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Major Article

Benznidazole therapy for Chagas disease in asymptomatic *Trypanosoma cruzi*-seropositive former blood donors: evaluation of the efficacy of different treatment regimens

***André Pires Antunes*[1], *Antônio Luiz Pinho Ribeiro*[2], *Ester Cerdeira Sabino*[3], *Marise Fagundes Silveira*[1], *Cláudia Di Lorenzo Oliveira*[4] *and Ana Cristina de Carvalho Botelho*[1]**

[1]. Programa de Pós-Graduação em Ciências da Saúde, Departamento de Clínica Médica, Universidade Estadual de Montes Claros, Montes Claros, Minas Gerais, Brasil. [2]. Hospital das Clínicas, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil. [3]. Departamento de Doenças Infecciosas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brasil. [4]. Departamento de Medicina, Universidade Federal de São João del-Rei, Divinópolis, Minas Gerais, Brasil.

Abstract

Introduction: Chagas disease currently affects 5.7 million people in Latin America and is emerging in non-endemic countries. There is no consensus concerning the efficacy of trypanocidal therapy for patients with the chronic form of the disease. We evaluated cardiac function and sociodemographic, clinical, and serologic characteristics of a group of asymptomatic *Trypanosoma cruzi*-seropositive former blood donors, and compared the effects of benznidazole treatment applied for different lengths of time. **Methods:** Blood donors who screened positive for *T. cruzi* between 1998 and 2002 were recruited 10 years later for follow-up (n = 244); 46 individuals had received treatment. Three subjects had terminated treatment prematurely. The remaining 43 individuals were divided into two groups: individuals who had received benznidazole therapy for 50-60 days (n = 28; BT ≤60 group) or more than 60 days (n = 15; BT >60). Serologic assays, biochemical tests, electrocardiographic, echocardiographic, and clinical examinations were performed on all participants. Parasite loads were determined by qualitative and quantitative polymerase chain reaction. **Results:** Parasitemia was significantly reduced in the BT ≤60 and BT >60 groups compared with the untreated group. There were no differences in epidemiologic profiles or clinical, biochemical, electrocardiographic, or echocardiographic data between any of the groups. **Conclusions:** Despite elimination or significant reduction in parasitemia in patients with chronic Chagas disease who received benznidazole, there was no clinical difference between those who were treated for >60 days and those treated for a shorter duration. Furthermore, the adverse effects of benznidazole appear to be less severe than previous reports would suggest.

Keywords: Chagas disease. Blood donors. Benznidazole. Parasitemia. PCR assay.

INTRODUCTION

Chagas disease, also known as American trypanosomiasis, is an inflammatory disease caused by the flagellate protozoan *Trypanosoma cruzi* (Trypanosomatidae). The disease is endemic in Latin America and is estimated to affect some 5.7 million people[1], a large proportion (20-40%) of whom will gradually evolve to the chronic phase of the disease and exhibit myocardiopathy, megaesophagus, megacolon, or other associated conditions in later life[2]. Although the incidence of Chagas disease is declining in most Latin American countries following the establishment of public policies of surveillance, prevention, and control[3] [4], the number of cases is increasing in non-endemic regions such as North America, Europe, Asia, and Oceania as a result of the migration of infected individuals. Despite its detrimental impact on the world economy, trypanosomiasis is considered a neglected disease[5].

The classical form of transmission of Chagas disease is vectorial via the feces of triatomine bugs, although non-vectorial modes of transmission (oral, vertical or congenital, blood transfusion, organ transplantation, and laboratory accidents) have also been reported. In Brazil, for example, the epidemiology of trypanosomiasis has changed substantially over the years such that it now resembles that of other infectious diseases that are not vector-borne[1]. Indeed, oral transmission through the ingestion of contaminated food is currently the most frequent mode of infection of Chagas disease in Brazil (68.9%), while congenital transmission and transfusion-related transmission are a problem in non-endemic regions[6]. Hence, safeguards relating to all aspects of blood transfusion have been

Corresponding author: Dr. André Pires Antunes.
e-mail: andrecardio@gmail.com
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strengthened in many countries. As a result, donated blood is screened for known blood-borne infections, including Chagas disease. In the United States (US) for example, the Retrovirus Epidemiology Donor Study-II (REDS-II; 2004 - 2012) was established with the aim of improving blood product safety and availability[7] [8].

