Major Article

Correlation between the cytokine profile and anticongestive medication in patients with chronic chagasic cardiopathy

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Abstract

Introduction: Chronic chagasic cardiopathy (CCC) is essentially a dilated cardiomyopathy in which a subacute, but constant chronic inflammatory process causes progressive destruction of the heart tissue. The action of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and anti-inflammatory cytokines, like interleukin IL-10 and IL-17, plays a fundamental role in the immunopathogenesis and evolution of disease. Early anti-congestive therapy, aimed at changing the morbidity and mortality rate, has been shown to reduce disease progression and to alter patients' immune response pattern. **Methods:** This cross-sectional study aimed to evaluate the profile of Th1 and Th17 cytokines and IL-17, TNF-α, and IFN-γ expressions in different stages of CCC. Forty patients affected by chronic Chagas disease were divided into different groups according to the stage of the pathology. In agreement with the Brazilian consensus on Chagas disease, patients were classified as presenting an undetermined form, a cardiac form and a digestive form. Serum IFN-γ, TNF-α, IL-10, and IL-17 were evaluated. **Results:** Lower serum IFN-γ concentrations were detected in patients receiving angiotensin-converting enzyme inhibitors (p = 0.0182), but not in those using angiotensin receptor blockers (p = 0.0783). Patients using amiodarone and aldosterone antagonist presented higher serum TNF-α concentrations (p = 0.0106 and 0.0187, respectively). IL-10 and IL-17 levels did not differ between the study groups (p = 0.7273 and p = 0.6697, respectively). **Conclusions:** These results suggest that the cytokine profile and disease progression are altered by anti-congestive medications commonly prescribed for CCC.

Keywords: Chagas disease. Cytokines. Left ventricular function. Anti-congestive medications.

INTRODUCTION

Chagas disease is recognized by the World Health Organization as one of the 20 neglected tropical diseases worldwide and the leading parasitic disease of the Western Hemisphere. About 30% of the infected population develops clinical manifestations of the disease, which makes the pathology relevant in terms of public health and economic impact[1-4]. It presents an acute phase of short duration, which progresses to a chronic phase in most patients that can be digestive, cardiac, or both. Chronic chagasic cardiopathy (CCC) is essentially a dilated and arrhythmogenic cardiomyopathy in which a subacute, but constant, chronic inflammatory process usually causes progressive destruction of the heart tissue and extensive fibrosis. Several mechanisms such as cardiac dysautonomia, microvascular disorders, parasite persistence, and immune response contribute to the pathophysiology of these cardiac lesions and the subsequent appearance of several pathophysiological manifestations[5-9].

In the chronic phase of the infection, the cytokine profile is characterized by an increase in serum concentrations of tumor necrosis factor alpha (TNF-α) and cytokines produced by T helper 1 lymphocytes, such as interferon gamma (IFN-γ) and inhibition of cytokines produced by T helper 2 lymphocytes, such as interleukin 4 (IL-4). Previous evidence suggests that patients with elevated TNF-α levels present a more compromised heart[10,11], whereas a balanced immune response

during *Trypanosoma cruzi* infection is fundamental to control the parasitic load in the heart and digestive tissues[12-20].

Interleukin 17 (IL-17) is a proinflammatory cytokine that is produced mainly by CD4+ and CD8+ T lymphocytes and activated Natural killer cells[16,18]. Their response has been associated with the pathogenesis of various inflammatory, oncologic, parasitic, and autoimmune diseases[21]. Although IL-17 is associated with an inflammatory response and autoimmune diseases, experimental data on *T. cruzi* infection suggest that this cytokine correlates with a protective immune response in relation to the parasite[11,14,18,21-23].

