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The association of exercise test variables with long-term mortality in patients with chronic Chagas disease

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Background: The identification of variables obtained in the exercise test (ET) associated with increased risk of death is clinically relevant and would provide additional information for the management of Chagas disease (CD). The objective of the present study was to evaluate the association of ET variables with mortality in patients with chronic CD.

Methods: This retrospective longitudinal observational study included 232 patients (median age 46.0 years; 50% women) with CD that were followed at the Evandro Chagas National Institute of Infectious Diseases (Rio de Janeiro, Brazil) and performed an ET between 1989 and 2000. The outcome of interest was all-cause mortality.

Results: There were 103 deaths (44.4%) during a median follow-up of 21.5 years (IQR 25–75% 8.0–27.8), resulting in 24.5 per 1,000 patients/year incidence rate. The ET variables associated with mortality after adjustments for potential confounders were increased maximal (HR 1.02; 95% CI 1.00–1.03 per mmHg) and change (HR 1.03; 95% CI 1.01–1.06 per mmHg) of diastolic blood pressure (DBP) during ET, ventricular tachycardia at rest (HR 3.95; 95% CI 1.14–13.74), during exercise (HR 2.73; 95% CI 1.44–5.20), and recovery (HR 2.60; 95% CI 1.14–5.91), and premature ventricular complexes during recovery (HR 2.06; 1.33–3.21).

Conclusion: Our findings suggest that ET provides important prognostic value for mortality risk assessment in patients with CD, with hemodynamic (increased DBP during exercise) and electrocardiographic (presence of ventricular

arrhythmias) variables independently associated with an increased mortality risk in patients with CD. The identification of individuals at higher mortality risk can facilitate the development of intervention strategies (e.g., close follow-up) that may potentially have an impact on the longevity of patients with CD.

KEYWORDS

Chagas disease, mortality, prognosis, VO_2 max, maximal functional capacity

Introduction

Chagas disease (CD) is an important public health problem that affects ~6 million people worldwide, most of them living in Latin America, but also with cases in United States, Europe, and Oceania (1, 2). It is responsible for loss of more than 800,000 disability-adjusted life-years and a global economic loss of ~US\$ 850 million per year (3). The cardiac form, also known as chronic Chagas cardiomyopathy (CCC), is considered the most severe clinical presentation of chronic CD, being responsible for the highest morbidity and mortality rates associated with CD. CCC is characterized by decreased functional capacity, arrhythmias, thromboembolic events, and heart failure (4–6).

The exercise test (ET) is an important tool to assess functional capacity by estimated maximal oxygen uptake (VO_2 max), heart rhythm and hemodynamic variables during exercise, being associated with prognosis in both healthy individuals and patients with a variety of chronic conditions, with greater functional capacity associated with lower mortality rates (7–9). It is a feasible and low-cost evaluation method that can be easily implemented in clinical practice. Although ET is a well-recognized tool for risk stratification of patients with a wide variety of clinical conditions, studies evaluating the prognostic value of ET in patients with CD remain scarce, with most of them presenting conflicting results and limited by short-term follow-up, small sample sizes, and inclusion of exclusively patients with CCC (10).

The identification of mortality predictors obtained in the ET is clinically relevant and may provide additional information for the management of patients with CD (11, 12). Therefore, the objective of this study was to evaluate the association of ET variables with long-term mortality rates in patients with chronic CD. We hypothesized that ET could provide important prognostic information among individuals with CD, with decreased exercise capacity measured by estimated VO_2 max and presence of arrhythmias during exercise being associated with an increased risk of death.

Methods

This is a retrospective longitudinal observational study including patients with CD who were followed at the Evandro Chagas National Institute of Infectious Disease (INI/Fiocruz) outpatient clinic and underwent an ET between 1989 and 2000. The diagnosis of CD was made in the immunodiagnostic sector of INI/Fiocruz using the following tests: indirect immunofluorescence-IFI (Bio-Manguinhos, Fiocruz) and enzyme linked immunosorbent assay—ELISA (Wiener lab—Chagatest), both commercial tests. CD diagnosis was confirmed when both IFI and ELISA were reactive. The INI/Fiocruz is a national referral center for screening, treatment and research of infectious and tropical diseases in Brazil. Patients were admitted by spontaneous demand or were referred from other healthcare institutions or blood banks. At the time of enrollment of patient in the INI/Fiocruz clinic, participant underwent an initial evaluation protocol, which included clinical and epidemiological anamnesis, a physical examination focused on chronic CD-related cardiovascular and digestive signs and symptoms, a 12-lead electrocardiogram and a two-dimensional Doppler echocardiogram. Participants were indicated to perform ET according to the physician discretion during their medical appointments. Those who did not undergo at least one medical appointment after the ET were excluded from the analysis. This study was approved by the INI/Fiocruz Research Ethics Committee (CAAE: 27576620.0.0000.5262). Patients signed consent was waived due to the retrospective nature of the study.

