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ORIGINAL RESEARCH ARTICLE

Incidence and Predictors of Progression to Chagas Cardiomyopathy

Long-Term Follow-Up of *Trypanosoma cruzi*–Seropositive Individuals

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BACKGROUND: There are few contemporary cohorts of *Trypanosoma cruzi*–seropositive individuals, and the basic clinical epidemiology of Chagas disease is poorly understood. Herein, we report the incidence of cardiomyopathy and death associated with *T. cruzi* seropositivity.

METHODS: Participants were selected in blood banks at 2 Brazilian centers. Cases were defined as *T. cruzi*–seropositive blood donors. *T. cruzi*–seronegative controls were matched for age, sex, and period of donation. Patients with established Chagas cardiomyopathy were recruited from a tertiary outpatient service. Participants underwent medical examination, blood collection, ECG, and echocardiogram at enrollment (2008–2010) and at follow-up (2018–2019). The primary outcomes were all-cause mortality and development of cardiomyopathy, defined as the presence of a left ventricular ejection fraction <50% or QRS complex duration ≥120 ms, or both. To handle loss to follow-up, a sensitivity analysis was performed using inverse probability weights for selection.

RESULTS: We enrolled 499 *T. cruzi*–seropositive donors (age 48±10 years, 52% male), 488 *T. cruzi*–seronegative donors (age 49±10 years, 49% male), and 101 patients with established Chagas cardiomyopathy (age 48±8 years, 59% male). The mortality in patients with established cardiomyopathy was 80.9 deaths/1000 person-years (py) (54/101, 53%) and 15.1 deaths/1000 py (17/114, 15%) in *T. cruzi*–seropositive donors with cardiomyopathy at baseline. Among *T. cruzi*–seropositive donors without cardiomyopathy at baseline, mortality was 3.7 events/1000 py (15/385, 4%), which was no different from *T. cruzi*–seronegative donors with 3.6 deaths/1000 py (17/488, 3%). The incidence of cardiomyopathy in *T. cruzi*–seropositive donors was 13.8 (95% CI, 9.5–19.6) events/1000 py (32/262, 12%) compared with 4.6 (95% CI, 2.3–8.3) events/1000 py (11/277, 4%) in seronegative controls, with an absolute incidence difference associated with *T. cruzi* seropositivity of 9.2 (95% CI, 3.6–15.0) events/1000 py. *T. cruzi* antibody level at baseline was associated with development of cardiomyopathy (adjusted odds ratio, 1.4 [95% CI, 1.1–1.8]).

CONCLUSIONS: We present a comprehensive description of the natural history of *T. cruzi* seropositivity in a contemporary patient population. The results highlight the central importance of anti–*T. cruzi* antibody titer as a marker of Chagas disease activity and risk of progression.

Key Words: Chagas cardiomyopathy ■ Chagas disease ■ disease progression ■ mortality ■ serology ■ *Trypanosoma cruzi*

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ORIGINAL RESEARCH ARTICLE

Clinical Perspective

What Is New?

- The incidence of cardiomyopathy associated with *Trypanosoma cruzi* seropositivity has been steadily declining over the past decade with effective vector control.
- *T. cruzi*–seropositive individuals with normal left ventricular ejection fraction and QRS duration have identical mortality to *T. cruzi*–seronegative controls.
- *T. cruzi* antibody levels play a major role in predicting progression to cardiomyopathy.

What Are the Clinical Implications?

- *T. cruzi* seropositivity is a strong determinant of new-onset cardiomyopathy and death.
- Identification of *T. cruzi*–seropositive individuals at risk of developing cardiomyopathy would be useful to inform patient counseling, intensity of follow-up, and design of clinical trials of treatment.
- Patients with high levels of anti–*T. cruzi* serum antibodies may be considered for antitrypanosomal therapy.

Nonstandard Abbreviations and Acronyms

PCR	polymerase chain reaction
REDS	Recipient Epidemiology and Donor Evaluation Study

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi,* is the most common cause of infectious cardiomyopathy worldwide.[1,2] Despite substantial progress toward its control, Chagas disease remains a major public health problem in Latin America.[3,4] Over the past several decades, migration has spread the disease to nonendemic countries, becoming a global health concern. Current estimates of 6 million *T. cruzi*–seropositive people and 1.2 million cases of cardiomyopathy make Chagas disease the highest burden parasitic disease in the Americas.[5]

Chagas disease is often a lifelong infection in which most *T. cruzi*–seropositive people remain asymptomatic but at risk of progression to cardiac damage.[6,7] It is often quoted that one-third of seropositive individuals will develop Chagas cardiomyopathy over a lifetime.[8] This figure likely comes from early studies of the natural history of Chagas disease from hyperendemic rural populations with acute infections or ECG findings, but without the additional sensitivity of modern echocardiography to identify cardiac involvement.[9–12] Current transmission control, making new *T. cruzi* infection increasingly rare,[5] has produced a cohort effect whereby most individuals with Chagas disease are now in their fourth decade of life or older.[13,14] Therefore, the lifetime risk of Chagas cardiomyopathy, its incidence in a contemporary aging patient population, and risk factors for progression to cardiomyopathy remain poorly understood.

Two methodological issues make it challenging to study the natural history of Chagas disease. First, the protracted time frame over which cardiac damage accumulates, which can run into decades, necessitates many years of follow-up to detect incident cases of disease progression. Second, the current definition of Chagas cardiomyopathy on the basis of the presence of typical ECG changes in a *T. cruzi*–seropositive patient[15] is insufficient epidemiologically, because findings considered typical of Chagas disease are also prevalent in older adults without *T. cruzi* infection.[13,16] As such, to determine the *T. cruzi*–attributable incidence of cardiomyopathy, parallel and optimally blinded follow-up assessments of a group of matched seronegative controls are required.

Herein we present the 10-year follow-up results of the Brazilian NIH REDS (National Institutes of Health Recipient Epidemiology and Donor Evaluation Study) cohort, made up of 499 *T. cruzi*–seropositive blood donors identified in routine donor screening, 488 age- and sex-matched *T. cruzi*–seronegative blood donors, and 101 patients with established Chagas cardiomyopathy recruited from a tertiary outpatient service. Blood donors were further stratified according to the presence of cardiomyopathy, on the basis of ECG and echocardiographic findings. The present study aimed to determine the incidence of cardiomyopathy and death associated with *T. cruzi* seropositivity. Furthermore, on the basis of preliminary reports of the value of anti–*T. cruzi* antibody level and *T. cruzi* polymerase chain reaction (PCR) positivity in predicting cardiomyopathy, we also investigated the prognostic value of these parameters in a population of *T. cruzi*–seropositive blood donors.

To address these aims, we conducted 4 main analyses. First, we compared all-cause mortality between *T. cruzi*–seronegative blood donors and the 3 other study groups (*T. cruzi*–seropositive blood donors with and without cardiomyopathy at baseline, and patients with established Chagas cardiomyopathy). Second, we built a model to assess the association between total anti–*T. cruzi* antibody level and *T. cruzi* PCR positivity with mortality in the *T. cruzi*–seropositive blood donor group. Third, we estimated the incidence of new-onset cardiomyopathy associated with *T. cruzi* seropositivity among participants initially free of cardiomyopathy at the baseline visit. Fourth, we built a model to assess the independent associations between baseline antibody level and PCR positivity and development subsequent cardiomyopathy.

METHODS

The data, analytic methods, and study materials will not be made available to other researchers for the sole purpose of reproducing the study results.