Despite advances in the treatment of heart failure (HF), the syndrome remains a serious pathology affecting >23 million people worldwide. Patients survival at 5 years after diagnosis can be up 35%, with a prevalence increasing with age (approximately 1% in individuals aged from 55 to 64 years, reaching up to 17.4% in those older than 85 years). In Latin America, with its social, economic, and cultural peculiarities, a distinct clinical profile is found. Low investment in health, inadequate access to care, and insufficient follow-up in primary or tertiary level services are potential risk factors; consequently, numerous pathophysiological processes favor the development of HF. In Brazil, Chagas disease, although less prevalent than in the past, is still present. It has a severe clinical course and is responsible for approximately 21% of patients with HF that need outpatient care. Regarding drug therapy for CCC, most clinical treatments were extrapolated from data on HF induced by other causes. The vast majority of clinical trials showing the benefits of anti-congestive medications did not include patients with CCC; therefore, their safety and efficacy have not been adequately established in this group of patients with heart disease. Despite little evidence, currently available national and international guidelines for the treatment of chronic HF have extended the drug treatment to patients with CCC. This treatment is based on the following classes: angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), aldosterone antagonists, and beta-blockers[35,37,47,49]. The aim of this study was to evaluate the cytokine profile in patients with different stages of CCC receiving anti-congestive medication with the aim of reducing the morbidity and mortality.

METHODS

A cross-sectional, observational, and analytical study started once the approval was obtained from the Research Ethics Committee of the Federal University of Triângulo Mineiro, Uberaba - MG, Brazil. The study included patients with Chagas disease monitored and followed up at the Chagas Disease/ Federal University of Triângulo Mineiro outpatient clinic. Their diagnosis was confirmed by at least two serological tests: enzyme-linked immunosorbent assay, indirect immunofluorescence, and indirect hemagglutination. Patients of both sexes, aged between 18 and 65 years, that agreed to participate to the study after receiving sufficient information from the project team and signing the informed consent form were included. Patients classified as presenting the indeterminate form showed normal chest radiograph,

transthoracic echocardiogram at rest, electrocardiography and normal contrasted radiograph of the esophagus and colon. Those classified as presenting the cardiac form without left ventricular dysfunction showed an altered and/or transthoracic echocardiogram at rest, although their left ventricular ejection fraction was still >45%. Furthermore, those classified as presenting the cardiac form with left ventricular dysfunction showed an altered and/or transthoracic echocardiogram at rest, with left ventricular ejection fraction <45%. Finally, those classified as presenting the digestive form showed normal and transthoracic echocardiograms at rest but altered contrast esophageal and colon examinations. Patients were classified according to the criteria of the Brazilian Chagas Disease Consensus, 2005/2016[50]. All patients underwent anamnesis, physical examination, and data collection of continuously used medications, with emphasis on anti-congestive medications— ACE inhibitors, ARB, aldosterone antagonists, beta-blockers, and antiarrhythmic drugs (amiodarone). Overall, 40 patients, divided into four groups, were studied: 10 patients with indeterminate form, 11 patients with cardiac form without left ventricular dysfunction, 12 with cardiac form with left ventricular dysfunction, and 8 with digestive form.

Peripheral blood was collected via venipuncture according to patient safety standards. *T. cruzi* culture was performed from obtained samples. The crude antigen from *T. cruzi* strain Y for the flow cytometry assays was obtained from the cell bank of the Immunology course of the Federal University of Triângulo Mineiro. Separation and peripheral blood mononuclear cell culture and evaluation of IL-10, IL-17, IFN-γ, and TNF-α cytokine concentration were performed using the cytometric bead array technique (BD Biosciences, San Diego, CA, USA). Flow cytometry was then used to characterize CD4+ T lymphocyte subpopulations obtained from patients with different clinical forms of Chagas disease.

Obtained results were analyzed for statistical significance by Graph Pad Prism 5 (Graph Pad Software Inc., San Diego, CA, USA) and Statview 4.57 (SAS Institute Inc., Cary, NC, USA) programs. All data were submitted to the Shapiro-Wilk normality test and Levene homogeneity test. The data presenting a homogeneous Gaussian distribution were analyzed by the ANOVA test followed by the Tukey post-test. Box plot graphs were used to present mean ± standard deviation. On the other hand, data presenting a non-Gaussian distribution were analyzed by the Kruskal-Wallis test followed by the Dunn post-test. Results are represented in box plot graphs, using the median and 10–90th percentiles. The Pearson's chi-squared test or Fisher's exact test was used for categorical variables. The Statview 4.57 software allowed to plot the correlations determined using the Lowess technique. The Spearman's correlation coefficient was then used to compare serum cytokine concentrations and the percentage of cells producing cytokines according to left ventricular ejection fraction (LVEF). Results with p-values < 0.05 were considered statistically significant and indicated by an asterisk over the group that presented differences compared to the experimental control group.

