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Elevated IL-17 levels and echocardiographic signs of preserved myocardial function in benznidazole-treated individuals with chronic Chagas' disease

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ABSTRACT

Background: The immunological and clinical impact of trypanocidal treatment in chronic Chagas' disease (CCD) is unclear.
Methodology and findings: Several cytokines were measured in plasma of 66 patients with CCD. Thirty-three patients had been previously treated with benznidazole and 33 had never been treated. The treated group exhibited higher levels of IL-17 (median 142.45 × 1.22 pg/ml, P = 0.025), which was the only one significantly associated with Bz treatment, especially after adjusting for time of disease and NYHA class (P = 0.024; OR 1.006, 95% CI 1.001-1.010). Compared to untreated patients, the treated group exhibited higher median values of mitral annular E' lateral (13.0 × 10.0 cm/s, P = 0.038), S' infero-lateral (8.9 × 7.6 cm/s, P = 0.013), S' septal (8.5 × 7.4 cm/s, P = 0.034), mean S' (9.0 × 7.9 cm/s, P = 0.013) and tricuspid annular S' (13.3 × 11.1 cm/s, P = 0.001) and lower values of E/E' septal (7.2 × 9.5 cm/s, P = 0.049). After adjustment for time of disease and NYHA class, S' infero-lateral (P = 0.031), mean S' (P = 0.049) and S' tricuspid (P = 0.024) persisted as significantly associated with treatment.
Conclusion: The present findings suggest that the group of CCD patients treated with Bz displayed increased plasma levels of IL-17 and preserved myocardial function, reinforcing the idea that Bz treatment may be beneficial.

Introduction

Chagas' disease (CD) is a serious health problem, affecting 6 to 7 million people worldwide and leading to roughly ten thousand deaths every year (WHO, 2018). Although mortality due to CD has decreased (Da Nóbrega et al., 2014), it still causes several irreversible consequences to the cardiovascular systems of 20–30% of infected individuals long after the initial phase, including arrhythmia, heart failure, thromboembolism, stroke, heart block and sudden death (Rassi et al., 2012). It is estimated that over $1 billion/year is spent worldwide on morbidity associated with Chagas' cardiomyopathy (Dutra et al., 2014).

Chagas' cardiomyopathy is characterized by diffuse myocarditis in the presence of few parasites (Andrade and Andrade, 1955) but with the persistence of *Trypanosoma cruzi* antigens and nuclear DNA, suggesting occult *T. cruzi* infection (Jones et al., 1993). The recognition of either *T. cruzi* antigens or myocardial antigens stimulates T cell proliferation (Añez et al., 1999; Cunha-Neto et al., 1996) consequently triggering a persistent inflammatory response with a dominant Th1 cytokine pattern characterized by a high number of cells expressing TNF and IFN-gamma, associated with

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heart failure (Rocha Rodrigues et al., 2012). As persistence of *T. cruzi* may be implicated in persistent inflammatory activation, the etiologic treatment of CD during the chronic phase may have some impact on disease progression.

Anti-parasite treatment for CD is limited to benznidazole (Bz) or nifurtimox, and Bz is considered the first-line treatment due to better tolerance and fewer side effects (Bern et al., 2007). These trypanocidal drugs are effective during the acute and early chronic phases of CD with a high cure rate (Cançado, 2002; de Andrade et al., 1996; Sosa Estani et al., 1998). Despite an unclear indication of the effectiveness of trypanocidal drugs (Fabbro et al., 2007; Morillo et al., 2015; Viotti et al., 2006), the WHO recommends the etiological treatment of chronic Chagas' disease (CCD) patients, primarily those with no symptoms (WHO, 2017). It has been shown that Bz treatment in CCD may have an impact on B and T cell responses (Laucella et al., 2009; Sathler-Avelar et al. 2012, 2008, 2006; Vallejo et al., 2016), but these findings have not been related to clinical improvement. As the clinical advantage of the etiological treatment of CCD remains undefined, further investigation of its efficacy and its potentially protective mechanisms may help in establishing its usefulness.

