SUPPLEMENTAL MATERIAL

Supplemental Methods

Addressing loss to follow-up (*Text I*)

Of the 856 participants without cardiomyopathy at baseline visit, 285 (33.3%) did not return for the follow-up visit; 108 (28.1%) were seropositives and 177 (37.6%) were seronegative blood donors. To assess whether some patients may not have returned because they died, we linked each participant lost to follow-up with the National Mortality System (SIM) to determine mortality. For participants who were alive, our strategy for dealing with loss to follow-up on prediction of new-onset of cardiomyopathy was to compare all characteristics of the subjects deemed lost with those who participated in follow-up visit (Table III in the Data Supplement). There was a higher loss-to follow-up in São Paulo compared with Montes Claros among seropositive subjects. In seronegative subjects younger participants were more frequently lost to follow-up (Figure I in the Data Supplement). The model to predict loss to follow-up is shown in Table IV in the Data Supplement. The variables that predicted either loss to follow-up or new-onset cardiomyopathy were included in the final models shown in Table 5. The inclusion of these auxiliary variables in the maximum likelihood analyses considerably enhanced the missing at random (MAR) assumption.

Additionally, a sensitivity analysis for new-onset cardiomyopathy using inverse probability weights for selection was performed. The weights were obtaining from the logistic model in Supplementary Table IV using all variables found to be significantly associated with loss to follow-up. The weighted analysis was done using the *svyglm* function from the R package *survey*. A Table with sensitivity analysis is presented in Table V in the Data Supplement with no important changes in the results.

Supplemental Tables

Table I: Association between anti-*T. cruzi* antibody levels and mortality

Study group	Person-time at risk (person-years)	Number of deaths	Mortality per 1000 person-years (95% CI)
Seronegative donors	4,976	18	3.6 (2.1 – 5.7)
Seropositive donors (< 5)	1,108	4	3.6 (1.0 – 9.2)
Seropositive donors (5-5.9)	1,094	5	4.6 (1.5 – 10.7)
Seropositive donors (6-6.9)	1,773	10	5.6 (2.7 – 10.4)
Seropositive donors (≥ 7)	1,214	13	10.7 (5.7 – 18.3)

Anti-*T. cruzi* antibody levels at baseline (EIA); 3 patients with missing data on antibody level).

Table II: Cardiomyopathy by antibody level category among seropositive blood donors at baseline visit.

EIA category	Cardiomyopathy (n=111)*	Without cardiomyopathy (n=385)	Proportion of cardiomyopathy at baseline visit (%)
< 3	0	28	0
3 – 3.9	4	24	14
4 – 4.9	7	42	14
5 – 5.9	21	84	20
6 – 6.9	39	129	23
≥ 7	40	78	34

* 3 patients with missing data on antibody level.

Table III: Baseline characteristics (visit 1) of participants lost to follow-up compared with those who participated in follow-up visit.

		Participants (n=539)	Lost to follow-up* (n=285)	P value
Age, years		49 (42-57)	47 (38-56)	0.024
Male sex		251 (46.6)	148 (51.9)	0.143
Anti-*T. cruzi* serological test	Positive	262 (48.6%)	108 (37.9%)	0.002
	Negative	277 (51.4)	177 (62.1)	
Centers (cities)	Montes Claros	295 (54.7)	124 (43.5)	0.002
	São Paulo	244 (45.3)	161 (56.5)	
BMI, kg/m2		26.7 (24.4 - 29.4)	26.8 (24.0 - 30.0)	0.922
NYHA functional class	I	512 (95.0)	272 (95.3)	0.973
	II/III	27 (5.0)	13 (4.7)	
Smoking history	Never	308 (57.1)	154 (54.0)	0.596
	Past	165 (30.6)	90 (31.6)	
	Current	66 (12.2)	41 (14.4)	
Diabetes		29 (5.4)	11 (3.9)	0.246
Hypertension		125 (23.2)	70 (24.6)	0.449
Chronic kidney disease		12 (2.2)	12 (4.2)	0.254
Heart rate (bpm)		68 (60-72)	68 (60-72)	0.088
Systolic blood pressure (mmHg)		125 (113-140)	125 (115-140)	0.655
Diastolic blood pressure (mmHg)		75 (65-86)	80 (69-89)	0.033
Laboratory measurements				
Low-density lipoprotein, mg/dL)		122 (96-149)	123 (101-148)	0.962
High-density lipoprotein, mg/dL		47 (40-56)	47 (39-56)	0.859
Triglycerides, mg/dL		118 (86-171)	122 (87-179)	0.507
Glycemia, mg/dL		87 (81 - 96)	86 (79 - 96)	0.628

