Title

Response to Letters Regarding Article, "Ten-Year Incidence of Chagas Cardiomyopathy Among Asymptomatic, Trypanosoma cruzi–Seropositive Former Blood Donors"

Permalink

https://escholarship.org/uc/item/8m63n8hr

Journal

Circulation, 128(9)

ISSN

0009-7322

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Publication Date

2013-08-27

DOI

10.1161/circulationaha.113.004018

Peer reviewed

Coronary Heart Disease

Ten-Year Incidence of Chagas Cardiomyopathy Among Asymptomatic *Trypanosoma cruzi*–Seropositive Former Blood Donors

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Background—Very few studies have measured disease penetrance and prognostic factors of Chagas cardiomyopathy among asymptomatic *Trypanosoma cruzi*–infected persons.

Methods and Results—We performed a retrospective cohort study among initially healthy blood donors with an index *T cruzi*–seropositive donation and age-, sex-, and period-matched seronegatives in 1996 to 2002 in the Brazilian cities of São Paulo and Montes Claros. In 2008 to 2010, all subjects underwent medical history, physical examination, ECGs, and echocardiograms. ECG and echocardiogram results were classified by blinded core laboratories, and records with abnormal results were reviewed by a blinded panel of 3 cardiologists who adjudicated the outcome of Chagas cardiomyopathy. Associations with Chagas cardiomyopathy were tested with multivariate logistic regression. Mean follow-up time between index donation and outcome assessment was 10.5 years for the seropositives and 11.1 years for the seronegatives. Among 499 *T cruzi* seropositives, 120 (24%) had definite Chagas cardiomyopathy, and among 488 *T cruzi* seronegatives, 24 (5%) had cardiomyopathy, for an incidence difference of 1.85 per 100 person-years attributable to *T cruzi* infection. Of the 120 seropositives classified as having Chagas cardiomyopathy, only 31 (26%) presented with ejection fraction <50%, and only 11 (9%) were classified as New York Heart Association class II or higher. Chagas cardiomyopathy was associated ($P<0.01$) with male sex, a history of abnormal ECG, and the presence of an S_3 heart sound.

Conclusions—There is a substantial annual incidence of Chagas cardiomyopathy among initially asymptomatic *T cruzi*–seropositive blood donors, although disease was mild at diagnosis. ***(Circulation.* 2013;127:1105-1115.)**

Key Words: blood donors ■ Brazil ■ Chagas cardiomyopathy ■ Chagas disease ■ incidence

In endemic areas, the majority of patients with acute Chagas disease are asymptomatic, and most symptomatic patients present only minor clinical manifestations. Most untreated cases evolve into the so-called indeterminate form of chronic Chagas disease, namely seropositive but without cardiac or intestinal pathology.[1,2] With universal blood bank screening for Chagas disease now being practiced in many endemic and some nonendemic regions, asymptomatic seropositives are now being routinely detected.[3,4] Counseling those individuals is not easy because the natural history of the disease is poorly

understood. Several natural history studies are based on patients referred to hospitals, and studies based on population-based serological screening are >20 years old.[5–7]

The evolution from indeterminate status to clinical Chagas cardiomyopathy or gastrointestinal disease is thought to occur 10 to 20 years after acute infection in a slow and progressive fashion. Previous studies suggest that up to 5% of patients will evolve each year from the indeterminate form to a clinical form of the disease.[7]

However, data are lacking on clinical predictors for severe clinical disease that could guide early treatment. Current Centers for Disease Control and Prevention guidelines indicate antitrypanosomal treatment of all confirmed seropositives ≤18 years of age, strong consideration of treatment for those 19 to 50 years of age, and optional treatment for those >50 years of age.[8] In Brazil, however, cardiologists currently refrain from treating asymptomatic subjects because of the uncertain prognosis and the toxicity of available antitrypanosomal drugs.[2,9]

As part of the National Heart, Lung, and Blood Institute (NHLBI) Retrovirus Epidemiology Donor Study-II (REDS-II), we performed a retrospective cohort study to characterize the natural history of clinical Chagas disease in *Trypanosoma cruzi*–seropositive blood donors identified 10 years earlier. We now report the clinical cardiology outcomes of this well-characterized cohort.

Methods

Overall Study Design

This was a retrospective cohort study in which we planned to enroll 500 donors (250 from the city of São Paulo and 250 from the city of Montes Claros in the state of Minas Gerais) who were confirmed positive for *T cruzi* antibody at the time of blood donation in 1998 to 2002 and 500 seronegative donors matched by year of index donation, site, age, and sex. Recruited individuals were submitted to a questionnaire and a medical evaluation. The local laboratories conducted a lipid panel (high-density lipoprotein, low-density lipoprotein, total cholesterol, and triglycerides). An ECG and an echocardiogram were performed and interpreted by central reading centers.

Blood Bank Screening Procedures

In São Paulo, the Fundação Pro-Sangue blood center performed *T cruzi* antibody screening in 1996 to 2002 for *T cruzi* antibodies that consisted of 3 serological methods, ELISA, hemaglutination, and immunofluorescence, as previously described.[10,11] If reactive by any of the serological assays, the blood unit was discarded, and the donor was invited to return to repeat the same tests. For the purpose of this study, we included confirmed Chagas donors who were positive by all 3 assays at the time of donation and a separate sample obtained at the time of counseling after the index donation. A total of 255 index plasma units were available from eligible donors, and those donors were preferentially contacted for the study. In Montes Claros, the Hemominas blood center screened all donations with 2 serological assays: ELISA and hemaglutination. Donors reactive to both assays at the time of donation and counseling were considered eligible for this study.