The two antiparasitic drugs that could be employed in the treatment of *T. cruzi* infection (i.e. nifurtimox and benznidazole) have not been approved by the US Food and Drug Administration (FDA) but are available at the request of a physician for use under investigational protocols[9]. It is important to emphasize, however, that prolonged treatment with benznidazole is contraindicated because of the associated side-effects and safety risks. The Benznidazole Evaluation for Interrupting Trypanosomiasis (BENEFIT) project, described recently by Morillo et al.[10], was a multicenter, double-blinded, randomized, placebo-controlled trial involving the administration of benznidazole or matching placebo to 2,854 patients presenting with chronic Chagas cardiomyopathy. The results showed that benznidazole therapy did not reduce the clinical decline in cardiac function despite significant reductions in parasite load in the blood over the 5 years of follow-up. The authors concluded that there is a need to research the effects of benznidazole in patients with the indeterminate form of Chagas disease without cardiomyopathy and also the effects of prolonged benznidazole therapy[10].

A retrospective cohort study, conducted in collaboration with the international component of the REDS-II program, evaluated a population of supposedly healthy blood donors in the Brazilian cities of São Paulo and Montes Claros, and showed that 24% of 499 *T. cruzi*-seropositive individuals presented with cardiomyopathy within a 10-year follow-up period, while only 5% of the 488 seronegative subjects exhibited this condition within the same time period[11]. Interestingly, only 46 seropositive subjects reported prior administration of benznidazole and treatment periods ranged from 6 to 700 days. In the present study we investigated *T. cruzi*-seropositive blood donors identified in Montes Claros with the objectives of: I) evaluating the sociodemographic, clinical, serologic, electrocardiographic (ECG) and echocardiographic (ECHO) characteristics of the individuals, and II) comparing the effects of benznidazole treatment applied for different lengths of time, namely 50-60 days and >60 days. This is the first report describing treating blood donors with benznidazole; hence, it contributes to a better evidence-based judgment of the appropriateness of prolonged treatment with this drug.

METHODS

Study design

This cross-sectional study involved 244 supposedly healthy volunteers who were registered at a blood donation center in Montes Claros, Brazil, during the period 1998-2002 and had been selected 10 years later for follow-up examinations in a previous investigation[11]. The inclusion criteria were: I) positive serologic test results for Chagas disease based on enzyme-linked immunosorbent (ELISA) and hemagglutination assays for *T. cruzi* antibodies performed at the time of blood donation, II) absence of comorbidities such as diabetes and renal insufficiency, and III) a signed document giving informed consent for participation. Subjects were excluded if their serologic test results were not completed. During the period 2008-2010, potential participants in the follow-up study were recruited by either mail or telephone. New serologic assays were performed on blood samples of all participants selected. These tests were repeated in the US REDS-II Central Laboratory at the Blood Systems Research Institute [(BSRI); San Francisco, CA, US] in order to confirm the diagnosis using methodology and reagent kits (Ortho-Clinical Diagnostics, Raritan, NJ, US) approved by the FDA. The parasite load in the blood of each subject was determined by qualitative and quantitative polymerase chain reaction (PCR) assays. Participants also underwent standard biochemical testing, ECG and ECHO investigations, and clinical examination by a physician (non-blinded test), and were subsequently offered counseling if appropriate.

