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BNP, NTproBNP, CMBK, and MMP-2 predict mortality in severe Chagas cardiomyopathy

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Abstract

Background—Chagas cardiomyopathy is a chronic sequela of infection by the parasite, *Trypanosoma cruzi*. Advanced cardiomyopathy is associated with a high mortality rate, and clinical characteristics have been used to predict mortality risk. Though multiple biomarkers have been associated with Chagas cardiomyopathy, it is unknown how these are related to survival.

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Objectives—Our study aimed to identify biomarkers associated with mortality in individuals with severe Chagas cardiomyopathy in an urban Bolivian hospital.

Methods—The population included individuals with and without *T. cruzi* infection recruited in an urban hospital in Santa Cruz, Bolivia. Baseline characteristics, ECG findings, medications, and serum cardiac biomarker levels (BNP, NTproBNP, CKMB, troponin I, MMP-2, MMP-9, TIMP-1, TIMP-2, TGFb1, and TGFb2) were ascertained. Echocardiograms were preferentially performed on those with cardiac symptoms or electrocardiogram abnormalities. Participants were contacted by phone approximately 1 year after initial evaluation; deaths were reported by family members. Receiver operating characteristic curves were used to optimize cut-off values for each marker. For markers with area under curve > 0.55, Cox proportional hazards models were performed to determine the hazards ratio (HR) and 95% confidence interval (CI) for the association of each marker with mortality.

Results—The median follow-up time was 14.1 months (interquartile range 12.5- 16.7 months). Of 254 individuals with complete cardiac data, 220 (87%) had follow-up data. Of 50 patients with severe Chagas cardiomyopathy, 20 (40%) had died. Higher baseline levels of BNP (HR[95% CI]: 3.1 [1.2, 8.4]), NTproBNP (4.4[1.8,11.0]), CKMB (3.3[1.3, 8.0]), and MMP-2 (4.2[1.5, 11.8]) were significantly associated with subsequent mortality.

Conclusions—Severe Chagas cardiomyopathy is associated with high short-term mortality. BNP, NTproBNP, CKMB and MMP2 have added predictive value for mortality, even in the presence of decreased ejection fraction and other clinical signs of congestive heart failure.

Keywords

Chagas disease; Cardiomyopathy; Biomarkers; Mortality

Introduction

Chagas disease is caused by the protozoan *Trypanosoma cruzi*. Chagas disease has traditionally been a disease of rural Latin American communities where poor housing promotes human contact with infected vectors. However, Chagas disease can now be found in urban centers both in and outside of Latin America due to massive emigration from rural endemic areas, with an estimated 300,000 infected people in the United States alone (1). Vector elimination programs have successfully decreased transmission of Chagas disease, but Bolivia remains the country with the highest prevalence of Chagas disease in the world (2).

In the decades following acute infection, 20-30% of individuals will develop cardiac manifestations of disease, including conduction system abnormalities, arrhythmias, and late in the course of disease, congestive heart failure. Advanced heart failure of any etiology is associated with shortened survival, but multiple investigations have shown that heart failure from Chagas disease carries a particularly poor prognosis (3) (4) (5) (6) (7).

Observational studies suggest that antitrypanosomal treatment may improve the prognosis and decrease progression in *T. cruzi*-infected individuals with no or early signs of Chagas cardiomyopathy. This hypothesis is currently under study in the BENEFIT trial

(clinicaltrials.gov/NCT00123916). However, once present, cardiac structural damage is not reversible, and access to advanced heart failure therapies such as left ventricular assist devices, heart transplants, and implantable cardiac defibrillators is limited in communities that are not even able to meet the need for simpler devices such as pacemakers (8). Therefore, identifying biomarkers that are predictive of those at highest risk for fatal outcomes of CCM could allow limited resources to be targeted to those most in need (9).

