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ARTICLE

Dealing with initial inconclusive serological results for chronic Chagas disease in clinical practice

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Abstract Most guidelines for Chagas disease recommend the performance of two serological tests in order to detect it. However, inconclusive results may arise from this strategy. The aim was to describe whether serological follow-up together with the patient's clinical characteristics could clarify the outcome of patients with initial inconclusive test results. In this retrospective case series, all results of Chagas disease serological tests and outpatient visits recorded from 2004 to 2008 were screened for inclusion. The inclusion criterion was clinical suspicion of chronic Chagas disease and the exclusion criteria were previous diagnosis of Chagas disease, suspicion of acute Chagas disease, and serological tests with no corresponding medical evaluation. A total of 1,732 patients were analyzed. Chronic Chagas disease prevalence was 21.1%. After the initial set of serological tests, 2.9% of patients had inconclusive test results. Most of these patients had definite diagnosis after clinical follow-up and the repetition of serological tests in a new blood sample. Loss to follow-up while partaking in the diagnostic investigation reached 17.7%. The prevalence of initial inconclusive serological tests for chronic Chagas disease is low. Clinical evaluations and follow-up clarify the definite diagnosis. Noncompliance to follow-up is a frequent problem. Strategies to reduce inconclusive results and noncompliance are discussed.

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Background

The accuracy of parasitological tests for chronic Chagas disease diagnosis is not acceptable. Therefore, its diagnosis relies solely on serological tests [1–6]. Two of the following serological techniques are usually recommended: indirect immunofluorescence (IIF), enzyme-linked immunosorbent assay (ELISA), or hemagglutination (HA). Inconclusive diagnosis may arise if the serological tests results do not match or if the result of one of them is in an indeterminate range [1, 3, 6].

Chilean guidelines are based on two different techniques, ELISA and IIF, performed in parallel. If inconclusive results arise, additional tests are performed in new blood samples in a reference laboratory [2]. Spanish recommendations state that a patient will have the diagnosis of chronic Chagas disease confirmed if either a parasitological test or a pair of serological tests is positive. If inconclusive serological tests arise, Western blot (WB) should be performed [6].

North American systematic review also recommends two serological tests in parallel, ELISA and IIF. If these are discordant, a third assay may be performed or a new blood

sample should be collected on which to run new serological tests [3]. Similar to this review, El Salvador's guideline also recommends the use of ELISA and a second serological test, either IIF or HA, in parallel. If their results do not match, a third assay of different methodology should be performed [1].

Current Brazilian consensus recommends two serological tests performed in parallel. If their results match, the patient is classified as either with or without Chagas disease; if their results do not match, the result is classified as inconclusive. If this is the case, a second sample is collected and the same pair of serological tests could be performed. If the second pair of serological tests results remains inconclusive, a third sample is collected and either WB or polymerase chain reaction (PCR) should be performed [5].

PCR and WB for chronic Chagas disease diagnosis were mentioned in most guidelines, but their use is controversial, and the World Health Organization (WHO) Expert Committee considered these two tests as nonconventional techniques [4].

This report addressed how inconclusive serological results for chronic Chagas disease were handled in clinical practice at Instituto de Pesquisa Clínica Evandro Chagas (IPEC), where nonconventional or in-house techniques were not available at the time. The aim was to check the definite diagnosis of patients suspected of chronic Chagas disease with initial inconclusive serological test results, after clinical evaluations and serological follow-up. Additionally, the ability of clinical characteristics to predict the definite diagnosis of patients with initial inconclusive results was explored. Characteristics potentially related with inconclusive results were also explored.

Methods

This investigation is a retrospective case series and was conducted at IPEC, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil. IPEC is a reference center for infectious diseases, including Chagas disease. Patients come to IPEC's outpatient service spontaneously or are referred from other health units in order to be screened for Chagas disease and receive appropriate health care. IPEC is the only health unit specialized in Chagas disease in Rio de Janeiro state. The institutional review board and ethics committee approved this research on July 15th, 2009 with Sistema Nacional de Informação sobre Ética em Pesquisa envolvendo Seres Humanos (SISNEP) (http://portal2.saude.gov.br/sisnep/) number 0027.0.009.000.09.

Clinical routine for chronic Chagas disease diagnosis at IPEC was: (a) first outpatient visit: patients are interviewed by nurses and briefly evaluated by a physician, and a blood sample is collected for serological tests; (b) ELISA and IIF are performed and other complementary tests (such as electrocardiogram [ECG]) could be performed according to the physician's judgment; (c) second outpatient visit (20 to 30 days after the initial evaluation): the patient is reevaluated, serological findings are analyzed, and counseling, follow-up, or therapy are provided as appropriate.