Enrollment

Donors were initially contacted via an invitation letter using the address present in the blood bank database. Individuals who responded to the initial invitation were telephoned; numbers were updated from the city public telephone list. For eligible donors who could not be located or enrolled, vital status was determined from the national death index system, which has been available only since 2001 and includes deaths from 2001 to 2009 (national death index system version 11/01/2010, Brasilia, Brazil). Brazil does not have a unique national identity number, so searches for cause of death were performed with software to link names through the use of Soundex for Portuguese phonetics (Record Linkage III, version 3.0.4 4005, Rio de Janeiro, Brazil). The national death index records were searched using the subject's name, date of birth, mother's name, and sex for all eligible seropositive and seronegative donors who were unable to be located during the recruitment period (July 2008–October 2010) in both sites. In a preliminary study, the sensitivity and specificity of the record linkage method used were determined to be 93.9% (95% confidence interval, 89.6–96.8) and 91% (95% confidence interval, 86.2–94.6), respectively.

Validation Cases

To assess the sensitivity of our cardiac outcomes measurement, a total of 101 previously diagnosed cases of Chagas cardiomyopathy were enrolled from the Heart Institute of the University of São Paulo Medical School. Inclusion criteria included a physician diagnosis of Chagas cardiomyopathy, no previous treatment with benznidazole, and no comorbidity such as diabetes mellitus, hypertension, or renal failure. These individuals were recruited by letter and telephone call with the use of information in the hospital database.

Measurements

A face-to-face *T cruzi* risk factor and health history questionnaire was administered by a nurse at each site. The questionnaire collected detailed information on demographics, cities of residence, physical activity, medical history, exposure to *T cruzi*, previous Chagas disease diagnoses, cardiac and gastrointestinal symptoms, medical history, and medication history. All subjects received a physical examination by a nonblinded physician; height, weight, blood pressure, pulse, and physical examination findings were recorded. Subjects were assigned to a New York Heart Association functional class on the basis of their responses to the symptom and physical activity questionnaire.

Electrocardiograms

Resting 12-lead ECGs were recorded with the same model of machine at both sites (General Electric MAC 1200 electrocardiograph; GE Healthcare, Waukesha, WI) using strictly standardized procedures.[12,13] All ECGs were interpreted blindly by researchers at the central ECG laboratory at the Epidemiological Cardiology Research Center, Wake Forest University (Winston-Salem, NC), where they were visually inspected for technical errors and inadequate quality and processed with the 2001 version of the GE Marquette 12-SL program. ECGs were analyzed electronically, with manual overreading by trained cardiologists to ensure quality control. ECGs were classified by Minnesota code[13] criteria using variables that were derived from the median complex of the Marquette measurement matrix. In this study, major and minor ECG abnormalities were defined as previously established[12] and modified to include ECG abnormalities typical of Chagas cardiomyopathy with prognostic significance such as low voltage or frequent supraventricular or ventricular premature beats.[6]

Echocardiograms

Complete echocardiographic studies were performed with a Sequoia 512 ultrasound machine (Acuson, Mountain View, CA) at the São Paulo site and a GE Vivid3 (GE Healthcare) at the Monte Claros site. Two-dimensional guided M-mode measurements of the left ventricle (LV) were taken, and LV mass corrected by body surface area was calculated from the Devereux formula.[14] From the apical acoustic window, 2-dimensional images of the left atrium, right atrium, and LV were traced, and LV ejection fraction was calculated by the use of a modified form of the Simpson biplane

method.[15] LV regional wall motion was based on 17-segment model segmentation, and each segment was confirmed in multiple views as follows: normal or hyperkinesis, hypokinesis, akinesis, dyskinesis, and aneurysmal. Wall motion score index was derived as a sum of all scores divided by the number of segments visualized. Diastolic function was assessed from pulsed-wave Doppler of the transmitral in-flow velocities, with the sample volume at the mitral valve leaflet tips, and from tissue Doppler imaging of the septal and lateral mitral annulus, both at the apical 4-chamber view.[16] Mitral, tricuspid, aortic, and pulmonary regurgitations were qualitatively evaluated; continuous-wave Doppler tricuspid regurgitation peak velocity was measured; and the presence of pericardial effusion was evaluated. All studies were recorded in digital format, and all measurements were performed on digital loops with an offline Prosolv cardiovascular workstation (version 3.5 software, Fujifilm) at the Echocardiography Laboratory of the Cardiovascular and Pulmonary Medicine Branch of the NHLBI (Bethesda, MD) by researchers blinded to serostatus.

Outcome Adjudication

All data were centralized by the coordinating center. A predefined set of abnormalities in the echocardiogram or ECG measurements triggered the expert panel to review the donor's cardiac findings to determine whether they were attributable to Chagas disease (Figure 1). The expert panel was composed of 3 Brazilian cardiologists with expertise in the management of Chagas disease patients who received a summary of questionnaire clinical, laboratory, ECG,

and echocardiogram findings from the coordinating center. The physicians were blinded to the subject's serostatus and were asked to answer the following question: "If this patient were seropositive for *T cruzi*, how would you classify him/her: Chagas cardiomyopathy, probable Chagas cardiomyopathy, possible Chagas cardiomyopathy, or no Chagas cardiomyopathy?"

Responses from each expert were sent separately to the coordinating center. If the physicians did not agree, the data were reviewed in a phone call. The online-only Data Supplement summarizes the main findings considered to define the classification of the clinical status, including clinical examination, ECG, and echocardiogram findings. Although most decisions were based on these classification rules, the physicians' experience and clinical expertise were also essential to reach, by consensus, a final classification.

Statistical Analysis

Incidence rates of Chagas cardiomyopathy were calculated by dividing the number of incident cases by the person-years of follow-up calculated from the date of index donation until the date of clinical examination. Incidence rates, calculated separately for the *T cruzi* seropositives and seronegatives, were subtracted to obtain an incidence rate difference.