Exercise test

ET was preferentially performed using a treadmill, but patients with any walking limitation (e.g., osteoarticular pain) were advised to perform the test on the cycle ergometer. The ET protocols used during the treadmill tests were Bruce, Naughton, and Astrand, whereas the ET protocols used during the cycle ergometer tests were Bruce, Ramp, and Jones. The choice of ET protocol took into consideration the clinical and functional condition of each patient in order to achieve the maximal exercise effort in a period between 8 and 12 min (8).

Variables of interest were collected using the ET physical forms of each patient. The electrocardiographic findings were continuously assessed at rest for 5 min, before starting the ET, throughout ET and during 5 min of recovery to detect ST-segment abnormalities, premature atrial contractions (PACs), supraventricular tachycardia (SVT), premature ventricular complexes (PVCs), and ventricular tachycardia (VT) considering together sustained and non-sustained tachycardia, classified according to the Minnesota code (13, 14). Heart rate (HR) was recorded at rest, during maximal exercise (HRmax) and after 1-min recovery (HRrec). Change of HR during ET (Δ HR during exercise) was obtained subtracting HRmax by resting HR. The chronotropic deficit was determined by the inability to reach 85% of HRmax predicted for age following the Karvonen formula ($220 - \text{age}$) (15). A reduction in the peak exercise HR ≤ 12 beats after 1-min recovery was used to define decreased parasympathetic activity (16). Measures of systolic (SBP) and diastolic (DBP) blood pressure were obtained at rest, during peak exercise and through 5 min of recovery after exercise cessation. Change of SBP (Δ SBP during Exercise) and DBP (Δ DBP during Exercise) during ET was obtained subtracting maximal SBP and DBP by their respective resting values. The double product was calculated as the product of the SBP times HR at the peak of ET. The $\text{VO}_{2\text{max}}$ ($\text{mL.kg}^{-1}.\text{min}^{-1}$) was indirectly estimated by a specific formula depending on the ergometer and the protocol used during the test (17–21). The level of metabolic equivalents achieved (MET) at the peak of exertion was calculated as a multiple of the basal metabolic rate. Functional aerobic impairment (FAI) was calculated as the difference between the estimated and expected functional capacity for the age and sex, expressed in percentage. Categories of cardiorespiratory fitness (CF) according to age and sex were determined following the American Heart Association classification (22).

Outcome

The outcome of interest was all-cause mortality. Death status was ascertained until December 31st, 2021 using personal information (patient's and parents' name, date of birth, or the number of federal identification document) through review of the INI/Fiocruz electronic medical records, online consultation of the mortality database system of the Department of Justice and Department of Health (SES/RJ) from the State of Rio de Janeiro, and through telephone contact with patients or their relatives when available. Follow-up period was measured from the date of the ET until the occurrence of event (death) or the last medical appointment, including those lost to follow-up, at which point follow-up was administratively censored.

Covariates

Covariates were collected from INI/Fiocruz electronic medical records including demographic and clinical information at the time of ET. The main covariates obtained from medical records comprised age, sex, self-reported race, region of origin according to prevalence of CD, CD classification, left ventricular ejection fraction (LVEF), and comorbidities including arterial hypertension, diabetes mellitus, dyslipidemia, heart failure, stroke, and non-CD cardiomyopathy. Information about the region of origin considered the prevalence of chronic CD in Brazil, which was based on serological data from national surveys, being categorized as low ($<2\%$), medium (2–4%), high ($>4\%$), and non-endemic (States of Rio de Janeiro and Espírito Santo). The classification of CD (indeterminate, cardiac, and digestive forms) and the stages of cardiac form followed the recommendations from the 2nd Brazilian Consensus on Chagas disease (5). To facilitate data analysis and following a clinical rationale, patients were grouped as following: indeterminate (positive CD serology test without any CD-specific electrocardiographic and echocardiographic abnormalities), CCC without heart failure (positive CD serology test and CD-specific electrocardiographic and/or echocardiographic abnormalities but without symptoms of heart failure), CCC with heart failure (positive CD serology test and CD-specific electrocardiographic and/or echocardiographic abnormalities with symptoms of heart failure), and/or digestive forms (presence of esophagus and/or intestine peristalsis dysfunction). LVEF was measured with transthoracic echocardiography using the Teicholz method by a single trained evaluator within 6 months before or after the ET.

Statistical analysis

Continuous data were presented as median (interquartile range 25–75%) and categorical variables were presented as number of observations (percentages). Baseline comparisons between groups of patients were performed using the Mann-Whitney test for continuous and the Chi-squared test for categorical variables. Cumulative incidence (expressed by the number of patients that presented the outcome divided by the total number of patients during the follow-up) and the incidence rate (expressed as the number of patients that presented the outcome divided by the time of exposure of each individual) were calculated in the incidence analysis. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CI) for the association between ET variables and death. Models were fitted without adjustments, as well as adjusted for potential confounders including age, sex, race, CD classification (indeterminate/digestive, CCC without heart failure, and CCC with heart failure), LVEF, and

presence of comorbidities (hypertension, diabetes mellitus, dyslipidemia, non-CD cardiomyopathy, or stroke). Schoenfeld residuals tests were performed to evaluate proportional hazard assumptions. The predictive value of Cox survival models was assessed using the Harrell's C indexes. The variance inflation factor (VIF) for the covariates included in the adjusted models was 1.22, suggesting no multicollinearity problems. Sensitivity analyses including the type of ET ergometer (treadmill or cycle ergometer) as a covariate were also conducted.