The present study compared untreated to Bz-treated CD patients with long-term follow-up and used an analytical approach that integrated clinical features with electrocardiography and echocardiography parameters, and immune markers.

Methods

Study design and participants

Outpatients with CCD were randomly recruited between 2012 and 2013 from two reference centers in Bahia state, Brazil (University Hospital Prof. Edgard Santos and Climecar, an outpatient clinic in an endemic region for CD), to participate in a prospective cohort study, to investigate biomarkers of prognosis in Chagas' disease. In the current paper, we will compare patients treated with Benznidazole to those untreated in relation to cytokines and echocardiographic data, in a cross-sectional study. All patients underwent a detailed medical interview with a standard questionnaire, and their medical records were assessed to obtain clinical data. The treatment was considered complete if the patient had taken five mg/Kg/day for sixty days. Only patients with a complete treatment course confirmed by their medical records and those without prior treatment with benznidazole were included in the study. The treatment was performed several months before inclusion in the study (median 38 months, IQR 30.8–45.7 mo.). Most of the individuals from the treated and untreated groups were female (67% and 70%, respectively); no differences were observed in terms of gender or age (Table 1). The untreated group had been diagnosed with CD for a longer period than had the treated group (median 15 and 6 years, respectively, P = 0.001, Table 1). Furthermore, more dyspnea was reported in the untreated group vs. treated group (64% × 33%, P = 0.003), and more class I NYHA (New York Heart Association) was observed in the treated group than in the untreated group (63% x 33%, P = 0.014) (Table 1). Some NYHA class III patients were included in the study, although all of our patients, except one, had LV ejection fraction greater than 50% and none had advanced heart failure. No differences were observed in terms of other clinical parameters (palpitation, syncope, edema, hypertension, diabetes II and stroke) according to previous Bz treatment (Table 1). The electrocardiogram (ECG) obtained at the time of admission in the study indicated that the untreated group had more ventricular premature beats than did the treated group (27% x 0%, P = 0.016, Table 2); however, when adjusted for time of disease and NYHA class, the difference was not statistically significant. The other ECG parameters were similar between the study groups (Table 2). CD was confirmed by IgG and ELISA.

Individuals older than 70 years; those treated for more than eight years since the beginning of the study; patients with incomplete Bz treatment; patients with cardiac rhythm other than sinus, such as atrial fibrillation or atrial flutter; advanced atrioventricular block (> grade I); pacemaker users; heart disease other than Chagas; and patients with cancer or other chronic inflammatory diseases (such as connective tissue diseases and rheumatic diseases), were not included in the study.

Ethics statement

All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolling in the study. This study was approved by the Ethics Committee of the Complexo Hospitalar Professor Edgard Santos (protocol number: 77/10).

Table 1
Clinical and baseline characteristics of patients according to previous Chagas' disease treatment.

	Treated n = 33	Untreated n = 33	P
Female – n (%)	22 (67)	23 (70)	0.79
Age – median years (IQR)	51.0 (47–55)	51.5 (49–56)	0.537
Time of disease – median years (IQR)	6 (410.5)	15 (8–20)	0.001
Interval between treatment and inclusion in the study – median months (IQR)	38 (30.8–47.5)		
Dyspnea – n (%)	11 (33)	21 (64)	0.003
Palpitation – n (%)	19 (57)	22 (67)	0.35
Syncope – n (%)	11 (21)	12 (36)	0.196
Lower limb edema – n (%)	13 (39)	6 (18)	0.08
Hypertension – n (%)	19 (58)	21 (64)	0.61
Type II diabetes – n (%)	1 (3)	3 (9)	0.61
Previous stroke – n (%)	0 (0.00)	2 (6)	0.28
NYHA class – n(%)			0.029
I	21 (63)	11 (33)	0.014
II	10 (30)	17 (52)	0.080
III	2 (5)	5 (15)	0.230
ACEI/ARB	14 (42)	20 (61)	0.112
Calcium channel blockers	5 (15)	9 (27)	0.206
Statins	14 (42)	18 (55)	0.273

IQR: interquartile range; NYHA: New York Heart Association.