Test sensitivity of the ECG and echocardiogram was calculated as the number of test positives divided by the number of Chagas cardiomyopathy cases diagnosed by the expert panel; specificity was determined from the number of test negatives divided by the subjects without cardiomyopathy according to the expert. The positive
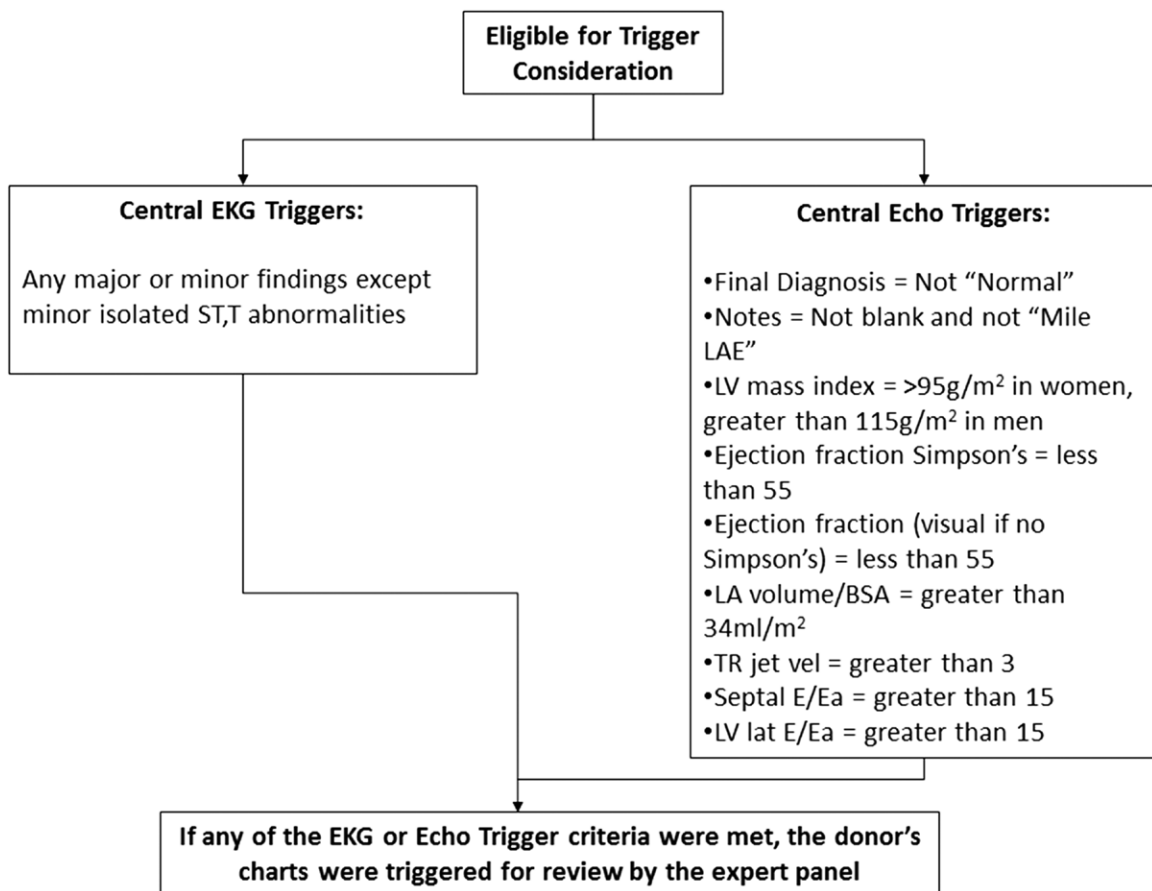
Figure 1. Expert panel review criteria. The Retrovirus Epidemiology Donor Study-II (REDS-II) Chagas study protocol included an expert panel review, tasked with determining whether a donor's cardiac findings were attributable to Chagas disease. Those records meeting the cardiac signs and symptoms trigger algorithm presented here were selected for review by the 3-member expert panel. Only records that had complete clinical examination, ECG, and echocardiography data were eligible for trigger consideration. The Appendix in the online-only Data Supplement gives additional information on how the expert panel designated a person as definite, probable, possible, or not Chagas cardiomyopathy. BSA indicates body surface area; LA, left atrial; LAE, left atrial enlargement; LV, left ventricular; and TR, tricuspid regurgitation.

predictive value was the number of Chagas cardiomyopathy cases divided by the number of test positives, and the negative predictive value was the number of subjects without cardiomyopathy divided by the number of test negatives.

Between Chagas cardiomyopathy cases and *T cruzi* seropositives without cardiomyopathy, categorical covariates were compared by use of χ^2 tests, and continuous covariates were compared by use of *t* tests. Logistic regression was used to model the probability of *T cruzi* serpositives being classified as definite Chagas cardiac disease by the expert panel. Unadjusted models show odds ratios relative to a reference group for categorical covariates and odds ratio per unit change for continuous covariates. Categorization of continuous covariates and other alternatives was considered, but none showed substantive difference in modeling. The final logistic regression model was developed with the use of a backward elimination approach. Variables with significant or nearly significant univariate associations and with biologically plausible involvement in pathophysiology were considered for inclusion; only covariates with values of *P*<0.05 were retained in the model. All statistical analyses were conducted with SAS (SAS 9.2; SAS Institute, Cary, NC).

Results

Study Population and Risk Factors

A total of 1327 and 1887 letters were sent to seropositive and seronegative blood donors respectively. Figure 2 summarizes the inclusion and exclusion into the cohort. The enrollment rate was slightly higher for seropositives (39%, 511 enrolled of 1327 recruited) compared with seronegatives (27%, 50 of 1887). The predominant reason for not enrolling in the study was the inability to locate the person; ≈59% of the seropositives and 67% of the seronegatives were not located. The death records search revealed that 65 seropositives (4.9%) were deceased of 1327 seropositives who had recruitment letters sent compared with 37 (1.96%) deceased among 1887 seronegatives who had recruitment letters sent (*P*<0.0001, χ^2).