Collection of data

Selected participants were called for an interview and were asked to complete a standardized questionnaire relating to sociodemographic data (age, sex, skin color, civil status, educational level, smoking and drinking habits, and physical activity), cardiovascular and digestive symptoms, and use of medicines. On the day of the interview, blood samples (20mL) for molecular tests were collected from participants and transferred to vials containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). Each sample was mixed immediately with an equal volume of a solution containing 6M guanidine hydrochloride in 0.2M EDTA and stored at room temperature. Samples were subsequently boiled for 15 min (to shear *T. cruzi* nuclear and kinetoplast deoxyribonucleic acid (kDNA), cooled, submitted to vortex mixing and distributed as 1mL aliquots into appropriately labeled cryovials. The vials were maintained in Brazil at -20°C until required for blind analysis by PCR or shipment to BSIR in containers cooled with dry ice (-70°C) for repeat blind testing.

The qualitative and quantitative analysis of blood parasite load was performed by targeted-capture real time polymerase chain reaction (TC-RT-PCR) following the methodology described by Virreira et al.[12] in which the target was kinetoplast DNA minicircles from *T. cruzi*. Specific DNA extraction was improved by the TC step employing a *T. cruzi*-specific 20-mer capture oligonucleotide bound to magnetic beads. TC-RT-PCR analysis was repeated four times for each sample and the result was considered positive when two or more tests detected the specific amplicon, or negative when none or only one of the tests detected the amplicon. According to Sabino et al.[13], this assay is able to detect a single parasite in 20mL of blood. The results of the PCR assays were sent to the US REDS-II Coordination Center (Westat; Rockville, MD, US) for decoding and analysis.

Blood samples (20mL) were also collected for the preparation of serum and plasma required for assessment of high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, total cholesterol, triglycerides, glycemia, troponin, myoglobin, N-terminal pro B-type natriuretic peptide

(NT-proBNP), creatine kinase fraction MB (CK-MB), and tumor necrosis factor-alpha (TNFα). The levels of troponin, CK-MB and NT-proBNP were determined using the VITROS® System (Ortho Clinical Diagnostics, Raritan, NJ, US), as approved by the FDA[11].

A resting ECG was performed using a General Electric Healthcare (Waukesha, WI, US) model MAC 1200 ECG instrument. ECG traces were analyzed by a local cardiologist and blind re-evaluations were carried out at the Epidemiological Cardiology Research Center in the Wake Forest University (Winston-Salem, NC, US). The Minnesota Code classification system was employed in the interpretation of ECG traces[14][15] considering the following parameters: QRS complex interval, corrected QT (QTc) interval, heart rate, PR interval, atrioventricular block (AVB), complete right bundle branch block (RBBB), complete left bundle branch block (LBBB), left anterior fascicular block (LAFB), ventricular premature beats (VPBs), Q wave abnormalities, and ST and T waves abnormalities. QRS ≥120ms and QTc >440ms were considered prolonged.

Echocardiographic assessment was performed using a General Electric Healthcare Vivid 3 ultrasound unit following the recommendations of the American Society of Echocardiography[16]. Images were recorded in digital format and analyzed at the Cardiovascular Branch of the Echocardiography Laboratory in the National Heart, Lung, and Blood Institute (NHLBI; Bethesda, MD, US). The parameters analyzed were: left ventricular ejection fraction (LVEF), left ventricular mass index (LVMI), wall motion score index (WMSI), left ventricular hypertrophy (LVH), Doppler-derived septal E/E' >15**,** and left ventricular diastolic diameter (LVDD). LVEF was calculated using the modified biplane Simpson's method[16]. LVEF <50% characterized left ventricular systolic dysfunction.

The results of all of the examinations and assays performed were sent to the US REDS-II centralized reading centers for blind evaluation.

Experimental groups

The initial study population (n = 244) was divided into two groups comprising subjects who had received treatment with benznidazole (BT group; n = 46) and non-treated subjects (NT group; n = 198). Subsequently, three members of the BT group were removed from the study because of premature termination of treatment (one after 6 days and two after 30 days). The remaining 43 members of the BT group were subdivided according to the duration of treatment, namely, 50-60 days (BT ≤60; n = 28) and more than 60 days (BT >60; n = 15).