Serological test results and outpatient visits recorded from 2004 to 2008 were retrospectively reviewed. The inclusion criteria were: (1) suspicion for chronic Chagas disease and (2) first serological investigation for Chagas disease. Patients were excluded if: (1) they had a previous diagnosis of Chagas disease; (2) they were under investigation for acute Chagas disease; (3) they were already under follow-up at IPEC due to other diseases; or (4) they had laboratory results without corresponding medical records.

The data of interest were as follows: Chagas disease serological tests findings, including optical densities or dilutions, as well as consecutive findings of the same patient (if more than one sample was collected); serological kit trademarks; clinical interpretations of test findings; history of possible exposure to *Trypanosoma cruzi* and signs or symptoms compatible with Chagas disease. All of these data were obtained from medical charts and laboratory registers.

Chagas disease was defined as a patient with two positive serological tests performed with the same blood sample. Chagas disease diagnosis was excluded when both serological tests were negative. Serological test result was defined as indeterminate if at least one of the serological tests presented within a not interpretable range (gray zone). The result was defined as discordant if one serological test was positive and the other was negative in the same blood sample. Patients with indeterminate or discordant serology findings, in which the assistant physician decided that they were not infected with *T. cruzi* and were discharged from the outpatient service were classified as "medical discharge without Chagas". Tests results were considered to be inconclusive when it was not possible to assign a diagnosis to a patient due to the findings of one of the serological tests within an indeterminate range or discordant findings from different serological tests. A noncompliant patient was defined as a patient that either collected a blood sample but missed medical follow-up, or had an inconclusive diagnosis but did not return to collect a new blood sample. Patients were considered to be from an endemic area if they were born in areas with active vector transmission at any time.

Between 2004 and 2008, the following serological tests were used at IPEC: EIE CHAGAS Bio-Manguinhos (Rio de Janeiro, Brazil; from 2004 to June 2006; informed sensitivity 100.00%, informed specificity 98.62%); Wiener Lab's ELISA (Rosário, Argentina; from June 2006 to

December 2008; informed sensitivity 98.04%, informed specificity 92.96%); Bio-Manguinhos' IIF Chagas (Rio de Janeiro, Brazil; from 2004 to January 2008; informed sensitivity 100.00%, informed specificity 100.00%); WAMA's Immuno-con Chagas (São Paulo, Brazil; from January 2008 to June 2008; informed sensitivity 100.00%, informed specificity 100.00%); and Biocientifica's Inmunofluor Chagas (Buenos Aires, Argentina; from June 2008 to December 2008, informed sensitivity 100.00%, informed specificity 100.00%). Neither PCR nor WB was available for clinical routine during this period.

These tests were used following the manufacturers' instructions. The cut-offs used were as follows: EIE CHAGAS Bio-Manguinhos: the mean optical density of positive controls plus the mean optical density of negative controls divided by two for each plate; Wiener Lab's ELISA: the mean optical density of negative controls plus 0.3; Bio-Manguinhos' and Wiener's ELISA indeterminate ranges were defined as from 1 to 1.2 times and 0.9 to 1.1 times the plate cut-off, respectively; and Bio-Manguinhos' IIF Chagas, Biocientifica's Inmunofluor Chagas and WAMA's Immuno-con Chagas: visualization of fluorescence only at 1:40 dilutions was considered to be an indeterminate test result.

Proportions equivalent to positive and negative predictive values for definite diagnosis were estimated. A logistic regression analysis was conducted to estimate the strength of association of several clinical characteristics to the initial inconclusive test results. Some nested models were fitted and compared through the Wald test. Among those with initial inconclusive test results, a multinomial regression analysis was conducted to explore the possible predictors for definite diagnosis. For these two analysis approaches, patients that were classified as "medical discharge" or "lost to follow-up" but had serological tests available with inconclusive results were classified as inconclusive in order to increase the sample size. All analysis was carried out using the R Project software [7].

Results

Initial screening included 5,570 medical records or laboratory tests; 229 patients were excluded because they had laboratory results but no correspondent medical record and 3,609 patients were excluded because they had a previous investigation for Chagas disease or were under the suspicion of acute Chagas diseases. The final study sample consisted of 1,732 patients.