Kaplan-Meier survival curves were constructed and compared using the log-rank test. Data analyses were performed using Stata 13.0 statistical software (College Station, TX: StataCorp LP) with 2-tailed significance level of $p \leq 0.05$ for all analyses.

Results

Of the 235 eligible patients, 3 were excluded following the pre-defined exclusion criteria, leaving a final sample of 232 patients. The characteristics of the study sample are depicted in [Tables 1, 2](#). The median age was 46.0 years. There was a balanced distribution of sex (50% women). White (54.3%) was the most common self-reported race. Most patients (48.7%) presented the indeterminate form of CD, followed by those with CCC without heart failure (45.7%). The median LVEF was 64.0%. The median time between INI-Fiocruz enrollment and ET performance was 0.6 years (IQR 25–75% 0.2–2.8). The median of estimated VO_2max was 31.3 $\text{mL.kg}^{-1}.\text{min}^{-1}$, with most patients (52.1%) presenting a good or excellent CF classification based on age and sex. Most ET were performed on a treadmill (85.8%, $n = 199$), 95.0% ($n = 189$) of those under Bruce protocol. Median of estimated VO_2max obtained from treadmill ET was slightly higher in comparison to those obtained on the cycle ergometer (31.5 vs. 30.0 $\text{mL.kg}^{-1}.\text{min}^{-1}$, respectively; $p = 0.05$), with a similar distribution of age ($p = 0.62$) and sex ($p = 0.19$). ST-segment abnormalities were observed in 8 patients (3.5%), of those 6 ST-segment depressions, 1 early repolarization, and 1 T-wave inversion. One-third of patients (34.1%) had arrhythmias at rest [6.5% PACs, 29.7% PVCs, and 2.2% VT (1.7% non-sustained VT and 0.5% sustained VT)], whereas 56.9% presented arrhythmias during effort [15.1% PACs, 0.4% SVT, 50.4% PVCs, and 9.5% VT (8.2% non-sustained VT and 1.7% sustained VT)], and 44.4% had arrhythmias during the recovery period [12.5% PACs, 0.9% SVT, 36.6% PVCs, and 6.5% VT (5.2% non-sustained VT and 1.3% sustained VT)].

The comparison of baseline characteristics according to survival status is also depicted in [Tables 1, 2](#). Patients who died were older (49.0 vs. 43.0 years; $p < 0.001$), more likely to be white (62.1 vs. 48.1%; $p = 0.03$), more likely to have diabetes (3.9 vs. 0.0%; $p = 0.024$) and heart failure (9.7 vs. 2.3%; $p = 0.015$), had a lower functional capacity (estimated VO_2max 30.8 vs. 34.1 $\text{mL.kg}^{-1}.\text{min}^{-1}$; $p = 0.01$ and METs 8.7

vs. 9.7; $p = 0.001$), lower percentage of individuals with good and excellent cardiorespiratory fitness (44.7 vs. 55.8%; $p = 0.04$), and lower resting and maximal HR (73.0 vs. 77.0; $p = 0.03$ and 150.0 vs. 160.0 bpm; $p = 0.008$, respectively). LVEF was lower among those who died (63.0 vs. 65.0%; $p = 0.01$). Deceased individuals also had a greater percentage of arrhythmias at rest (47.6 vs. 23.3%; $p < 0.001$), during exercise (66.0 vs. 49.6%; $p = 0.01$) and recovery (63.1 vs. 29.5%; $p < 0.001$), with a higher frequency of resting PVCs (41.7 vs. 20.2%; $p < 0.001$), exercise PVCs (58.2 vs. 44.2%; $p = 0.03$) and VT (14.6 vs. 5.4%; $p = 0.018$), and recovery PVCs (54.4 vs. 22.5%; $p < 0.001$).

The associations between ET variables with death are depicted in [Table 2](#). There were 103 deaths (44.4%) during a median follow-up of 21.5 years (IQR 25–75% 8.0–27.8), resulting in 24.5 per 1,000 patients/year incidence rate. The cause of death was due to heart failure ($n = 17$; 16.5%), sudden death ($n = 26$; 25.3%), stroke ($n = 1$; 1.0%), not related to CD ($n = 19$; 18.5%), and unknown ($n = 40$; 38.8%). Fifty-four patients (23.2%) were censored due to loss of follow-up. The characteristics of patients according to loss to follow-up status is demonstrated in [Supplementary Table 1](#). Overall, losses to follow-up had a lower follow-up time, were younger, with a greater functional capacity, and lower percentage of resting PVCs. The median follow-up time of those lost to follow-up was 5.1 years.

