

## Evaluation of Asymptomatic Patients with Chronic Chagas' Disease through Ambulatory Electrocardiogram, Echocardiogram and B-Type Natriuretic Peptide Analyses

Divina Seila de Oliveira Marques, Manoel Fernandes Canesin, Flavio Barutta Júnior, Claudio José Fuganti, Antonio Carlos Pereira Barretto

*Hospital de Clínicas da Faculdade de Medicina da Universidade Estadual de Londrina e Instituto do Coração do Hospital das Clínicas da FMUSP - Londrina, PR – São Paulo, SP - Brazil*

### OBJECTIVE

To evaluate asymptomatic patients with chronic Chagas' disease to determine prevalence of ventricular arrhythmias, left ventricular dysfunction, and B-type natriuretic peptide (BNP) plasma levels.

### METHODS

One hundred and six patients from the Chagas' disease outpatient clinic underwent clinical evaluation, electrocardiogram (ECG), cardiothoracic index (CTI), ambulatory electrocardiogram (Holter monitoring), echocardiogram, and BNP measurement and then were distributed into three groups: GI, with normal ECG (n = 50); GIIA, with ECG changes characteristic of Chagas' disease (n = 31); and GIIB, with other ECG changes (n = 25).

### RESULTS

The most common electrocardiographic changes were the following. GIIA: complete right bundle branch block (35%), left anterior hemiblock (35%), and electrically inactive areas (32%); GIIB: inferolateral repolarization change (28%), and left ventricular overload (24%). Mean CTI index values were similar ( $p = 0.383$ ). Ventricular arrhythmia prevalence was higher in the GIIA (77%) and GIIB (75%) groups than in the GI group (46%) ( $p = 0.002$ ). Ventricular dysfunction was more prevalent in the GIIA (52%) and GIIB (32%) groups than in the GI group (14%) ( $p = 0.001$ ). Systolic dysfunction was more prevalent in the GIIA group (29%) than in the GIIB (20%) and GI groups (2%) ( $p < 0.001$ ). Diastolic dysfunction was more prevalent in the GIIA (42%) and GIIB (28%) groups than in the GI group (12%) ( $p = 0.005$ ). Mean B-type natriuretic peptide levels were  $30 \pm 88$  pg/mL in the GI group,  $66 \pm 194$  in the GIIA group and  $24 \pm 82$  for the GIIB group ( $p = 0.121$ ), respectively.

### CONCLUSION

Arrhythmias and left ventricular dysfunction are more prevalent in asymptomatic patients with chronic Chagas' disease and abnormal ECG than in patients with normal ECG. Plasma BNP levels were similar among the groups.

### KEY WORDS

Chagas' disease, ambulatory electrocardiography, Doppler echocardiography, brain natriuretic peptide.

Chagas' disease is characterized by a large clinical diversity. It is one of the major public health problems in Brazil, with an estimated 3 million people infected<sup>1-3</sup>.

Approximately 60% of the infected individuals will remain in the indeterminate form (IF); 25% to 35% will develop heart disease, 10% of them severely. Chagas' disease manifests itself as arrhythmias and/or conduction disturbances, heart failure, thromboembolic accidents and sudden death<sup>4,5</sup>.

It is known that, in the natural history of Chagas' disease, electrocardiographic changes precede symptoms' onset, as well as physical examination and chest radiography abnormalities<sup>6</sup>. Since the clinical significance of ECG changes in asymptomatic individuals has not yet been well established, they may be found in patients who have recently entered the classic chronic phase and show either slight or more advanced electrocardiographic changes<sup>7</sup>.

In addition to ECG and chest radiography, the use of ambulatory electrocardiogram allows a better understanding of ventricular arrhythmias, detected since the first reports on the cardiac form of Chagas' disease. Although ventricular arrhythmias are associated with higher incidence of sudden death<sup>8</sup>, some characteristics, such as their presence in the different clinical forms of Chagas' disease and their relationship with symptoms and ventricular dysfunction, are not yet well defined and differ from one study to another, depending on the sample population and methodology employed<sup>9</sup>.