NT-ProBNP, pg/mL	38.0 (22.7 - 65.2)	40.1 (23.3 - 65.5)	0.596
Medications			
Amiodarone	2 (0.4)	2 (0.7)	0.516
ACE inhibitors	52 (9.6)	25 (8.8)	0.681
Beta blockers†	25 (4.6)	19 (6.7)	0.218
Antiplatelet‡	19 (3.5)	5 (1.8)	0.151
Benznidazole	26 (4.8)	12 (4.2)	0.690
ECG and echocardiographic data			
QRS duration (ms)	88 (82 - 94)	88 (82 - 94)	0.987
PR duration (ms)	156 (142 - 168)	156 (142 - 170)	0.736
QTc calculated (ms)	426 (411 - 441)	424 (405 - 441)	0.149
Low QRS amplitude	17 (3.2)	7 (2.5)	0.571
Sinus bradycardia ≥40 bpm	163 (30.4)	87 (30.6)	0.947
Minor isolated ST-T abnormalities	57 (10.6)	23 (9.7)	0.248
LV ejection fraction (%)	63 (60 - 65)	63 (60 - 65)	0.509
LV mass, g/m^2	78 (65 - 89)	77 (65 - 89)	0.716
LA volume, mL/m^2	27 (24- 33)	27 (23 - 32)	0.236
E/e' ratio	6 (5 - 8)	6 (5 - 7)	0.098

*Participants who were alive but did not return for cardiovascular assessment at visit 2.

† Beta blockers included atenolol, propranolol and carvedilol.

‡ Only aspirin was used.

ACE inhibitors = angiotensin-converting-enzyme inhibitors

Table IV: Multivariable model to predict loss to follow-up

Variables		Betas	OR (95% CI)	P value
Age, years		-0.032	0.969 (0.952 - 0.985)	0.001
Female sex		-0.131	0.877 (0.642 - 1.198)	0.410
Center (São Paulo)		0.458	1.581 (1.154 - 2.172)	0.004
T. cruzi-seropositives		-0.431	0.650 (0.479 - 0.879)	0.005
Obesity		0.154	1.167 (0.813 - 1.667)	0.399
Dyslipidemia		-0.051	0.950 (0.697 - 1.294)	0.747
Diabetes		-0.444	0.641 (0.291 - 1.322)	0.246
Hypertension		0.293	1.340 (0.910 - 1.968)	0.136
Smoking history	Current	0.041	1.041 (0.658 - 1.629)	0.860
	Past	0.071	1.074 (0.761 - 1.511)	0.684

Table V: Predictors of new-onset cardiomyopathy or death at long-term follow-up (sensitivity analysis using inverse probability weights for selection)

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									
Male sex		227 (45.8)	43 (57.3)	1.61 (0.97-2.69)	0.066	1.78 (1.06-2.99)	0.030	1.86 (1.10 - 3.15)	0.022
Age, years		49 (42-56)	53 (44-61)	1.04 (1.01-1.07)	0.009	1.04 (1.01-1.07)	0.004	1.05 (1.02-1.08)	0.003
T. cruzi serological test									
Negative		266 (53.6)	28 (37.3)	Reference		Reference		Reference	
Positive		230 (46.4)	47 (62.7)	1.97 (1.18 - 3.29)	0.010	2.28 (1.36 - 3.81)	0.002	2.30 (1.37-3.87)	0.002
Centers	MOC	274 (55.2)	30 (40.0)	Reference		Reference		Reference	
	SP	222 (44.8)	45 (60.0)	1.66 (1.00 - 2.76)	0.050	1.28 (0.74 - 2.22)	0.373	1.28 (0.73 - 2.23)	0.387
***T. cruzi* seropositives without cardiomyopathy at baseline visit**									
		(n=230)	(n=47)						
Male sex		99 (43.0)	26 (55.3)	1.73 (0.90-3.32)	0.101	1.88 (0.96-3.65)	0.065	2.05 (1.03-4.07)	0.041
Age, years		48 (41-56)	50 (41-59)	1.02 (0.99-1.06)	0.172	1.03 (0.99-1.07)	0.109	1.04 (1.00-1.06)	0.075
Benznidazole use†		23 (10.0)	5 (10.6)	0.88 (0.31-2.47)	0.808	1.07 (0.37-3.04)	0.905	1.05 (0.35-3.12)	0.932
T. cruzi DNA detected by PCR									
Negative		129 (56.1)	23 (48.9)	Reference		Reference		Reference	