Of those who enrolled in the study, a total of 499 seropositives and 488 seronegatives had complete data and could be submitted for the expert panel review process (ie, trigger consideration). Table 1 summarizes the demographic characteristics of those eligible for trigger consideration. Approximately 50% of the seropositives and seronegatives were from São Paulo and 50% were from Montes Claros. Similarly, ≈50% of the seropositives and seronegatives were male, and the distributions of age and skin color (Brazilians do not measure race) were similar. Seropositives were more likely to be of lower educational attainment, consistent with the recognized concentration of *T cruzi* infection among those of lower socioeconomic status. Various risk factors for *T cruzi* infection were also reported more commonly among seropositives, as was length of exposure to *T cruzi* risk factors (*P*<0.0001). Seropositives and seronegatives were similar in terms of physical examination and laboratory measurements.

Cardiac Outcomes

All 101 validation cases of Chagas cardiomyopathy were triggered, and 99 were classified as Chagas cardiomyopathy by the expert panel (sensitivity, 98%). The 499 seropositives and 488 seronegatives with complete data who were eligible for trigger consideration were not significantly different in terms of their past diagnoses and medication use, except that the use of furosemide, spironolactone, captopril, aspirin, and benznidazole was reported more frequently by the seropositives (Table 2).

Of the 499 seropositives and 488 seronegatives eligible for the expert panel review process, 63% and 48%, respectively, presented abnormalities and were triggered for review. Table 2 summarizes the expert panel results for cases and controls. Twenty-four controls (5%) were classified as having Chagas cardiomyopathy (specificity, 95% for the expert panel process). Among the 499 *T cruzi* seropositives, 315 (63%) were referred to the expert panel, and 120 (24%) had Chagas cardiomyopathy. There was no difference in the prevalence of probable Chagas cardiomyopathy (3%) or possible Chagas cardiomyopathy (10%–11%) between seropositives and seronegatives. Of the 120 seropositives classified as having Chagas cardiomyopathy, only 31 (26%) presented with ejection fraction <50%, and only 11 (9%) were classified as New York Heart Association functional class II or higher.

The mean follow-up time between the index donation and outcome assessment was 10.5 years for the seropositives and 11.1 years for the seronegatives (range, 7–14 years). If we assume that all Chagas positive donors were without cardiomyopathy at the time of donation, the cardiomyopathy incidence attributable to *T cruzi* infection would be 1.85 per 100 person-years.

Most of the 24 *T cruzi*–seronegative subjects who were misclassified as having Chagas cardiomyopathy had at least 1 typical finding, as defined in the online-only Data Supplement: global LV hypocontractibility in 15 patients, right bundle-branch block in 3 patients (1 patient also with LV hypocontractibility), and a combination of typical abnormalities in the other 7 patients. These findings were likely due to other types of cardiac disease, including coronary artery disease.

Performance of Screening Tests

Retrospective analysis of the algorithmic trigger for referral to the expert panel showed that of the 99 previously diagnosed Chagas cardiomyopathy cases, 1 (1%) was triggered by echocardiogram alone, none by ECG alone, and 98 (99%) by both echocardiogram and ECG. Of 120 *T cruzi*–seropositive cardiomyopathy cases, 6 (5%) were triggered by echocardiogram alone, 45 (38%) by ECG alone, and 69 (58%) by both echocardiogram and ECG. Finally, of the 24 *T cruzi*–seronegative cardiomyopathy cases, 8 (33%) were triggered by echocardiogram alone, 1 (4%) by ECG alone, and 15 (63%) by both echocardiogram and ECG. Thus, for abnormal ECG, the sensitivity was 95%, specificity was 60%, positive predictive value was 43%, and negative predictive value was 97%. For abnormal echocardiogram, the sensitivity was 63%, specificity was 80%, positive predictive value was 49%, and negative predictive value was 87%.