Clinical findings characteristic of advanced cardiomyopathy and congestive heart failure (NYHA class III or IV, large cardiothoracic ratio on chest radiography, and varied electrocardiogram findings including atrial fibrillation, multiple premature ventricular complexes, ventricular conduction deficits, low voltage, and pathologic Q waves and low QRS voltage) are well known as predictors of mortality in Chagas disease (10-12) (13) (14, 15) (9). Other less consistently identified risk factors include older age and male sex (11) (12) (14) (13) (16) (17). In recent retrospective study including only individuals with severe cardiomyopathy, low ejection fraction did not show further predictive value for mortality within this severely ill group (18) (19).

We measure 10 serum biomarkers in this study, Brain Natriuretic Peptide (BNP), N-terminal brain natriuretic peptide (NTproBNP), Troponin-1, Creatine kinase-MB (CKMB), matrix metalloproteinase (MMP)-2, MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1, TIMP-2, and transforming growth factor beta (TGF β)1 and TGF β 2. Prior work has shown that serum cardiac markers including BNP, NTproBNP, CKMB, MMP-2, and TIMP-2 are associated with mortality in heart failure unrelated to Chagas disease (20-23). The association of TIMP-1 with mortality is controversial (21, 24) (25) (22). To date, only BNP had been shown to predict mortality in Chagas cardiomyopathy (26), (27), (28). The objective of the current analysis was to evaluate the additive predictive value of serum biomarkers within the Chagas disease patient group already known to be at high risk of short-term mortality based on clinical and echocardiographic signs of heart failure.

Materials and Methods

Ethics Statement

The study protocol was approved by the Institutional Review Boards of Universidad Catolica Boliviana (Santa Cruz, Bolivia), PRISMA (Lima, Peru), and Johns Hopkins Bloomberg School of Public Health (Baltimore, Maryland). All participants provided written informed consent.

Study Population and data collection

Participants were drawn from a cross-sectional study of serum biomarkers for Chagas cardiomyopathy (23). Briefly, we recruited patients attending the inpatient and outpatient services, as well as visitors to San Juan de Dios Hospital, the largest general public hospital in Santa Cruz, from September 2012 to April 2013. Our aim was to recruit *T. cruzi*-infected and uninfected individuals spanning the spectrum from no heart disease to severe cardiomyopathy. Baseline demographic, electrocardiographic, echocardiographic, and laboratory data were collected as previously described (23). A total of 409 participants were

recruited into the study, of whom 254 had full cardiac evaluations and were eligible to be included in the current analysis. The limiting factor was our capacity to perform echocardiograms, To ensure echocardiograms were performed on individuals in all severity stages, participants were grouped based on history of heart failure and presence of ECG abnormalities. Within each group, individuals were randomly chosen for an echocardiogram appointment until all available appointments were filled. One to three individual or household phone numbers were recorded at the time of baseline data collection. Study personnel attempted to contact all participants by phone from February 2014 to March 2014. Phone numbers were tried three times before coding the individual as lost to follow-up. If the individual was deceased, date of death and available information about the cause was collected from family members.

Clinical Classification

Participants were assigned cardiac disease severity stages based on electrocardiogram (ECG) and echocardiogram results at the time of enrollment (23). In the current analysis, participants with clinically severe disease who died before receiving an echocardiogram were excluded. Criteria for the stages were as follows:

- Stage A: *T. cruzi*-infected (Tc +) with normal ECG (no abnormalities suggestive of Chagas heart disease) and normal echocardiogram
- Stage B: *T. cruzi*-infected with characteristic ECG findings (RBBB, LBBB, LAFB, any AV conduction block, multiple PVCs, bradyarrhythmias (HR \leq 50), and/or atrial fibrillation) and normal echocardiogram
- Stage C: *T. cruzi*-infected with mild to moderate systolic dysfunction (EF 40-54%)
- Stage D: *T. cruzi*-infected with severe systolic dysfunction by ejection fraction (EF) $<$ 40% and/or left ventricular end diastolic dilatation (LVEDD) \geq 57 mm

T. cruzi-uninfected (Tc-) individuals assigned similar categories to provide healthy controls (Tc-, normal ECG) and Tc- individuals with heart failure (systolic dysfunction, severe LVEDD) for comparison.