Positive		101 (43.9)	24 (51.1)	1.20 (0.62-2.86)	0.594	1.16 (0.60-2.24)	0.653	1.16 (0.60- 2.24)	0.666
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	0.88 (0.43-1.81)	0.739	0.86 (0.42-1.77)	0.687
Antibody against *T. cruzi*									
EIA (S/C)		6.1 (4.8-6.8)	6.5 (5.2-7.3)	1.28 (0.98-1.68)	0.068	1.32 (1.00-1.74)	0.049	1.32 (1.00-1.75)	0.051
Antibody EIA quartiles	1st	72 (31.3)	10 (21.3)	Reference		Reference		Reference	
	2nd	60 (26.1)	12 (25.5)	1.27 (0.50-3.24)	0.619	1.22 (0.46-3.24)	0.695	1.29 (0.48-3.51)	0.613
	3rd	58 (25.2)	9 (19.1)	1.14 (0.42-3.09)	0.797	1.17 (0.44-3.08)	0.759	1.06 (0.40-2.83)	0.911
	4th	40 (17.4)	16 (34.0)	2.46 (0.99-6.11)	0.053	2.73 (1.07-6.95)	0.033	2.87 (1.08-7.63)	0.035

Data are expressed as the absolute numbers (percentage) or median (interquartile range-IQR)

*Risk factors: Diabetes, hypertension, dyslipidemia, and body mass index. S/C: absorbance/cut off

† Benznidazole was the antitrypanosomal medication

Abbreviations: MOC: Montes Claros; SP: São Paulo

Supplemental Figures and Figure Legends

Figure I

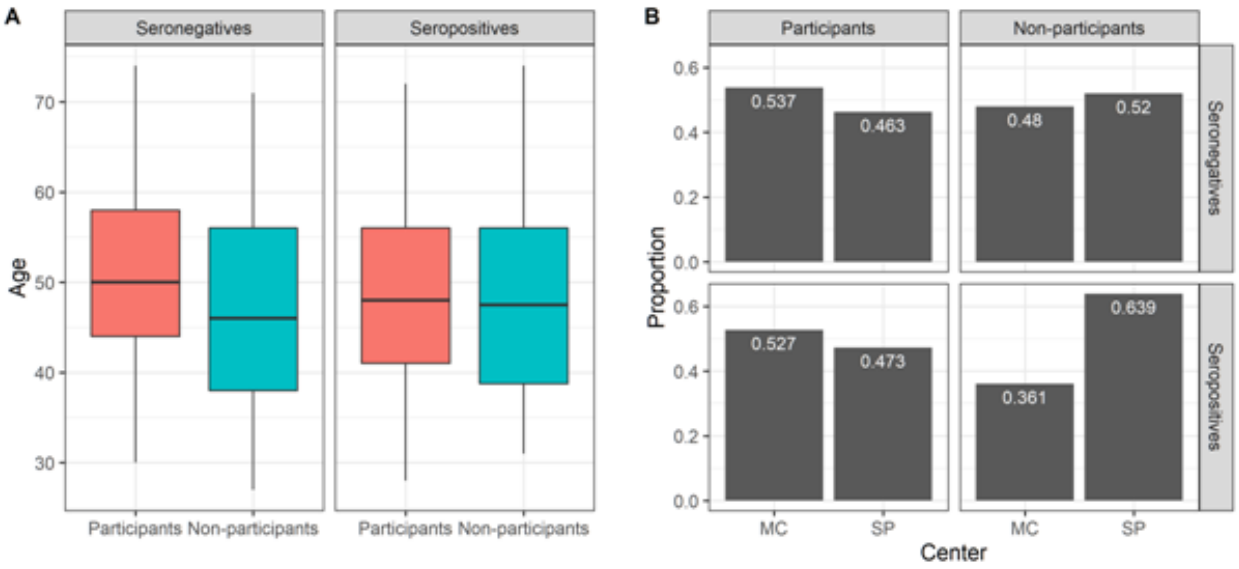

Figure I: Differences of serological status according to age at enrollment (panel A), and the center of recruitment (panel B) between participants and non-participants at follow-up visit.

In seronegative participants, younger individuals were more frequently lost to follow-up. There was a higher loss-to follow-up in São Paulo compared with Montes Claros among seropositive participants.

MC = Montes Claros

SP = São Paulo