RESULTS

Regarding the general characteristics of included patients, there was general a predominance of men over women (56% vs. 43%) (p = 0.0464). Only in the undetermined disease form group women prevailed [7 (80%)]. In the other groups, there was a predominance of men: 9 (64%) in the group of patients presenting a cardiac form without dysfunction; 7 (70%) in the group of patients presenting a cardiac form with dysfunction, and 5 (62%) in the group of patients presenting a digestive form. LVEF analysis by groups showed that patients presenting indeterminate and digestive forms had a higher median LVEF (70% and 66%, respectively) than those with and without left ventricular dysfunction (40.5% and 60%, respectively). There was a statistical difference in LVEF between patients presenting an indeterminate form and those presenting the cardiac form with and without ventricular dysfunction between those presenting a digestive form and those presenting the cardiac form with left ventricular dysfunction (p < 0.0001). Regarding the analysis of the left ventricular end-diastolic diameter, patients with an undetermined form presented smaller mean diameters when compared to patients presenting the cardiac form with and without left ventricular dysfunction and patients presenting the digestive form had smaller diameters than those with the cardiac form with left ventricular dysfunction (both p < 0.0001). The analysis of the left atrium size showed that patients presenting the cardiac form with left ventricular dysfunction showed significantly higher mean values (47.70 ± 13.40 mm) than those presenting the indeterminate and digestive form (p = 0.0018). The general data of the study population is shown in **Table 1**.

Symptoms compatible with HF and the use of anti-congestive medications were evaluated in the different study groups and are shown in **Table 2**.

Serum concentrations of proinflammatory cytokines, such as IFN-γ and TNF-α, anti-inflammatory cytokines, like IL-10 and IL-17, in different study groups were analyzed. Serum IFN-γ production did not differ between the groups (p = 0.6652). Patients presenting cardiac forms, with and without left ventricular dysfunction, tended to have a higher serum TNF-α production (12.93 pg/mL and 12.17 pg/mL, respectively) than the other groups. However, no statistical difference was observed. Further, serum IL-10 and IL-17 production did not differ between the study groups (p = 0.7273 and p = 0.6697, respectively).

Serum IFN-γ levels were significantly lower (0 pg/mL) in patients who used ACE inhibitors than in those who did not use that medication (0.6069 pg/mL) (p = 0.0182) (**Figure 1**). No differences in the serum concentrations of other cytokines was observed between the two groups.

TABLE 1: Distribution of the parameters that compose the general characteristics of the studied population.

	Clinical forms of Chagas disease				
	Indeterminate n (%)	Cardiac without dysfunction n (%)	Cardiac with dysfunction n (%)	Digestive n (%)	Total
Patients	10	12	10	08	40
Sex					
Male	02 (20)	09 (75)	07 (70)	05 (63)	23
Female	08 (80)	03 (25)	03 (30)	03 (37)	17
Age (years)					
Median (IQR)	56 (45–64)	60 (38–65)	63 (43–65)	53.5 (39–64)	60 (38–65)
LVEF (%)					
Median (IQR)	70 (65–78)	60 (48–69)	39.5 (13–44)	66 (61–78)	65 (13–78)
LVDD (mm) Mean ± standard deviation	46.74 ± 3.086	55.45 ± 7.967	62.25 ± 7.479	51.13 ± 2.416	53.63 ± 8.008
LA (mm) Mean ± standard deviation	33.15 ± 3.215	40.18 ± 6.809	47.70 ± 13.40	35 ± 5.099	39.24 ± 9.72

n: number of patients; **LVEF**: left ventricular ejection fraction; **LVDD**: left ventricular diastolic diameter; **LA**: left atrium size; **IQR**: Interquartile range.

TABLE 2: Distribution of clinical parameters in the study population.