Laboratory Methods

T. cruzi infection status was determined using two commercial IgG serological tests (enzyme-linked immunoassay and indirect hemaagglutination test); those with discordant results were tested by trypomastigote excreted-secreted antigens (TESA)-blot as described previously (23). Confirmed infection was defined by positive results by at least two assays.

We measured 10 serum biomarkers: BNP, NTproBNP, CKMB, troponin I, MMP-2, MMP-9, TIMP-1, TIMP-2, TGFb1, and TGFb2. Biomarker measurement was performed in sera by multiplex bead assays as previously described (23). Brain natriuretic protein, NTproBNP, CKMB, and troponin I were measured using the Milliplex Map Human Cardiovascular Disease Magnetic Bead Panel. Other biomarker levels were measured using Milliplex MAP Human MMP Magnetic Bead Panel 2, Milliplex Map Human TIMP Magnetic Bead Panel 1, and Milliplex MAP TGF-B1,2,3 Plex Magnetic Bead Panel (all kits from Millipore, Billerica, Massachusetts).

Statistical Analysis

Categorical variables are reported as percentages and compared using the Chi-square or Fisher exact test. Normality of variable distribution was determined by the Shapiro-Wilke test. Normal variables are reported by mean, standard deviation, and Student's T-test for comparisons. Continuous non-normal variables are reported by median and IQR and compared using Wilcoxon rank sum. Receiver operating characteristic (ROC) curves were created for each biomarker to optimize the cut-point that best discriminates death at follow-up. Markers with an area under the curve (AUC) value of >0.55 were selected for evaluation of their association with mortality, and a cut-point was defined based on the maximum sensitivity and specificity identified in the ROC analysis. Kaplan–Meier methods were used to estimate the proportion of participants surviving through follow-up. Univariate and multivariate Cox proportional hazards models were performed to determine the hazards ratio (HR) and 95% confidence interval (CI) for the association of each marker with mortality. Multivariate cox proportional hazards models were adjusted for age and sex. All statistical analyses were performed using Stata 12.0 with a two-tailed $p \leq 0.05$ considered to be significant.

Results

Among the 254 individuals with full cardiac evaluations at baseline, follow-up information was available for 220 (87%) individuals, with no differences in completeness of follow-up by *T. cruzi* infection status or severity stage (Table 1). The median time to outcome was 424 days (14.0 months). Among *T. cruzi*-infected individuals, mortality was highest in Stage D (20/50; 40%) followed by Stage C (2/16; 13%). Individuals with Stage D disease at baseline had a significantly lower overall probability of survival as compared to individuals in earlier stages of cardiac disease across follow-up ($p < 0.001$) (Figure 1). No deaths occurred among participants in Stage B. The single death in Stage A was due to a stroke in an 82 year-old man. In uninfected individuals, the only deaths occurred in Stage D, with a 50% (4/8) mortality rate. Stage D had the shortest time to outcome due to the deaths included. Among those with severe cardiac disease, follow-up information was available for 86% and 89% of *T. cruzi*-infected and uninfected individuals, respectively. Mortality was also high (50%) among uninfected individuals in Stage D, but the denominator was only 8, providing insufficient power for Stage D comparisons based on infection status. Therefore, further analyses focused on the 50 *T. cruzi*-infected Stage D patients with complete data. Among these patients, those who were still alive at the time of follow-up had a higher body mass index than those who died (Table 2). Other baseline characteristics at the time of recruitment, including age, sex, self-reported medical history, medication use, and ECG findings were not significantly different for those who died compared to those who were still alive.