Table 2
Electrocardiogram parameters according to previous treatment.

	Treated n = 33	Untreated n = 33	P	Adjusted P[#]
ECG normal	21 (64)	10 (30)	0.643	0.554
Heart rate – mean (SD)	65 (13.1)	69 (13.6)	0.235	0.218
Left ventricular hypertrophy – n (%)	3 (9)	3 (9)	0.500	0.500
Supraventricular premature beats – n (%)	1 (3)	0 (0.00)	1.00	0.235
Ventricular premature beats – n (%)	0 (0.00)	9 (27)	0.016	0.513
Atrioventricular block (grade I) – n (%)	0 (0.00)	2 (6)	0.592	0.307
Right bundle branch block – n (%)	7 (21)	10 (30)	0.589	0.791
Left bundle branch block – n (%)	0 (0.00)	1 (3)	1.00	0.321
Left anterior hemiblock – n (%)	4 (12)	8 (24)	0.533	0.649
Left posterior hemiblock – n (%)	0 (0.00)	0 (0.00)	1.000	1.000
ST-T wave changes – n (%)	14 (42)	14 (42)	1.00	0.466

[#]P adjusted for time of disease and NYHA class.

Plasma measurements

The collection of clinical and laboratory data began at a median of 38 months (IQR 30.8–47.5) after treatment with benznidazole. Blood was obtained by venipuncture, and heparinized plasma was separated by centrifugation and stored at −70° C until use in immunoassays. We measured circulating levels of several cytokines and chemokines, including IL-1β (assay sensitivity: 0.6 pg/ml), IL-2 (1.6 pg/ml), IL-4 (0.7 pg/ml), IL-5 (0.6 pg/ml), IL-6 (2.6 pg/ml), IL-7 (1.1 pg/ml), IL-8 (1.0 pg/ml), IL-10 (0.3 pg/ml), IL-12 p70 (3.5 pg/ml), IL-13 (0.7 pg/ml), IL-17 (3.3 pg/ml), IFN-γ (6.4 pg/ml), TNF (6.0 pg/ml), CCL2 (1.1 pg/ml), CCL4 (2.4 pg/ml), GCSF (1.7 pg/ml), and GMCSF (0.2 pg/ml). Samples were measured in duplicate using a single multiplex assay according to the manufacturer's protocol (BIO-RAD, Hercules, CA, USA).

Electrocardiogram (ECG) and Doppler echocardiography (ECHO)

A 12-lead electrocardiogram (ECG) was performed at admission to the study and it was read by two independent and blinded cardiologists. Two-dimensional echocardiograms with conventional Doppler and tissue Doppler (2D Echo) systematized for the study, without knowledge to which group of treatment the patient belonged, were performed in 37 (56%) of the patients (20 treated and 17 untreated group) with a Samsung Sonoview machine, soon after inclusion in the study. Previously performed echocardiograms, not standardized for this study, were not considered for statistical analysis. The 2D Echo parameters were obtained according to the American Society of Echocardiography recommendations (Lang et al., 2015). Left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV) and ejection fraction (EF) were obtained using the biplane Simpson's method. E and A waves and the E/A ratio were measured from the mitral inflow by pulsed Doppler. E'(diastole), and S'(systole) velocities were obtained by tissue Doppler at the medial (septal), lateral and infero-lateral mitral annulus and the lateral tricuspid annulus. The E/E' ratio from each mitral annulus, the mean E/E' ratio from the three mitral annular measurements, and the mean S' were calculated (Lang et al., 2015).

Data analysis

In the exploratory analysis of the data, frequency tables were constructed and Chi-square or Fisher exact tests were applied to evaluate the association between qualitative variables. The quantitative variables were tested for Gaussian distribution within the total sample using D'Agostino and Pearson omnibus normality tests. Mann-Whitney test was used to assess the differences between the two clinical groups. Receiver operator characteristic (ROC) curves for immune and Doppler echocardiography

Table 3
Cytokine and chemokine levels according to previous treatment.