Statistical analysis

The Kolmogorov-Smirnov test was used to test the normality of the distributions. Normally distributed variables were expressed in terms of the mean ± standard deviation (SD) while continuous variables that were not normally distributed were expressed in terms of the median and interquartile range (IQR). The statistical significance of differences between groups was assessed using the Mann-Whitney or Kruskal-Wallis tests. Categorical variables were expressed as numbers and percentages, and differences were determined using the χ^2 or Fisher's exact tests. For all tests, the level of significance was set at $p < 0.05$. The strength of the relationship between parasite load and duration of treatment was evaluated using the Spearman correlation coefficient. All statistical analyses were performed with the aid of Statistical Package for the Social Sciences (SPSS) version 18 software (Chicago: SPSS Inc.).

Ethical considerations

The study was submitted to and approved by the National Research Ethics Commission (protocol no. CONEP 3 1312/2006) and all procedures followed the ethical principles for medical research involving human subjects proposed by the Declaration of Helsinki. Written informed consent was obtained from all participants.

RESULTS

The initial study population (n = 244) had an average age of 46.7 years and a male preponderance (56.6%). Most subjects had partners (73.1%), had achieved a basic level of education (75.6%), and were sedentary (77%), non-smokers (62.3%) and non-drinkers (52.2%). The majority of participants declared themselves to be of mixed race (67.5%) or black (9.5%), while 23% stated that they were non-colored (**Table 1**).

The BT group comprised only 18.9% of the study population (n = 46). There were no differences between the BT and NT groups (Not Treated) with respect to sociodemographic variables. When the duration of treatment was taken into consideration, the sociodemographic characteristics of the NT, BT 60, and BT >60 groups were similar, except for a significantly higher frequency of patients who reported having drunk alcohol in the previous month among individuals in the BT ≤60 group (**Table 1**).

The three groups were also similar with respect to the biochemical variables (**Table 2**) and clinical, ECG, and ECHO characteristics (**Table 3**). However, quantitative PCR revealed that the parasite load of the NT group was significantly higher than that of the BT groups ($p < 0.05$) (**Table 4**). Qualitative PCR showed that the parasite load of the NT group was significantly higher ($p < 0.05$) than that of the BT ≤60 group but was not significantly higher than that of the BT >60 group (**Table 4**). Qualitative and quantitative PCR demonstrated that there were no significant differences in parasite load between the BT ≤60 and BT >60 groups.

The duration of benznidazole therapy received by the two treatment groups was highly variable as demonstrated by the distribution: 6 days (n = 1), 30 days (n = 2), 50 days (n = 5), 60 days (n = 23), 90 days (n = 4), 100 days (n = 4), 120 days (n = 3), 180 days (n = 1), 200 days (n = 1), 250 days (n =1), and 700 days (n =1). The three participants whose treatment terminated prematurely (i.e. <50 days) were not included in the BT ≤60 group. **Figure 1** shows that the correlation between parasite load (as determined by quantitative PCR) and duration of treatment was inverse, as demonstrated by the negative value of the Spearman correlation coefficient, but not significant ($r = -0.13$; $p = 0.401$). Skin allergic reactions induced by benznidazole were reported by 19.6% (9/46) of the blood donors.

TABLE 1

Sociodemographic characteristics of former blood donors registered in Montes Claros, State of Minas Gerais, Brazil.

Variables	NT group (n = 198)		BT ≤60 group (n = 28)		BT >60 group (n = 15)		P value
Age (mean ± SD)	47.2 ± 10.1		45.2 ± 10.9		42.7 ± 7.1		0.202
Sex (n; %)							0.137
female	117	59.1	11	39.3	08	53.3	
male	81	40.9	17	60.7	07	46.7	
Skin color (n; %)							0.173
male							
non-colored	43	21.8	10	35.7	02	13.3	
mixed race/black	154	78.2	18	64.3	13	86.7	
Civil status (n; %)							0.793
single	53	26.9	08	29.6	03	20.0	
married/cohabiting	144	73.1	19	70.4	12	80.0	
Educational level (n; %)							0.497
elementary	149	75.6	19	70.4	13	86.7	
secondary or higher	48	24.4	08	29.6	02	13.3	
Smoking habits (n; %)							0.172
non-smoker	122	61.6	21	75.0	07	46.7	
smoker/former smoker	76	38.4	07	25.0	08	53.3	
Alcohol last month (n; %)							0.037*
yes	88	46.8[a]	18	66.7[b]	04	26.7[a]	
no	100	53.2	09	33.3	11	73.3	
Physical activity (n; %)							0.626
Yes	45	22.7	06	21.4	05	33.3	
no	153	77.3	22	78.6	10	66.7	