	Clinical forms of Chagas disease				
	Indeterminate n (%)	Cardiac without dysfunction n (%)	Cardiac with dysfunction n (%)	Digestive n (%)	P-value*
Patients	10 (100)	12 (100)	10 (100)	08 (100)	
Dyspnea*	02 (22)	09 (64)	07 (77)	05 (63)	**0.0039**
Heart beats	02 (20)	04 (33)	06 (60)	02 (25)	0.25
Fatigue*	02 (20)	05 (41)	08 (80)	02 (25)	**0.0316**
Edema*	02 (20)	03 (25)	08 (80)	02 (25)	**0.0158**
Ventricular arrythmia*	0 (0)	6 (50)	8 (80)	0 (0)	**0.0002**
Previous use of benznidazole	4 (40)	4 (33)	5 (50)	1(12)	0.4083
ACEI*	5 (50)	3 (25)	6 (60)	0 (0)	**0.0356**
ARB*	1 (10)	9 (75)	4 (40)	1 (12)	**0.0056**
Beta-blocker*	0 (0)	6 (50)	8 (80)	1 (12)	**0.0008**
Aldosterone antagonist*	0 (0)	4 (33)	7 (70)	0 (0)	**0.0011**
Amiodarone*	0 (0)	4 (33)	7 (70)	0 (0)	**0.0011**

n: number of patients; **ACEI:** angiotensin-converting enzyme inhibitors; **ARB:** angiotensin receptor blockers. *p < 0.05.
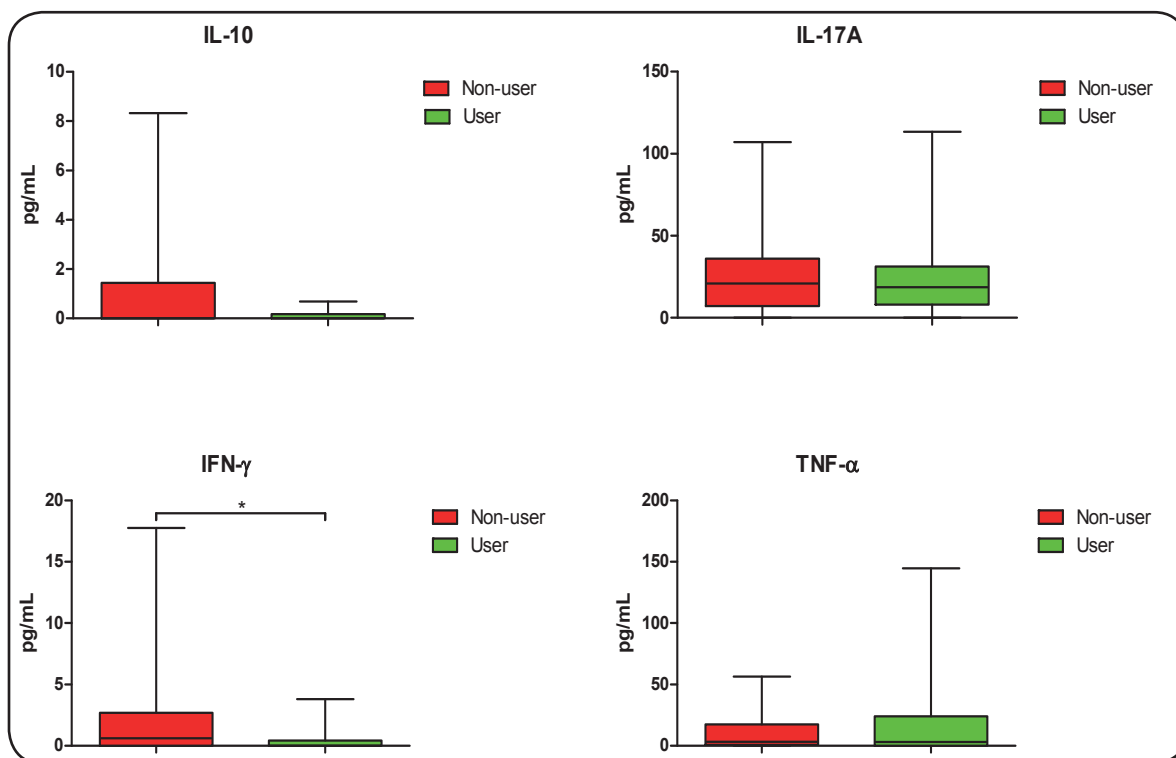