Echocardiograms are helpful in that they make it possible to diagnose and follow heart involvement, particularly ventricular dysfunction and, especially, systolic ventricular dysfunction, known to be the main prognostic factor of chagasic cardiomyopathy<sup>10,11</sup>. The prognostic value of the diastolic ventricular dysfunction that usually precedes systolic dysfunction is yet to be defined. Novel methods, such as tissue Doppler imaging to quantify myocardial flow velocity, have shown to be invaluable tools, because they are more sensitive in detecting pseudonormal patterns of diastolic dysfunction than pulsed Doppler mitral flow<sup>12,13</sup>.

Measurement of brain natriuretic peptide (BNP), also referred to as B-type natriuretic peptide, is useful in the diagnosis of left ventricular systolic and diastolic dysfunctions<sup>14,15</sup>. The BNP is a hormonally active peptide secreted by the heart ventricles, and its plasma concentrations increase according to the severity of the heart failure (HF) based on the New York Heart Association classification<sup>16</sup>. Few studies have been conducted to evaluate this peptide plasma levels in Chagas' disease patients<sup>17,18</sup>.

We aimed at prospectively evaluating ventricular arrhythmia and left ventricular dysfunction prevalence, as well as BNP plasma levels, in asymptomatic patients with chronic Chagas' disease and abnormal ECG, divided into two groups: GIIA (ECG with changes characteristic of Chagas' disease) and GIIB (ECG with changes non-characteristic of Chagas's disease), compared with a group of chagasic patients with normal ECG (GI).

## METHODS

In this prospective, cross-sectional study approved by the institution's Research Ethics Committee, 329 patients seen at the Chagas' disease outpatient clinic from April to November 2002 and confirmed seropositive for *Trypanosoma cruzi* infection by at least two of three different techniques (indirect hemagglutination, indirect immunofluorescence, and ELISA) were evaluated. All patients underwent history taking and physical examination after the following exclusion criteria were applied: 1) younger than 18 or older than 50; 2) symptoms suggestive of heart failure<sup>19</sup> and/or arrhythmia;<sup>20</sup> 3) arterial hypertension – defined as previous history, use of antihypertensive drugs or, at the time of inclusion, diastolic blood pressure (DBP) > 89 and/or systolic blood pressure (SBP) > 139;<sup>21</sup> 4) diabetes mellitus – defined as fasting glucose  $\geq$  126 mg/dL or regular use of hypoglycemic agents;<sup>22</sup> 5) history of chronic obstructive pulmonary disease; (6) endocrine dysfunctions; 7) heart disease of other etiologies; 8) pregnancy at the time of inclusion. One hundred and six patients were selected and, after being fully informed about the purpose of the study, they signed an informed consent.

After electrocardiogram (ECG) analysis, patients were distributed into three different groups: 1) GI – patients with normal ECG; 2) GIIA – patients with characteristic ECG changes (atrioventricular blocks, complete right bundle branch block, and/or left anterior hemiblock in the His bundle; electrically inactive areas; sinus bradycardia accompanied by ventricular extrasystoles or primary changes in ventricular repolarization);<sup>23</sup> and 3) GIIB – patients with ECG changes non-characteristic of Chagas' disease.

Next, the following examinations were performed: a) Chest radiography and cardiothoracic index measurement (CTI) according to the technique already described<sup>24</sup>; b) A 24-hour ambulatory electrocardiogram using a two-channel portable tape recorder Dynamis 4000 (Cardios Sistemas, São Paulo, Brazil) and analysis using Premier 4 holter software (Diagnostic Monitoring Software, Nevada, USA). Evaluation included the presence and number of ventricular extrasystoles (VES) in 24 hours: sustained ventricular tachycardia (SVT), defined as three or more consecutive ventricular ectopies lasting 30 seconds or longer, and nonsustained ventricular tachycardia (NSVT), defined as three or more consecutive ventricular ectopies lasting less than 30 seconds<sup>25</sup>; c) Echocardiogram using a "Vivid 3" echocardiograph (GE Medical Systems, Milwaukee, EUA), following the American Society of Echocardiography criteria.