NT: non-treated; **BT:** benznidazole-treated; **SD:** standard deviation. Data expressed in number and percentage, and used $\chi 2$ or Fisher's exact tests. Values followed by different lowercase superscript letters (^{a,b}) are significantly different. *Statistically significant (χ^2 test). Age expressed in mean and SD, and used Kruskal-Wallis test.

TABLE 2

Biochemical profile of former blood donors registered in Montes Claros, State of Minas Gerais, Brazil.

Variable	NT group (n = 198)	BT ≤60 group (n = 28)	BT >60 group (n = 15)	P value
HDL cholesterol (mg/dL)	46.5 (40.0–55.0)	44.4 (40.0–57.5)	51.5 (45.3–55.8)	0.521
LDL cholesterol (mg/dL)	119.0 (97.0–147.3)	117.5 (72.7–163.5)	109.0 (95.5–186.5)	0.894
Total cholesterol (mg/dL)	193.5 (165.0–225.3)	201.5 (143.7–247.2)	175.5 (164.0–265.7)	0.986
Triglycerides (mg/dL)	117.5 (85.5–182.0)	145.5 (78.7–197.5)	108.0 (71.0–168.0)	0.435
Glucose (mg/dL)	90.0 (83.8–97.3)	88.5 (78.2–96.5)	90.0 (82.2–107.5)	0.829
Troponin (ng/mL)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.306
Myoglobin (ng/mL)	36.6 (30.5–44.4)	37.2 (30.9–50.9)	38.9 (34.2–55.9)	0.311
NT-proBNP (pg/mL)	49.4 (27.9–98.4)	39.6 (18.1–55.7)	40.3 (23.3–85.7)	0.271
CKMB (ng/mL)	0.76 (0.50–1.21)	0.63 (0.38–0.96)	0.87 (0.49–1.11)	0.286
TNFα (pg/mL)	3.2 (1.8–4.9)	3.7 (1.9–5.7)	3.7 (2.0–4.6)	0.825

NT: non-treated; **BT:** benznidazole-treated; **HDL:** high density lipoprotein; **LDL:** low density lipoprotein; **NT-proBNP:** N-terminal pro B-type natriuretic peptide; **CKMB:** creatine kinase fraction MB; **TNFα:** tumor necrosis factor alpha. Data are expressed as median (interquartile range) and were compared using Kruskal-Wallis test.

TABLE 3

Clinical, ECG and ECHO alterations of former blood donors registered in Montes Claros, State of Minas Gerais, Brazil.