FIGURE 1: Box plots representing medians, maximum, and minimum values of the serum concentrations of IL-10, IL-17A, IFN-γ, and TNF-α in patients using angiotensin-converting enzyme inhibitors. The asterisk represents significant statistical difference (p = 0.0182). **IL:** interleukin; **IFN:** interferon; **TNF:** tumor necrosis factor.
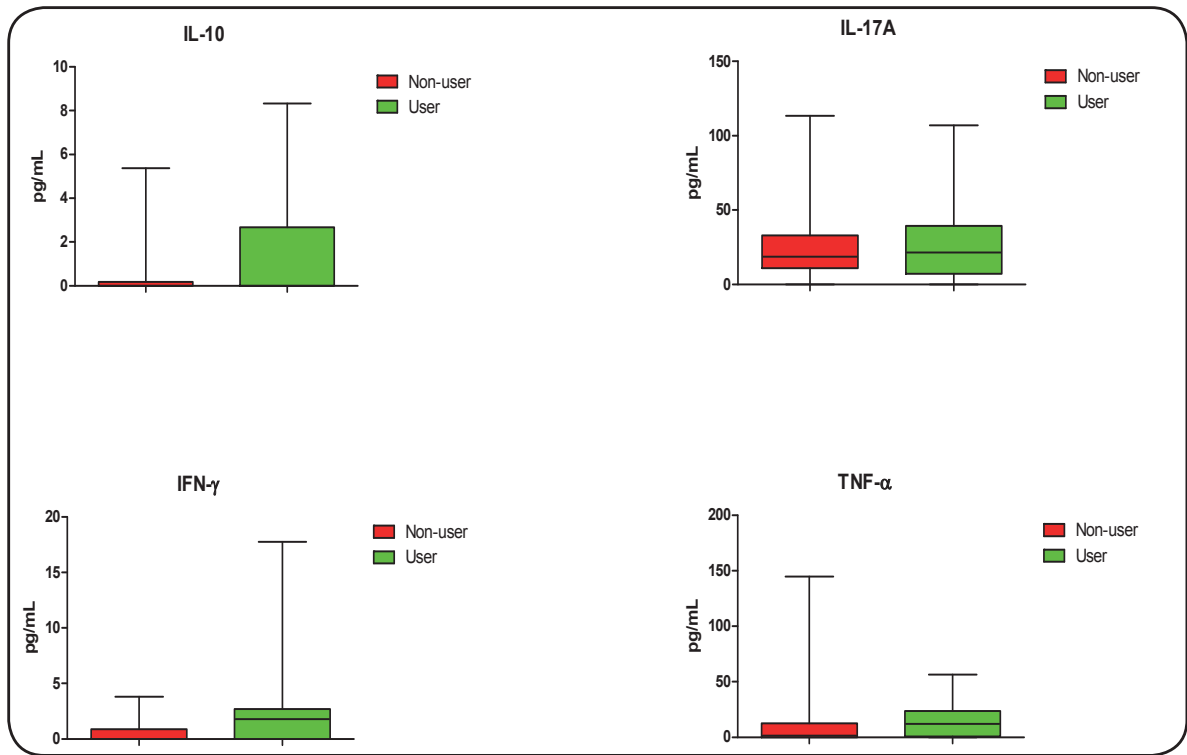
FIGURE 2: Box plots representing medians, maximum, and minimum values of the serum concentrations of IL-10, IL-17A, IFN-γ, and TNF-α in patients using angiotensin receptor blockers. **IL:** interleukin; **IFN:** interferon; **TNF:** tumor necrosis factor.
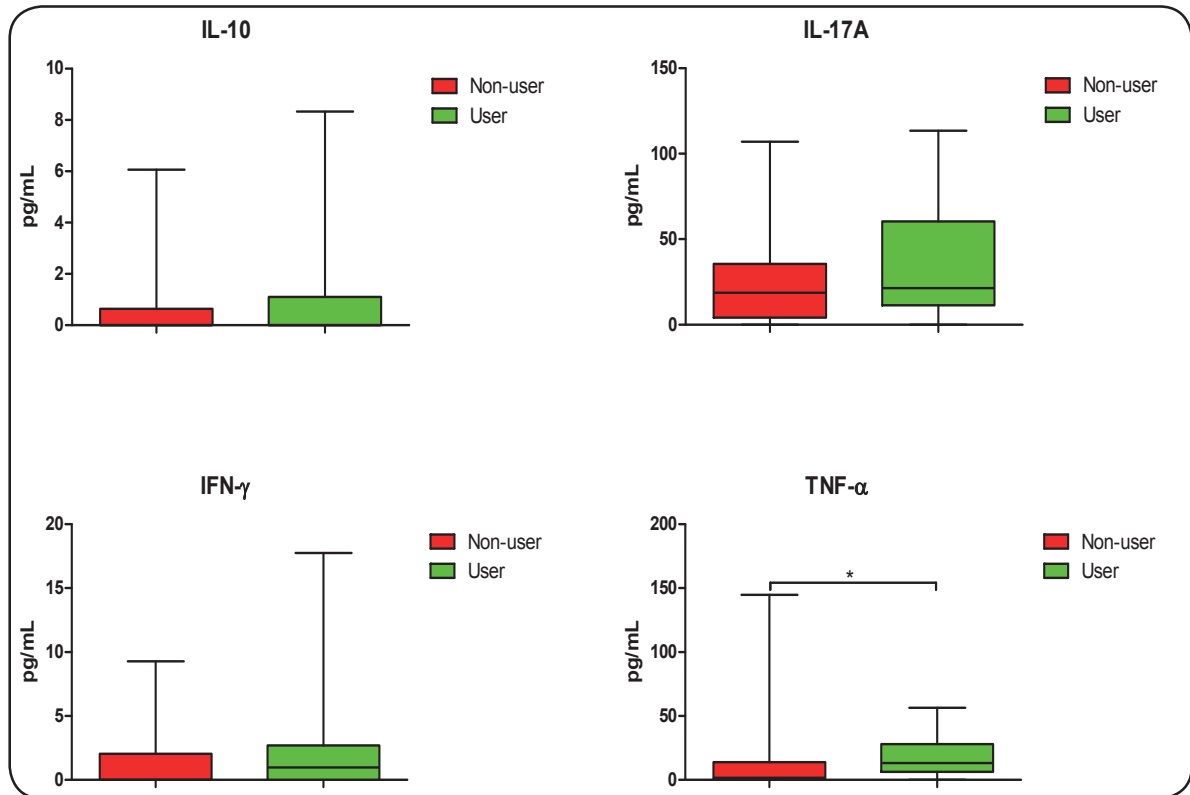