Chronic Chagas disease prevalence was 20.7% in the first blood sample and 21.1% after definite diagnosis (Fig. 1). Most of the patients who did seek diagnosis at IPEC were female (52.0%); the median age of the patients was 49 (mean 47.5) years and the age range was 0 to 88 years (Table 1). Physicians referred the majority of patients that came to the IPEC's outpatient service (41.6%) (Table 1). Among patients referred by a physician, the most common indication was heart disease, followed by esophagus disease (Table 1). Most were born in rural areas (80.9%) and stated that their mother did not have Chagas disease (47.9%), did not receive blood transfusion in the past (58.9%), and did not have systemic hypertension (60.5%). Only 1% of the patients reported a history of a previous stroke. Complaints compatible with cardiac involvement, such as palpitations, syncope in the past 3 months, or dyspnea on exertion, were reported by 27% of the patients, whereas 4.5% of the patients reported symptoms of esophagus involvement, such as dysphagia, and 2% of the patients reported intestinal symptoms such as persistent constipation. Patients were born in all 27 Brazilian states, but were living in the Rio de Janeiro metropolitan area at the time of the medical evaluation.

The mean (standard deviation) time between the first and the second blood collections among those with initial inconclusive result was 133.8 (120.3) days when a second sample was necessary, and the mean (standard deviation) time between the first and the third blood collections among those with initial inconclusive results was 253.6 (36.0) days when a third sample was necessary. Most of the patients had definite diagnosis after two blood samples and all had definite diagnosis after four consecutive blood samples (Fig. 1). Following current Brazilian recommendations, 8 (0.5%) patients would need either PCR or WB tests to clarify their diagnosis. However, it is possible that some or all of the patients classified as "medical discharge" would be submitted to nonconventional tests if they were available. Therefore, up to 36 (2.1%) patients could have needed nonconventional tests. Inconclusive results summed 2.9% in the first sample and 13.0% in the second sample (Fig. 1).

The main outcomes of the serological investigation were that 16.6% of patients did not return for follow-up after the first blood sample and 25% of the patients did not return after the second blood sample (Fig. 1). The definite diagnosis identified 1,061 patients (61.3%) without Chagas disease, 365 patients (21.1%) with Chagas disease, and 306 patients (17.7%) were lost to follow-up (Table 1).

Fourteen patients from those that were initially inconclusive were submitted to a different set of serological test trademarks in the second sample. Specific serology kits' trademark combinations were the only possible reasons associated with initial inconclusive test results (Table 2). The following adjusted odds ratios for initial inconclusive test results were found: 2.8 for ELISA CHAGAS Bio-Manguinhos (reference Wiener Lab's ELISA) and 3.1 for WAMA's Immuno-con Chagas (reference Bio-Manguinhos'