Study Design and Data Collection

The REDS study has been described in detail elsewhere.[17] Briefly, healthy blood donors were recruited between 1996 and 2002 from 2 donation centers in Brazil (Fundação Pró-Sangue in São Paulo and Hemominas blood center in Montes Claros). Participants were selected on the basis of the results of routine *T. cruzi* serology screening performed at the time of blood donation, confirmed by contemporary ELISA, hemaglutination, and immunofluorescence. A total of 1327 seropositive and 1887 seronegative blood donors were invited to participate in the study. Of those blood donors who were initially eligible, 499 *T. cruzi*–seropositive and 488 *T. cruzi*–seronegative blood donors matched by age, sex, and period of donation had complete clinical, ECG, and echocardiography data and were enrolled in the study (Figure 1).

In addition, 101 *T. cruzi*–seropositive participants with established Chagas cardiomyopathy, defined by the presence of left ventricular dilatation with systolic dysfunction, were recruited from a tertiary cardiology outpatient service (InCor Hospital das Clínicas, São Paulo) for management of heart failure. This group was included to compare mortality rates with asymptomatic blood donors identified through serological screening.

The baseline visit was conducted between 2008 and 2010. All participants underwent a medical history and physical examination, 12-lead ECG, and echocardiogram. Baseline characteristics of this population have been described previously.[17] The follow-up study visit was performed between 2018 and 2019. All participants were invited for a second cardiovascular evaluation, including blood collection, ECG, and echocardiographic assessments.

Cardiomyopathy among *T. cruzi*–seropositive and –seronegative blood donors was defined as the presence of a left ventricular ejection fraction <50% or QRS complex duration ≥120 ms.[18] All patients with established cardiomyopathy recruited from the outpatient service had left ventricular systolic dysfunction.

Clinical and Laboratory Evaluation

All individuals underwent a clinical examination by a cardiologist, and demographic data were recorded. Cardiovascular history and risk factors, including hypertension, dyslipidemia, diabetes, and previous history of ischemic heart disease or revascularization procedures were also recorded. New York Heart Association functional class was assessed on the basis of symptoms and physical activity questionnaire. Serum lipids and other biochemical blood measurements were determined in the local laboratories using standard laboratory procedures. Cardiac injury markers including troponin I, creatine kinase isoenzyme MB, and myoglobin were measured at the central laboratory. NT-proBNP (N-terminal pro-B-type natriuretic peptide) was also measured.

For *T. cruzi*–seropositive participants, parasite detection in blood by PCR and the evaluation of semiquantitative antibody results by ELISA were obtained. Levels of antibodies were reported as the ratio of signal-to-cutoff, which is a function of the amount of anti–*T. cruzi* antibody present in the test sample (Ortho *T. cruzi* ELISA test system, Raritan, NJ).

ECG and Echocardiographic Examination

ECGs were recorded at both sites during the 2 visits using standardized procedures.[17,19] All ECGs were interpreted by trained cardiologists who were blinded to study group at a central reading center, and were classified according to the Minnesota code criteria.[13]

Comprehensive Doppler-echocardiographic examinations were performed at enrollment and at follow-up using a commercially available ultrasound system at each site. All images were stored digitally and analyzed offline by central reading centers. The echocardiographic measurements were performed according to the recommendations of the American Society of Echocardiography by independent investigators who were blinded to study group.[20] Left ventricular ejection fraction was calculated according to the modified Simpson method. A comprehensive examination from multiple windows was performed to detect wall motion abnormalities and apical aneurysms.

Diastolic function was assessed by pulsed-wave Doppler examination of mitral inflow, and by tissue Doppler imaging. Early diastolic velocity (e') at septal and lateral mitral annulus was obtained, and the ratio between peak mitral E and e' (E/e') was calculated.[20] Left atrial volume was assessed

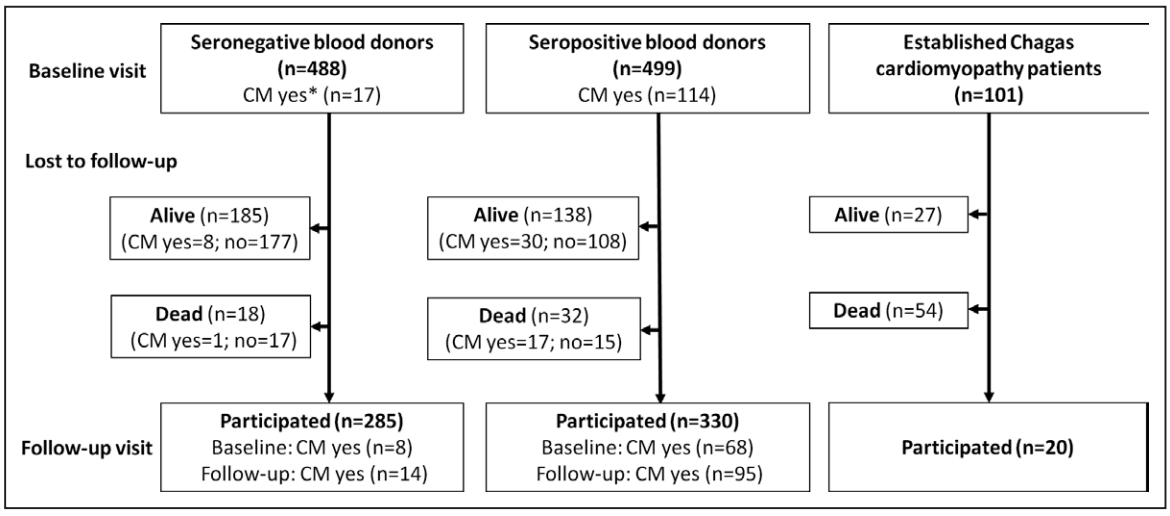

Figure 1. Study population flow chart.
*CM, cardiomyopathy, which was defined as left ventricular ejection fraction <50% or QRS complex duration ≥120 ms.

by the biplane area-length method from the apical 4- and 2-chamber views.

Outcome Definition and Analytic Groups

The main outcomes were all-cause mortality and development of cardiomyopathy at follow-up visit. The date and occurrence of death were determined first by direct interview with participants' relatives when contact was possible. We also conducted a probabilistic linkage with the Brazilian National Mortality System (Sistema de Informação sobre Mortalidade) using full name, date of birth, mother's name, and municipality of residence as matching variables. The linkage algorithm has been previously validated with a sensitivity and specificity of 94% (95% CI, 90%–97%) and 91% (95% CI, 86%–95%), respectively.[21] Patients not identified in the mortality database were censured at June 1, 2020, the date the linkage was performed. As such, vital status was determined for all participants irrespective of whether contact was possible at the follow-up visit.

New-onset cardiomyopathy was defined as the presence of left ventricular systolic dysfunction (left ventricular ejection fraction <50%) or QRS complex duration ≥120 ms in participants undergoing cardiovascular assessment at the follow-up visit, but in whom cardiomyopathy was absent at the baseline visit.[18] Using this definition, our study design is at risk of a survivorship bias, whereby participants with new-onset cardiomyopathy were more likely to die and thus not attend the follow-up visit. This would lead to an underestimate of the true incidence of cardiomyopathy because of differential loss to follow-up. As such, we conducted a sensitivity analysis in which we assumed all deaths represented cardiomyopathy cases, and as such, we used a combined outcome of death or new-onset cardiomyopathy.

Statistical Analysis

Continuous variables were expressed as medians with interquartile ranges, and categorical variables were presented as numbers and percentages proportions. Clinical characteristics were compared across the groups using the χ^2 test, unpaired Student's *t* test, Mann-Whitney test, 1-way ANOVA, or Kruskal-Wallis tests, according to the pattern of variable distributions.

