Autonomic Dysfunction and Anti-M2 and Anti- β1 Receptor Antibodies in Chagas Disease Patients

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Abstract

Background: Sudden death is the leading cause of death in Chagas' disease, affecting patients even in the early stages of the disease. The impairment of the autonomic nervous system in this disease has been recognized, as well as its potential as a trigger for malignant arrhythmias when associated with structural or metabolic changes.

Objective: We sought to identify, in Chagas patients with preserved systolic function, the impairment of the autonomic nervous system and its association with functionally active anti-m2 and anti-β1 receptor antibodies.

Methods: Using spectral analysis of RR variability during passive tilt test, chronic chagasic patients were compared with healthy controls matched for age. Subsequently, the association of autonomic dysfunction with functionally active antibodies with anti-m2 and anti-β1 action was investigated by the Langendorf method.

Results: We observed that patients with Chagas disease without ventricular dysfunction express parasympathetic activity against a vagal stimulus, however with less intensity compared to controls. Chagasic patients with anti-m2 or anti-β1 antibodies showed a further significant reduction of the vagal response during respiratory sinus arrhythmia, regardless of the presence of structural lesion. However, the association of both factors promoted response to vagal stimulation similar to that seen in Chagas disease without their presence.

Conclusion: The lower vagal reserve in Chagas patients with preserved function was associated with functionally active anti-m2 or anti-β1 antibodies, and not with the presence of structural heart lesion. (Arq Bras Cardiol 2012;99(2):732-739)

Keywords: Chagas disease; autonomic denervation; antibodies.

Introduction

One hundred years after its description by Carlos Chagas in 1909, Chagas Disease (CD) remains a challenge to Health Organizations. According to the World Health Organization (WHO), although the vector transmission has been interrupted in countries such as Brazil, Chile and Uruguay, the prevalence of patients with chronic Chagas disease was estimated at 18 to 20 million individuals in Latin America, with 300,000 new cases each year and 50,000 deaths a year associated with the disease[1]. In Brazil, chronic disease cases currently predominate, with approximately three million infected individuals[2].

There is not enough evidence to support the need for immediate treatment in all chronic chagasic patients with preserved function, although there are studies suggesting an increase in morbidity and mortality in this group of patients[3-11]. A recent review has suggested that in patients with this form of heart disease (ECG alterations and regional alterations in the echocardiogram, but with preserved ventricular function), the need for further evaluation should be judged on an individual basis[11]. The I Latin American Guidelines for the diagnosis and treatment of Chagasic cardiopathy, recently published, also recognizes the existence of this group of patients and its prognostic value for sudden death[12].

In recent decades, several studies have elucidated an association between the Autonomic Nervous System (ANS) and immune function, and have helped to disclose some mechanisms involved in this interaction. Our group has had active participation in relation to chronic chagasic cardiopathy[13-18]. It is possible that functionally active circulating antibodies against G protein-coupled receptors, capable of interfering with receptors of both, parasympathetic and sympathetic systems, can physiologically affect the cardiac autonomic behavior and modulate electrophysiological properties involved in mechanisms of complex ventricular arrhythmias[18-20]. These

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antibodies are not just epiphenomenon of the destruction of the autonomic nervous system and are independent from cardiac lesions[14,20-23]. We recently showed that in chagasic patients with preserved systolic function, cardiac sympathetic autonomic modulation is affected. This was demonstrated by cardiac nerve destruction that occurred in the ventricular context demonstrated by ^{123}I-MIBG[23].

Studies of RR variability (RRV) have been established as a real and reproducible marker of cardiovascular autonomic function[24]. Among the maneuvers to assess the parasympathetic response, the Respiratory Sinus Arrhythmia (RSA) has been shown to be the most potent stimulus to evoke vagus nerve action[25,26]. In Chagas disease, in the group with no ventricular dysfunction, the evaluation of the autonomic nervous system through the RRV by different methods has shown variable impairment of parasympathetic and sympathetic modulation[27-30]. The present study aims to identify, in patients with Chagas disease without ventricular dysfunction, those with alterations in vagal autonomic modulation on the RRV in response to the RSA and at the physiological sympathetic stimulation maneuver (passive tilt at 70°), and correlate it to the presence of functionally active antibodies against cardiac muscarinic m2 and adrenergic β1 receptors.

Methods

This is a case series study carried out between October/2009 and August/2010. Patient selection was performed at the chagasic cardiopathy outpatient clinic of Hospital Universitário Clementino Fraga Filho - UFRJ / HUCFF. Of 310 regularly monitored individuals, 76 patients whose sera were characterized for the presence of functionally active antibodies against G-protein coupled receptors (anti-m2 and/or anti-β1) were selected. The study excluded 35 patients with chronic diseases, especially cardiovascular ones; those with ejection fraction (EF) < 50%; frequent cardiac arrhythmias and/or non-sinus rhythms; diabetes mellitus, Parkinson's disease or any neuropathy; patients on medications that could interfere with the cardiovascular system; chronic obstructive pulmonary disease, kidney or thyroid dysfunction, pregnancy, alcoholism, smoking and those using steroid drugs.