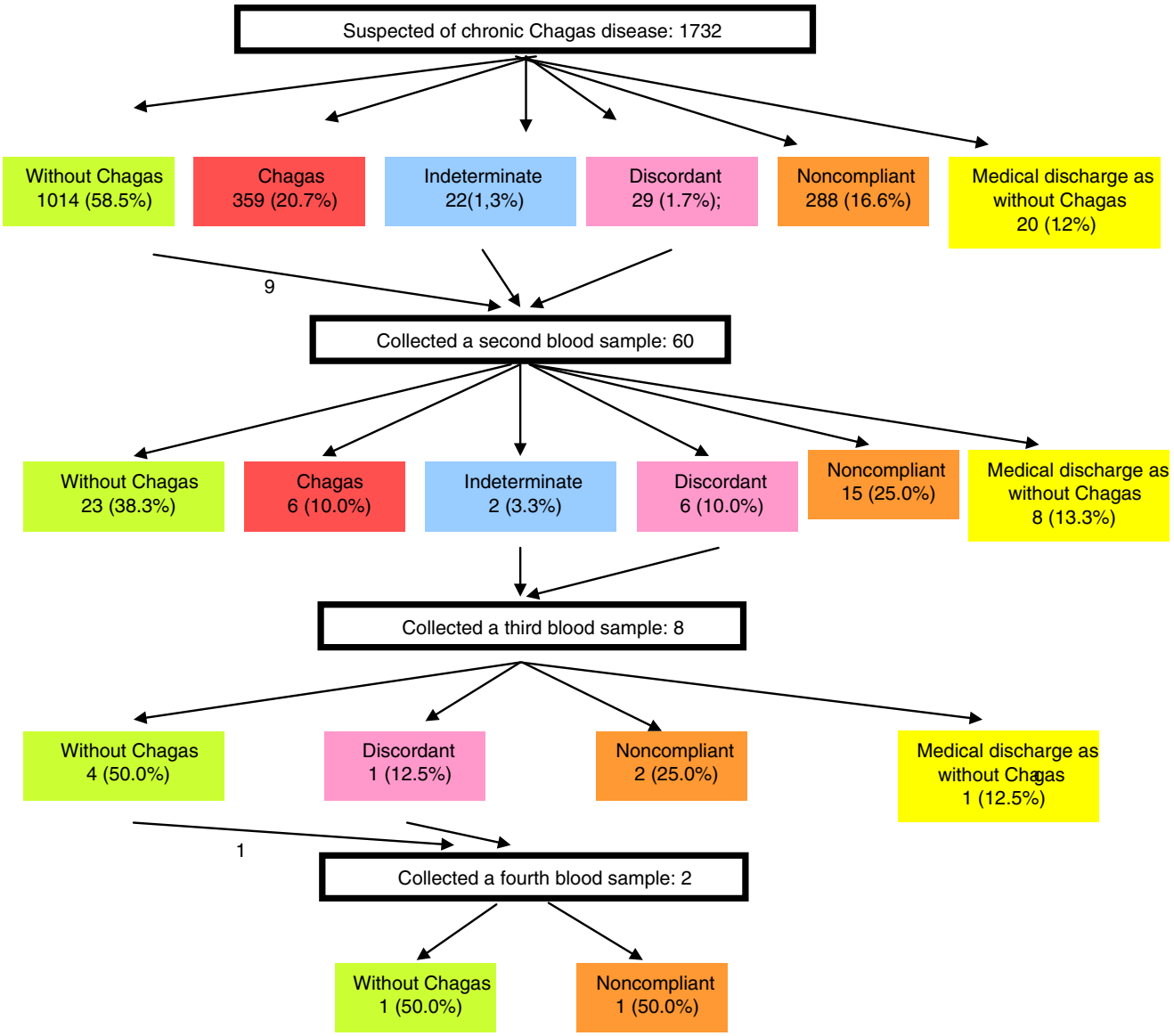

Fig. 1 Applied serologic diagnostic algorithm for chronic Chagas disease

IIF). Bio-Manguinhos' IIF and Biocientifica's IIF showed similar adjusted odds ratios. The serology kits combination that resulted in less inconclusive results was Wiener Lab's ELISA with Bio-Manguinhos' IIF.

After the first blood sample, 51 patients were considered to have inconclusive results and a second sample was requested. Also, another nine patients, whose initial results ruled out Chagas disease, collected a second sample. Definite diagnosis of those with initial inconclusive results was as follows: 6 (11.8%) had Chagas disease, 28 (54.9%) did not have Chagas disease, and 17 (33.3%) were lost to follow-up. Indeterminate test result, in both ELISA and IIF, were more likely to come from subjects without Chagas disease (Table 3). Most definite diagnoses confirmed the findings of the initial ELISA, mainly when the initial ELISA was negative (Table 3). Patients lost to follow-up were more common in the subgroup with initial inconclusive results than in the whole population. Patients discharged as without Chagas disease had this diagnosis relied mostly on the particular combination of serology results rather than on clinical characteristics.

The clinical characteristics (those shown in Table 1) of those initially inconclusive patients were very similar to those who had definite diagnosis with only one sample. There was only a small increase in the prevalence of inconclusive test results in the group of patients referred from physicians due to esophagus findings (7.41% vs. 4.30%) and a small decrease in the prevalence of

Table 1 Clinical characteristics and their respective frequencies according to the definite diagnosis of chronic Chagas disease