Clinical Characteristics of Chagas Cardiomyopathy Diagnoses

Among seropositives, Chagas cardiomyopathy was significantly associated with male sex (odds ratio, 1.97; 95% confidence interval, 1.23–3.16), a history of an abnormal ECG (odds
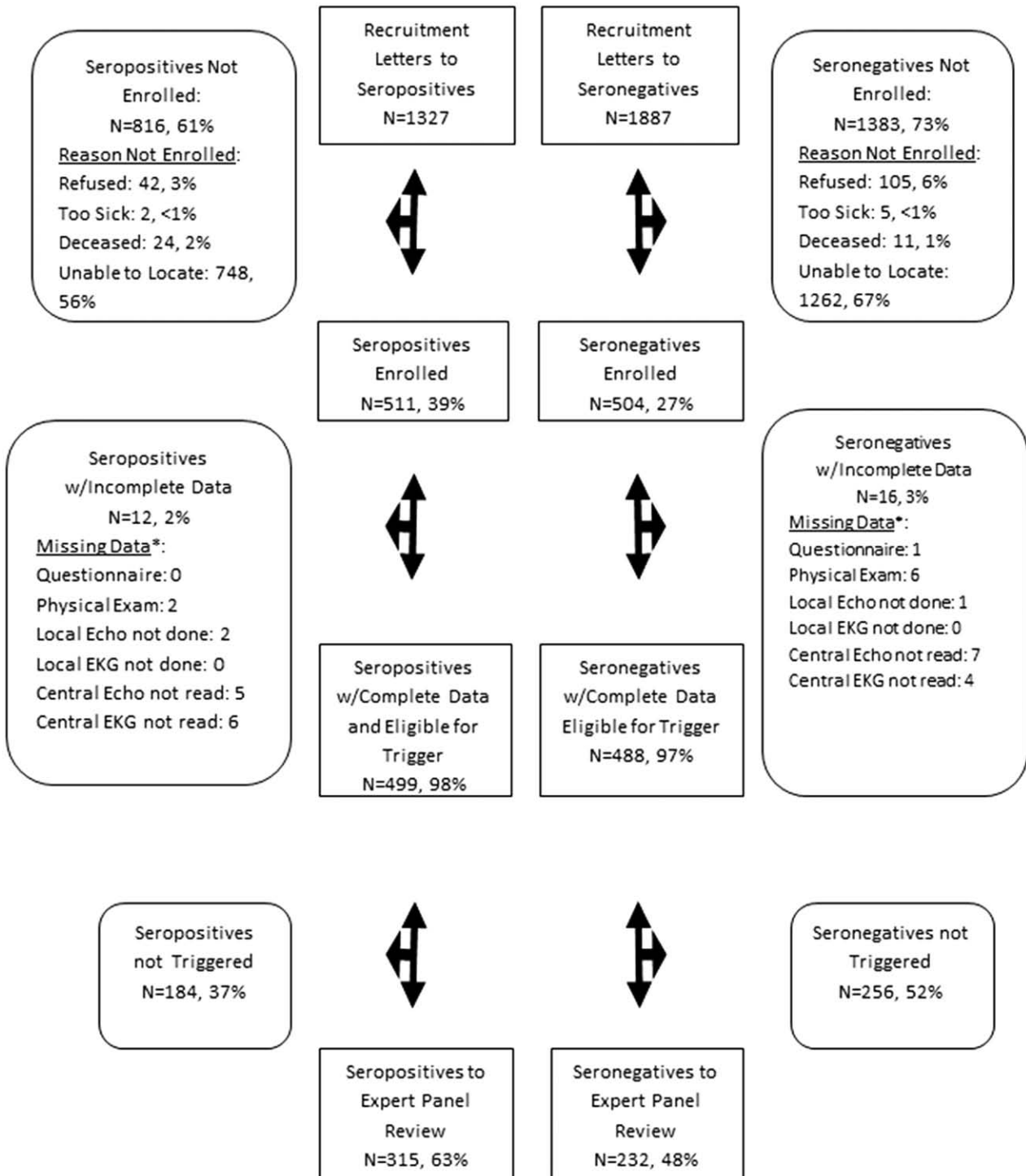
Figure 2. Donors included and excluded in the Retrovirus Epidemiology Donor Study-II (REDS-II) Chagas study. A total of 1327 seropositive and 1887 seronegative blood donors were recruited for this study. In total, 39% of the seropositives and 27% of the seronegatives enrolled. Ninety-eight percent of the enrolled seropositives and 97% of the enrolled seronegatives had complete clinical, ECG, and echocardiography data and were considered eligible for trigger consideration. Sixty-three percent of the seropositives and 48% of the seronegatives met the trigger criteria, and their cardiac findings were reviewed by the expert panel.

ratio, 6.09; 95% confidence interval, 3.64–10.19), and S_3 heart sound on physical examination (odds ratio, 6.01; 95% confidence interval, 3.00–12.05) but not with city, age, race, cigarette smoking, alcohol intake, or other medical diagnoses (Table 3).

Forty-nine of *T cruzi* seropositives (10%) reported that they had received any prior benznidazole treatment, although it was not clear if they had received a full course of treatment. There was no difference in the incidence of Chagas cardiomyopathy among seropositives previously treated with

Table 1.   Demographic, Exposure, and Medical Characteristics of the Study Population by Antibody Status

	Trypanosoma cruzi Seropositive (n=499)	T cruzi Seronegative (n=488)
Site, n (%)		
Sao Paulo	255 (51)	239 (49)
Montes Claros	244 (49)	249 (51)
Sex, n (%)		
Male	261 (52)	241 (49)
Female	238 (48)	247 (51)
Age, n (%)		
20–29 y	4 (0.8)	4 (0.8)
30–39 y	110 (22)	90 (18)
40–49 y	163 (33)	151 (31)
50–59 y	142 (28)	152 (31)
≥60 y	80 (16)	91 (19)
Skin color, n (%)		
White	155 (31)	203 (42)
Black	56 (11)	28 (6)
Mixed	274 (55)	249 (51)
Other	11 (2)	7 (1)
Refused to answer/missing	3 (1)	1 (<1)
Education, n (%)		
Adult alphabet or no school	46 (9)	11 (2)
Did not complete elementary school	239 (48)	134 (27)
Completed elementary school	87 (17)	60 (12)
Completed high school	108 (22)	171 (35)
Completed college, technical, or above	17 (3)	112 (23)
Missing	2 (<1)	0
Chagas risk factors (answered "yes" to the following), n (%)		
Ever lived in a rural area?	464 (93)	248 (51)
Ever lived in an area where the kissing bug was present?	441 (88)	191 (39)
Know how to recognize a kissing bug?	390 (78)	247 (51)
Ever bitten by a kissing bug?	249 (50)	17 (3)
Know someone bitten by a kissing bug?	327 (66)	251 (51)
Relative/friend bitten by kissing bug?	326 (65)	248 (51)
House of wood or mud/thatched roof?	395 (79)	153 (31)
Any relative ever had Chagas disease?	322 (65)	117 (24)
Exposure to Chagas	Mean±SD	Mean±SD
Time exposed to Chagas (mean±SD), y	18 (13)	8 (14)
Age at first exposure to Chagas, y	1 (3)	1 (4)
Age at last exposure to Chagas, y	21 (13)	23 (16)
Physical examination outcomes		
Height, cm	164 (9)	165 (10)
Weight, kg	72 (12)	76 (14)
Body mass index, kg/m^2	27 (4.0)	28 (5)
Pulse, bpm	66 (9)	68 (9)
Systolic blood pressure, mm Hg	124 (18)	125 (19)
Diastolic blood pressure, mm Hg	76 (14)	77 (16)
		(continued)

