4,363	33	756 (499-1013)	3	69 (0-147)	1	23 (0-68)	5	115 (14-215)
Sex									
Female	73,630	87	118 (93-143)	21	29 (16-41)	3	4 (0-9)*	56	76 (56-96)
Male	113,340	126	111 (92-131)	28	25 (16-34)	15	13 (7-20)	123	109 (89-128)
Education									
<High school	12,817	60	468 (350-586)*	11	86 (35-137)*	2	16 (0-37)	9	70 (24-116)
High school	16,913	39	231 (158-303)	6	35 (7-64)	2	12 (0-28)	15	89 (44-134)
Completed 3 years of college	62,486	29	46 (30-63)	6	10 (2-17)	2	3 (0-8)	33	53 (35-71)
College +	15,566	3	19 (0-41)			1	6 (0-19)	11	71 (29-112)
Type of donation									
Community	100,092	105	105 (85-125)	22	22 (13-31)	10	10 (4-16)	85	85 (67-103)
Replacement	86,843	108	124 (101-148)	27	31 (19-43)	8	9 (3-16)	94	108 (86-130)
Location									
Recife	57,921	27	47 (29-64)*	8	14 (4-23)*	8	14 (4-23)	26	45 (28-62)
Belo Horizonte	43,871	45	103 (73-133)	7	16 (4-28)	5	11 (1-21)	87	198 (157-240)
Sao Paulo	85,178	141	166 (138-193)	34	40 (27-53)	5	6 (1-11)	66	77 (59-96)

* $p < 0.05$.

TABLE 4. Confirmed or PP Chagas infection by demographic characteristics among first-time donors

Characteristics	Serologic pattern: Number of donations	CP Chagas or PP Chagas: Number positive	CP Chagas or PP Chagas: Prevalence/100,000 donations (95% CI)
Overall	186,970	262	140 (123-157)
Age (years)			
<25	73,596	17	23 (12-34)*
≥25-<35	61,857	60	97 (72-125)
≥35-<45	31,329	82	262 (205-318)
≥45-<55	15,761	67	425 (323-527)
≥55	4,363	36	825 (557-1094)
Sex			
Female	73,630	108	147 (119-174)
Male	113,340	154	136 (114-157)
Education			
<High school	12,817	71	554 (425-682)*
High school	16,913	45	266 (188-344)
Complete 3 years of college	62,486	35	56 (37-75)
College +	15,566	3	19 (0-41)
Type of donation			
Community	100,092	127	127 (105-149)
Replacement	86,843	135	155 (129-182)
Location			
Recife	57,921	35	60 (40-80)*
Belo Horizonte	43,871	52	119 (86-151)
Sao Paulo	85,178	175	206 (175-236)

* $p < 0.05$.

TABLE 5. Summary of the results obtained among the 42 cases initially considered as seroconverters

Serologic pattern	Total	Excluded based on previous donation tested positive	Previous donation negative	Returned for follow-up*	Results at follow up
CP	5	4	1	1	Negative
PP	11	1	10	7	5 negative 2 gray zone
POPI	26	7	19	14	8 negative 3 gray zone 3 EIA reactive

* Subsets of previous donation negative group.

DISCUSSION

This report describes the serologic patterns of Chagas disease serologic markers among 877 *T. cruzi* EIA-reactive donations detected after screening of more than 600,000 allogeneic donations given to three large blood centers in Brazil over a 2-year period. Diagnostic confirmation of Chagas disease is difficult due to the lack of widely available and well-validated confirmatory assays (e.g., radioimmunoprecipitation assay) or accepted gold standard supplemental testing algorithms.[4,15,18] Inconclusive results are commonly reported and have been systematically described and analyzed by several groups with different conclusions regarding the interpretation of these reactivity patterns.[4,15,18] Remesar and coauthors,[14] analyzing data from a highly endemic area for Chagas disease in Argentina, recently showed that of the 20% of the units discarded due to Chagas disease screening test reactivity, one-third (6% of donations overall) had an inconclusive result based on combinations of results from different assays. In our study, inconclusive results were also common, with 104 PP or POPI results relative to 218 CP results, a similar ratio (approx. one in three) to that documented in the highly endemic donor population in Argentina.