Clinical characteristic	Without Chagas, *n* (%)	Chagas, *n* (%)	NC, *n* (%)	Total, *n* (%)
Sex				
Male	489 (60.8)	160 (19.9)	155 (19.3)	804 (100)
Female	572 (63.5)	204 (22.6)	125 (13.9)	901 (100)
Ignored	0 (0)	1 (3.7)	26 (96.3)	27 (100)
Age (years)				
Median (IQR)	48 (34–60)	52 (40.5–61)	43 (30.5–57.5)	49 (36–60)
Referral for diagnosis				
Own will	74 (90.2)	6 (7.3)	2 (2.4)	82 (100)
Relatives	262 (78.7)	69 (20.7)	2 (0.6)	333 (100)
Physician	561 (77.9)	147 (20.4)	12 (1.7)	720 (100)
Blood bank	79 (36.6)	134 (62)	3 (1.4)	216 (100)
Others	22 (91.7)	0 (0)	2 (8.3)	24 (100)
Ignored	63 (17.6)	9 (2.5)	285 (79.8)	357 (100)
Retest executed in BB				
No	25 (38.5)	40 (61.5)	0 (0)	65 (100)
Yes	25 (26.9)	68 (73.1)	0 (0)	93 (100)
Not applicable	982 (64.8)	231 (15.2)	303 (20)	1,516 (100)
Ignored	29 (50)	26 (44.8)	3 (5.2)	58 (100)
Retest result in BB				
Negative	3 (75)	1 (25)	0 (0)	4 (100)
Positive	19 (23.8)	61 (76.2)	0 (0)	80 (100)
Indeterminate	2 (66.7)	1 (33.3)	0 (0)	3 (100)
Not applicable	1,036 (63.2)	297 (18.1)	306 (18.7)	1,639 (100)
Ignored	1 (16.7)	5 (83.3)	0 (0)	6 (100)
Physician referral indication				
Ignored	36 (83.7)	7 (16.3)	0 (0)	43 (100)
Heart	374 (78.6)	97 (20.4)	5 (1.1)	476 (100)
Esophagus	56 (72.7)	19 (24.7)	2 (2.6)	77 (100)
Intestine	28 (75.7)	9 (24.3)	0 (0)	37 (100)
Endemic area	32 (80)	6 (15)	2 (5)	40 (100)
Other	21 (67.7)	7 (22.6)	3 (9.7)	31 (100)
Transplant screening	14 (87.5)	2 (12.5)	0 (0)	16 (100)
Not applicable	499 (49.4)	218 (21.6)	294 (29.1)	1,011 (100)
Born in endemic area				
No	301 (90.9)	23 (6.9)	7 (2.1)	331 (100)
Yes	760 (54.2)	342 (24.4)	299 (21.3)	1,401 (100)
Born in rural area				
No	208 (89.7)	21 (9.1)	3 (1.3)	232 (100)
Yes	471 (66.3)	228 (32.1)	11 (1.5)	710 (100)
Ignored	382 (48.4)	116 (14.7)	292 (37)	790 (100)
Lived in mud houses				
No	354 (84.1)	59 (14)	8 (1.9)	421 (100)
Yes	501 (65.7)	250 (32.8)	11 (1.4)	762 (100)
Ignored	206 (37.5)	56 (10.2)	287 (52.3)	549 (100)
Mother with Chagas diseased				
No	610 (73.5)	206 (24.8)	14 (1.7)	830 (100)
Yes	142 (82.6)	27 (15.7)	3 (1.7)	172 (100)
Ignored	309 (42.3)	132 (18.1)	289 (39.6)	730 (100)

Table 1 (continued)

Clinical characteristic	Without Chagas, *n* (%)	Chagas, *n* (%)	NC, *n* (%)	Total, *n* (%)
Previous blood transfusion				
No	743 (72.8)	262 (25.7)	15 (1.5)	1,020 (100)
Yes	98 (71.5)	38 (27.7)	1 (0.7)	137 (100)
Ignored	220 (38.3)	65 (11.3)	290 (50.4)	575 (100)
Previous blood transfusion before 1991[a]				
No	22 (73.3)	8 (26.7)	0 (0)	30 (100)
Yes	54 (70.1)	22 (28.6)	1 (1.3)	77 (100)
Not applicable	963 (60.4)	327 (20.5)	305 (19.1)	1,595 (100)
Ignored	22 (73.3)	8 (26.7)	0 (0)	30 (100)
Previous blood donation				
No	408 (70.1)	164 (28.2)	10 (1.7)	582 (100)
Yes	201 (66.1)	100 (32.9)	3 (1)	304 (100)
Ignored	452 (53.4)	101 (11.9)	293 (34.6)	846 (100)
Positive screening in previous blood donation				
No	164 (80.8)	37 (18.2)	2 (1)	203 (100)
Yes	37 (37)	62 (62)	1 (1)	100 (100)
Not applicable	859 (60.2)	265 (18.6)	303 (21.2)	1,427 (100)
Ignored	1 (50)	1 (50)	0 (0)	2 (100)
Systemic arterial hypertension				
No	748 (71.4)	283 (27)	17 (1.6)	1,048 (100)
Yes	291 (77.6)	79 (21.1)	5 (1.3)	375 (100)
Ignored	22 (7.1)	3 (1)	284 (91.9)	309 (100)
Coronary artery disease				
No	962 (72.7)	341 (25.8)	21 (1.6)	1,324 (100)
Yes	75 (80.6)	18 (19.4)	0 (0)	93 (100)
Ignored	24 (7.6)	6 (1.9)	285 (90.5)	315 (100)
Previous stroke				
No	972 (73.6)	330 (25)	19 (1.4)	1,321 (100)
Yes	8 (44.4)	9 (50)	1 (5.6)	18 (100)
Ignored	81 (20.6)	26 (6.6)	286 (72.8)	393 (100)
Previous leishmaniasis				
No	999 (73.5)	341 (25.1)	20 (1.5)	1,360 (100)
Yes	3 (60)	2 (40)	0 (0)	5 (100)
Ignored	59 (16.1)	22 (6)	286 (77.9)	367 (100)
Cardiac symptoms				
No	825 (74.5)	267 (24.1)	16 (1.4)	1,108 (100)
Yes	179 (69.1)	76 (29.3)	4 (1.5)	259 (100)
Ignored	57 (15.6)	22 (6)	286 (78.4)	365 (100)
Esophagus symptoms				
No	951 (74.4)	310 (24.2)	18 (1.4)	1,279 (100)
Yes	53 (60.2)	33 (37.5)	2 (2.3)	88 (100)
Ignored	57 (15.6)	22 (6)	286 (78.4)	365 (100)
Intestinal symptoms				
No	975 (74.1)	321 (24.4)	20 (1.5)	1,316 (100)
Yes	30 (58.8)	21 (41.2)	0 (0)	51 (100)
Ignored	56 (15.3)	23 (6.3)	286 (78.4)	365 (100)
ELISA initial results				
Indeterminate	3 (37.5)	2 (25)	3 (37.5)	8 (100)