The ROC analyses identified 4 serum markers (BNP, NTproBNP, CKMB and MMP-2) with AUCs above 0.55 (Table 3). The AUCs for the other biomarkers fell below the 0.55 cut-off (AUC[95% CI]: troponin 0.45[0.28, 0.61], MMP9 0.47[0.29, 0.65], TIMP1 0.48[0.29, 0.68], TIMP2 0.53[0.34, 0.71], TGFb1 0.51[0.33, 0.68] and TGFb2 0.51[0.33, 0.69]). Based on the optimal cut-offs in the ROC curves for each of these markers, Kaplan-Meier curves were

constructed. All four biomarkers were elevated at the time of recruitment in individuals who died compared to those who survived (Figure 2). Elevations in these biomarker levels were significantly associated with mortality in Cox regression models analysis adjusted for age and sex (Table 3).

Discussion

Advanced Chagas cardiomyopathy is associated with very high short-term mortality (10-12). Both heart failure and sudden cardiac death are known causes of mortality in CCM. Consistent with the literature, we found the highest rates of mortality in patients in Stage D, the most severe stage of cardiomyopathy based on left ventricular dilatation and ejection fraction. Stage D *T. cruzi* infected individuals experienced 40% mortality over 14 months, similar to other studies of severe CCM (19). By restricting the analysis to patients in Stage D, we effectively examined the additive predictive value of the serum biomarkers beyond the clinical indicators already identified in many previous studies (10-12) (13) (14, 15) (9).

Of the 10 serum markers we examined in this analysis, four (BNP, NT-pro BNP, CKMB and MMP2) were significantly associated with mortality among patients with severe Chagas cardiomyopathy. Interestingly, troponin-I in a conventional assay provided no additional predictive value in this study group. However, the sensitivity of the assay we used is much lower than the newly available high sensitivity troponin assays now being used in tertiary care centers in the United States (29).

BNP and NT-pro BNP were identified as strong predictors of mortality in our study. These markers are some of the most well characterized markers used to guide treatment and predict outcomes in heart failure. In heart failure from all etiologies BNP-guided therapy reduces all-cause mortality (30). Serum BNP levels were significantly positively correlated with 5-year mortality in elderly adults from Switzerland in those with and without identified cardiovascular disease (20). Furthermore, individuals with Chagas cardiomyopathy, even in the absence of systolic dysfunction, had higher BNP levels than healthy controls (26). For those with heart failure due to Chagas cardiomyopathy, BNP has previously been shown to be a strong predictor of mortality (27) (26) (28) (31). NT-pro BNP levels are correlated with severity of LV dysfunction in Chagas disease (32). Our results suggest that NT-pro BNP may be a better predictor of mortality than BNP, possibly related to the longer half-life of NT-pro BNP compared to BNP. While our data support using these markers as a predictor of mortality among those with known heart failure, their significance in predicting death among *T. cruzi*-infected individuals without heart failure is unknown.

CKMB is a well-known biomarker for acute cardiac muscle damage, and in the setting of myocardial infarction has a rapid evolution over hours to days (33-35). Elevated CKMB has been shown to be associated with poor prognosis and shortened survival after surgical interventions such as coronary artery bypass (36), but has not previously been examined as a prognostic marker in Chagas cardiomyopathy. We did not find a significant association of CKMB levels with Chagas cardiomyopathy stage in our previous analysis (23), but in this restricted sub-population, elevated CKMB levels predicted shortened survival. CKMB is likely acting as a non-specific marker of cardiac myocyte injury, and as in the setting of

myocardial infarction (33), higher levels indicate more extensive damage and therefore worse prognosis.