	Treated pg/ml – median (IQR)	Untreated pg/ml – median (IQR)	P	[#]P adj	O.R.	95% C.I.
IL-1β	45.74 (1.27–77.96)	1.27 (1.27–54.64)	0.284	0.212		
IL-2	38.03 (0.46–81.90)	0.46 (0.46–56.83)	0.135	0.590		
IL-4	37.20 (23.29–49.94)	30.44 (19.39–49.94)	0.423	0.597		
IL-5	84.52 (7.66–111.88)	54.41 (1.44–95.70)	0.439	0.407		
IL-6	90.81 (1.04–143.86)	41.93 (1.04–153.86)	0.495	0.501		
IL-7	84.31 (28.35–127.93)	57.98 (28.35–90.65)	0.197	0.082		
IL-8	67.64 (8.60–125.50)	48.16 (0.77–125.50)	0.687	0.990		
IL-10	13.80 (6.11–23.87)	13.80 (6.11–23.87)	0.882	0.874		
IL-12p70	79.54 (8.36–241.48)	79.54 (8.36–156.31)	0.627	0.512		
IL-13	204.60 (77.65–354.3)	204.62 (103.13–346.27)	0.756	0.794		
IL-17	142.45 (1.22–287.13)	1.22 (1.22–142.45)	0.025*	0.024*	1.006	1.001–1.010
IFN-γ	1888.76 (854.6–2781.4)	1888.76 (854.62–3236.27)	0.821	0.962		
TNF	195.57 (77.98–382.02)	143.67 (58.29–252.85)	0.232	0.119		
CCL2	75.30 (42.43–107.69)	42.43 (8.38–115.73)	0.316	0.178		
CCL4	62.13 (16.94–80.18)	41.95 (1.05–75.77)	0.059	0.165		
GCSF	243.31 (133.85–438.43)	198.04 (154.55–338.35)	0.188	0.069	1.003	0.999–1.007
GMCSF	0.51 (0.51–59.48)	0.51 (0.51–3.24)	0.186	0.249		

IQR: interquartile range; [#]P Adjusted for time of disease and NYHA class. Binary logistic regression analysis (stepwise forward method) was used.
* $P < 0.05$.

measurements were employed to test the ability to distinguish the different groups. Binary regression analysis (stepwise forward method) was performed to test for independent association of the cytokines and Doppler echocardiography markers with treatment, controlled by the time of disease and by NYHA class. Each of the cytokines and each marker of the echocardiogram were tested separately. The statistical analyses were performed using the programs GraphPad Prism 6.0 (GraphPad Software Inc., USA), SPSS 19.0 (IBM, Armonk, NY, USA), and JMP 11.0 (SAS, Cary, NC, USA). A two-tailed P value less than 0.05 was considered statistically significant.

Results

Levels of immune markers according to previous Bz treatment

Compared to the untreated group, treated patients exhibited significantly higher levels of IL-17 (median, IQR 142.45, 1.22–287.13 versus 1.22, 1.22–142.45, P = 0.025).

Significant differences between study groups, in terms of time of disease and NYHA class, could be influencing the results due to different stages of CD. Than, the statistical significance for each of the cytokines/chemokines was adjusted for time of disease and for