Table 1 (continued)

Clinical characteristic	Without Chagas, *n* (%)	Chagas, *n* (%)	NC, *n* (%)	Total, *n* (%)
Not reactive	1,040 (78.5)	2 (0.2)	282 (21.3)	1,324 (100)
Reactive	18 (4.5)	361 (90.2)	21 (5.2)	400 (100)
IIF initial results				
Indeterminate	31 (60.8)	6 (11.8)	14 (27.5)	51 (100)
Not reactive	1,001 (78.9)	7 (0.6)	261 (20.6)	1,269 (100)
Reactive	29 (7)	352 (85.4)	31 (7.5)	412 (100)
Total	1,061 (61.3)	365 (21.1)	306 (17.7)	1,732 (100.0)

BB - blood banks, ELISA - enzyme-linked immunosorbent assay, IIF – indirect immunofluorescence, IQR – interquartile range, NC – noncompliant

[a] Brazilian regulation for blood screening for Chagas disease started in 1991

inconclusive test results in the group of patients referred from physicians due to heart findings (23.46% vs. 27.70%). The multinomial regression analysis strategy did not identify possible diagnosis predictors among those with initial inconclusive results. However, this finding was not interpretable, as it was not possible to perform a regression analysis with multiple predictors due to the small number of initial inconclusive results.

The amount of overall missing information about the clinical characteristics (Table 1) did not allow the clinical characteristics' prediction ability to be evaluated appropriately. Nevertheless, previous stroke had the highest positive predictive value (50%) and not born in endemic or rural areas had the highest negative predictive value (90%).

Considering only patients from blood banks, disease prevalence was higher; the proportion of inconclusive results was similar and the proportion of patients lost to follow-up was smaller than in the whole population (Fig. 2).

Discussion

The main findings of this investigation were as follows: (a) the prevalence of initial inconclusive serology results during Chagas disease investigation is low in clinical practice; (b) the need for nonconventional tests can be replaced by serological follow-up; (c) in this study, it was not possible to adequately explore clinical characteristics as predictors of the definite outcome of inconclusive results; (d) serology trademark combinations might reduce inconclusive tests results; (e) the majority of patients with initial inconclusive serological test results are ultimately classified as without Chagas disease; (f) the major pitfall limiting the success of the serological follow-up strategy is the significant number of patients lost to follow-up.

As far as we know, the prevalence of inconclusive results in the serological investigation of chronic Chagas disease in clinical settings was never estimated, and neither are there data available about chronic Chagas disease diagnostic algorithms evaluation in clinical settings. Nevertheless, algorithms were developed and are considered to be appropriate to rule out the risk of Chagas disease transmission through blood products [8–11]. However, the main concerns of blood banks screening are to rule out disease in blood products and to deal with indeterminate and false-positive results after serological screening. Inconclusive and all positive test results (false-positive or not) are referred to diagnosis; thus, these algorithms cannot be applied to the diagnostic clinical scenario. Although there was a major concern regarding inconclusive results and the inability to make a correct diagnosis of Chagas disease in the past [12–16], the low prevalence of this problem found in this research reveals that the actual need for nonconventional tests would be low. Nevertheless, reports and guidelines suggested several approaches to deal with inconclusive results or with clinical scenarios where Chagas disease is the most likely diagnosis and serology does not

Table 2 Logistic regression output showing the possible explanations for inconclusive results

Factor	Effect	SE	OR	Lower Cl	Upper Cl
ELISA Bo-Manguinhos + Wiener Lab	1.02	0.24	2.77	1.71	4.47
IIF Biocientifica + Bio-Manguinhos	−0.05	0.61	0.95	0.29	3.13
IIF Wama + Bio-Manguinhos	1.14	0.41	3.11	1.40	6.91