The following parameters were analyzed: left ventricular ejection fraction (LVEF) assessed by Simpson's biplane method, with a normal value of 50% or more<sup>26</sup>; regional left ventricular contraction, using the 16-segment model recommended by the American Society of Echocardiography and classified as normal and abnormal (hypokinetic, akinetic or dyskinetic)<sup>27</sup>; E' wave (peak velocity of the mitral annulus motion during early diastole) to A' wave (peak velocity of the mitral annulus motion during end-diastole) ratio (E'/A') using tissue Doppler imaging of mitral annulus motion on the septal wall in the apical four-chamber view,

with normal value  $\geq 1^{28}$ . Ventricular dysfunction was considered in the presence of left ventricular systolic and/or diastolic dysfunction. Ventricular systolic dysfunction was defined in the presence of global and/or regional ventricular dysfunction. Global ventricular systolic dysfunction was defined in the presence of LVEF  $< 50\%$ . Diastolic ventricular dysfunction was defined in the presence of  $E' / A' < 1$ .

d) BNP: five-mL blood samples were collected and drawn into tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. After centrifugation, plasma was separated and stored at  $-20^{\circ}\text{C}$ . The Triage® BNP fluorescence immunoassay test was used for quantitative measurement (Biosite, San Diego, USA) after the plasma sample was equilibrated to room temperature and mixed thoroughly.

Statistical analyses were performed using Epi Info 6 version 6.04 and SAS – Statistical Analysis System version 6.11. Continuous variables were expressed as mean and standard deviation (SD), and categorical variables were expressed as absolute and relative frequencies. The chi-square test or Fisher's exact test was performed to test the groups' homogeneity relative to proportions. One-factor analysis of variance was used for the hypothesis of equality among the three groups, with the multiple comparison procedure employing the Bonferroni method. When the assumption of data normality was rejected, the Kruskal-Wallis non-parametric test was applied, using Dunn's method of multiple comparisons. The significance level was set at 5%.

## RESULTS

The most frequent ECG changes found in the GIIA group were right bundle branch block and left anterior hemiblock, with 35% each, followed by electrically inactive areas in 32%. In the GIIB group they were inferolateral repolarization changes and left ventricular overload, with 28% and 24%, respectively.

Mean CTI values were similar among the groups. GI,  $0.41 \pm 0.05$ ; GIIA,  $0.43 \pm 0.05$ ; and GIIB,  $0.43 \pm 0.05$  ( $p = 0.383$ ).

Among the 106 patients, 102 had ambulatory electrocardiograms that could be analyzed: 48 (96%) in

the GI group, 30 in the GIIA group (97%), and 24 (96%) in the GIIB group. Ventricular arrhythmia prevalence was similar between groups with ECG changes (GIIA e GIIB) and significantly higher than in the group with normal ECG (GI) (Fig. 1).

Mean ventricular extrasystole in the 24 hours (24H/VES) was significantly higher in the GIIA group ( $1138.00 \pm 2779$ ) than in the GI ( $51.68 \pm 161$ ) and GIIB groups ( $115.40 \pm 297$ ) ( $p < 0.05$ ). Six study participants experienced NSVT, one (2%) from the GI group and five (16.6%) from the GIIA group.

Ventricular dysfunction was more prevalent in the GIIA and GIIB groups and significantly higher than in the GI group (Figure 2). In the univariate analysis of relative risk (RR) a positive association between ventricular dysfunction and the GIIA and GIIB groups was found compared to the GI group. Patients in the GIIA group showed a positive RR of 3.69 (95% CI = 1.71-7.94;  $p < 0.001$ ), and patients in the GIIB group showed a positive RR of 2.72 (95% CI = 1.13-6.54;  $p = 0.024$ ).

Ventricular systolic dysfunction (SD) was found in one patient (2%) of the GI group, 9 patients (29%) of the GIIA group, and 5 patients (20%) of the GIIB group ( $p < 0.001$ ) (Fig. 3).

No patient in the GI group showed global systolic dysfunction, found in four patients (13%) of the GIIA group and two patients (8%) of the GIIB group ( $p = 0.020$ ).