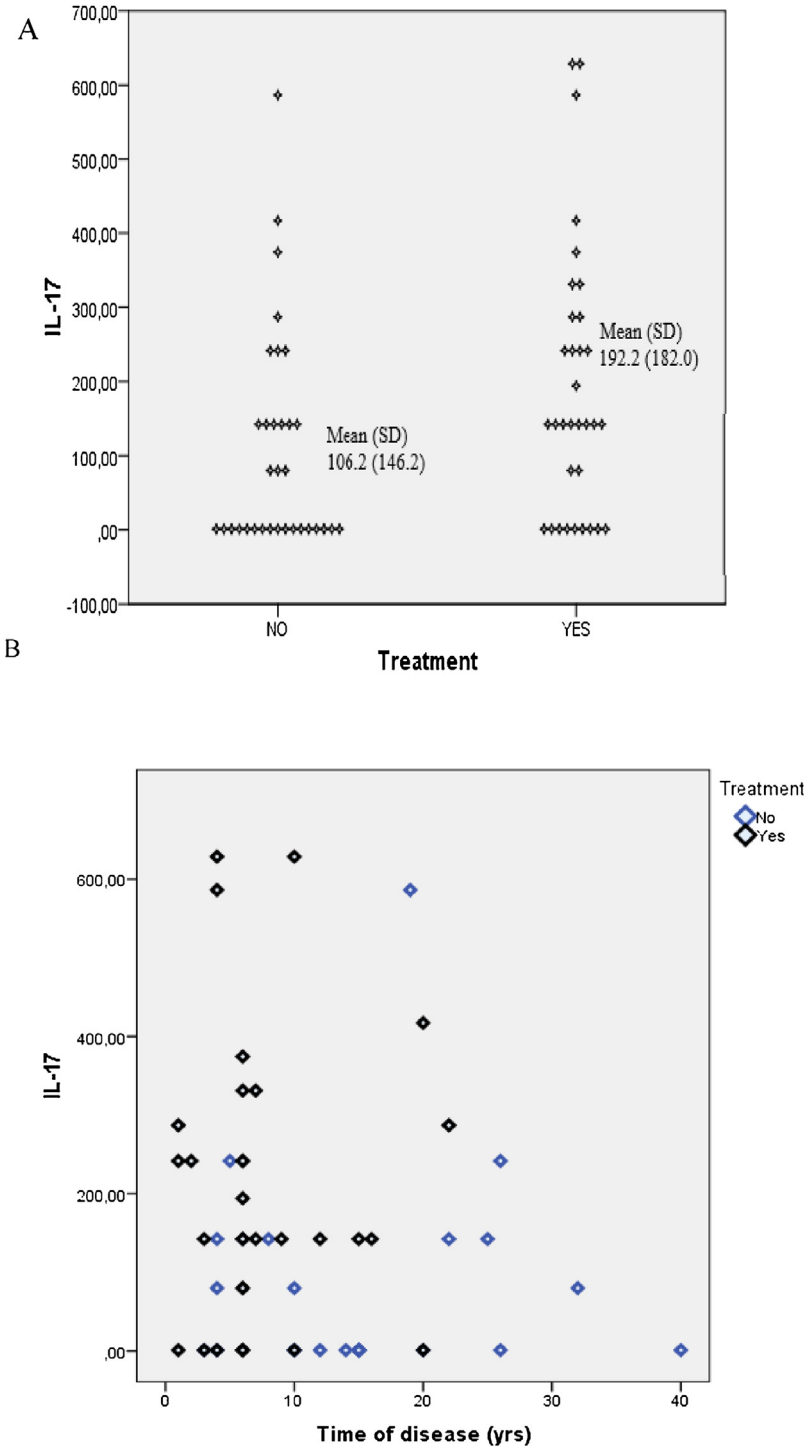

Figure 1. Scatter plot for IL-17: A- according to treatment; B- according to treatment and time of disease.

NYHA Class, using binary logistic regression analysis, stepwise forward method.

After adjustment, IL-17 persisted as the only immune marker exhibiting an independent association with Bz treatment. This cytokine was higher in the treated group than in the untreated group (OR: 1.006, 95% CI: 1.001–1.010, P = 0.024) – Table 3, Figure 1.

ROC curves of the several cytokines, with or without treatment, are shown in Table 4. IL-17 had a larger AUC and was the only cytokine with statistical significance (AUC 0.655, C.I. 0.522–0.787, P = 0.031).

ECHO parameters according to previous Bz treatment

Echocardiograms were performed in 37 patients (56%), 20 of the treated group and 17 of the untreated group, with similar NYHA class and clinical data, as well as time of disease and interval between treatment and inclusion in the study.