The mortality rate, incidence of new-onset cardiomyopathy, and ncidence of the combined outcome (death or new-onset cardiomyopathy) were calculated by dividing the number of incident events by the person-years of follow-up calculated from the date of visit 1 until either the date of death or the date of cardiovascular assessment at the follow-up visit. Absolute incidence differences were calculated with the *T. cruzi*–seronegative group as a reference. Exact Poisson 95% CIs were calculated.

A Cox proportional hazards regression model was performed to identify the predictors of mortality in the *T. cruzi*–seropositive group. Separate multivariable models were built to assess the association between mortality and the 2 predictors of interest: anti–*T. cruzi* antibody level and *T. cruzi* PCR result (positive or negative). PCR and antibody level were not included in the same model for statistical and theoretical reasons, because they are highly collinear, and both are indirect indicators of parasite burden. Covariates tested in the model included age, sex, previous benznidazole treatment, and traditional cardiovascular risk factors.

We built a multivariable logistic regression model including *T. cruzi*–seropositive and –seronegative blood donors without cardiomyopathy at baseline to determine the independent contribution of *T. cruzi* serostatus to the development of new-onset cardiomyopathy. C-statistic and the integrated discrimination improvement were used to determine the added value of serostatus in predicting new-onset cardiomyopathy. Subsequently, we developed a multivariable logistic regression model including only *T. cruzi*–seropositive blood donors without cardiomyopathy at baseline to determine the independent contribution of anti–*T. cruzi* antibody level and *T. cruzi* PCR result in predicting new-onset cardiomyopathy.

To handle loss to follow-up, we assumed missing data are missing at random, which is a requirement for the validity of the maximum likelihood-based regression methods applied.[22] To identify the variables that make the missing at random assumption plausible, we built logistic regression models to predict loss to follow-up. These predictive models included demographic and clinical characteristics that could influence missingness. Main effects and interaction terms were tested. Variables that were found to predict the missingness pattern were included in the regression models predicting new-onset cardiomyopathy, to minimize potential bias caused by differential loss to follow-up. In addition, a sensitivity analysis for new-onset cardiomyopathy was performed using inverse probability weights for selection. The weights were obtaining from the logistic model using all variables found to be significantly associated with loss to follow-up.

Statistical analysis was performed in the Statistical Package for Social Sciences for Windows, version 22.0 (SPSS Inc., Chicago, IL) and R for Statistical Computing version 4.0.3 (R Foundation, Vienna, Austria) using the packages *tidyverse* (data manipulation), *survey* (weighted analysis), *survival* (survival analysis), *epiR* (incidence calculations, and *PredictABEL* (risk reclassification analysis).

Ethics

The study was approved by the Brazilian National Institutional Review Board, No. 179.685/2012. In this study, written informed consent was obtained from all participants at baseline visit.

RESULTS

Study Population Characteristics

The study population flow chart is shown in Figure 1. After initial evaluation, 114 *T. cruzi*–seropositive blood donors met criteria for cardiomyopathy (23%). Sixty-six percent (330/499) of *T. cruzi*–seropositive blood donors and 58% (285/488) of *T. cruzi*–seronegative blood donors participated in the follow-up visit. Among the 101 patients with established Chagas cardiomyopathy who were in heart failure treatment, 20 (19.8%) underwent the second cardiovascular evaluation at follow-up. The median [interquartile range] time between visit 1 and visit 2 was 8.7 [8.3–9.2] years. Demographic and clinical characteristics of the cohort at baseline and follow-up visits are shown in Table 1.

Table 1. Clinical Characteristics of Study Participants at Baseline and at Follow-Up

Participant characteristics	Visit 1 (baseline)				Visit 2 (follow-up)			
	T. cruzi–seronegative (n=488)	*T. cruzi*–seropositive (n=499)	Chagas cardiomyopathy (n=101)	*P* value	*T. cruzi*–seronegative (n=285)	*T. cruzi*–seropositive (n=330)	Chagas cardiomyopathy (n=20)	*P* value
Age, y	49 (42–58)	48 (40–57)	48 (42–54)	0.237	59 (52–66)	56 (50–65)	55 (50–61)	0.054
Male sex	241 (49.4)	261 (52.3)	60 (59.4)	0.172	140 (49.1)	159 (48.2)	13 (65.0)	0.344
Clinical history								
Diabetes	24 (4.9)	27 (5.4)	6 (5.9)	0.870	38 (13.3)	42 (12.7)	3 (15)	0.504
Hypertension	119 (24.4)	113 (22.6)	36 (35.6)	0.072	102 (35.8)	128 (38.8)	9 (45)	0.648
Chronic kidney disease	15 (3.1)	15 (3.0)	10 (9.9)	0.009	15 (5.3)	21 (6.4)	2 (10)	0.793
Suspected CAD*	5 (1.0)	3 (0.6)	12 (11.9)	0.001	10 (3.5)	11 (3.3)	2 (10)	0.039
Symptoms–New York Heart Association functional class								
Class I	469 (96.1)	461 (92.4)	60 (59.3)	<0.001	262 (91.9)	298 (90.3)	9 (45.0)	<0.001
Class II	18 (3.7)	35 (7.0)	27 (26.7)		20 (6.9)	24 (7.3)	8 (40.0)	
Class III/IV	1 (0.2)	3 (0.6)	14 (13.9)		3 (1.2)	8 (2.4)	3 (15.0)	
Smoking history								
Never	255 (52.2)	283 (56.7)	47 (46.5)	0.014	165 (57.9)	215 (65.2)	9 (45.0)	0.119
Past	158 (32.4)	161 (32.3)	46 (45.5)		77 (27.0)	65 (19.7)	6 (30.0)	
Current	75 (15.4)	55 (11.0)	8 (8.0)		43 (15.1)	50 (15.2)	5 (25.0)	
BMI, kg/m^2	27 (25–30)	26 (24–29)	26 (23–28)	<0.001	27 (25–31)	27 (25–30)	26 (23–31)	0.307
Obesity (BMI>30)	127 (26.0)	94 (18.8)	14 (13.9)	0.002	85 (29.8)	80 (24.2)	5 (25.0)	0.274
Heart rate, bpm	70 (60–75)	65 (60–70)	60 (58–70)	<0.001	73 (65–80)	70 (63–78)	67 (59–72)	0.002
SBP, mm Hg	125 (115–140)	125 (114–140)	122 (107–134)	0.003	130 (120–145)	130 (116–143)	110 (102–118)	<0.001
DBP, mm Hg	79 (65–88)	76 (65–85)	80 (69–90)	0.088	80 (70–88)	80 (70–85)	71 (61–80)	0.004
NT-proBNP, pg/mL	38 (23–65)	48 (27–90)	746 (335–2267)	<0.001	37 (23–61)	46 (24–84)	360 (129–550)	<0.001

Data are median (interquartile range) or n (%). BMI indicates body mass index; CAD, coronary artery disease; DBP, diastolic blood pressure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and SBP, systolic blood pressure.

*Chest pain suggestive of ischemic heart disease.

Overall Mortality

The greatest mortality was observed in patients with established cardiomyopathy at the baseline visit, with 54 deaths (53%) and a mortality rate of 80.9 deaths/1000 person-years follow-up (Figure 2, Table 2). There were 17 deaths (15%) among seropositive blood donors meeting the definition of cardiomyopathy at baseline visit (114/499), equating to a mortality rate of 15.1 deaths/1000 person-years follow-up. There was no difference in the mortality between seropositive donors without cardiomyopathy at the baseline visit and seronegative controls, with incidence rates of 3.7 and 3.6 events/1000 person-years, respectively.