FIGURE 3: Box plots representing medians, maximum and minimum values of the serum concentrations of IL-10, IL-17A, IFN-γ, and TNF-α in patients using aldosterone antagonists. The asterisk represents a significant statistical difference (p = 0.0187). **IL:** interleukin; **IFN:** interferon; **TNF:** tumor necrosis factor.

Among patients using ARB, serum IL-10 and IL-17A concentrations did not differ. Regarding IFN-γ and TNF-α, patients receiving that medication tended to have higher serum levels (1.788 pg/mL and 12.17 pg/mL) than those did not use that medication (p = 0.0783 and p = 0.1092, respectively) (**Figure 2**).

Patients who received aldosterone antagonists showed significantly higher serum TNF-α concentrations (13.09 pg/mL) than those who did not receive that medication (1.544 pg/mL) (p = 0.0187). Serum concentrations of other cytokines did not show any statistical difference (**Figure 3**).

Serum cytokine concentrations did not differ among beta-blocker users. There was a higher tendency of TNF-α production (13.09 pg/mL) among patients using beta-blockers than among those who did not use that medication (1.544 pg/mL) (p = 0.0613). Patients who used amiodarone presented significantly higher concentrations of TNF-α (19.12 pg/mL) than those who did not use that medication (0.1554 pg/mL) (p = 0.0106). Dosages of other serum cytokines did not differ between the groups. Benznidazole-treated patients showed significantly lower serum TNF-α concentrations (0.7829 pg/mL) than untreated patients (7.723 pg/ml) (p = 0.0451).

DISCUSSION

The inflammatory response in the pathophysiology of Chagas disease is still subject of discussion. It has a fundamental role in *T. cruzi* infection by participating in the control or evolution of the disease[8]. Proinflammatory cytokines (IL-12, TNF-α, and IFN-γ) promote the activation of the inflammatory response; however, this process is also regulated by the action of anti-inflammatory cytokines (IL-4 and IL-10). These cytokines promote a Th2 response, which regulates the Th1 response. Thus, they are involved in both the resistance and immunopathology-related mechanisms of the disease[8,24-26].