The measures of the left atrium (LA), the E/E' septal ratio (E/E' SEP), the mean of the E/E' septal and E/E' lateral ratios (MEAN E/E'), the E/E' lateral ratio (E/E' LAT), and the A-wave on mitral (A-WAVE), exhibited higher values in the untreated group, suggesting worse myocardial function in this group (Table 5). By contrast, the septal S'-wave (S' SEP), lateral S' wave (S' LAT), infero-lateral S' wave (S' INF-LAT) on the mitral annulus (MA), mean of the septal, lateral and infero-lateral S'-waves (MEAN S'), and the S'-wave on basal tricuspid annulus (S' TRIC), exhibited higher values in the treated group, suggesting better myocardial function in this group (Table 5).

Because the statistically significant differences between study groups in terms of time of disease and NYHA class could possibly influence results because of different stages of CD, Doppler echocardiogram and tissue Doppler variables were adjusted for these two variables using binary logistic regression analysis, forward conditional method. After adjustment, LVEDD, LVESD, S' TRIC, S' INF-LAT and MEAN S' were independently associated with treatment (Table 5).

ROC curves of the mitral and tricuspid annular S' waves from tissue Doppler, with or without treatment, are shown in Table 6. S'

Table 4
ROC curves of cytokines.

Variables	AUC (area under curve)	Standard model	P	95% C.I.	
				Inferior limit	Superior limit
GCSF	.594	.071	.191	.454	.733
GMCSF	.615	.070	.108	.479	.752
IFN-G	.516	.072	.822	.374	.658
IL1-B	.573	.071	.308	.434	.712
IL-2	.601	.070	.158	.463	.739
IL-4	.557	.072	.427	.416	.698
IL-5	.555	.071	.445	.415	.694
IL-6	.548	.072	.505	.406	.689
IL-7	.592	.072	.200	.451	.733
IL-8	.528	.072	.691	.387	.670
IL-10	.511	.072	.883	.370	.651
IL-12	.534	.073	.631	.392	.677
IL-13	.522	.072	.758	.381	.663
IL-17	.655	.068	.031*	.522	.787
MCP-1	.571	.071	.320	.432	.710
MIP-1B	.634	.069	.062	.499	.768
TNF-A	.585	.072	.236	.444	.726

Results of ROC curves of the cytokines and chemokines, with or without treatment, are shown.
* P < 0.05.

Table 5
Doppler echocardiogram parameters in patients according to previous Chagas' disease treatment.

	Treated median (IQR)	Untreated median (IQR)	P value	#Adjusted P	OR	95% CI
LA (mm)	33.0 (29.0–35.3)	36.0 (32.0–37.0)	0.073	0.615		
LVEDD (mm)	48.8 (46.8–50.0)	47.0 (44.0–52.0)	0.486	0.301		
LVEDDind.	28.6 (25.5–30.8)	27.6 (25.5–30.4)	0.630	0.508		
LVESD (mm)	29.0 (27.0–31.6)	27.5 (25.0-31.6)	0.361	0.483		
Ejection fraction	72.5 (70.0–76.0)	69.0 (66.0–74.0)	0.145	0.201		
E-wave	80.0 (69.0–87.5)	78.0 (70.0–96.3)	0.852	0.279		
A-wave	65.9 (58.0–77.0)	70.0 (60.0–90.5)	0.170	0.102		
E/A	1.17 (1.10–1.35)	1.14 (0.88–1.29)	0.363	0.471		
E' septal	11.0 (9.8–12.0)	10.0 (7.0–12.0)	0.114	0.436		
E' lateral	13.0 (11.2–14.9)	10.0 (8.0–13.5)	0.038	0.409		
E/E' septal	7.2 (6.0–8.4)	9.5 (7.5–10.0)	0.049	0.051	0.57	0.33–1.00
E/E' lateral	5.7 (5.2–7.4)	7.1 (5.7–9.3)	0.094	0.070		
Mean E/E'	6.4 (5.5–8.0)	8.0 (6.4–9.6)	0.083	0.058	0.50	0.24–1.02
S' Septal	8.5 (7.7–9.5)	7.4 (6.1–8.7)	0.034	0.061	2.30	0.96–5.51
S' Lateral	8.9 (8.4–12.1)	8.2 (7.2–10.5)	0.074	0.115		
S' Infero-lateral	8.9 (8.5–9.3)	7.6 (6.5–8.7)	0.013	0.031##	3.99	1.14–13.98
MEAN S'	9.0 (8.2–10.4)	7.9 (6.0–8.2)	0.013	0.049##	3.05	1.00–9.30
S' tricuspid	13.3 (12.7–14.2)	11.1 (9.8–12.2)	0.001	0.024##	2.55	1.13-.5.74