We found elevated MMP2 levels to predict mortality in our study patients, suggesting that activation of the cardiac remodeling process is associated with negative outcomes in severe cardiomyopathy. This finding is consistent with studies of heart failure from other etiologies as well (21) (37) (38). In heart failure, MMP-2 mRNA expression and blood protein levels are increased (38). Higher levels of MMP-2 are markers of diastolic heart failure and predict poor outcomes (21) (37). Higher levels of MMP-2 and MMP-9 correlate with more severe Chagas heart disease (39). Elevated MMP-2 levels have been reported to be associated with mortality in acute experimental Chagas disease, but have not been previously studied for this purpose in chronic Chagas cardiomyopathy in humans (40). Interestingly, among patients with heart failure and high BNP levels, MMP-2 was a better predictor of mortality than BNP level alone, consistent with our findings (41).

Previously identified factors associated with longer survival include being overweight and being on beta-blockers (42) (43). We found an elevated BMI to be associated with survival, consistent with prior studies (44). Though not well explained, this may be influenced by a cachectic state that can occur in end stage disease. We did not find significant associations between medications and outcome, but this analysis may have been hindered by the limited number of individuals in our study on optimal heart failure medication regimens.

Limitations of Study

The major limitation was the relatively small sample size, which impeded more in-depth analysis of *T. cruzi*-infected individuals, and precluded analysis of the uninfected group. In addition, those classified as having Stage D Chagas cardiomyopathy in this study were patients already presenting with clinical heart failure necessitating hospitalization. Therefore, this group may not be representative of all individuals with severe cardiomyopathy in Chagas disease. We had a thirteen percent loss to follow-up for the entire population, and a twelve percent loss to follow-up among those in group D. Individuals who were lost to follow-up may have had a higher mortality rate than those who remained in the study. We focused on individuals with advanced heart failure which may limit the generalizability of our results to less severe stages of Chagas disease. A longer follow-up period is necessary to investigate associations of biomarkers in early stages of disease with subsequent mortality. Finally, clinical data, such as the presence of co-morbid conditions and routine medications, were based on patient self-report and may have been subject to reporting biases based on the patient's overall health state and their comprehension of specific medical diagnoses. However, these data were collected at baseline and should not have biased the associations between biomarkers and subsequent mortality over the follow-up period.

Future Areas of Research

Longitudinal studies of longer duration would be valuable to examine a wider range of outcomes in a more representative group of Chagas disease patients. Biomarkers capable of identifying individuals at risk of cardiac progression (short of mortality) could enable

targeted early interventions. A comparison between patients with Chagas and other cardiomyopathies could help elucidate common or specific pathways of pathogenesis. Finally, the biomarkers we identified may have additional predictive value when combined with existing risk stratification based on clinical cardiological evaluations (10) (45).

Conclusion

Chagas cardiomyopathy leads to significant morbidity and mortality in *T. cruzi* infected individuals. Insufficient information exists to anticipate which infected individuals will develop cardiomyopathy, and identify those at risk of fatal outcomes. We found that elevated serum levels of BNP, NT-pro BNP, CKMB, and MMP-2 were associated with an increased of death among individuals with advanced Chagas cardiomyopathy. Further research is needed to better qualify the trend of these markers throughout the different stages of the disease process.

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Highlights

- Our study followed patients with severe Chagas cardiomyopathy who had a 40% mortality at a 1-2 year follow up.
- For those with severe Chagas cardiomyopathy, the characteristics between those who died and those who survived to follow up were similar, except body mass index which was significantly higher among the survivors.
- BNP, NT-pro BNP, CKMB, and MMP-2 were significantly higher in the subgroup that died compared to those who survived.