The associations between ET variables and all-cause mortality are depicted in [Table 3](#). Higher estimated VO_2max was significantly associated with lower mortality rates in unadjusted analysis (HR 0.97; 95% CI 0.95–0.99 per $\text{mL.kg}^{-1}.\text{min}^{-1}$). However, this association was not further significant after adjustments for potential confounders. A greater mortality risk was observed among those with increased maximal DBP (HR 1.02; 95% CI 1.00–1.03 per mmHg; C-index 0.75) and ΔDBP (HR 1.03; 95% CI 1.01–1.06 per mmHg; C-index 0.75) during ET in the adjusted analysis. A higher mortality risk was observed among those with PACs during recovery (HR 1.78; 95% CI 1.04–3.04 per presence) in the unadjusted model, that did not remain statistically significant after adjustments for potential confounders. Ventricular arrhythmias (both PVCs and VT) at rest, during exercise and recovery were statistically associated with a greater mortality risk in the unadjusted analyses. However, only VT at rest (HR 3.95; 95% CI 1.14–13.74 per presence; C-index 0.74), during exercise (HR 2.73; 95% CI 1.44–5.20 per presence; C-index 0.75), and during recovery (HR 2.60; 95% CI 1.14–5.91 per presence; C-index 0.74), and PVCs during recovery (HR 2.06; 95% CI 1.33–3.21 per presence; C-index 0.75) maintained statistical significance after adjustments for potential confounders. The Harrell's C-index for the adjusted Cox models ranged from 0.73 to 0.75. Sensitivity analysis including the type of ET ergometer in the adjusted model provided similar estimates for all studied variables ([Supplementary Table 2](#)). The influence of resting, exercise, and recovery ventricular arrhythmias on mortality are illustrated in Kaplan-Meier Survival curves ([Figure 1](#)).

TABLE 1 Baseline demographic and clinical characteristics of patients (total and stratified by survival status) ($n = 232$).

Variable	Median (IQR 25–75%) or frequency (%)			<i>p</i> -value*
	Total	Deceased ($n = 103$; 44.4%)	Alive ($n = 129$; 55.6%)	
Age (years)	46.0 (39.0–52.0)	49.0 (43.0–55.0)	43.0 (35.0–50.0)	<0.001
Women	116 (50.0)	50 (48.5)	66 (51.2)	0.692
Race				
White	126 (54.3)	64 (62.1)	62 (48.1)	0.033
Non-white	106 (45.7)	39 (37.9)	67 (51.9)	
Region of origin according to prevalence				
Non-endemic Chagas disease area	11 (4.8)	5 (3.9)	6 (5.9)	0.477
Low Chagas disease prevalence area	12 (5.2)	9 (7.0)	3 (2.9)	0.170
Medium Chagas disease prevalence area	72 (31.2)	35 (27.1)	37 (36.3)	0.136
High Chagas disease prevalence area	136 (58.9)	80 (62.0)	56 (54.9)	0.275
Body mass index (Kg/m ²) ($n = 148$)	24.7 (22.4–27.5)	24.1 (23.0–26.9) $n = 57$	25.6 (22.3–27.7) $n = 91$	0.329
Arterial hypertension	57 (24.6)	29 (28.2)	28 (21.7)	0.257
Diabetes mellitus	4 (1.7)	4 (3.9)	0 (0.0)	0.024
Dyslipidemia	37 (16.0)	15 (14.6)	22 (17.0)	0.607
Non-CD cardiomyopathy	9 (3.9)	4 (3.9)	5 (3.9)	0.998
Heart failure	13 (5.6)	10 (9.7)	3 (2.3)	0.015
Stroke	1 (0.4)	1 (1.0)	0 (0.0)	0.262
Clinical presentation of CD				
Indeterminate	113 (48.7)	42 (40.8)	71 (55.0)	0.031
Cardiac without CCC	106 (45.7)	51 (49.5)	55 (42.6)	0.296
Cardiac with CCC	13 (5.6)	10 (9.7)	3 (2.3)	0.015
Digestive	5 (2.2)	3 (2.9)	2 (1.5)	0.478

CCC, chronic Chagas cardiomyopathy; VO₂, Oxygen Consumption; AHA, American Heart Association; ΔHR, heart rate change; ΔSBP, Systolic Blood Pressure Change; ΔDBP, Diastolic Blood Pressure Change; LVEF, Left Ventricular Ejection Fraction.

Estimates in **bold** were statistically significant.

Discussion

The present study demonstrated an important prognostic value of ET in patients with CD, with DBP during exercise (both maximal and Δ), presence of VT (considering together sustained and non-sustained arrhythmias) at rest, during exercise, and recovery, and PVCs during recovery independently associated with high mortality rates in a long-term follow-up (median of 21 years). Surprisingly, higher estimated VO₂max was not independently associated with a mortality risk reduction contrary to what we previously hypothesized.

ET is a widely used method to evaluate clinical, hemodynamic, and electrocardiographic responses to physical effort, providing important prognostic information in a variety of clinical conditions (7, 16, 23, 24). However, only few studies have been conducted in patients with CD, mostly limited by small sample sizes, short-term follow-up, and restricted to patients with CCC (10). A recent study including 49 CCC patients with impaired LVEF found that a decreased VO₂max was significantly associated with an increased cardiovascular

mortality during a mean follow-up period of 39 months. This study suggested a VO₂ cut-point of 25 mL.kg⁻¹.min⁻¹, but with no significant difference for cardiovascular mortality between those below and above this cutoff point in the Kaplan Meier survival analysis (p -value = 0.36) (25). Conversely, a later prospective observational longitudinal study with 75 CCC patients conducted by the same research group did not find any significant association between VO₂max and a composite outcome of cardiovascular events including cardiovascular death, heart transplantation, or ischemic event in a 41 months follow-up (26), similar to the findings observed in our study.