#Adjusted P value for time of disease and NYHA class. Binary logistic regression analysis was used. LA = left atrium; LV = left ventricle; RV = right ventricle; E wave, A wave and E/A ratio from mitral inflow with pulsed Doppler; E' and S' from mitral annular velocities with tissue Doppler; S' tricuspid from tricuspid annular velocities with tissue Doppler; mean E/E' = the mean of septal plus lateral E/E'; mean S' = mean of septal plus lateral plus infero-lateral S'.
P < 0.05

Table 6
ROC curves of mitral annular S' from tissue Doppler analysis.

Variables	AUC (area under curve)	Standard model	P	95% C.I.	
				Inferior limit	Superior limit
S' tric	.866	.090	.003*	.689	1.000
S' sept	.755	.108	.036*	.544	.966
S' lat	.709	.122	.085	.470	.949
S' in_lat	.761	.107	.031*	.552	.971
Mean S'	.765	.110	.029*	.550	.979

Results of ROC curves of the mitral and tricuspid annular S' waves from tissue Doppler, with or without treatment, are shown.
* $P < 0.05$.

tricuspid had the largest AUC (0.866, C.I. 0.689–1.000, P = 0.003), followed by mean S' (AUC 0.765, C.I. 0.550–0.979, P = 0.029), S' infero-lateral (AUC 0.761, 0.552–0.971, P = 0.031) and S' septal (AUC 0.755, C.I. 0.544–0.966, P = 0.036).

Discussion

In the current study, we observed better myocardial function and interestingly higher IL-17 levels in Chagas' disease patients treated with benznidazole compared to untreated ones, even after adjusting for the disease stage. This appears to argue for the notion that Bz treatment may be beneficial in CD patients. To the best of our knowledge, this is the first study to compare several cytokines and chemokines in the indeterminate and chronic cardiac phase of Chagas' disease in patients treated with benznidazole versus those untreated, and simultaneously to investigate myocardial function in this population with tissue Doppler.

IL-17 is a proinflammatory cytokine and is the central component of the Th17 response. It stimulates production of GM-CSF, IL-6, and TNF and induces neutrophil migration (Miyazaki et al., 2010). IL-17 has a protective function in experimental mice BALB/c infected with *T. cruzi*. IL-17 neutralization resulted in increased levels of IL-12, IFN and TNF, increased myocarditis and premature mortality, despite parasitological reduction (Da Matta Guedes et al., 2010). Similarly, mice without the receptor subunit for IL17 also showed an amplified inflammatory response and increased mortality (Boari et al., 2012). Furthermore, high IL-17 expression was related to improved cardiac function as determined by left ventricular ejection fraction and left ventricular end-diastolic diameter (Magalhães et al., 2013). In another study in humans, higher numbers of CD4 + IL-17+ cells were found in patients without or with mild cardiomyopathy than in subjects with severe disease (Guedes et al., 2012). A previous study with a follow-up of 18 months examining 14 Bz-treated CD patients in indeterminated phase reported increased numbers of Th17 cells in the peripheral circulation following treatment (Vallejo et al., 2016). The present study confirmed that IL-17 serum levels were higher in the treated group than in the untreated group. We expanded these observations by showing that IL-17 was the only cytokine that remained significantly different after adjustment for the disease stage, identified here by the time of disease and the NYHA functional class. In a different way, an article with children with chronic CD in Mexico (different population from our study) showed that Th17 circulating cytokines (IL-17, IL-6 and IFN) are increased in children with severe cardiac manifestations (Alba-Alvarado et al. 2018).