Variable	NT group (n = 198)	BT ≤60 group (n = 28)	BT >60 group (n = 15)	P value
Clinical				
BMI [kg/m^2; median (IQR)]	26.2 (23.7–28.6)	26.8 (24.9–29.4)	26.5 (22.0–32.5)	0.925
SBP [mm Hg; median (IQR)] †	120.0 (110–125)	120.0 (110.0–125.0)	110.0 (110.0–125.0)	0.837
DBP [mm Hg; median (IQR)]	65 (60–70)	70.0 (60.0–70.0)	67.5 (60.0–74.3)	0.481
Altered heart sounds [n (%)]	29 15.0	3 11.1	2 13.3	0.856
ECG alterations				
QTc interval [>440ms; n (%)]	58 29.3	6 21.4	4 26.7	0.681
Heart rate [bpm; median (IQR)] †	63.0 (57.8–70.0)	62.0 (56.0–69.0)	62.5 (58.0–69.3)	0.974
PR interval [≥200ms; n (%)]	15 7.7	1 3.7	1 6.7	0.747
Third degree AVB [n (%)]	1 0.5	0 0.0	0 0.0	0.897
RBBB [n (%)]	35 17.7	5 (17.9)	1 (6.7)	0.545
LAFB [n (%)]	42 21.5	5 (17.9)	1 (6.7)	0.375
Frequent VPBs [n (%)]	5 2.5	0 0.0	0 0.0	0.574
Major Q wave abnormalities [n (%)]	6 3.0	0 0.0	0 0.0	0.513
Major isolated ST and T waves abnormalities [n (%)]	6 3.0	2 (7.1)	0 0.0	0.398
ECHO alterations				
LVEF [<50%; n (%)]	16 8.1	1 3.6	0 0.0	0.388
LVMI [g/m^2; median (IQR)] †	79 (66–93.8)	78.5 (68.5–88.3)	68.5 (63.5–89.5)	0.546
Abnormal WMSI [n (%)]	17 8.7	1 3.6	0 0.0	0.357
LVH [n (%)]	2 1.0	1 3.6	1 6.7	0.174
Doppler-derived septal E/E' >15 [n (%)]	4 2.1	0 0.0	0 0.0	0.637
LVDD [mm; median (IQR)] †	45.0 (42.0–48.0)	45.0 (43.0–48.0)	44.0 (40.7–48.5)	0.865

ECG: electrocardiographic**; ECHO:** echocardiographic; **NT:** non-treated; **BT:** benznidazole-treated; **BMI:** body mass index; **IQR:** interquartile range; **SBP:** systolic blood pressure; **DBP:** diastolic blood pressure; **QTc:** corrected QT interval; **PR:** PR interval ; **AVB:** atrioventricular block; **RBBB:** right bundle branch block; **LAFB:** left anterior fascicular block; **VPBs:** ventricular premature beats; ST: ST-segment; T: T wave; **LVEF:** left ventricular ejection fraction; **LVMI:** left ventricular mass index to body surface area; **WMSI:** wall motion score index; **LVH:** left ventricular hypertrophy; **LVDD:** left ventricular diastolic diameter.

Data expressed in number and percentage, and used $\chi 2$ test used to compare, excepted † data expressed in median and interquartile range, and Kruskal-Wallis test used for comparison, as appropriate.

TABLE 4

Parasite load as determined by quantitative and qualitative PCR.

Variable	NT group (n = 198)	BT ≤60 group (n = 28)	BT >60 group (n = 15)	P values
Quantitative PCR*; median (interquartile range)]	0.08 (0–0.88)	0 (0–0.05)	0 (0–0.03)	0.003*** (NT vs. BT≤60) 0.005*** (NT vs. BT>60) 0.839 (BT≤60 vs. BT 60)
Qualitative PCR** [n (%)]	112 (57.4)	6 (22.2)	5 (33.3)	0.001*** (NT vs. BT≤60) 0.070 (NT vs. BT>60) 0.433 (BT≤60 vs. BT>60)

PCR: polymerase chain reaction; **NT:** non-treated; **BT:** benznidazole-treated. *Kruskal-Wallis test; **χ^2 test; ***Statistically significant (for two groups: Mann-Whitney test and Fischer's exact test).