Effect – regression coefficient, ELISA – enzyme-linked immunosorbent assay trademark, IIF – indirect immunofluorescence trademark, Lower Cl – 95% OR inferior confidence limits, OR – odds ratio, SE – standard error, Upper Cl – 95% OR superior confidence limits

Table 3 Frequencies of serological test result combinations and definite diagnosis of Chagas disease from patients with initial inconclusive tests

ELISA/IIF	Without Chagas, *n* (%)	Chagas, *n* (%)	NC, *n* (%)	Total, *n* (%)
Indeterminate/not reagent	0 (0)	0 (0)	3 (100)	3 (100)
Indeterminate/reagent	3 (60)	2 (40)	0 (0)	5 (100)
Not reagent/indeterminate	29 (69)	0 (0)	13 (31)	42 (100)
Not reagent/reagent	12 (52.2)	0 (0)	11 (47.8)	23 (100)
Reagent/indeterminate	1 (50)	1 (50)	0 (0)	2 (100)
Reagent/not reagent	2 (33.3)	3 (50)	1 (16.7)	6 (100)
Total	47 (58.0)	6 (7.4)	28 (34.6)	81 (100.0)

ELISA – enzyme-linked immunosorbent assay, IIF – immunofluorescence, NC – noncompliant

confirm it, such as: running a third test with the same sample [1–3, 6, 14]; serological follow-up [2, 3, 5, 14]; the use of WB [5, 6, 14, 15]; the use of PCR [5, 6, 14]; and other nonconventional tests [3, 12, 14, 16]. From these options, serological follow-up proved to be feasible and appropriate in most cases, and may prove to be a good strategy in places where nonconventional tests are not available.

Many risk factors or predictors were explored and found to be associated with chronic Chagas disease among blood donors [9, 12, 17–22] and in rural area settings [23–28]. However, as far as we know, no previous investigation addressed which clinical characteristics could predict chronic Chagas disease diagnosis among those with initial inconclusive serology results in the clinical setting. This study showed that a few clinical characteristics could have a potential role in predicting the definite diagnosis in this setting, but this was not possible to confirm in the regression analysis with multiple predictors. The small sample size of inconclusive serology results, together with the amount of missing clinical data in this investigation, probably compromised this evaluation.