Previous studies showed that in CD, TNF correlated with both trypanocidal action (Lima et al., 1997) and cardiac tissue injury (Ferreira et al., 2003; Pérez-Fuentes et al., 2003). However, the TNF levels in the current study were not different between the treated and untreated CD groups after adjustment for time of disease and NYHA class.

Some studies comparing the long-term outcomes of Bz-treated patients with nonacute disease, indicated that the treatment prevents the progression of disease, and may lead to negative seroconversion (Fabbro et al., 2007; Viotti et al., 2006; Lana et al., 2009). However, the effectiveness of trypanocidal treatment remained unclear in other research groups. A Brazilian study showed no effectiveness of nitroderivative therapy for CCD during a follow-up period of 10 years (Lauria-Pires et al., 2000). The BENEFIT study (a trial involving 2854 patients in 5 countries in Latin America), despite showing a reduction in serum parasite detection and seronegative conversion 5 years after treatment with Bz, did not show significant clinical impact (Morillo et al., 2015). However, in the BENEFIT trial there were geographical differences, and in Brazil (country with a different strain of *T. cruzi*) the efficacy of Bz-treatment differed significantly from other countries (Rassi et al., 2017). In Brazil, there was a strong trend to avoid an overall primary clinical endpoint (based in morbidity and mortality), as seen in BENEFIT trial (Rassi et al., 2017). Additionally, in a post-hoc analysis there were fewer hospital admissions due to cardiovascular causes in Bz-treated patients (Morillo et al., 2015; Rassi et al., 2017). Thus the BENEFIT study is not enough to abolish the trypanocidal treatment in non-acute phase, especially in Brazil. More recently, another study which observed in a follow up of two years 1,959 patients with chronic CD in Brazil, found that patients who previously received Bz treatment had less mortality, lower rates of parasitemia and less ECG abnormalities, suggesting a clinical and parasitological benefit of etiological treatment in the early phase chronic CD in Brazil (Cardoso et al., 2018). In the perspective of no new treatments for CD, and also knowing that Bz is a safe drug, with tolerable side effects and has an affordable price, Bz-treatment is still recommended for the treatment of nonacute CD by the WHO (WHO, 2018). Further studies should address the trypanocidal clinical efficacy of Bz during CCD.

Chagas' disease patients with no or only mild cardiac involvement did not differ significantly from controls using classical echocardiographic parameters including ejection fraction, chamber dimensions and Doppler (Barbosa et al., 2014; Lima et al., 2016; Moreira et al., 2017; Regueiro et al., 2013). More recently, tissue Doppler and speckle-tracking strain demonstrated the possibility of early detection of left ventricular contractility abnormalities (Barbosa et al., 2014; Barros et al., 2001; García-Álvarez et al. 2011; Gomes et al., 2016; Lima et al., 2016; Moreira et al., 2017; Nascimento et al., 2013; Regueiro et al., 2013; Sánchez-Montalvá et al. 2016). Our study compared untreated patients and patients treated with a trypanocidal drug using tissue Doppler, and showed that treated patients had better preservation of left and right ventricular function. Our findings of increased levels of IL-17, a protective factor in the same population, together with better myocardial function by tissue Doppler parameters, reinforced the notion of beneficial effects of Bz in the treatment of Chagas' disease, even in patients with mild cardiac abnormalities.

This was not a randomized and placebo-controlled study, and treated patients had less time of disease evolution than did untreated subjects, a confounder effect for which we made statistical adjustment. In addition, detection of blood parasite DNA was not performed, and thus efficacy of Bz treatment was not

assessed; therefore a direct relationship of variables with reduced *T. cruzi* parasitism could not be ascertained.

The present findings suggested that the group of CD patients that were subject to trypanocidal treatment with Bz displayed increased plasma levels of IL-17, less severe NYHA class and improved myocardial function as displayed by the analysis with tissue Doppler, reinforcing the idea that Bz treatment may be beneficial to CD patients.

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Conflict of interest

The authors declare that they have no conflict of interests.

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Ethical approval

This study was approved by the Ethics Committee of the Complexo Hospitalar Professor Edgard Santos (protocol number: 77/10).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2018.11.369.

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