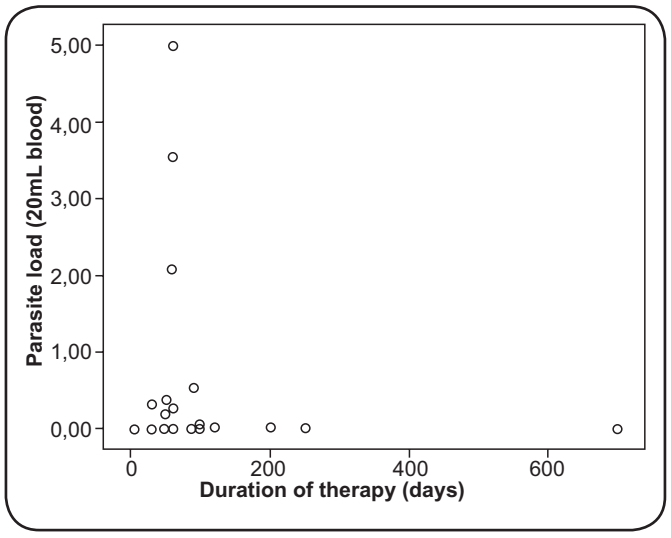

FIGURE 1. Correlation between parasite load as estimated by quantitative PCR and duration of benznidazole treatment. **PCR:** polymerase chain reaction**.** Spearman correlation coefficient r = 0.13; p = 0.401.

DISCUSSION

Chagas disease, described more than 100 years ago[17], remains a potentially life-threatening condition that negatively impacts on the quality of life of millions of people in Latin America, with attendant economic consequences. Infection is mediated mainly by vectors, but the epidemiologic profile of the disease has changed markedly in Brazil over recent years such that ingestion of contaminated food is currently the most important mode of transmission. Moreover, while the disease was originally confined to Latin American countries, its prevalence in the US, Canada, Europe, and some Western Pacific countries is on the increase owing mainly to population mobility[3].

Treatment of adults with the indeterminate form of Chagas disease remains controversial chiefly because of the absence of appropriate blinded randomized clinical trials. In the past, parasite persistence was not thought to be relevant to the physiology of complications in Chagas disease. Many other reasons also motivated the paradigm of not treating Chagas disease[18]. Despite the current recommendations, treatment is not implemented in >99% of infected individuals[2]. In the present study, the proportion of seropositive blood donors who had been treated with benznidazole was also modest (18.9%; 46/244) while the duration of treatment varied markedly and ranged between 6 and 700 days.

Few studies have focused on the efficacy of different durations of benznidazole therapy in the treatment of Chagas disease. Thirty years ago, Coura et al.[19] compared the effectiveness of 30- and 60-day treatments, but observed no statistical difference between the two regimens regarding the control of parasitemia, thus suggesting that there was no advantage in opting for a longer therapy. However, in a more recent report, Coura et al.[20] suggested a treatment regimen comprising 30 days of therapy repeated every two months over a 6 to 12 month period. In contrast, the study conducted by Morillo et al.[10] for the BENEFIT project involved benznidazole treatment administered for a single period of 40 to 80 days.

In a systematic review and meta-analysis of benznidazole therapy applied to patients with chronic Chagas disease who were in the indeterminate phase or had visceral involvement, Pérez-Molina et al.[21] considered 696 reports and selected for detailed analysis three clinical trials and six observational studies of which six involved adults. In these studies, the duration of treatment varied widely encompassing regimens of 30[22], 45-60[23] and 60[24] days, while the follow-up periods were also somewhat disparate, ranging from 12 months to 24 years (median 4 years)[21]. Regarding the efficacy of benznidazole administered in the late chronic phase of the disease, the authors of the meta-analysis concluded that the efficacy of therapy was doubtful and that the beneficial effect was marginal[21]. Uncertainty appeared to be even greater in asymptomatic individuals and those older than 50 years; this was largely due to differences in the study populations, endpoints, and durations of follow-up. However, the non-randomized and non-blinded prospective study conducted by Viotti et al. [22] confirmed clinical improvement and increased seroreversion rates of benznidazole-treated subjects compared with their non-treated counterparts (n = 283 in each group). Moreover, Fabbro et al.[23] reported improvement of the ECG profiles of benznidazole-treated patients, while Fragata-Filho et al.[25] found that 79.1% (263/310) of benznidazole-treated patients and 46.8% (47/310) of those untreated had normal ECG findings after a follow-up period of around 20 years. The occurrence of ECG abnormalities and clinical events (heart failure, stroke, mortality, and death) was less prevalent in treated patients. In contrast, Morillo et al.[10] concluded that therapy with benznidazole in patients with established Chagas cardiomyopathy reduced significantly serum parasite load but did not significantly reduce clinical cardiac deterioration through five years of follow-up.