Studies that directly evaluated VO₂max by gas exchange analysis during cardiopulmonary exercise testing (CPET) in patients with CCC demonstrated a beneficial association of higher VO₂max and lower incidence of adverse health outcomes (27–29). However, CPET is a relatively expensive technique that requires specialized equipment and personnel, limiting its application in low socioeconomic settings, a common characteristic of patients with CD that live in endemic regions (30, 31). On the other hand, standard ET

TABLE 2 Exercise test characteristics of patients (total and stratified by survival status) ($n = 232$).

Variable	Median (IQR 25–75%) or frequency (%)			p -value*
	Total	Deceased ($n = 103$; 44.4%)	Alive ($n = 129$; 55.6%)	
VO ₂ max (mL.kg ⁻¹ .min ⁻¹)	31.3 (24.6–39.3)	30.8 (24.6–37.5)	34.1 (27.9–41.4)	0.002
FAI (%)	0.9 (–15.7 to 16.9)	4.7 (–14.7 to 22.5)	–1.5 (–15.7 to 14.8)	0.446
METs	8.9 (7.0–11.3)	8.7 (7.0–10.7)	9.7 (8.0–11.8)	0.001
Resting HR (bpm)	75.0 (67.0–85.0)	73.0 (65.0–82.0)	77.0 (69.0–87.0)	0.027
Maximal HR (bpm)	155.0 (138.0–170.0)	150.0 (130.0–165.0)	160.0 (141.0–173.0)	0.008
ΔHR during exercise	78.0 (59.0–93.0)	77.0 (57.0–90.0)	79.0 (60.0–94.0)	0.224
Chronotropic deficit (HRmax < 85%)	84 (36.2)	38 (36.9)	46 (35.7)	0.846
Recovery HR at 1 st minute (bpm)	121.0 (104.0–137.0)	121.0 (103.0–135.0)	121.0 (105.0–141.0)	0.358
ΔHR during recovery at 1 st minute (bpm)	–30.0 (–41.0 to –21.0)	–29.0 (–38.0 to –20.4)	–32.0 (–44.0 to –22.0)	0.099
ΔHR during recovery ≤ 12 bpm	19 (8.3)	11 (10.9)	8 (6.3)	0.213
Resting SBP (mmHg)	120.0 (110.0–140.0)	130.0 (120.0–140.0)	120.0 (110.0–130.0)	0.064
Resting DBP (mmHg)	80.0 (80.0–90.0)	80.0 (80.0–90.0)	80.0 (70.0–90.0)	0.327
Maximal SBP (mmHg)	167.5 (150.0–182.5)	170.0 (150.0–195.0)	160.0 (150.0–180.0)	0.393
Maximal DBP (mmHg)	80.0 (80.0–90.0)	82.5 (80.0–90.0)	80.0 (75.0–90.0)	0.006
ΔSBP during exercise (mmHg)	40.0 (30.0–60.0)	40.0 (30.0–60.0)	40.0 (30.0–60.0)	0.960
ΔDBP during exercise (mmHg)	0.0 (0.0–10.0)	0.0 (0.0–10.0)	0.0 (–5.0 to 10.0)	0.088
Double Product (mmHg.bpm)	26,000 (21,000–30,000)	25,500 (20,720–29,520)	26,250 (21,920–30,240)	0.371
Cardiorespiratory fitness level (AHA)				
Very poor and poor	43 (18.5)	23 (22.3)	20 (15.5)	0.184
Regular	68 (29.3)	34 (33.0)	34 (26.4)	0.269
Good and excellent	121 (52.1)	46 (44.7)	75 (58.1)	0.041
LVEF (Teicholz, %)	64.0 (58.0–69.0)	63.0 (50.0–68.0)	65.0 (61.0–69.0)	0.010
ST-segment abnormalities	8 (3.5)	3 (2.9)	5 (3.9)	0.689
Supraventricular arrhythmia				
Resting PACs	15 (6.5)	8 (7.8)	7 (5.4)	0.471
Resting SVT	0 (0.0)	0 (0.0)	0 (0.0)	1.00
Exercise PACs	35 (15.1)	15 (14.6)	20 (15.5)	0.842
Exercise SVT	1 (0.4)	1 (1.0)	0 (0.0)	0.262
Recovery PACs	29 (12.5)	16 (15.5)	13 (10.1)	0.212
Recovery SVT	2 (0.9)	1 (1.0)	1 (0.8)	0.873
Ventricular arrhythmia				
Resting PVCs	69 (29.7)	43 (41.7)	26 (20.2)	<0.001
Resting VT	5 (2.2)	4 (3.9)	1 (0.8)	0.105
Exercise PVCs	117 (50.4)	60 (58.2)	57 (44.2)	0.033
Exercise VT	22 (9.5)	15 (14.6)	7 (5.4)	0.018
Recovery PVCs	85 (36.6)	56 (54.4)	29 (22.5)	<0.001
Recovery VT	15 (6.5)	8 (7.8)	7 (5.4)	0.471

FAI, Functional Aerobic Impairment; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HR, Heart Rate; MET, Metabolic Equivalent; PACs, Premature atrial contractions; SVT, Supraventricular tachycardia; PVCs, Premature ventricular complexes; VT, Ventricular tachycardia. Estimates in **bold** were statistically significant.

is a relatively low cost and accessible method that allows a reliable and inexpensive evaluation of hemodynamic and electrocardiographic responses to physical effort that can be easily implemented in clinical facilities located in low-income areas where CPET is not available.