The predictors of death among *T. cruzi*–seropositive blood donors are shown in Table 3. Among 499 participants, 32 died at a median follow-up of 10.8 years (range, 5.3 months to 11.8 years). After adjusting for age and sex, positive *T. cruzi* PCR at baseline was associated with greater mortality (hazard ratio [HR], 2.4 [95% CI, 1.1–5.3]). Similarly, higher anti–*T. cruzi* antibody level detected by the semiquantitative ELISA at baseline was associated with greater mortality (HR, 1.5 for each unit increase [95% CI, 1.1–2.0]). *T. cruzi*–seropositive individuals with low anti–*T. cruzi* antibody level had identical mortality rate to *T. cruzi*–seronegative controls (Table S1 in the Supplemental Material). As antibody *T. cruzi* titers increased, mortality rate also increased.

This association between antibody level and mortality was attenuated after additional adjustment for the presence of cardiomyopathy at baseline, which was the strongest predictor of death in seropositive individuals (unadjusted HR, 4.1 [95% CI, 2.0–8.2]). Multivariable analysis identified age (HR=1.1 per year), antibody level (HR=1.4 per unit increase in signal-to-cutoff), and cardiomyopathy (HR=3.0) as independent predictors of mortality. PCR positivity lost statistical significance in the fully adjusted model.

Cardiomyopathy Development

For the purpose of assessing the incidence and risk factors for new-onset cardiomyopathy, only individuals in whom no cardiomyopathy was detected at baseline visit were included. After initial evaluation, 114 *T. cruzi*–seropositive individuals (23%) and 17 seronegative

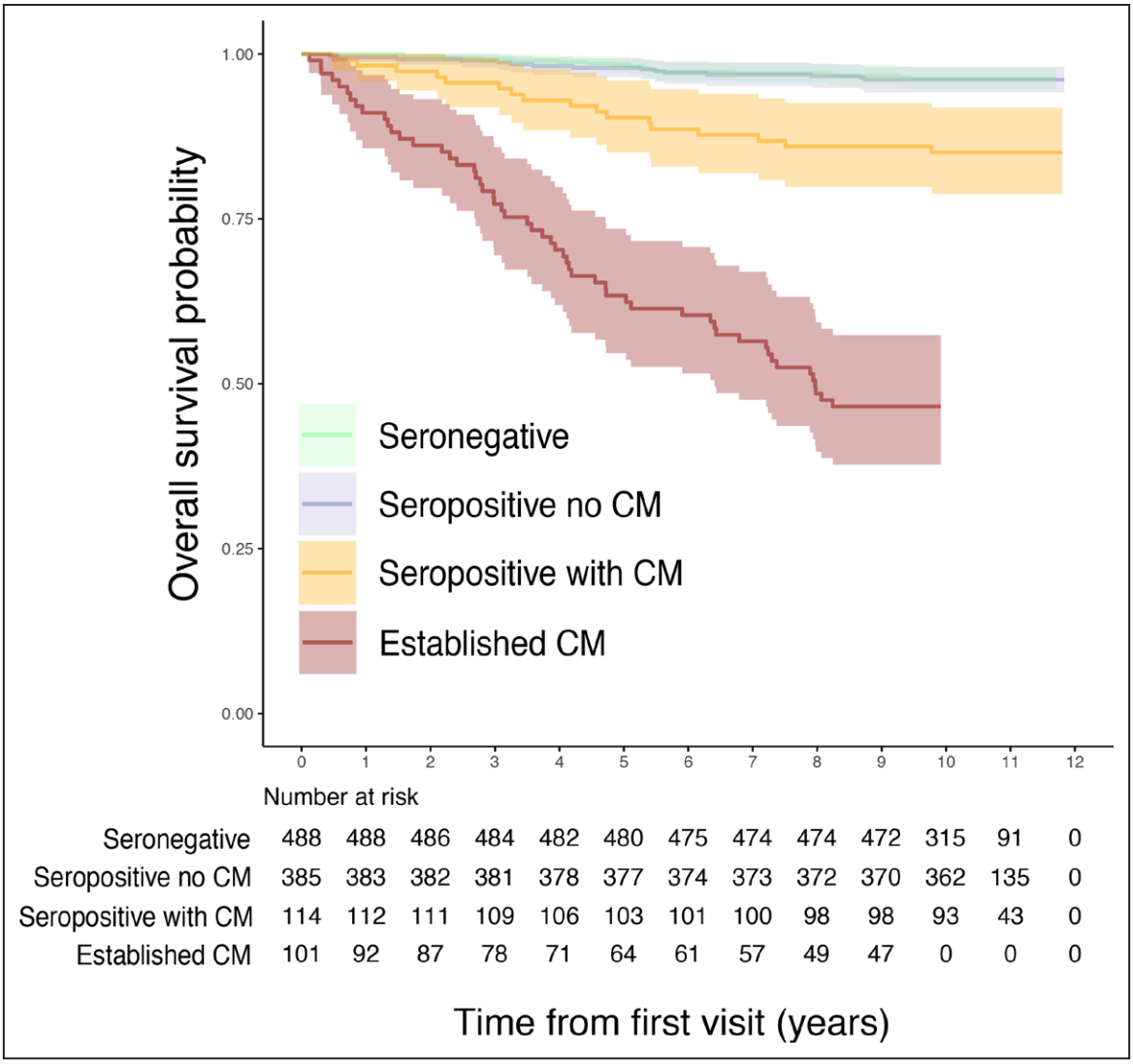

Figure 2. Long-term survival curves according to the presence of cardiomyopathy and *T. cruzi* seropositivity.
The greatest mortality was found among patients with established cardiomyopathy at baseline, with a mortality rate of 80.9 deaths/1000 person-years follow-up (red line). The mortality rate among *T. cruzi*–seropositive blood donors without cardiomyopathy at baseline and *T. cruzi*–seronegative blood donors was similar (light green and dark blue lines are overlapping). CM indicates cardiomyopathy.

controls (3.5%) were excluded from the analysis of progression (Figure 3). The baseline characteristics of the 385 *T. cruzi*–seropositive and 471 *T. cruzi*–seronegative blood donors without cardiomyopathy at the baseline visit are shown in Table 4. The clinical characteristics of these participants were not different. Of these participants, 262 of the seropositive donors and 277 of the seronegative donors attended the follow-up visit for cardiovascular assessment, meaning that 108 (28.1%) seropositive and 177 (37.6%) seronegative donors could not be classified for this outcome (Figure 3). For assessment of new-onset cardiomyopathy, some strategies were used to deal with loss to follow-up (Text S1 in the Supplemental Material).