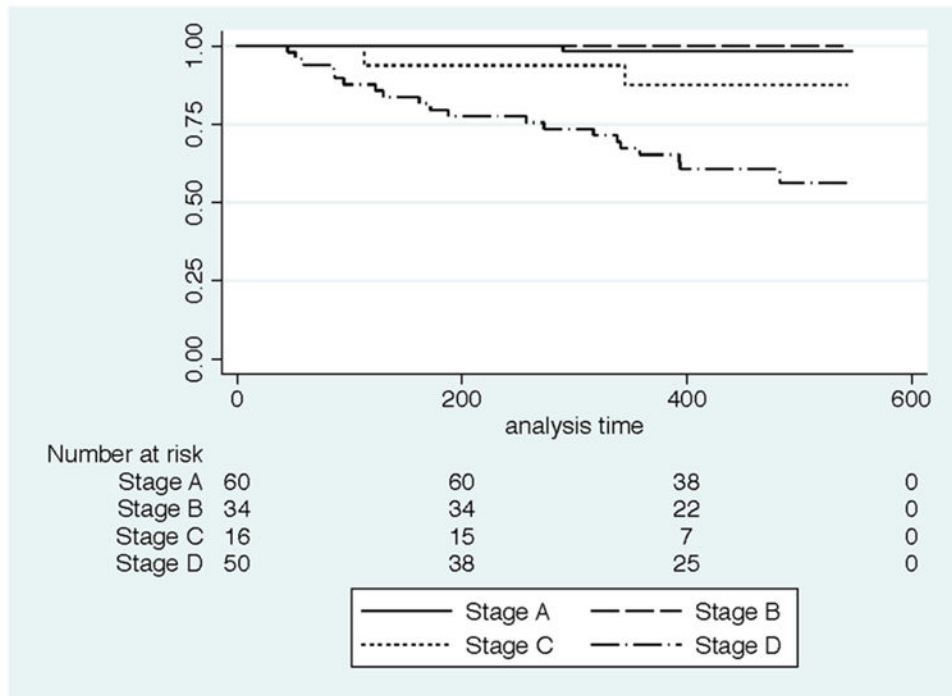


Figure 1. Kaplan Meier Curves for *T. cruzi*-infected individuals by severity stage

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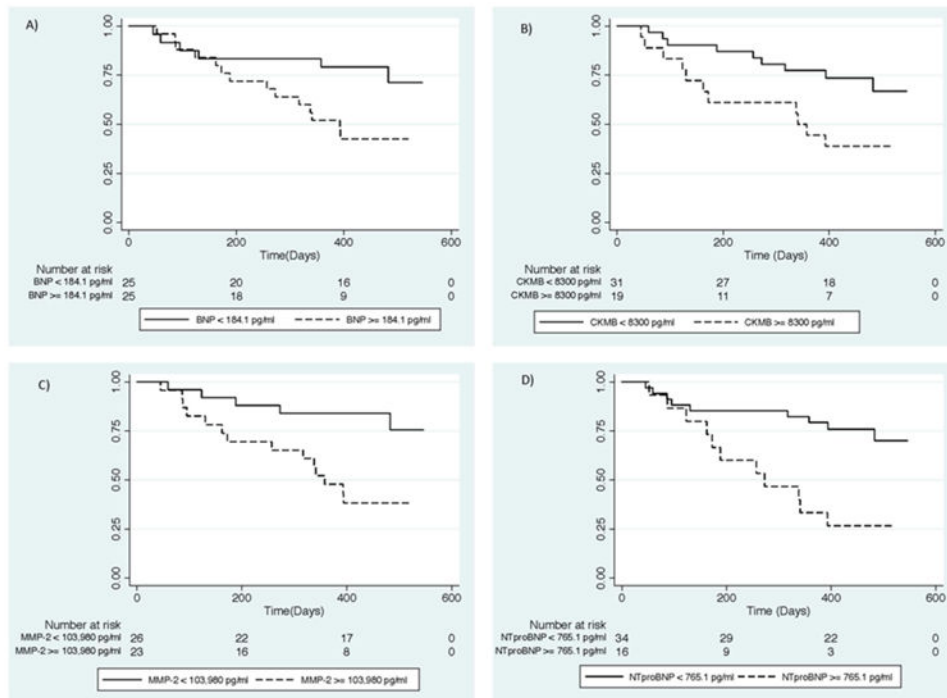