The median estimated VO₂max in the present study was higher in comparison to other studies that included CCC patients with similar age and sex distribution. This difference may be attributed to our inclusion of a large proportion (~50%) of patients with the indeterminate form of CD, without any

TABLE 3 Unadjusted and adjusted associations between ET variables and all-cause mortality.

Variable	Unadjusted			Adjusted*			Harrel's C-index**
	HR	95% CI	p-value	HR	95% CI	p-value	
VO ₂ max (mL.kg ⁻¹ .min ⁻¹)	0.97	(0.95–0.99)	0.014	0.98	(0.95–1.00)	0.158	0.73
FAI (%)	1.00	(0.99–1.01)	0.819	1.01	(1.00–1.02)	0.132	0.73
Resting HR (bpm)	0.99	(0.98–1.00)	0.175	1.00	(0.99–1.01)	0.930	0.73
Maximal HR (bpm)	0.99	(0.99–1.00)	0.123	1.00	(0.99–1.01)	0.778	0.73
ΔHR during exercise (bpm)	1.00	(0.99–1.00)	0.472	1.00	(0.99–1.01)	0.823	0.74
Chronotropic deficit (HRmax < 85%)	0.98	(0.66–1.47)	0.936	0.94	(0.62–1.43)	0.773	0.74
Recovery HR at 1st minute (bpm)	1.00	(0.99–1.00)	0.512	1.00	(0.99–1.01)	0.443	0.74
ΔHR during recovery at 1st minute	1.01	(1.00–1.02)	0.133	1.00	(0.99–1.02)	0.432	0.74
ΔHR recovery ≤ 12 bpm	1.22	(0.65–2.28)	0.540	1.25	(0.65–2.40)	0.504	0.74
Resting SBP (mmHg)	1.01	(1.00–1.02)	0.161	1.00	(0.99–1.02)	0.364	0.74
Resting DBP (mmHg)	1.01	(0.99–1.03)	0.322	1.00	(0.98–1.02)	0.915	0.74
Maximal SBP (mmHg)	1.00	(0.99–1.01)	0.756	1.00	(0.99–1.01)	0.647	0.74
Maximal DBP (mmHg)	1.01	(1.00–1.02)	0.115	1.02	(1.00–1.03)	0.040	0.75
ΔSBP during exercise (mmHg)	0.99	(0.98–1.00)	0.230	0.99	(0.98–1.00)	0.372	0.74
ΔDBP during exercise (mmHg)	1.01	(0.99–1.03)	0.254	1.03	(1.01–1.06)	0.006	0.75
Double product (mmHg.bpm)	1.00	(0.99–1.00)	0.257	1.00	(0.99–1.00)	0.961	0.74
Cardiorespiratory fitness level (AHA)							
Very poor and poor		Reference			Reference		0.74
Regular	0.93	(0.55–1.58)	0.795	0.94	(0.54–1.62)	0.81	
Good and excellent	0.68	(0.41–1.12)	0.131	0.66	(0.39–1.12)	0.12	
ST-segment abnormalities	0.92	(0.29–2.91)	0.890	0.88	(0.27–2.82)	0.825	0.73
Supraventricular arrhythmias							
Resting PACs	1.91	(0.93–3.93)	0.079	1.42	(0.63–3.18)	0.394	0.74
Resting SVT [†]	–	–	–	–	–	–	–
Exercise PACs	1.00	(0.58–1.73)	1.000	0.98	(0.55–1.74)	0.951	0.74
Exercise SVT	2.12	(0.29–15.25)	0.456	1.69	(0.21–13.30)	0.617	0.74
Recovery PACs	1.78	(1.04–3.04)	0.034	1.21	(0.68–2.11)	0.495	0.74
Recovery SVT	1.64	(0.23–11.80)	0.624	1.35	(0.17–10.54)	0.775	0.74
Ventricular arrhythmias							
Resting PVCs	1.98	(1.34–2.93)	0.001	1.31	(0.84–2.02)	0.231	0.74
Resting VT	26.89	(8.30–87.17)	<0.001	3.95	(1.14–13.74)	0.030	0.74
Exercise PVCs	1.66	(1.12–2.46)	0.011	1.05	(0.69–1.60)	0.810	0.74
Exercise VT	4.17	(2.37–7.33)	<0.001	2.73	(1.44–5.20)	0.002	0.75
Recovery PVCs	3.05	(2.06–4.51)	<0.001	2.06	(1.33–3.21)	0.001	0.75
Recovery VT	3.65	(1.74–7.63)	0.001	2.60	(1.14–5.91)	0.023	0.74

VO₂, Oxygen Consumption; FAI, Functional Aerobic Impairment; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HR, Heart Rate; MET, Metabolic Equivalent; AHA, American Heart Association; ΔHR, heart rate change; ΔSBP, Systolic Blood Pressure Change; ΔDBP, Diastolic Blood Pressure Change; LVEF, Left Ventricular Ejection Fraction; PACs, Premature atrial contractions; SVT, Supraventricular tachycardia; PVCs, Premature ventricular complexes; VT, Ventricular tachycardia.