Regarding serum concentrations of cytokines evaluated in the present study, although cytokine production was observed in all groups, no significant differences in their concentrations were observed. TNF-α had a tendency to be higher in patients presenting the cardiac form with and without left ventricular dysfunction than in the other groups. Previous studies on Chagas disease immunopathogenesis have described that patients with early forms of the disease had a predominance of immunoregulatory response, with a higher production of anti-inflammatory cytokines, such as IL-10, which alter disease progression[14]. Despite this fact, IL-10 production was also found in patients with advanced forms. However, in those individuals, there is a predominance of inflammatory response with a higher production of TNF-α and IFN-γ[14,27-29]. One of the hypotheses for insufficient IL-10 production to prevent disease progression is the presence of a genetic polymorphism in the IL-10 gene effector region, which is likely to cause a lower production of this cytokine. The deficient production of IL-10 would then favor the inflammatory response and lead the disease to evolve toward severe forms of heart disease[14,30]. Regarding the studied inflammatory cytokines, TNF-α and IFN-γ, high concentrations of these compounds correlate with an inflammatory immune response, which is responsible for

rapid disease progression toward severe forms. However, some studies have reported TNF-α production also during the early stages of heart disease[14,19,20,27,28]. However, other studies have found that high IFN-γ concentrations could be related to the parasitological cure and that IFN-γ expression could be inversely proportional to disease severity[14,30,31].

Regarding the drugs used to treat CCC, serum cytokine concentrations were analyzed among patients using the main classes of drugs. We observed that serum IFN-γ concentrations were significantly lower in patients using ACE inhibitors than in those who did not use ACE inhibitors. There were no changes in the serum concentrations of other cytokines. Perhaps the small number of patients per group and the need to increase the sample size may have influenced obtained results, especially regarding serum IL-10 and IL-17 concentrations. A tendency toward a higher production of IFN-γ and TNF-α was observed in patients receiving ARB than in those who were not under that medication. In previous studies, ACE inhibitors were shown to reduce the inflammatory response at the cardiac level, thereby improving the course of the disease. This inflammatory response is controlled by increased IL-17 expression, reduced lymphocyte activation, and diminished markers of myocardial lesions[32]. In experimental models, the use of ACE inhibitors alone or in combination with benznidazole promoted a decrease in the local inflammatory response of *T. cruzi* infected mice, mainly by reducing the production of cytokines, such as TNF-α and IFN-γ, activating lymphocytes in the myocardial tissue, and decreasing collagen production[33,34]. Such action may justify the better evolution of patients receiving this drug, who present an increased survival, reduced mortality, and improved left ventricular function[35,36]. Regarding the use of ARB, no studies have shown a better effect in relation to ACE inhibitors as for the reduction of cardiovascular outcomes in patients with heart disease. Their use should be indicated only when the patient is intolerant to ACE inhibitors[35,36,37]. Perhaps an explanation for the findings in our study is the excessive use of this medication in patients with heart disease due to dosage and low cost convenience, as these patients take a large number of prescription drugs, and not necessarily due to ACE inhibitor intolerance.

The analysis of patients receiving beta-blockers showed no statistical difference between the groups. A tendency toward a higher TNF-α production was observed, although no statistical difference was detected. In experimental studies, as well as in humans, it has been demonstrated that beta-blockers prevent heart disease progression via a myocardial anti-remodeling effect, subsequently improving the ejection fraction and survival[35,37,38]. Carvedilol reduced serum TNF-α concentration and oxidative stress in patients with chronic HF, when used alone or in combination with vitamins E and C[38-41].

Higher serum TNF-α concentrations were observed in patients who used aldosterone antagonist and amiodarone than in those who did not take those medications. Amiodarone, an antiarrhythmic drug that is widely used in patients with the arrhythmogenic cardiac form of Chagas disease, has been studied for its possible antiparasitic effect inside the cells, which

reduces the parasitic load via its action against the amastigote form of the parasite. As for the effects of amiodarone on TNF-α concentration, inhibition of this cytokine production was observed, suggesting that this is due to a decrease in potassium, achieved by blocking intracellular potassium channels. However, mechanisms by which amiodarone modulates cytokine production need to be further clarified[42,43].