Figure 2. Kaplan Meier survival analyses stratified by biomarker results above and below the cut-offs chosen based on receiver operating characteristic curves. Survival of *T. cruzi* infected patients in Stage D by results of (A) Brain Natriuretic Peptide (BNP); (B) Creatine kinase-MB (CKMB); (C) Matrix metalloproteinase-2 (MMP2); (D) N-terminal brain natriuretic peptide (NTproBNP)

Table 1
Overall study population recruited in the Hospital San Juan de Dios, Santa Cruz, Bolivia, with follow-up data and mortality by stage of disease and *T. cruzi* infection status

Study population (N)	Mean age (SD)	Male N (%)	Inpatients	Successful Follow Up N (%)	Follow-up time, days Median (IQR)	Mortality N (%)
Screened for enrollment (409)	57.7 (12.7)	200 (48.9)	119 (29.1)	NA	NA	NA
Completed cardiac evaluation (254)	57.0 (12.7)	123 (48.4)	77 (30.3)	220 (87)	424 (374-501)	27 (12)
<i>T. cruzi</i> infected (183)	58.2 (12.3)	92 (50.2)	61 (33.3)	160 (89)	394 (352-475)	23 (14)
<i>T. cruzi</i> uninfected (71)	53.9 (13.2)	31 (43.7)	16 (22.5)	60 (85)	462 (384-504)	4 (7)
Stage and <i>T. cruzi</i> infection status (N)						
A (114)						
<i>T. cruzi</i> -infected (66)	55.6 (12.7)	29 (43.9)	5 (7.6)	60 (91)	426 (382-497)	1 (2)
Uninfected (48)	53.2 (12.8)	18 (37.5)	3 (6.3)	39 (81)	469 (393-511)	0 (0)
B (50)						
<i>T. cruzi</i> -infected (41)	58.2 (12.7)	19 (46.3)	8 (19.5)	34 (83)	431 (385-509)	0 (0)
Uninfected (9)	53.8 (15.6)	4 (44.4)	3 (33.3)	9 (100)	495 (378-518)	0 (0)
C (23)						
<i>T. cruzi</i> -infected (18)	59.4 (12.6)	8 (44.4)	10 (55.6)	16 (89)	395 (367-488)	2 (13)
Uninfected (5)	51.4 (13.0)	2 (40.0)	3 (60.0)	4 (80)	480 (405-538)	0 (0)
D (67)						
<i>T. cruzi</i> -infected (58)	60.8 (11.0)	36 (62.1)	38 (65.5)	50 (86)	404 (257-483)	20 (40)
Uninfected (9)	59.1 (14.1)	7 (77.8)	7 (77.8)	8 (89)	360 (253-461)	4 (50)

“Follow-up time” based on either last date of contact alive or date of death
 NA = not applicable; SD=standard deviation; IQR=interquartile range

Table 2
Demographic information, clinical history, medications, ECG and echocardiographic findings for *T. cruzi*-infected Stage D at time of recruitment