Table 1.   (Continued)

	Trypanosoma cruzi Seropositive (n=499)	T cruzi Seronegative (n=488)
Total cholesterol, mg/dL	199 (42)	204 (51)
High-density lipoprotein cholesterol, mg/dL	50 (14)	48 (13)
Low-density lipoprotein cholesterol, mg/dL	122 (37)	126 (39)
Triglycerides, mg/dL	137 (89)	151 (100)
Diagnosed with the following health problems, n (%)		
Diabetes mellitus	27 (5)	24 (5)
Kidney problems	15 (3)	15 (3)
Coagulation problems	2 (0.4)	1 (0.2)
Heart attack	3 (0.6)	5 (1)
Hypertension	113 (23)	119 (24)
Glucose	90 (22)	93 (27)
Medication use, currently taking or ever taken, n (%)		
Digoxin	4 (1)	0
Amiodarone	5 (1)	2 (<1)
Furosemide	14 (3)	2 (<1)
Hydrochlorothiazide	42 (8)	41 (8)
Spironolactone	12 (2)	1 (<1)
Captopril	46 (9)	25 (2)
Enalapril	15 (3)	20 (4)
Losartan	11 (2)	13 (3)
Carvedilol	7 (1)	3 (1)
Atenolol	14 (3)	15 (3)
Propranolol	10 (2)	8 (2)
Hydralazine	0	1 (<1)
Isosorbide mononitrate	1 (<1)	3 (1)
Amlodipine	9 (2)	9 (2)
Warfarin	4 (1)	2 (<1)
Aspirin	28 (6)	14 (3)
Nifurtimox	1 (<1)	0
Benznidazole	49 (10)	0

benznidazole (22%) compared with those who had received no treatment (24%).

Discussion

This 10-year retrospective cohort study provides strong evidence for a moderate rate of progression to cardiomyopathy (1.85%/y) among persons infected with *T cruzi* but without cardiomyopathy at baseline. The inclusion of a *T cruzi*–seronegative comparison cohort allowed measurement and elimination of competing morbidity resulting from other cardiovascular disease. We found relatively few demographic or clinical prognostic factors other than sex, a history of cardiac arrhythmia, and S_3 gallop on physical examination. This study yielded valuable information on the performance of our screening algorithm and suggests that initial screening by ECG followed by echocardiogram on those with abnormal ECGs is a viable strategy. Finally,

Table 2. Chagas Cardiomyopathy: Past Diagnoses and Expert Panel Review

	Trypanosoma cruzi Seropositive (n=499), n (%)	T cruzi Seronegative (n=488), n (%)	P
Current diagnosis by expert panel			<0.0001
Not triggered for expert review	184 (37)	256 (52)	
Expert panel review decision			
Definite Chagas cardiac disease	120 (24)	24 (5)	
Probable Chagas cardiac disease	15 (3)	14 (3)	
Possible Chagas cardiac disease	53 (11)	49 (10)	
Not Chagas cardiac disease	127 (26)	145 (30)	
New York Heart Association functional class (definite Chagas only)			0.76
1=Ordinary physical activity does not cause dyspnea	107 (90)	23 (96)	
2=Slight limitation of ordinary activity	10 (8)	1 (4)	
3=Marked limitation of ordinary activity	1 (<1)	0	
Missing	2 (2)	0	
Ejection fraction (definite Chagas only)			0.09
≥50%	88 (74)	13 (57)	
<50%	31 (26)	10 (43)	

treatment with benznidazole was rare in this cohort, and we found no evidence that cardiomyopathy was reduced in the few subjects who were treated.

Because the subjects in the study had to pass blood donor eligibility criteria that would have excluded those with symptomatic cardiac disease from blood donation, we are confident that the cohort did not have clinically significant cardiomyopathy at baseline. Thus, we believe that cases detected an average of 10 years later by our diagnostic algorithm represent truly incident cases, allowing the calculation of the incidence rate of 1.85 per 100 person-year. This incidence rate, if summed over 10 years of follow-up, is consistent with previous estimates of a 20% to 30% rate of disease penetrance, lending credence to our findings.[8,17] Differences in disease penetrance estimates between studies may be due to study population, definition of disease progression, loss to follow-up, and enrollment bias. In regard to enrollment bias, we saw a higher death rate among nonenrolled the seropositives compared with nonenrolled seronegatives, so our incidence may be slightly underestimated. The incidence rate from the present study may be used by physicians to give patients prognostic information when they present with *T cruzi* seropositivity of unknown duration. It may also be used by public health authorities to estimate the future burden of Chagas cardiomyopathy in Brazil and to model the cost and efficacy of treatment interventions.

Our diagnostic algorithm used a stratified screening procedure that included physician examination, ECG, and echocardiogram. The sensitivity and specificity of the algorithm were very good, as estimated by its detection of cardiomyopathy in 99 of 101 cases of previously diagnosed Chagas cardiomyopathy (sensitivity, 98%) and false-positive diagnosis of Chagas cardiomyopathy in only 24 of 488 *T cruzi* seronegatives (specificity, 95%). However, our data showed that echocardiography added little to the sensitivity of detection among previous cardiomyopathy cases or *T cruzi* seropositives and contributed to worse specificity, as evidenced by triggering 8 *T cruzi* seronegatives (33%). The high negative predictive value of ECG suggests that future studies could potentially use ECG alone for screening and perform echocardiograms only on patients with abnormal ECG findings. Because it reveals left atrial volume and LV function, the echocardiogram may remain more important in predicting disease progression and death among patients already diagnosed with cardiomyopathy.[6,18]