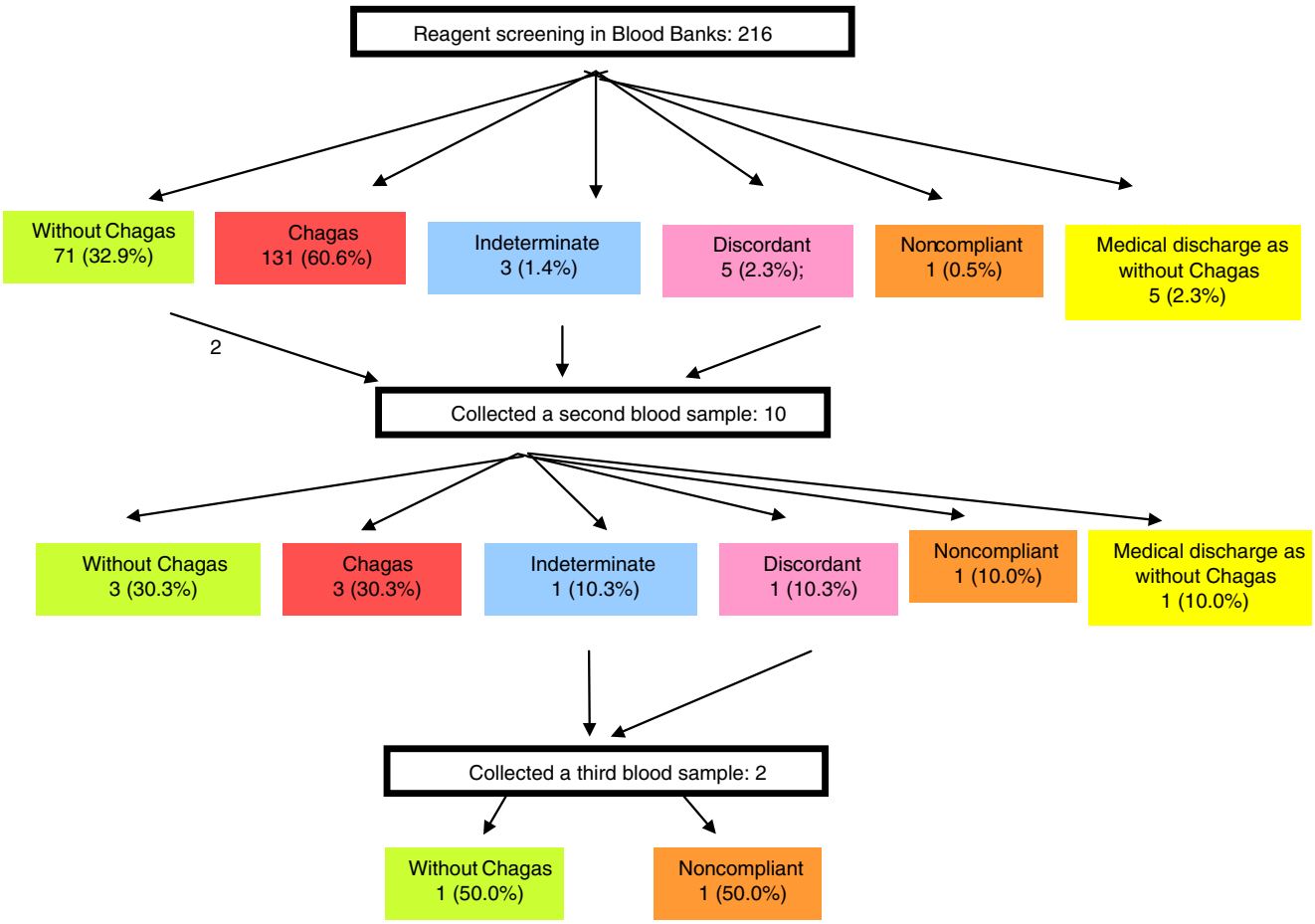

Fig. 2 Applied serologic diagnostic algorithm for chronic Chagas disease in patients referred from blood banks

Regression analysis showed that specific serology trademark combinations were associated with inconclusive results. Therefore, the test accuracy may depend on some quality issue related to the trademark and a particular trademark combination could be used to reduce the incidence of inconclusive results in clinical practice. In most cases, the ELISA's initial result was confirmed after serological follow-up. Thus, in several cases, the inconclusive results will be attributed to IIF's lack of reliability or accuracy, and the IIF's utility in the diagnostic strategy may be far less important than the ELISA's. If a single ELISA test for chronic Chagas disease diagnosis is used, the number of inconclusive results may be substantially reduced. Some authors have used a third test (either a different method or similar techniques with different antigens, such as ELISA with recombinant antigens) in the first sample, where the patient needs to have two reagent tests out of three in order to be considered for Chagas disease, thus, avoiding discordant results. Despite the great concern about how to handle inconclusive test results, a major problem found during this retrospective analysis was the number of patients lost to follow-up. It is important to establish strategies to reduce the noncompliance to diagnostic investigation, as patients that do not know their final diagnosis may miss the opportunity of appropriate health assistance or may later apply for blood donation. All current guidelines [1–3, 5, 6] state recommendations to proceed in case of inconclusive results, but none state any consideration about investigation noncompliance.

One possible strategy to reduce noncompliance, although less feasible, is to perform nonconventional tests earlier in the diagnostic algorithm. If the initial test results are inconclusive, a third test could be performed using the same blood sample before the results are disclosed to the patient. In this approach, at least two out of three reagent tests are necessary so as to classify the patient as having Chagas disease. However, nonconventional tests, such as PCR or WB, are not easily accessed, as no commercial PCR or WB is currently available, and these nonconventional tests are only available at research or reference centers. Also, a third test could be a commercially available test, such as hemagglutination, or a similar test with different antigens, such as ELISA with recombinant antigens. Other alternatives to reduce noncompliance should be considered, such as: using a single ELISA, systematic phone or mail contact to warn about medical appointments, home visits, or incentives (e.g., bus tokens), and reducing the time between the first and the second tests.

This research has potential limitations that should be considered. Disease prevalence among the patients investigated at our health unit is expected to be higher than in the general population, as IPEC is a reference center to where blood donors and patients from other health units are referred. The patients' clinical characteristics may also be quite different from other health units, due to the same reasons. Another possible limitation is that IPEC has a very experienced immunodiagnostic service, thus, the prevalence of inconclusive serological test results may be lower than elsewhere.

Conclusions

Despite current Brazilian guidelines recommending nonconventional tests in order to clarify persistent inconclusive serology results, serological follow-up associated with medical evaluation was able to clarify the definite diagnosis of all patients that were not lost to follow-up. Enzyme-linked immunosorbent assay (ELISA) negative results in an initial inconclusive diagnosis are usually confirmed after serological follow-up and clinical evaluation. However, the follow-up strategy leads to a significant number of patients lost in the diagnostic investigation, because they either do not return to collect new blood samples or do not return to a new medical visit. Therefore, strategies to reduce serological investigation noncompliance should be considered and some of them could be easily implemented.

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Conflicts of interest No conflicts of interest to declare.

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