We have developed a classification scheme for inconclusive samples based primarily on reactivity to a widely available recombinant *T. cruzi* antigen–based EIA assay, which sorts these samples into PP cases that we hypothesized represent remote *T. cruzi* exposure and POPI cases (samples nonreactive by the recombinant EIA) that we suspect are attributable to cross-reactivity resulting from past *Leishmania* infections.[11] We then evaluated if these two categories differ in terms of epidemiologic characteristics, relative to the characteristics of donors with CP and FP reactivity patterns. The PP and CP samples had very similar age, educational level, and geographical distributions (PP/CP ratio), supporting our hypothesis that PP are predominantly related to *T. cruzi* infection and perhaps reflect remote infections that had resolved and in which antibody titers are waning. In contrast, the POPI/CP ratio was significantly different in each region suggestion that they are not related to the same biologic phenomenon. The sex distribution of POPI cases (male predominance) was also different from that of the PP and CP equal male and female rates). *Leishmania* is endemic in Belo Horizonte and is increasing in incidence in Pernambuco state (where Recife is located), but is not common in Sao Paolo city. Acute or chronic *Leishmania* infections are well known to induce antibodies that cross-react with Chagas lysate EIAs, but not with recombinant antigen EIAs. Unfortunately, there are no commercially available *Leishmania*-specific antibody assays to allow us to confirm the proportion of donations with POPI reactivity that represent *Leishmania* antibodies versus other parasite exposures or nonspecific reactivity to two lysate EIAs with or without *T. cruzi* IFA reactivity.

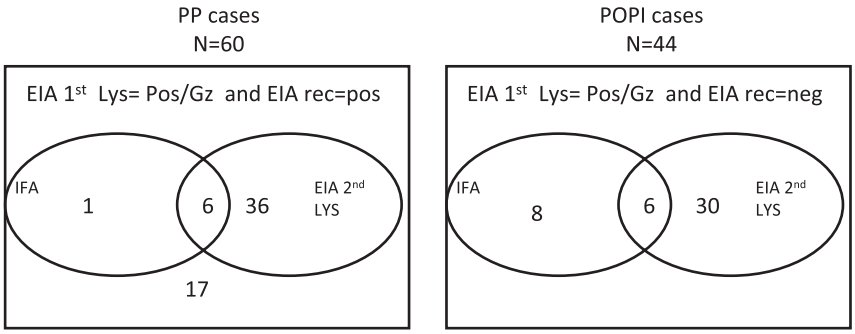


Fig. 2. Summary of test results obtained on cases classified as PP and POPI. The samples positive or indeterminate by IFA and positive (Pos) or gray zone (Gz) for second EIA test are summed inside the corresponding circles. PP = Pos/gz for screening EIA and Pos for recombinant EIA; POPI = Pos/Gz for screening EIA negative (neg) for recombinant EIA and Pos/Gz for EIA 2nd Lyz; EIA 1st Lys = EIA based on parasite lysate used for routine screening; EIA 2nd Lys = EIA based on parasite lysate used as a supplemental test; EIA Rec = EIA based on parasite recombinant DNA expressed proteins; Gz = gray zone.

A previous study from our group has shown that donors with low-reactive Chagas results had epidemiologic evidence of higher rates of exposure to the Chagas' disease vector compared to negative controls.[15] Levy and colleagues[19] studying Chagas-reactive samples in Peru also concluded that samples with discrepant results are geographically associated with confirmed Chagas cases. A more recent study from an Argentinean group in the Chaco region has shown that among low-reactive samples, those reactive in a recombinant assay had epidemiologic evidence of Chagas exposure, where as those who tested nonreactive on that test did not.[20]

If all 60 PP samples represent true infection it is important to note that there were 36 cases that were negative by IFA and 17 cases that were negative by the second lysate-based EIA and by IFA. Hence it is important to recognize that there are true low-reactive *T. cruzi*–seropositive cases that are missed by both current screening and confirmatory assays. Indeed, studies based on a well-characterized panel that include low-reactive samples show that most EIAs and confirmatory assays do not achieve 100% sensitivity.[4,14,18] Low seroreactivity may correlate with spontaneous parasite clearance and represent evolving seroreversion, and hence such cases may not present serious concerns for blood safety, although this issue will require further study.