Twelve patients (11.3%) showed regional systolic dysfunction: one patient (2%) of the GI group, seven patients (23%) of the GIIA group and four patients (16%) of the GIIB group ( $p = 0.005$ ). In the GI group, regional systolic dysfunction was located in the apical region. Figure 4 shows the absolute frequency and site of regional systolic dysfunction in the GIIA and GIIB groups. The apical region was the most affected.

Diastolic dysfunction prevalence was similar in the GIIA and GIIB groups and significantly higher than in the GI group ( $p = 0.009$ ) (Fig. 5).

No statistically significant difference was found when mean BNP plasma level was compared among the groups ( $p = 0.121$ ).

Descriptive analysis of BNP values in the groups studied is shown in Table 2.

**Table 1 – Clinical and demographic variables of 106 patients with chronic Chagas' disease**

Groups (N)	GI (50)	GIIA (31)	GIIB (25)	p*
<b>Variable</b>				
Age (M $\pm$ SD) (years)	41 $\pm$ 5.6	43 $\pm$ 4.6	42 $\pm$ 6.8	0.429
(Male gender [N/%])	21 (42%)	19 (61%)	11 (44%)	0.215
HR (bpm)	62 $\pm$ 7.1	63 $\pm$ 10.1	63 $\pm$ 12.5	0.852
SBP (mmHg)	115.7 $\pm$ 12.0	117.4 $\pm$ 11.4	118.2 $\pm$ 12.0	0.645
DBP (mmHg)	75.8 $\pm$ 6.6	77.1 $\pm$ 8.4	77.2 $\pm$ 6.5	0.623
BMI (Kg/m <sup>2</sup> )	26.5 $\pm$ 4.7	26.8 $\pm$ 4.6	26.8 $\pm$ 4.6	0.797
<i>M <math>\pm</math> SD- mean <math>\pm</math> standard deviation; HR- heart rate; bpm- beats per minute; SBP- systolic blood pressure; DBP- diastolic blood pressure; BMI- body mass index. P*- One-factor analysis of variance; chi-square or Fisher's exact test.</i>				

**Table 2 – Distribution of B-type natriuretic peptide (BNP) plasma levels in pg/ml according to the groups studied**

Groups (N)	M ± SD median percentiles minimum maximum (25%-75%)
GI (50)	30.28 ± 88.4 8.3 5.0 – 12.9 5.0 460.0
GIIA (31)	65.65 ± 193.9 12.3 5.0 – 20.1 5.0 946.0
GIIB (25)	24.15 ± 82.4 5.0 5.0 - 10.3 5.0 419.0

Variable analyzed using log transformation; One-factor analysis of variance –  $p = 0.121$ ; N- number of participants in each group M ± SD mean ± standard deviation, pg/mL- picograms per milliliter.

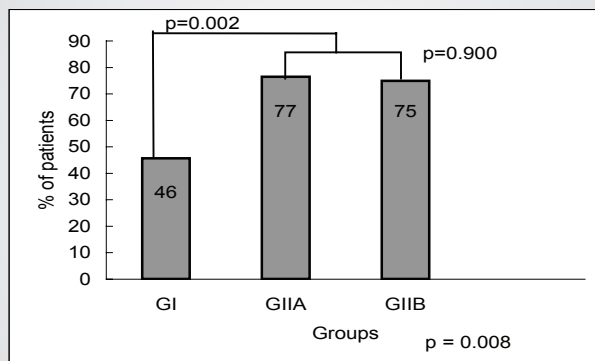


Fig. 1 – 24-hour ambulatory electrocardiogram analysis of ventricular arrhythmia prevalence, according to the groups studied.

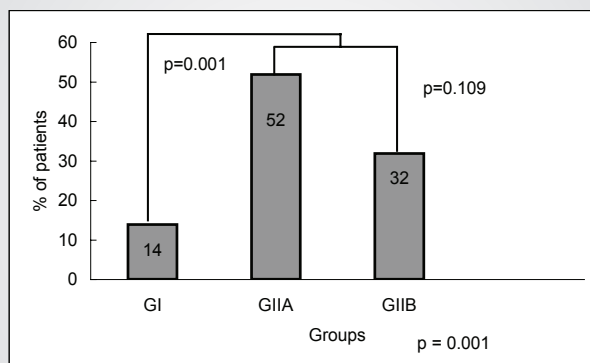


Fig. 2 – Ventricular dysfunction prevalence by echocardiogram according to the groups studied.