*Cox regression model adjusted for age, sex, race, classification of CD (indeterminate, CCC without heart failure, and CCC with heart failure), LVEF, and presence of comorbidities (arterial hypertension, diabetes mellitus, dyslipidemia, non-CD cardiomyopathy, or stroke).

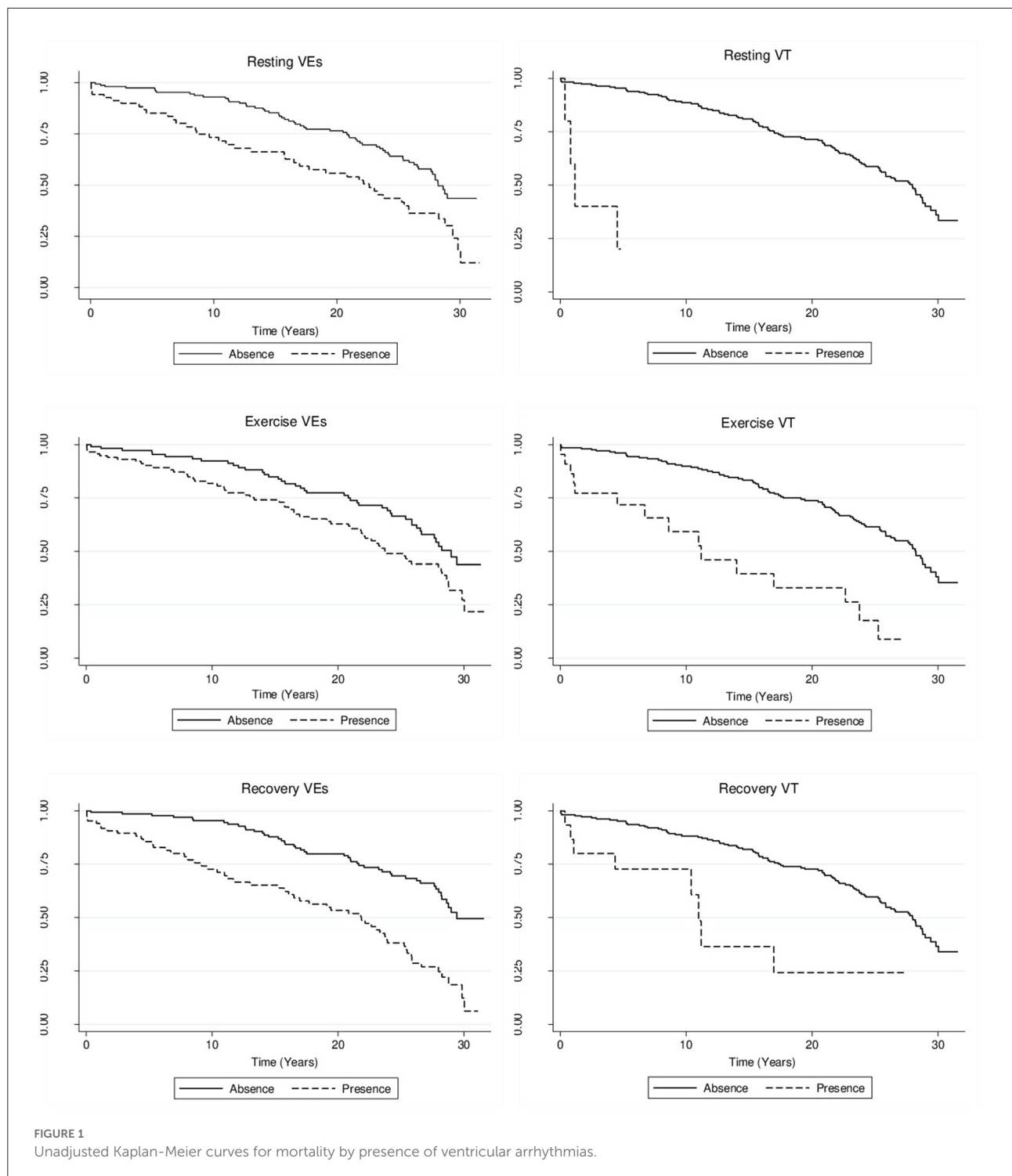
**Harrel's C-Index for the adjusted model.

[†]Non-valid HR estimates due to zero counts in at least one of the groups.

Estimates in **bold** were statistically significant.

structural cardiac disease that negatively impacts functional capacity, partially explaining the absence of association between VO₂max and mortality. The results from the present study may provide a more reliable estimate about the prognostic effects of

ET in the overall CD population, not limited to those patients with structural cardiomyopathy, considering that most patients in the overall CD population present the indeterminate form (32, 33).