We found few demographic or clinical predictors of cardiomyopathy among *T cruzi* seropositives. Our associations between cardiomyopathy and previously reported heart or ECG abnormalities and S_3 gallop are more likely to represent symptoms and signs of disease rather than true prognostic factors. An association between male sex and the progression of cardiomyopathy or death has previously been reported,[5,19,20] but to the best of our knowledge, this is the first report of an association between male sex and the onset of cardiomyopathy. That lower body mass index was associated with cardiomyopathy in the unadjusted model but not final model may be explained by a previous observation that B-type natriuretic peptide, a marker of severity of Chagas cardiomyopathy, is inversely related to body mass index and other anthropometric measures.[21] It was surprising that older age at baseline and length of exposure to *T cruzi* did not predict incident disease because previous studies have implicated age as a risk factor.[7,22] Our epidemiological data and *T cruzi* eradication campaigns over the last 20 years in Brazil suggest that infection occurred in most subjects during childhood or young adulthood.[23] Thus, the lack of an age association suggests that variation in host genetics or immunology, rather than duration of infection, is more important in determining disease risk.

The premise that our cases were newly incident is supported by the relatively mild disease severity that we observed among the cases of Chagas cardiomyopathy. In contrast to case series enrolled by cardiology clinics,[24–26] most were New York Heart Association class I and only a few were class II. In addition, only 26% of *T cruzi*–seropositive cardiomyopathy cases had LV ejection fractions of <50%. To confirm the impression that we diagnosed cases early in the course of Chagas disease, we intend to follow up the cohort for both new incident cases and disease progression in those with diagnosed disease.

The study was not designed to evaluate the effect of treatment, and among the small number of the seropositives who had previously been treated with benznidazole, there appeared to be no difference in the incidence of cardiomyopathy. Because we had no information on the timing or duration of benznidazole treatment, treatment could have been initiated after the onset of clinical symptoms and therefore would be unlikely to be effective. Treatment is still recommended for

Table 3. Demographic Characteristics, Symptoms, and Signs Predicting Chagas Disease: Seropositives Only

	Definite Chagas Cardiac Disease (n=120)	No Definite Chagas Cardiac Disease (n=379)	Unadjusted Odds Ratio	P	Adjusted Odds Ratio*	P
Site, n (%)				0.73		
Sao Paulo	63 (53)	192 (51)	1			
Montes Claros	57 (48)	187 (49)	0.93			
Sex, n (%)				0.01		0.01
Male	75 (63)	186 (49)	1.73		1.97 (1.23–3.16)	
Female	45 (38)	193 (51)	1		1	
Age in 10 y, n (%)				0.73		
20–29 y	1 (<1)	3 (<1)	0.78			
30–39 y	25 (21)	85 (22)	0.69			
40–49 y	39 (33)	124 (33)	0.73			
50–59 y	31 (26)	111 (29)	0.65			
≥60 y	24 (20)	56 (15)	1			
Mean (SD) age, y	49 (11)	48 (10)	…			
Skin color, n (%)				0.87		
White	34 (28)	121 (32)	1			
Black	16 (13)	40 (11)	1.42			
Mixed	66 (55)	208 (55)	1.13			
Other	4 (3)	7 (2)	2.03			
Refused to answer/missing	0	3 (<1)	…			
Weight, kg	70 (11)	72 (12)	0.99	0.23		
Height, cm	165 (8)	164 (9)	1.01	0.31		
Body mass index, n (%)				0.62		
<25 kg/m2	45 (37)	132 (35)	1			
≥25 kg/m2	75 (62)	245 (65)	0.90			
Laboratory results, n (%)						
Systolic blood pressure, mm Hg	122 (18)	124 (18)	0.99	0.31		
Diastolic blood pressure, mm Hg	75 (14)	76 (13)	0.99	0.58		
Pulse, bpm	67 (10)	66 (8)	1.00	0.50		
High-density lipoprotein, mg/dL	52 (15)	50 (13)	1.01	0.06		
Low-density lipoprotein, mg/dL	121 (36)	122 (38)	1.00	0.79		
Total cholesterol, mg/dL	199 (38)	199 (43)	1.00	0.89		
Triglycerides, mg/dL	132 (72)	139 (94)	1.00	0.48		
Glycemia, mg/dL	89 (22)	91 (22)	1.00	0.51		
Previous physician diagnoses, n (%)						
Symptomatic	52 (43)	121 (32)	1.63	0.02		
Cardiac presentation (observed by a clinician)	44 (37)	74 (20)	2.39	0.0002		
Gastrointestinal presentation (observed by a clinician)	17 (14)	68 (18)	0.76	0.34		
Asymptomatic	66 (55)	249 (66)	0.64	0.03		
Medical history, n (%)						
Diabetes mellitus	7 (6)	20 (5)	1.11	0.82		
Kidney problems	5 (4)	10 (3)	1.61	0.39		
Coagulation problems	0	2 (<1)	…	0.98		
Heart attack	2 (2)	1 (<1)	6.36	0.13		
Hypertension	24 (20)	89 (23)	0.82	0.44		
Symptoms, n (%)						
Do you feel unable to climb 2 flights of stairs without resting?	18 (15)	41 (11)	1.46	0.21		
Do you have heart palpitations?	40 (33)	108 (29)	1.25	0.32		

(continued)

Table 3.   (Continued)