There were 32 new-onset cardiomyopathy cases among 262 seropositive blood donors that attended the follow-up visit, equating to an incidence of 13.8 (95% CI, 7.6–16.5) events/1000 person-years. Among the 277 seronegative blood donors, there were 11 cases of new-onset cardiomyopathy, or 4.6 (95% CI, 2.3–8.3) events/1000 person-years. The absolute incidence difference associated with *T. cruzi* seropositivity was 9.2 (95% CI, 3.6–15.0) events/1000 person-years follow-up (P=0.001) (Table 2). We next conducted a sensitivity analysis assuming all deaths to represent incident cardiomyopathy cases. There were 47 progression events (15 deaths, 32 new-onset cardiomyopathy) among 277 *T cruzi*–seropositive blood donors attending the second visit or dying during follow-up, equating to 19.6 (95% CI, 14.5–26.3) events/1000 person-years (Figure 3). Among the 294 seronegative donors, there were 28 events (17 deaths, 11 new-onset cardiomyopathy), giving an incidence of 11.4 (95% CI, 7.6–16.5) events/1000 person-years with absolute incidence difference associated with

Table 2. Overview of Mortality and Disease Progression Among the Overall Study Population

Definition of event and participants at risk at baseline visit	Number of participants at risk at baseline visit	Follow-up time* (person-years)	Number of events N (n deaths, n new-onset cardiomyopathy)	Incidence events/1000-person-years (95% CI)	Absolute incidence difference/1000 person-years (95% CI)
Overall mortality (all participants at baseline visit)					
Seronegative donors	488	4977	18 (18, 0)	3.62 (2.14 to 5.72)	Reference
Seropositive donors without cardiomyopathy	385	4091	15 (15, 0)	3.67 (2.05 to 6.04)	0.05 (−2.45 to 2.54)
Seropositive donors with cardiomyopathy	114	1128	17 (17, 0)	15.1 (8.79 to 24.1)	11.5 (4.1 to 18.8)
Patients with Chagas cardiomyopathy	101	667	54 (54, 0)	80.9 (60.8 to 105.6)	77.3 (55.7 to 98.9)
New-onset cardiomyopathy or death (participants without cardiomyopathy at baseline visit)					
Seronegative donors	294	2465	28 (17, 11)	11.4 (7.6 to 16.5)	Reference
T. cruzi–seropositive donors	277	2393	47 (15, 32)	19.6 (14.5 to 26.3)	8.2 (1.3 to 15.4)
New-onset cardiomyopathy (participants without cardiomyopathy at baseline visit who underwent the second cardiovascular evaluation)					
Seronegative donors	277	2376	11 (0, 11)	4.6 (2.3 to 8.3)	Reference
T. cruzi–seropositive donors	262	2326	32 (0, 32)	13.8 (9.5 to 19.6)	9.2 (3.6 to 15.0)

Cardiomyopathy was defined as left ventricular ejection by echocardiography <50% or QRS complex duration ≥120 ms.

*For overall mortality analysis, follow-up time was contributed by each participant evaluated at baseline visit (visit 1) until the date of death or until the date of linkage with the national mortality system (Sistema de Informação sobre Mortalidade) on the June 1, 2020. For new-onset cardiomyopathy, follow-up time was contributed either until the date of death or until the date of assessment at follow-up visit (visit 2).

seropositivity of 8.2 events/1000 person-years (95% CI, 1.3–15.4) (Table 2).

In the overall population (seropositive and seronegative blood donors) without cardiomyopathy at baseline visit, the predictors of new-onset cardiomyopathy are shown in Table 5. After adjusting for age, sex, comorbidities, and variables associated with loss to follow-up, *T.cruzi*–seropositive blood donors carried twice the odds of new-onset cardiomyopathy compared with seronegative blood donors. Specifically, the inclusion of a positive serological test in a model with traditional risk factors for cardiovascular disease resulted in significant improvement in model performance with the integrated discrimination improvement of 0.014 (95% CI, 0.001–0.026; *P*=0.031). The C-statistic increased from 0.611 (95% CI, 0.570–0.651) to 0.678 (95% CI, 0.619–0.736).

Predictors of New-Onset Cardiomyopathy in *T. cruzi*–Seropositive Blood Donors

In the subset of *T. cruzi*–seropositive blood donors without cardiomyopathy at baseline, total anti–*T. cruzi* antibody level was associated with new-onset cardiomyopathy, with an adjusted odds ratio of 1.4 (95% CI, 1.1–1.8; *P*=0.011) per unit increase in assay signal-to-cutoff and an adjusted odds ratio of 3.2 (95% CI, 1.3–7.9; *P*=0.012) comparing the highest and lowest antibody quartiles (Table 5). We also found a strong association between antibody levels and the presence of cardiomyopathy using the cross-sectional data at visit 1 (Table S2 in the Supplemental Material). PCR status was not retained in the final adjusted model. When we performed the sensitivity analysis adjusting for missing data, the results remained unchanged. We found an association of new-onset cardiomyopathy with antibody level (adjusted OR, 2.9 [95% CI, 1.1–7.6]; *P*=0.035).

DISCUSSION

We have reported the long-term follow-up of the NIH REDS Chagas disease cohort. The main findings were as follows. First, the mortality rate among *T. cruzi*–seropositive blood donors with cardiomyopathy was 15.1 deaths/1000 person-years, whereas seropositive blood donors without heart involvement had 4 times lower mortality, dying at the same rate as *T. cruzi*–seronegative individuals. Second, anti–*T. cruzi* serum antibodies level, a marker of parasite burden, was associated with both new-onset cardiomyopathy and mortality. Third, the rate of incident cardiomyopathy associated with *T. cruzi* seropositivity was 9.2 (95% CI, 3.6–15.0) events/1000 person-years. The risk was 2 times higher compared with seronegative individuals, after adjusting for age, sex, and other cardiovascular risk factors. The odds of incidence cardiomyopathy were 3-fold higher in *T. cruzi*–seropositive individuals with the highest antibody titres. Taken together, our study findings reinforce the concept that the presence of cardiomyopathy in chronically seropositive individuals is a major determinant of survival in Chagas disease. Subclinical cardiac dysfunction induced by *T. cruzi* infection precedes the development of heart failure and death. In addition, our results corroborate growing evidence that the degree of parasite burden, as indirectly measured by quantitative serology, plays a major role in the pathogenesis of Chagas cardiomyopathy.

We previously estimated that the incidence of cardiomyopathy associated with *T. cruzi* seropositivity was 18.5 cases/1000-person years.[17] However, this estimation

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Table 3. Univariate and Age- and Sex-Adjusted Associations With Overall Mortality Among 499 *T. cruzi*–Seropositive Participants