	Alive N=30	Died N=20	P-value
Demographics			
Age, mean (SD)	62.6 (11.2)	60.3 (9.6)	0.45
Sex, male	16 (53 %)	14 (70 %)	0.24
BMI, mean (SD) *	27.1 (4.4)	24.4 (4.1)	0.04
Overweight or obese *	20 (69 %)	10 (50 %)	0.18
Inpatient at recruitment	21 (70 %)	10 (50 %)	0.15
Medical History by Self-Report **			
Hypertension	16 (53 %)	7 (35 %)	0.20
Diabetes Mellitus	3 (10 %)	2 (10 %)	1.00
Coronary Artery Disease	7 (23 %)	2 (10 %)	0.29
Heart Failure	22 (73 %)	14 (70 %)	1.00
Pacemaker	4 (13 %)	2 (10 %)	1.00
Medications**			
Beta Blocker	6 (20 %)	4 (20 %)	1.00
ACE inhibitor	10 (33 %)	10 (50 %)	0.24
Angiotensin II Receptor Blockers	3 (10 %)	1 (5 %)	0.64
Anticoagulant	3 (10 %)	2 (10 %)	1.00
Aspirin	21 (70 %)	10 (50 %)	0.15
Nitroglycerin	1 (3 %)	2 (10 %)	0.56
Amiodarone	8 (27 %)	3 (15 %)	0.49
Calcium Channel Blocker	2 (7 %)	1 (5 %)	1.00
Diuretics	11 (37 %)	8 (40 %)	0.81
Digoxin	11 (37 %)	5 (25 %)	0.39
Spirolactone	5 (17 %)	6 (30 %)	0.27
Any medication	26 (87 %)	16 (80 %)	0.70
ECG **			
Isolated right bundle branch block	2 (7 %)	5 (25 %)	0.10
RBBB and LAFB	3 (10 %)	1 (5 %)	0.64
Left Bundle Branch Block	4 (13 %)	1 (5 %)	0.33
Atrial fibrillation or flutter	9 (33 %)	9 (45 %)	0.28
Multiple PVC	5 (17 %)	3 (15 %)	0.60
Low voltage	1 (3 %)	1 (5 %)	0.65
Normal ECG	1 (3%)	1 (5%)	0.76
Other Findings			
NYHA III or IV	15 (50 %)	12 (60 %)	0.49
Ejection Fraction, median(IQR)	27.5 (20-35)	20 (20-30)	0.13
Segmental Score, median(IQR)	2.0 (1.6-2.3)	2.3 (1.8-2.4)	0.09

BMI = body mass index; RBBB=right bundle branch block; LAFB=left anterior fascicular block; NYHA = New York Heart Association; PVC = premature ventricular contraction. Overweight and obese defined as BMI 25 - 29.9 and 30, respectively.

* Weight data missing for one surviving participant

** Adds up to >100% because some patients had more than one abnormality or drug.

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Table 3
Results of analyses of the association of candidate biomarkers with mortality among patients with advanced Chagas cardiomyopathy (Stage D) using receiver operating characteristic curves and Cox regression models

Biomarker	AUC ¹	Cut-off ¹ (pg/ml)	Status	Median (IQR) biomarker level in pg/ml	N (%) above cutoff	Crude model		Adjusted model ²	
						P	HR ¹ (95% CI)	P	HR (95% CI)
BNP ³	0.61	184.1	Alive	109 (9-263)	11 (37%)	0.039	2.8 (1.1-7.2)	0.025	3.1 (1.2-8.4)
			Died	267 (37-347)	14 (70%)				
NTproBNP ³	0.66	765.1	Alive	425 (288-740)	5 (16.7 %)	0.002	4.1 (1.6-10.0)	0.002	4.4 (1.8-11.0)
			Died	777 (423-1310)	11 (55%)				
CKMB ³	0.56	8300	Alive	3994 (3447-7487)	5 (17%)	0.005	3.5 (1.6-8.5)	0.009	3.3 (1.3-8.0)
			Died	7534 (2960-11,438)	10 (50%)				
MMP-2 ³	0.72	103980	Alive	94,680 (75,549-111,884)	9 (30%)	0.008	4.0 (1.4-11.2)	0.007	4.2 (1.5-11.8)
			Died	126,828 (96,148-164,256)	14 (74 %) ⁴				

¹ Area under the curve (AUC), cutoffs and hazard ratios (HRs) based on receiver operating characteristic (ROC) curves. Hazard ratios were only calculated for those with AUC greater than 0.55.

² Model adjusted for age and sex.

³ BNP, Brain Natriuretic Peptide; NTproBNP, N-terminal brain natriuretic peptide; CKMB, Creatine kinase-MB; MMP2, Matrix metalloproteinase 2.

⁴ MMP2 data were missing for one individual who died