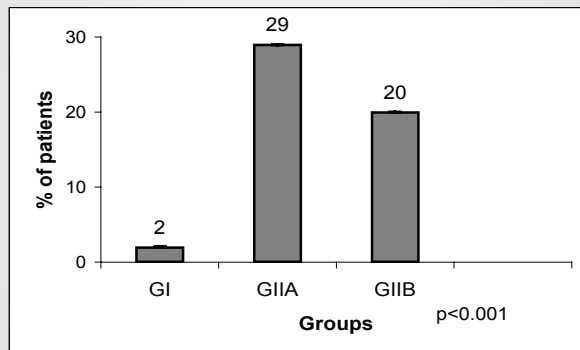


Fig. 3 – Systolic ventricular dysfunction prevalence by echocardiogram according to the groups studied.

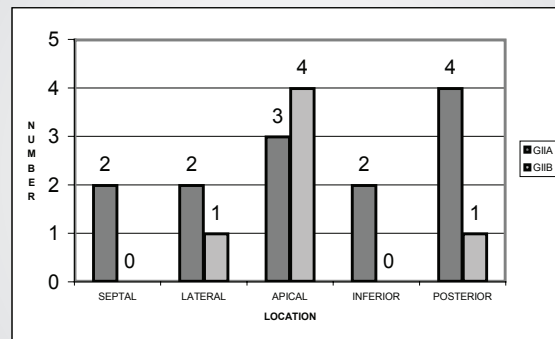


Fig. 4 – Distribution of absolute frequency and site of regional systolic dysfunctions in the GIIA and GIIIB groups.

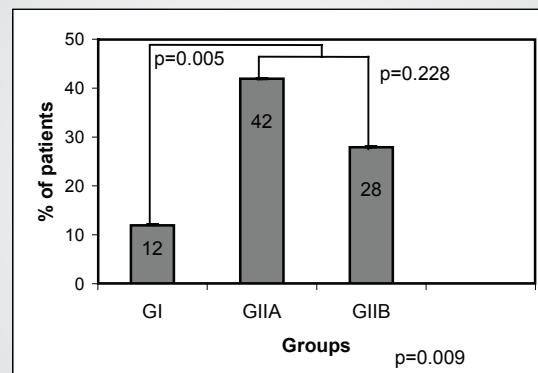


Fig. 5 – Prevalence of diastolic ventricular dysfunction by echocardiogram according to the groups studied.

## DISCUSSION

Current challenges in the management of infected patients are early diagnosis and halting the progression of Chagas's disease clinical forms in the chronic phase, indicating the appropriate treatment and preventing complications<sup>29</sup>.

This study, in which asymptomatic patients with no previous diagnosis of heart disease were assessed, sought to evaluate the relationship between ECG changes and prevalence of arrhythmias, ventricular dysfunction and BNP plasma concentrations. The starting point in this evaluation was electrocardiographic changes analysis, because, despite all technological advances, ECG remains the basic examination required in the baseline evaluation of Chagas' disease patients, and may be the only complementary propaedeutic in most healthcare services, especially in poverty-stricken regions where there is usually a greater predominance of infected patients.

The usefulness of ECG in identifying systolic ventricular dysfunction leading to heart failure was evaluated by Davie et al<sup>30</sup>, who observed a 35% positive predictive value and a 98% negative predictive value for the diagnosis of systolic ventricular dysfunction in patients with abnormal ECG. Studying ECG abnormalities and their

relationship with ventricular systolic function (ventricular volumes and EF by angiography) in Chagas' disease, Casado et al<sup>31</sup> found a relationship between decreased left ventricular systolic function and the progression and association of ECG abnormalities.