Variable*		Alive (n= 467)	Died (n= 32)	Unadjusted		Adjusted for age and sex		Adjusted for age, sex, antibody,† and cardiomyopathy	
				HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Sex	Female	225 (48.2)	13 (40.6)	Reference		Reference		Reference	
	Male	242 (51.8)	19 (59.4)	1.35 (0.67–2.74)	0.399	1.53 (0.75 –3.11)	0.242	1.57 (0.72 – 3.39)	0.250
Age, y		47 (40–56)	56 (44–64)	1.05 (1.02–1.09)	0.005	1.05 (1.02 –1.09)	0.004	1.05 (1.01– 1.09)	0.009
<40		122 (26.1)	5 (15.6)	Reference		Reference			
40–49		142 (30.4)	8 (25.0)	1.36 (0.44–4.14)	0.594	NA			
50–59		135 (28.9)	7 (21.9)	1.25 (0.39–3.94)	0.703				
≥60		68 (14.6)	12 (37.5)	4.08 (1.44–11.59)	0.008				
BMI, kg/m^2									
<24.9		159 (34.0)	13 (40.6)	Reference		Reference			
25–29.9		218 (46.7)	15 (46.9)	0.81 (0.39–1.71)	0.586	0.77 (0.37–1.63)	0.498		
≥30		90 (19.3)	4 (12.5)	0.53 (0.17–1.64)	0.273	0.59 (0.19–1.82)	0.354		
Benznidazole use		46 (9.9)	3 (9.4)	0.90 (0.28–3.04)	0.899	1.11 (0.34–3.65)	0.868		
Diabetes		25 (5.4)	2 (6.3)	1.09 (0.26–4.55)	0.908	0.85 (0.20–3.62)	0.828		
Hypertension		106 (22.7)	7 (21.9)	1.21 (0.52–0.80)	0.655	0.79 (0.33–1.88)	0.587		
NYHA FC									
I		424 (90.8)	26 (81.3)	Reference		Reference			
II		28 (6)	6 (18.8)	2.18 (1.02–4.66)	0.045	2.34 (1.06–5.18)	0.035		
Smoking									
Never		272 (58.2)	11 (34.4)	Reference		Reference			
Past		144 (30.8)	17 (53.1)	2.78 (1.30–5.94)	0.008	2.17 (0.98–4.80)	0.055		
Current		51 (10.9)	4 (12.5)	1.89 (0.60–5.94)	0.275	1.84 (0.57–5.97)	0.308		
T. cruzi DNA detected by PCR‡									
Negative		215 (96.4)	8 (3.6)	Reference		Reference		Reference	
Positive		246 (91.1)	24 (8.9)	2.56 (1.15–5.70)	0.021	2.38 (1.07–5.31)	0.035	NA	
Antibody against *T. cruzi*									
EIA (S/C)		6.3 (5.2–6.9)	6.9 (5.8–7.3)	1.45 (1.07–1.97)	0.017	1.48 (1.08–2.02)	0.015	1.40 (1.01–1.95)	0.047
Antibody EIA quartiles									
First		116 (24.8)	6 (18.8)	Reference		Reference		Reference	
Second		125 (26.7)	4 (12.5)	0.63 (0.18–2.21)	0.467	0.54 (0.15–1.94)	0.348	0.55 (0.15–1.99)	0.363
Third		118 (25.2)	8 (25.0)	1.29 (0.45–3.72)	0.638	1.26 (0.44–3.65)	0.667	1.19 (0.40–3.54)	0.750
Fourth		108 (23.3)	14 (43.8)	2.39 (0.92–6.21)	0.075	2.44 (0.93–6.42)	0.070	2.06 (0.74–5.73)	0.165
Cardiomyopathy at baseline visit									
No		370 (79.2)	15 (46.9)	Reference		Reference		Reference	
Yes		97 (20.8)	17 (53.1)	4.07 (2.03–8.15)	<0.001	3.73 (1.83–7.57)	<0.001	3.01 (1.45– 6.21)	0.003

BMI indicates body mass index; EIA, enzyme immunoassay; HR, hazard ratio; NA, not applicable; NYHA FC, New York Heart Association functional class; and S/C, absorbance/cutoff, unless otherwise stated.

*Data are expressed as the absolute numbers (percentage) or median (interquartile range).

†Continuous or stratified as quartiles.

‡PCR was not included simultaneously with antibody in the multivariable model.

was based only on cross-sectional data from the baseline visit. The key assumption was that all seropositive blood donors were free of cardiomyopathy at their index donation, approximately 10 years before the baseline visit. Although this was the best approximation at the time, it is likely that some participants with cardiomyopathy were prevalent at index donation, thus causing an overestimation of the true incidence. Now, given 2 time points with comprehensive cardiovascular assessment (baseline and follow-up visits), and also including seronegative blood donors as a control group, we were able to exclude all prevalent cases, resulting in the lower incidence estimate associated with *T. cruzi* seropositivity of 9.2 cases/1000-person years, approximately half of our previous estimate.

Previous studies of Chagas cardiomyopathy have mostly relied on clinical and ECG markers of cardiac

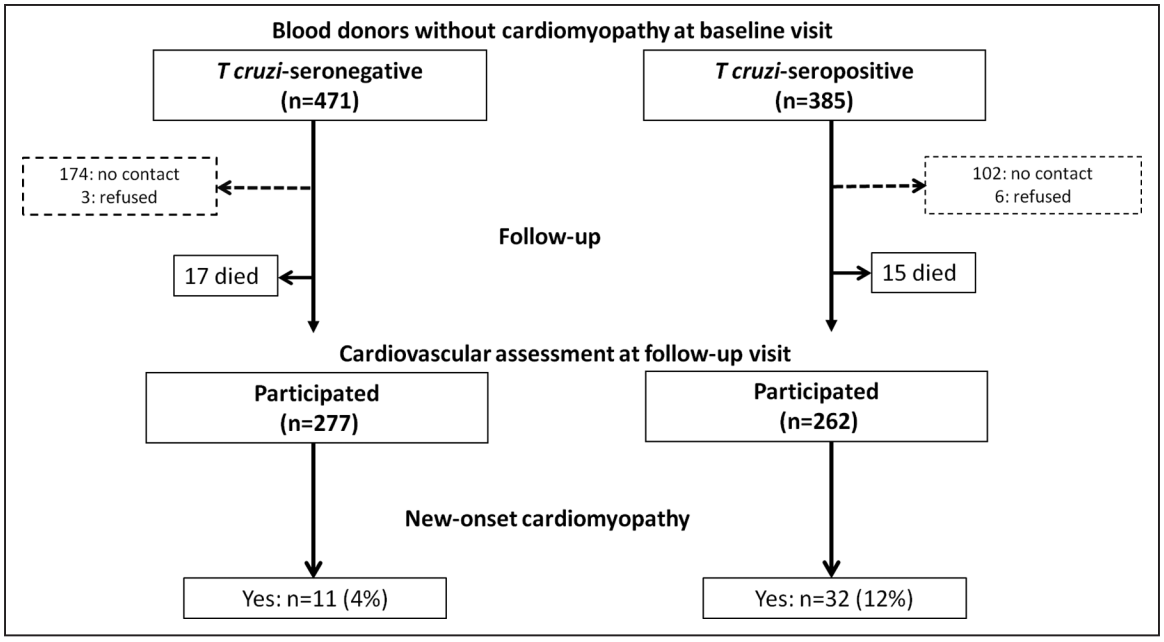

Figure 3. Disease progression according to *T. cruzi* serological tests among blood donors without cardiomyopathy at the baseline visit.

After initial evaluation, 471 *T. cruzi*–seronegative and 385 *T. cruzi*–seropositive blood donors were included. Disease progression was defined as either new-onset cardiomyopathy or death.

involvement with a paucity of information about progression of ventricular dysfunction.[10,23,24] In a systematic review and meta-analysis of 23 studies, the estimated annual rate of cardiomyopathy was 1.9% among patients with the indeterminate form.[9] Most of these studies were conducted in Brazil or Argentina between 1960 and 2005, with a mean participant age of 31 years. More recently, a study showed an annual progression rate of 1.48%[25] but without comparing with a control group. A key methodological strength of our cohort, compared with these other studies, was the inclusion of a matched *T. cruzi*–seronegative control group drawn from a similar population (blood donors), with a blinded parallel clinical, ECG, and echocardiographic assessment of all participants at the baseline visit to rule out subclinical ventricular dysfunction. This allowed all prevalent cases to be excluded at baseline and the *T. cruzi*–attributable incidence of cardiomyopathy to be calculated. It is likely for these reasons that the value of 9.2 cases/1000-person years (0.92% annual rate) is lower than these existing estimates.