High serum aldosterone stimulates fibroblast production and increases myocardial, perivascular, and perimicocytic fibrosis content, thereby causing muscle stiffness and dysfunction. In addition, it induces vascular damage by decreasing arterial compliance and modulates fibrinolysis balance by increasing plasminogen activator inhibitor 1, thus predisposing to ischemic events. Further, it can cause baroreceptor dysfunction and sympathetic activation, aggravate myocardial dysfunction and, consequently, promote heart disease progression. In contrast, it causes water and sodium retention, causing potassium and magnesium loss, thereby increasing adrenergic neurohormone release and the risk for cardiac arrhythmias and sudden death. Hence, its blockage can reduce collagen synthesis and deposition, which then improve myocardial function[37,44,48]. The aldosterone antagonist available in our country, spironolactone, is used in symptomatic patients who present with the cardiac form with left ventricular dysfunction, despite the optimal use of ACE inhibitors (or ARBs) and beta-blockers. In combination with these drugs, it has been shown to reduce cardiac remodeling and morbidity and mortality[35,37,44].

Our results showed that patients receiving amiodarone and aldosterone antagonists presented higher serum levels of proinflammatory cytokines, namely TNF-α. Considering that patients with higher serum levels of this cytokine have worse clinical outcome and in view of the fact that these drugs are indicated at an advanced stage of heart disease, where there is already a considerable tissue damage, we believe that the higher serum TNF-α production is a marker of disease severity[13-14,28,30].

There are still few studies evaluating immune changes in the chronic form of Chagas disease after benznidazole treatment. It is the most indicated drug in Latin American countries and the most used in Brazil for antiparasitic treatment in Chagas disease. The mechanism of action of benznidazole is related to the reduction of nitrogen from the parasite components and binding of nuclear DNA, k-DNA metabolites, lipids, and proteins[45]. Coura et al. (1997) demonstrated that benznidazole was more effective than nifurtimox in suppressing parasitemia during the chronic phase of Chagas disease[51]. The results showed a greater suppressive effect, but similar side effects. Despite the trypanocidal effectiveness of benznidazole during the acute phase of the disease, its efficacy is not well established in the chronic phase of Chagas disease. Previous publications have shown that while not eradicating the parasite in the chronic phase, antiparasitic treatment contributed to reducing parasitemia and modulating the host immune response, thereby leading to a balanced inflammatory response, with the production of proinflammatory cytokines, such as TNFα and IFN-γ, and anti-inflammatory cytokines, such as IL-10,

which are essential for disease control[29,46]. Pissetti et al. (2009), verifying the association among serum IFN-γ, TNF-α, and IL-10 concentrations in patients with different clinical forms, revealed that patients presenting higher IFN-γ and TNF-α concentrations also had higher IL-10 concentrations[15]. The BENEFIT (BENznidazole Evaluation For Interrupting Trypanosomiasis) study by Morillo et al. (2015) assessed the efficacy of etiological treatment of patients presenting the chronic cardiac form of Chagas disease. This study found no statistical difference in terms of disease progression between patients treated or not with benznidazole. The results of the study show that despite the reduction in parasitemia, there was no reduction in disease progression, morbidity, and mortality, suggesting that other mechanisms are associated with the evolution of CCC[52].

In the present study, patients who underwent etiological treatment with benznidazole for *T. cruzi* had lower serum TNF-α concentrations than those not subjected to the treatment. We believe that these patients have a balanced immune response, avoiding the inflammatory response and secondary tissue aggression to prevail.

Other factors such as the time period they were treated (none of the patients studied were recently treated) and the use of drugs against HF could modify the host immune response.

Our study had some limitations. First, only patients living in endemic areas, mostly in advanced stages of the disease and without adequate treatment, were referred to our specialty outpatient clinic. Second, other factors, such as advanced age and the small number of patients per group, might influence obtained results. However, our results suggest that cytokine profiles and disease progression may be altered by commonly prescribed anti-congestive medications for CCC. Advances are needed to better evaluate the application of cytokines as predictors of CCC progression in clinical practice.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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