In this study, prevalence of ventricular arrhythmias and ventricular dysfunction was found to be similar in the abnormal-ECG groups and significantly higher than in the normal-ECG group. Carrasco et al<sup>32</sup> analyzed four groups of patients - with normal ECG, with normal ECG and regional segmental dysfunction, with abnormal ECG and global ventricular dysfunction, and with abnormal ECG and heart failure - and found prevalence of ventricular arrhythmia in 30%, 53%, 86%, and 99% of these patients, respectively. Rassi Junior et al<sup>9</sup> found prevalence of 85% in the normal population, 74% in the indeterminate form (IF) and 88% in the cardiac forms, but did not specify whether they were symptomatic or asymptomatic.

When VES was analyzed in the three groups, mean VES in 24 hours was significantly higher in the GIIA group than in the GIIIB and GI groups. Similar VES incidence, number and complexity were found in literature among individuals in the indeterminate form and individuals with no heart disease. As arrhythmia occurrence increases proportionally to the degree of involvement

on resting ECG, the VESs are less frequent in patients with chronic Chagas' disease and minimal ECG changes and more frequent and complex when the patient shows characteristic changes, especially when associated with left ventricular dysfunction<sup>33</sup>.

NSVT prevalence was 16.6% in the GIIA group and 2% in the GI group, lower than those observed by Rassi Junior et al<sup>9</sup>, who found 42% in the chagasic group with characteristic ECG, 16% in patients with non-characteristic ECG, and 1% among the IF patients.

In our study, univariate analysis showed that the relative risk (RR) of experiencing ventricular dysfunction is 3.69-fold in patients with electrocardiographic changes characteristic of Chagas's disease (GIIA) and 2.72-fold in patients with electrocardiographic changes non-characteristic of Chagas's disease (GIIB) than in patients with normal ECG (GI). Six patients showed global ventricular systolic dysfunction, characterized by LVEF > 50%, four in the GIIA group and two in the GIIB group. The LVEF was lower than 40% in two patients (6.5%) of the GIIA group.

Ventricular systolic dysfunction, present in 2% to 4% of the general population, is the anatomical substrate for around two-thirds of patients with heart failure (HF)<sup>34</sup>.

Mady et al<sup>11</sup> evaluated patients with HF in NYHA functional class II or III secondary to Chagas' disease and found that LVEF is an important index for survival analysis in these groups. According to literature, systolic dysfunction in Chagas' disease, measured by echo, varies depending on the clinical form of the disease and the methodology followed in the many studies. In regard to the indeterminate form, reports range from normal ventricular systolic function<sup>35</sup> to significant increases in LV dimension and volume and decrease in EF compared to a control group<sup>36</sup>. Ianni et al<sup>37</sup> compared the IF in patients with minimal ECG changes and found no significant difference between groups regarding LV fractional shortening. However, Pereira-Barretto et al.<sup>38</sup> found a 4.5% incidence of depressed ventricular function in patients with normal ECG and 45.7% and 66% of ventricular dysfunction in patients with abnormal ECG, depending on the electrocardiographic change. Perez et al<sup>39</sup> reported a 29% incidence of global ventricular dysfunction in a non-selected group of chagasic patients.

Regional systolic dysfunction was more frequent in the GIIA group than in the GIIB and GI groups (23%, 16% and 2%, respectively). The apical region was the most affected in all patients. Segmental changes in contractility are found in up to 74% of chagasic patients and are typical of Chagas' disease, especially when located in the apical region, in the absence of ischemic heart disease<sup>40</sup>. Aparício et al<sup>41</sup> found 12% of interventricular septum hypokinesia in asymptomatic patients with normal ECG measured by M-mode ECHO. In the IF, Ortiz et al<sup>42</sup> found a 26% segmental change incidence, restricted to the posterior apical region. Câmara<sup>43</sup> evaluated patients with abnormal ECG and found a 68% segmental change frequency, 64% of them in the apical region, and also the presence of apical aneurysm in 42% of the patients.

Diastolic dysfunction was more common than systolic dysfunction, and was significantly more frequent in the GIIA group (41,9%) than in the other groups.