Previous studies assessing the risk of cardiomyopathy in *T. cruzi*–seropositive individuals and its predictors had several limitations.[9] The main criterion used to determine the onset of cardiomyopathy was typical ECG abnormalities, which may overestimate the risk of progression because ECG changes can occur in the general population not infected with *T. cruzi* because of aging and comorbidities.[16,25,26] On the other hand, other studies that considered disease progression were based on the development of heart failure symptoms or the presence of complications associated with advanced cardiomyopathy, which reflects late disease process.[9,27,28] In addition, the higher rates of progression seen in earlier studies, which were conducted in *T. cruzi*-endemic areas, may have been partly due to recurrent infections with persistent exposure to *T. cruzi*-infected vectors.[10–12,23,24,29] Last, previous studies were conducted in different epidemiological settings and included small samples, younger populations, and variable follow-up durations, which could have accounted for differences in the rates of progression among studies.[9]

We defined Chagas cardiomyopathy as the presence of prolonged QRS duration or left ventricular systolic dysfunction on echocardiography. This definition reproduced, with 95% accuracy, the diagnosis of cardiomyopathy in participants at the baseline visit, which was made by a panel of expert cardiologists.[19] QRS duration ≥120 ms encompasses right bundle-branch block, which is the most typical finding associated with *T. cruzi* seropositivity.[13,19] We have shown that *T. cruzi*–seropositive individuals with normal QRS duration and left ventricular ejection fraction have an identical 10-year mortality compared with *T. cruzi*–seronegative individuals. In contrast, seropositive cases meeting this definition of cardiomyopathy have a relative mortality risk of 4.2 compared with seronegative controls (15.1 versus 3.62 deaths/1000-person years). The strong association between patient outcome validates these criteria as a simple definition of cardiomyopathy for use in epidemiological studies of Chagas disease. Furthermore, the presence of these ECG and echocardiographic findings can be used for risk stratification and patient counseling.

Table 4. Characteristics of Seropositive and Seronegative Blood Donors Without Cardiomyopathy at the Baseline Visit

	T. cruzi–seronegative donors (n=471)	*T. cruzi*–seropositive donors (n=385)	*P* value
Age, y*	49 (42–58)	48 (40–56)	0.128
Male sex	231 (49.0)	187 (48.6)	0.890
Body mass index, kg/m²	26.9 (25–30)	26.6 (24–29)	0.014
NYHA functional class			
I	454 (96.4)	357 (92.8)	0.020
II/III	17 (3.6)	28 (7.2)	
Heart rate, bpm	68 (60–75)	65 (60–72)	0.005
Systolic blood pressure, mm Hg	125 (114–140)	125 (114–140)	0.943
Diastolic blood pressure, mm Hg	79 (65–88)	76 (68–86)	0.740
Laboratory measurements			
Low-density lipoprotein, mg/dL	124 (100–151)	118 (96–145)	0.087
High-density lipoprotein, mg/dL	46 (38–55)	48 (41–58)	0.015
Triglycerides, mg/dL	125 (89–176)	116 (80–167)	0.024
Glycemia, mg/dL	86 (80–97)	87 (79–95)	0.676
Myoglobin, ng/mL	35.5 (30.0–50.0)	36.2 (29.3–44.3)	0.913
Troponin-I, ng/dL	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.195
CK-MB, ng/dL	0.68 (0.41–1.18)	0.78 (0.47–1.25)	0.043
NT-proBNP, pg/mL	36.6 (23–61)	42.9 (24–72)	0.009
ECG parameters			
QRS duration, ms	88 (82–94)	86 (80–94)	0.209
PR duration, ms	156 (142–168)	158 (142–174)	0.252
QTc calculated, ms	427 (410–441)	425 (409–441)	0.564
Low QRS amplitude	9 (1.9)	15 (3.9)	0.080
First-degree AV block	3 (0.6)	10 (2.6)	0.020
Sinus bradycardia ≥40 bpm	133 (28.2)	125 (32.5)	0.176
Minor isolated ST-T abnormalities	37 (7.9)	47 (12.2)	0.033
Isolated ventricular premature beats	3 (0.6)	2 (0.5)	0.826
Echocardiographic data			
LV end-diastolic diameter, mm	45 (41–49)	45 (42–49)	0.639
LV end-systolic diameter, mm	29 (27–32)	30 (27–33)	0.993
LV ejection fraction, %	63 (60–65)	63 (60–65)	0.274
LA diameter, mm	34 (32–37)	35 (32–37)	0.201
LA volume, mL/m²	26.5 (22.9–30.6)	28.7 (22.9–30.6)	0.001
LV mass, g/m²	76 (64–90)	80 (67–89)	0.013
Mitral inflow E, cm/s	70 (58–80)	68 (56–82)	0.818
Mitral inflow A, cm/s	57 (48–70)	59 (48–71)	0.394
Deceleration time, ms	197 (166–233)	194 (159–227)	0.220
E/A ratio	1.2 (1.0–1.5)	1.2 (1.0–1.4)	0.672
E/e' ratio	6 (5–8)	6 (5–7)	0.352
Right atrial area, cm²	14 (12–15)	14 (12–15)	0.554

Data are expressed as absolute numbers (percentage) or median (interquartile range). AV indicates atrioventricular; CK-MB, creatine kinase isoenzyme MB; E/e', ratio of the early diastolic transmitral flow velocity to early diastolic mitral annular velocity (average at septal and lateral mitral annulus); LA, left atrial; LV, left ventricular; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and NYHA, New York Heart Association.

*Age at the time of the cardiovascular assessment (baseline visit).

Predictors of Chagas Disease Progression

Various factors have been reported to be associated with cardiomyopathy onset, including the *T. cruzi* genotype, persistent tissue or blood parasitism, abnormal immune responses, male sex, oral acquisition of infection, and recurrent infections.[17,30–32] There is a growing consensus

Table 5. Predictors of New-Onset Cardiomyopathy or Death at Long-Term Follow-Up

Disease status at follow-up	Did not progress (n=496)	Progressed (n=75)	Unadjusted		Adjusted for age and sex		Adjusted for age and sex, and risk factors*	
			OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Overall participants without cardiomyopathy at baseline visit (n=571)								
Male sex	227 (45.8)	43 (57.3)	1.59 (0.98–2.60)	0.063	1.73 (1.05–2.85)	0.031	1.89 (1.13 – 3.16)	0.016
Age, y	49 (42–56)	53 (44–61)	1.04 (1.01–1.06)	0.004	1.05 (1.02–1.07)	0.002	1.05 (1.02–1.08)	0.001
T. cruzi serological test								
Negative	266 (53.6)	28 (37.3)	Reference		Reference		Reference	
Positive	230 (46.4)	47 (62.7)	1.94 (1.18 – 3.20)	0.009	2.25 (1.34 – 3.76)	0.002	2.24 (1.33–3.77)	0.002
Centers								
MOC	274 (55.2)	30 (40.0)	Reference		Reference		Reference	
SP	222 (44.8)	45 (60.0)	1.85 (1.13 – 3.03)	0.015	1.47 (0.87 – 2.48)	0.144	1.47 (0.86 – 2.50)	0.156
T. cruzi–seropositive donors without cardiomyopathy at baseline visit (n=277)								
	(n=230)	(n=47)						
Male sex	99 (43.0)	26 (55.3)	1.63 (0.87–3.08)	0.125	1.73 (0.91–3.28)	0.094	1.98 (1.02–3.84)	0.043
Age, y	48 (41–56)	50 (41–59)	1.02 (0.99–1.05)	0.257	1.02 (0.99–1.05)	0.185	1.03 (0.99–1.06)	0.153
Benznidazole use†	23 (10.0)	5 (10.6)	1.07 (0.38–2.98)	0.895	1.26 (0.44–3.58)	0.664	1.51 (0.48–4.69)	0.480
T. cruzi DNA detected by PCR								
Negative	129 (56.1)	23 (48.9)	Reference		Reference		Reference	
Positive	101 (43.9)	24 (51.1)	1.34 (0.71–2.51)	0.367	1.32 (0.70–2.49)	0.387	1.42 (0.74– 2.69)	0.291
Centers								
MOC	123 (53.5)	23 (48.9)	Reference		Reference		Reference	
SP	107 (46.5)	24 (51.1)	1.20 (0.64 – 2.25)	0.570	0.98 (0.50–1.91)	0.955	0.96 (0.49–1.89)	0.916
Antibody against *T. cruzi*								
EIA (S/C)	6.1 (4.8–6.8)	6.5 (5.2–7.3)	1.34 (1.06–1.70)	0.015	1.38 (1.08–1.77)	0.010	1.37 (1.07–1.76)	0.011
Antibody EIA quartiles								
1st	72 (31.3)	10 (21.3)	Reference		Reference		Reference	
2nd	60 (26.1)	12 (25.5)	1.44 (0.58–3.57)	0.430	1.41 (0.56–3.53)	0.464	1.39 (0.55–3.47)	0.486
3rd	58 (25.2)	9 (19.1)	1.12 (0.43–2.93)	0.822	1.16 (0.44–3.06)	0.770	1.11 (0.42–2.96)	0.829
4th	40 (17.4)	16 (34.0)	2.88 (1.20–6.94)	0.018	3.23 (1.31–7.94)	0.011	3.18 (1.29–7.85)	0.012