One consequence of the lack of sensitivity of existing screening assays is the detection of apparent seroconversions (i.e., detection of reactivity in subsequent donations by donors who previously tested negative) when a different screening assay with a similar or higher sensitivity is implemented. However, despite the change in screening assays, we did not detect any CP seroconversions when such cases were carefully analyzed. Instead, we found that the apparent "seroconversions" in the three centers in this study showed only the PP or POPI pattern. We believe that even these cases do not represent new *T. cruzi* infections, but rather long-standing (possibly reverting) *T. cruzi* or *Leishmania* seroreactivity that had been missed by a previous screening kit

Overall the results on demographics of *T. cruzi* infection in donors document a dramatic decreasing prevalence by age, consistent with the findings of other studies. The prevalence of CP among donors below 25 years old was 75-fold lower than that observed in the oldest donor strata (15/100,000 vs. 756/100,000), and there was clear trend with age across all strata. Since the 1970s, Brazil has implemented a program to eradicate *T. cruzi* vectorial transmission in houses.[21] The decline in donor seroprevalence rates may be explained by a combination of successful vector control along with increasing urbanization of the population in Brazil, such that younger donors have not lived in rural settings with an increased risk of exposure.

The lack of observed seroconversions in our repeat donors, combined with the dramatic decline in prevalence in younger first-time donors, suggests that the acquisition of new *T. cruzi* infection in Brazil is rare. The data also imply that travel to Brazil is very unlikely to result in *T. cruzi* infection and therefore indicate that there is no justification for other countries to defer potential donors who travel to urban areas in Brazil. Furthermore, in countries such as the United States with a selective *T. cruzi* antibody testing policy, there is no reason to retest donors after travel or even prolonged residence in urban areas of Brazil.

Our data also indicate a very low risk of *T. cruzi* window phase transmission within Brazil. However, residual risk could exist if low-level-reactive samples that are not consistently detected by the primary screening EIAs represent cases of active parasitemic infection with weak antibody responses. However, we believe that most such cases represent resolved infections and evolving seroreversion, as has been reported by others among the control group arm of benzonidazole clinical trials.[22,23] Cases of transfusion-transmitted *T. cruzi* infection in Brazil have been exceedingly rare since screening procedures were made mandatory in the 70s. Recently, Souza and colleagues[24] described one case of transmission of Chagas disease to a liver transplant recipient. The donor tested negative for Chagas antibodies by IFA. However, a sample from the organ donor was not available for further testing to determine whether it was from a low-level

antibody-reactive individual or was due to an error in performing the IFA (the only test used for screening in this case). Further studies including performance of sensitive PCR on follow-up samples are warranted to address these issues. Recently, Cooley and coauthors[25] have cloned and screened 400 new proteins for diagnostic purposes, and it is possible that these new antigens may help in improving serodiagnosis of Chagas disease and will allow discrimination of active from resolved infections.

In conclusion, we have proposed a classification algorithm that may have practical importance for donor counseling and for epidemiologic analyses of Chagas disease. Importantly, we believe that the sensitivity of *T. cruzi* assays should be defined based on their capacity to detect both low- and high-level-reactive true *T. cruzi*–seroreactive samples and not samples that are cross-reacting to other parasitic infections. Such borderline-reactive samples are frequent and were responsible for all our of the apparent incident donor cases in our study. It is important to continue to study donors with borderline reactivity, including determination if this group includes cases that are parasitemic for *T. cruzi* by PCR analysis and thus represent active infections that would drive the development of new screening assays to consistently interdict these donations.

CONFLICT OF INTEREST

No conflict of interest.

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