In the present study, although the length of treatment varied considerably, two groups with different benznidazole treatment regimens could be defined. While parasite load was significantly reduced in the BT group compared with the NT group, no significant differences were detected between BT ≤60 and BT >60 groups. Quantitative and qualitative PCR revealed a significant reduction in the parasite load of the BT 60 group compared with NT. However, an analogous reduction in the BT >60 group could only be confirmed by quantitative PCR, probably because this group was small and the statistical power to detect differences by qualitative PCR was not so strong. Regarding the clinical cardiac conditions, no alterations were detected in the study population, regardless of group. It is important to emphasize, however, that the frequencies of use of cardiovascular drugs by the study population at the time of evaluation were 23.2, 25.0 and 6.7% for the NT, BT ≤60, and BT >60 groups, respectively, although the differences were not statistically significant (p = 0.313).

It is of interest to note that in the study conducted by Morillo et al.[10], which involved only patients with established

Chagas cardiomyopathy, the efficacy of treatment as assessed by conversion to negative PCR results was 66.2% at the end of treatment and 46.7% after 5 years or more of follow-up. This finding suggests that the standard regimen of benznidazole may be less effective in patients with cardiomyopathy. In contrast, the preliminary results of a randomized placebo-controlled evaluation of benznidazole treatment on long-term disease progression in adults with chronic Chagas disease (the TRAENA study)[26][27] showed that rates of negative parasitemia increased with time after treatment from 55.97% after two months, to 62.59% after 8-16 months, and 72.81% after 9-11 years.

In our study, side effects (allergic reactions) to the drug were detected in 19.5% of individuals, and therapy was discontinued in only 6.5% (3/46) of cases. Morillo et al.[10] reported that 14.4% of patients permanently discontinued treatment with benznidazole because of adverse events, a rate that is lower than that previously reported in observational and small randomized trials. These authors recommended that a dose of 300mg/day should not be surpassed since low doses appeared to increase tolerance to the drug.

New evidence supports the idea that the level of circulating parasites is relevant to the pathogenesis of chronic Chagas disease[11]. Furthermore, the association between parasite load (as measured by PCR) and the severity of clinical conditions has been confirmed[12]. In addition, various blinded randomized studies have demonstrated the efficacy of benznidazole therapy and that earlier treatment is associated with a more successful outcome[9][18][28][29][30]. Such findings have encouraged some agencies, including members of the network Nuevas Herramientas para el Diagnóstico y la Evaluación del Paciente con Enfermedad de Chagas (NHEPACHA; New Tools for the Diagnosis and Evaluation of Chagas Disease Patients), the World Health Organization and the Centers for Disease Control and Prevention, to recommend antiparasitic treatment for all *T. cruzi*-infected individuals in the chronic phase, even though this remains controversial in current medical practice. The need for a paradigm shift becomes stronger when one considers that delay in treatment while *awaiting further studies* leaves most of the infected population without any treatment.

Limitations of the present study include recall bias and the absence of information in the study population relating to dosage and the length of time after treatment completion. Moreover, employing positive ELISA results in the inclusion criteria may have led to the exclusion from the study of serologically cured patients, thereby reducing the ability to detect differences favoring treatment. In addition, the small sample population probably reduced the statistical power to detect differences. A strength of the study is that sampling a population of blood donors allowed inclusion of asymptomatic individuals, thus minimizing the effect of referral bias.

In conclusion, our study revealed that parasite load was significantly lower in the benznidazole-treated group than the non-treated group. However, comparison between individuals treated for 50-60 days and those treated for a more extended period (>60 days) showed that prolonged treatment conferred no advantage. Since the majority of blood donors are asymptomatic they are ideal targets for etiologic treatment in specialized health facilities. However, treatment regimens are currently inconsistent and this problem needs to be addressed with dosage, duration, and treatment intervals specified according to age and clinical groups. Although our results indicate that the duration of treatment does not influence the parasite load, the study was not designed to draw conclusions about the most appropriate therapeutic regimen for individuals with chronic asymptomatic or indeterminate Chagas disease.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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