Data are expressed as the absolute numbers (percentage) or median (interquartile range), unless otherwise stated. EIA indicates enzyme immunoassay; MOC, Montes Claros; OR, odds ratio; S/C, absorbance/cutoff; and SP, São Paulo.

*Risk factors: diabetes, hypertension, dyslipidemia, and body mass index.

†Benznidazole was the antitrypanosomal medication.

that parasite persistence is required for the development of cardiomyopathy, and consequently, antiparasitic treatment may have an effect on the clinical course of the disease.[33–36] The disappearance of *T. cruzi* antibodies is considered the best evidence for a parasitological cure. However, there is still no robust evidence that seroreversion is a surrogate of clinical outcome, or that it reflects a halting of disease progression.[37,38]

There was a biological gradient whereby the prevalence of cardiomyopathy increased at higher antibody levels (Table S1 in the Supplemental Material). Individuals with higher antibody level at baseline were more likely to develop cardiomyopathy or to die during the follow-up period. Those with antibody levels in the highest quartile carried odds of 3.2, compared with the lowest quartile, for this outcome. Serology assays that detect total antibody against *T. cruzi* cell lysate reflect the overall humoral immune response against *T. cruzi*. In patients with greater parasite burden, experiencing more frequent parasitemia, antigenic immune stimulation by *T. cruzi* is thought to be greater, resulting in higher total antibody levels.[39,40] Because the development of cardiomyopathy seems to be driven by parasite persistence, the association between antibody levels, a marker of parasite burden, and incident cardiomyopathy is expected mechanistically, but has not previously been demonstrated. This observation may be useful in counseling patients about their long-term prognosis and risk of cardiomyopathy. It may also be a useful criterion for enrollment in clinical trials of antitrypansomal treatment. By selecting individuals without cardiac disease but with high antibody titers, the event rate, and therefore power,

will be higher. Furthermore, patients with higher parasite burden, as assessed by antibody level, may stand to benefit the most from etiologic treatment, although this is unproven.

Parasite detection in blood by PCR has also been used to assess parasite load and treatment effectiveness. Previous studies demonstrated the role of PCR in predicting progression.[12,40] However, a negative PCR does not mean absence of parasite, as false negatives occur beause of fluctuations in parasitemia, the intrinsic limit of detection of PCR, quantitative PCR techniques, and other factors that contribute to the overall performance of PCR assays.[41] Therefore, because of frequent false negatives, PCR is a relatively poor indicator of parasite burden, and the association with disease outcome would be expected to be biased toward the null. It makes sense, therefore, that PCR status was not retained within the fully adjusted models, but antibody level, which is a more stable indicator of parasite burden, did remain significantly associated with death and new-onset cardiomyopathy.

Study Limitations

Loss to follow-up may lead to bias, which may affect the inferences drawn from the study. To ensure that those lost to follow-up did not have higher mortality rate than those who complete the study, the vital status of each participant lost to follow-up was determined from the National Mortality System. The linkage method used has high sensitivity and specificity to detect deaths. To assess new-onset of cardiomyopathy among participants who were alive at the time of the linkage, we compared well-established risk factors for cardiovascular diseases between those lost to follow-up with those who returned for follow-up visit to complete the study (Text S1, Figure S1, and Table S3 in the Supplemental Material). In addition, a model to predict loss to follow-up was built including variables that predict either loss to follow-up and progression (Table S4 in the Supplemental Material). Last, a sensitivity analysis for new-onset cardiomyopathy was performed using inverse probability weights for selection (Table S5 in the Supplemental Material) with no important changes in inferences. The effect of the variables that we previously found to be associated with new-onset cardiomyopathy (Table 5), including antibody levels, remained statistically significant.

Our approach was intended to be explorative and may be useful for inferences about possible selection bias in new-onset of cardiomyopathy measured after loss to follow-up has occurred. After careful evaluation, we assumed that loss to follow-up depended on a missing at random mechanism, in which the probability of a participant remaining in the study depends on the exposure or confounders, but not on nonobserved outcomes.[42] The missing at random mechanisms provide an unbiased estimate of effect because collected variables can explain the potential bias by controlling for the covariates that are associated with loss to follow-up in multivariable analysis.[42]

To assess the effect of changes in life expectancy observed in *T. cruzi*–seropositive individuals, other associated comorbidities must be considered to increase the risk of cardiovascular events and death.[26,43] Over the past few decades, the migration from rural areas to large urban centers has exposed these individuals to a lifestyle that predisposes to atherosclerosis and cardiovascular diseases. Comorbidities become increasingly more frequent as the population with Chagas disease ages.[43] As such, in an aging cohort of individuals with Chagas disease, the incidence of cardiomyopathy associated with *T. cruzi* is expected to fall, whereas cardiac disease with other causes is expected to increase. However, after controlling for age and comorbidities by including seronegative individuals, *T. cruzi* seropositivity remained an important determinant of cardiomyopathy even among the elderly in our study.

The pathophysiology of myocardial damage in Chagas cardiomyopathy is complex and multifactorial. Although we found an association between antibody levels and cardiomyopathy, pathogenic differences in *T. cruzi* strains and host genetic and immunologic susceptibility factors are also likely to play roles in disease progression.[44] Therefore, the progression to cardiomyopathy likely results from multiple factors linked to the parasite, the host, and the interaction between them, which cannot be determined from our study.

Last, we recommend caution in extrapolating these findings to *T. cruzi*–seropositive individuals living in nonendemic countries. It is possible that in nonendemic settings, which present little or no risk of reinfection and higher socioeconomic conditions, the progression to cardiomyopathy is delayed.

CONCLUSIONS

We have reported a comprehensive description of the natural history of *T. cruzi* seropositivity in a large contemporary cohort of blood donors with detailed cardiovascular evaluation. *T. cruzi* seropositivity was a strong determinant of new-onset cardiomyopathy and death. *T. cruzi* antibody level, an indirect measure of parasite burden, was associated with disease progression. Our data highlight the central importance of total anti–*T. cruzi* antibody titer as a marker of Chagas disease activity and risk of progression.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Materials

Text S1 (addressing loss to follow-up)

Figure S1

Data Supplement Tables S1–S5

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