	Definite Chagas Cardiac Disease (n=120)	No Definite Chagas Cardiac Disease (n=379)	Unadjusted Odds Ratio	P	Adjusted Odds Ratio*	P
Do you have difficulty breathing when lying down?	6 (5)	21 (6)	0.91	0.84		
Have you experienced swelling or puffiness in your feet in the morning?	11 (9)	23 (6)	1.56	0.25		
Does your heartbeat sometimes race when resting?	33 (28)	82 (22)	1.38	0.18		
Have you noticed any visible neck veins when standing up or sitting in front of a mirror?	6 (5)	8 (2)	2.42	0.11		
Has your doctor told you that you have heart abnormalities or rhythm problems on ECG?	51 (43)	41 (11)	6.07	<0.0001	6.09 (3.64–10.19)	<0.0001
Have you been told that your heartbeat is not regular?	23 (19)	36 (10)	2.24	0.006		
Do you have a pacemaker?	6 (5)	1 (<1)	19.81	0.006		
Have you ever noticed your heart racing or beating abnormally?	42 (35)	104 (28)	1.42	0.12		
Have you ever awakened during the night with shortness of breath or unable to breathe?	15 (13)	35 (9)	1.42	0.29		
Do you feel shortness of breath when you have to use physical strength, eg, climbing up stairs or hills?	36 (30)	72 (19)	1.83	0.01		
Do you feel chest pain?	41 (43)	111 (29)	1.28	0.27		
Have you ever fainted or lost consciousness?	11 (10)	31 (9)	1.16	0.69		
Physical examination, n (%)						
Jugular vein engorgement	5 (4)	11 (3)	1.45	0.50		
Liver enlargement	3 (3)	5 (1)	1.94	0.37		
Edema of legs	9 (8)	16 (4)	1.84	0.16		
S3 heart sound	26 (22)	20 (5)	5.09	<0.0001	6.01 (3.00–12.05)	<0.0001
Rales on chest examination	1 (1)	5 (1)	1.60	0.67		
New York Heart Association functional class, n (%)				0.79		
1=Ordinary physical activity does not cause dyspnea	107 (89)	343 (91)	1			
2=Slight limitation of ordinary activity	10 (8)	24 (6)	1.33			
3=Marked limitation of ordinary activity	1 (<1)	2 (<1)	1.60			
4=Inability to carry out any physical activity	0	0	…			
Missing	2 (2)	10 (3)	…			

*Stepwise logistic regression retained all covariates with P<0.05 (and excluded all others from the final model).

specific patient subgroups, although there is a lack of consensus,[1,2] and a randomized trial of benznidazole is underway.[27] A companion analysis of the present report will examine the effects of treatment on parasitemia as measured by polymerase chain reaction.

One strength of this study is its inclusion of a *T cruzi*–seronegative cohort. This allowed the determination of true Chagas cardiomyopathy diagnoses against a background of cardiac disease resulting from other causes, the incidence of which is increasing in *T cruzi*–endemic areas as a result of economic development and subsequent increases in atherosclerotic cardiovascular disease. Our blood donor sampling frame allowed the inclusion of asymptomatic persons at baseline, thus minimizing the effect of referral bias if the study had been conducted at a cardiology referral center. The follow-up period of 10 years was long enough to allow a sufficient number of incident cases of cardiomyopathy to be diagnosed. Finally, the diagnostic algorithm including expert panel adjudication had very good sensitivity and specificity for Chagas cardiomyopathy.

Limitations of the study include the enrollment of prevalent rather than incident *T cruzi* infections. Formation and evaluation of incident cohorts are extremely difficult, and we attempted to estimate the duration of infection using epidemiological analysis of risk factors and geography. The lack of cardiac evaluation at baseline means that some asymptomatic cases of cardiomyopathy could have been included, thereby inflating our incidence estimate. We believe that the blood donor sampling frame minimized the effect of this bias. Finally, the differential diagnosis of Chagas cardiomyopathy is difficult, and we could have misdiagnosed Chagas disease. This shortcoming is shared by many clinical studies of Chagas disease.

Conclusions

There was a moderate (1.85%) annual incidence of Chagas cardiomyopathy among initially asymptomatic *T cruzi*–seropositive blood donors, although disease was generally mild at diagnosis. Prognostic factors for cardiomyopathy, including male sex, a history of ECG abnormalities, and S₃ gallop,

if confirmed by other studies, may be useful in deciding which seropositives are at highest risk of cardiomyopathy and potentially eligible for treatment. The false-positive diagnoses of Chagas-like cardiomyopathy among 5% of *T cruzi* seronegatives illustrate the difficulty of distinguishing Chagas from other cardiac diseases, leading to overestimation of Chagas disease incidence in uncontrolled seropositive cohorts.

Acknowledgments

Sources of Funding

Disclosures

None.

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CLINICAL PERSPECTIVE

This study presents the results from a large, retrospective cohort study of Chagas cardiomyopathy incidence from a National Heart, Lung, and Blood Institute–funded multicenter study in Brazil. After an average follow-up of 11 years, cohorts of previously healthy *Trypanosoma cruzi*–seropositive and –seronegative blood donors were subjected to comprehensive cardiology evaluations. We estimated a cardiomyopathy incidence of 1.85 per 100 person-years attributable to *T cruzi* infection. These results are important on several levels. First, in terms of knowledge about the pathogenesis of Chagas disease, we present well-controlled data on cardiomyopathy incidence among previously asymptomatic *T cruzi* seropositives. Previous studies have relied on case series derived from referral centers, so our incidence estimates better represent those of seropositives in the general population. Second, the inclusion of *T cruzi* seronegatives as control subjects allowed us to validate our screening diagnostic algorithm by estimating the fraction of cardiomyopathy cases resulting from other causes that may be erroneously attributed to Chagas disease. This is important in that endemic countries in Latin America attain living standards that bring with them an increasing incidence of atherosclerotic cardiovascular disease. Third, the data will allow public health authorities to better estimate the potential disease burden and to calculate the costs and benefits of antitrypanosomal treatment in asymptomatic seropositives. Finally, these data will be of interest to the practicing cardiologist in the United States who may increasingly encounter *T cruzi*–seropositive patients among immigrants from Latin America. They will allow her/him to better estimate prognosis and to better consider treatment.