Tissue Doppler was used, because in addition to being more sensitive in detecting diastolic dysfunction than the classic method of transmittal flow analysis by pulsed Doppler, it differentiates the pseudonormal pattern<sup>12,28</sup>. It is known that transmitral flow is affected by a host of factors, among them left atrial pressure. Increases in the left atrial pressure above blood pressure increase as the result of ventricular relaxation often lead to a pseudonormalization of the transmittal flow.

Diastolic dysfunction in the non-selected general population is more prevalent than systolic dysfunction, about 11%<sup>44</sup>. In chagasic patients, however, it ranges from 27.6% to 83%, according to the evaluation method employed and the clinical form of the disease<sup>45</sup>. Early studies on diastolic dysfunction in Chagas' disease through non-invasive methods used M-mode ECHO associated with phonocardiogram and apexcardiogram followed by two-dimensional echocardiogram coupled with Doppler flow studies and, finally, tissue Doppler. Regardless of the method used, evidence exists that diastolic dysfunction is common and may be present in asymptomatic patients, preceding systolic dysfunction; and its prevalence increases according to the progression of chronic Chagas cardiomyopathy<sup>12,43,45,46</sup>.

Natriuretic peptides are neurohormones produced by the heart cells with natriuretic, diuretic and vasodilatory properties<sup>16</sup>.

Studies suggest that serum or plasma BNP measurements may be useful to confirm HF diagnosis, assess the degree of left ventricle involvement, quantify the functional class, estimate the prognosis and anticipate cardiac events, and evaluate the therapeutic efficacy in HF<sup>15,47-49</sup>.

In this study, patients were asymptomatic at the time of inclusion and were divided according to the ECG changes found. The highest mean BNP plasma level was found in the GIIA group, compared with the GI and GIIB groups, although the difference did not achieve statistical significance.

In published studies evaluating Chagas' disease patients, studied population was classified according to the degree of LV systolic function involvement and compared with a control group, regardless of the presence of ECG changes<sup>17,18</sup>.

Ribeiro et al<sup>17</sup> found similar mean BNP values between the control group and patients with LVEF > 40%, and significantly lower than those of patients with LVEF < 40%. Setting the cut-off point at 60.7 pmol/L, a sensitivity of 80% and specificity of 93% were found for LVEF < 40% in patients with abnormal ECG ou CTI. Walther et al<sup>18</sup>, in a similar study, found significant lower values in the control group than in the group of chagasic patients even with LVEF > 40%. Using a cut-off point of 18.1 pmol/L, the sensibility was 86% and the specificity was 91% for the diagnosis of patients with LVEF < 40%.

In the present study, results might have been affected by the sample size and criteria that allowed the inclusion only of asymptomatic patients with low frequency of more marked ventricular systolic dysfunction. The two patients with LVEF < 40% showed the highest BNP plasma levels: 589 pg/mL and 946 pg/mL.

Another peculiarity was that, in four patients (all female), BNP levels were higher than 100 pg/mL, and these women showed no ventricular dysfunction; and in four patients with slight ventricular dysfunction, BNP levels were lower than 100 pg/mL. Of these, three were male and one was female. Previous studies showed that BNP levels are affected by gender both in normal population and HF population. The female gender is associated with higher BNP levels than the male gender<sup>50</sup>.

All patients with global systolic dysfunction received standard drug treatment with captopril at the maximum tolerated dose, and for two patients carvedilol was also associated. Three patients who experienced ventricular dysfunction and NSVT were treated also with amiodarone.

Our results demonstrate that in patients with Chagas' disease and electrocardiographic changes, even those who are asymptomatic, other diagnostic methods such as echocardiogram and 24-hour ambulatory electrocardiogram, in addition to standard electrocardiogram and chest radiography, play key roles in the initial evaluation. Study limitations include the small number of patients due to the strict inclusion and exclusion criteria, absence of a control group of non-infected individuals, and lack of follow-up.

As Chagas' disease is a slow-progressing disease and patients enrolled in this study will remain under follow-up, it is believed that, after an adequate period, some pending issues may be clarified. Among them, the long-term evolutive aspects of Chagas' disease patients and non-characteristic changes in ECG, and the prognostic value of diastolic ventricular dysfunction and BNP levels in the groups studied.

#### Potencial Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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