A variable that was independently associated with greater mortality rate was an increased DBP during exercise. Sheps et al. (34) demonstrated that an abnormal DBP response during treadmill exercise was associated with a greater frequency of coronary artery disease and LV contraction abnormalities.

However, other studies investigating the prognostic value of abnormal DBP responses to exercise did not demonstrate any significant association with mortality. For example, Sydó et al. (35) studied 20,000 patients with no previous history of cardiovascular diseases and found no association between DBP

responses during the ET and mortality over an average follow-up of 12 years. This result is in accordance with those observed by Myers et al. (7) in healthy men and patients with cardiovascular diseases. Increased DBP responses to exercise may be a result of increased arterial stiffness and endothelial dysfunction, which are considered early signs of atherosclerotic vascular disease (36, 37). Considering the change in the epidemiological profile of patients with CD over recent decades, in which most of CD individuals migrated from rural to urban areas, facilitating their exposure to inadequate lifestyle habits not previously common in this population, it is reasonable to speculate that an exaggerated increase in DBP during exercise could be associated with early vascular abnormalities that increased their risk of death (38).

The results from the present study indicates that VT (considering together sustained and non-sustained arrhythmias) at rest, during exercise, and recovery were important independent mortality predictors in patients with CCC. Previous studies have demonstrated the adverse prognostic value of VT in patients with CCC, with non-sustained VT in a 24-h Holter monitoring being one of the variables included in a validated risk score for predicting death in CCC (39). The reentrant circuit that causes VT and cardiac dysautonomia, a typical finding in CD, may be related to the pathogenesis and increased mortality risk associated with VT at rest in CD (40).

Conversely, the prognostic value of exercise-induced ventricular arrhythmias, especially in patients with CD, is less clear (41–44). Similar to our results, the presence of VT during the ET was associated with an increased risk of sudden death in a previous study including 69 CCC patients followed over a mean period of 24 months (45), being sudden death more frequently observed among patients with VT during the ET ($n = 44$) in comparison to those without VT (16 vs. 0%; $p < 0.05$).

During recovery from ET, our study demonstrated that ventricular arrhythmias (both VT and PVCs) were associated with increased mortality, which has already been previously demonstrated in non-CD individuals (46). Frolkis et al. investigated the association between ventricular arrhythmias during exercise and recovery with all-cause mortality in 29,244 patients with known or suspected coronary artery disease that underwent an ET. During a mean follow-up of 5.3 years, there were 1,862 deaths. After adjustment for potential confounders, ventricular arrhythmia during recovery was associated with a 60% increase on all-cause mortality risk, with no significant association with ventricular arrhythmias during exercise (47). Therefore, in this large cohort study, ventricular arrhythmia during recovery was a better predictor of an increased risk of death than ventricular arrhythmia during exercise.

The present study has some limitations. The retrospective design precluded us from obtaining information of some potential covariates that may result in potential residual confounding. In addition, censored information due to losses to follow-up (~20%) may have introduced some bias. However,

the results obtained in the present study are in accordance with others that previously investigated the prognostic value of ET in other populations, reinforcing the importance of exercise-induced arrhythmias on prediction of mortality risk. Moreover, information about factors that may have changed over the time were not obtained (e.g., availability and access of healthcare, behavioral and sociodemographic risk factors, alternative treatments and point of intervention). However, we believe that these changes would have occurred in a similar frequency in the entire cohort, providing a non-differential error. Despite the relatively large sample size, the small number of some arrhythmias precluded us from performing a more detailed analysis about the influence of arrhythmias subtypes during the ET on mortality rates in CD patients (e.g., comparing sustained vs. non-sustained VT). In addition, the small percentage of patients with HF may indicate some degree of selection bias, limiting the extrapolation of the results to HF patients. Finally, the indirect evaluation of VO_2 max using both treadmill and cycle ergometer likely increases measurement error and may have contributed to the lack of association between functional capacity and mortality, even though most patients had performed the ET on a treadmill. On the other hand, our study is strengthened for long-term follow-up and the relatively high sample size, allowing a more reliable evaluation of the prognostic value of ET in patients with CD.

To conclude, our findings suggest that ET has important prognostic value for assessment of mortality risk in patients with CD. Increased DBP during exercise, presence of VT at rest, during exercise, and recovery, and PVCs during recovery were the variables independently associated with an increased mortality risk in patients with CD. The identification of individuals at higher mortality risk can facilitate the development of intervention strategies (e.g., close follow-up) that may potentially have an impact on longevity of patients with CD. Considering the potential prognostic value of ET in patients with CD, studies are necessary to assess if the inclusion of regular ET assessments would be beneficial for monitoring those with indeterminate or CCC to monitor for clinical evidence of worsening disease.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by Evandro Chagas National Institute of Infectious Disease Ethical Committee. The Ethics Committee waived the requirement of written informed consent for participation.

Author contributions

RSS and MM contributed to the study design and drafted the manuscript. FM, IX, AS, RMS, AH-M, RSS, and MM contributed to the data acquisition. RSS, MM, and JF contributed to the data analysis and interpretation. All authors revised and approved the final version and agree with all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2022.972514/full#supplementary-material>

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