The remaining 41 patients (17 men, aged between 26 and 75 years) with a definite diagnosis of Chagas disease (two different serological tests with positive reaction to *T. cruzi*) had normal history, physical examination and the following tests: CBC, thyroid-stimulating hormone (TSH), free-T4, glucose, potassium, urea and creatinine. All patients had 24-hour Holter monitoring free of frequent arrhythmias, normal or abnormal echocardiography (ECHO) (with abnormal ECHO understood as the presence of any detectable segmental alteration, but with EF > 50%), normal or abnormal 12-lead electrocardiogram (ECG) (with abnormal ECG defined by the presence of sinus bradycardia - HR < 50 bpm, complete or incomplete block of any branch, any degree of atrioventricular block, monomorphic or polymorphic ventricular extrasystoles, electrically inactive area and nonsustained ventricular tachycardia); normal chest x-ray and absence of myocardial ischemia and/or stress-induced ventricular arrhythmias during exercise test.

Forty-four healthy volunteers (nine men, aged 15-75 years) were controls. All controls and chagasic patients assessed were in good physical and mental shape, had no history of sedentary lifestyle and performed regular daily activities, without any drugs. Chagasic patients did not receive specific anti-*T. cruzi* therapy. The study was approved by the Ethics Committee in Research (ECR) of the institution under No. 233/09 and all patients signed an informed consent form in accordance with current Brazilian law and the rules of the ECR of HUCFF-UFRJ.

Both groups were evaluated for RRV through passive tilt test, in an air-conditioned and soundproof room, after fasting for four hours.

The test was performed in three stages - RSA, rest and passive tilt at 70°. The first step was to inhale and exhale at 12 breaths/minute for two minutes between the 10th and 12th minutes. The second step was performed at rest - in which the RRV was analyzed without any stimulus, between the 15th and 20th minutes. The third and last phase began in the 20th minute, when patients were passively tilted at 70° and the complexes were evaluated until the 25th minute[26].

The RRV was analyzed in the frequency domain. We evaluated the High Frequency (HF) in percentage terms - which represents the vagal component of the cardiovascular autonomic nervous system, and the low/high ratio (L/H ratio) - which expresses the balance between the sympathetic and parasympathetic components. The high frequency and the low frequency were measured in normalized units that indicate the relative value of each component relative to the total power of the spectrum. The vagal reserve was assessed by the increase in HF in percentage terms during the RSA, comparing it to rest. The vagal stimulation in the presence of the sympathetic stimulus was calculated by the increase in the L/H ratio in the first five minutes after passive inclination, compared with rest - in which great differences express a better response.

The presence of functionally active circulating antibodies against G-protein coupled cardiac receptors (anti-M2 and anti-β1) was investigated only in the serum of patients with Chagas disease using the Langendorf method in isolated rabbit hearts[13]. The presence of anti-M2 was defined when a 10% reduction in the sinus heart rate occurred *in vitro*, as compared to control or some degree of intra-atrial or atrioventricular block reversed after atropine addition. The presence of anti-β1 was defined when a 5% increase in sinus HR occurred in vitro compared to control, reversed after addition of propranolol[19,31].

The separation of the IgG fraction with adrenergic and muscarinic action was performed by affinity column purification. The IgG fraction was separated from the serum of patients by precipitation in $(NH_4)\ 2SO_4$ at 50% and extensive dialysis against PBS, pH 7.4, during 24 hours at 4 °C. Immunoglobulin thus prepared was loaded onto a column of Sepharose 4B gel activated with cyanogen bromide (CNBr) (Pharmacia), to which the peptide corresponding to the second extracellular loop of the receptor (m2 or β1) was covalently bonded. After washing with PBS, the fraction of the immunoglobulin that bound to the column was eluted with 3M potassium thiocyanate (pH 7.4), quickly followed by extensive dialysis against PBS. This fraction thus obtained was used in the isolated rabbit heart to characterize the serum, following the methodology described above[13].

Statistical analysis

Statistical analysis was performed using the Stata software (release 8.0, 2003) and CIA software. Medians and their respective interquartile values were reported due to the asymmetrical distribution of data. Variables were compared by Student's *t* test. We also present the 95% confidence interval for the different medians. A p value < 0.05 was considered significant for all analyses in the study.

Results

We evaluated 41 patients with Chagas disease (17 men and 24 women) and 44 controls (9 men and 35 women). In the chagasic group, age ranged between 26 and 75 years, median of 57 years and in the control group it ranged between 15 and 75 years, with a median of 38 years. The level of physical activity was similar between groups. Chagasic patients had a mean BMI of 23.3, mean hemoglobin of 13 g/dL and mean leukocyte count of 12.9 mil/mm^3. All had TSH levels within the normal range, with a mean value of 2.92 mIU/L.

The most common findings observed in the electrocardiogram and transthoracic echocardiogram of patients with Chagas disease are shown in Table 1. Only two patients (4.9%) had mild dysfunction - the mean ejection fraction was 64.9% (Teicholz). All control patients had normal ECG. Echocardiography was not performed in the control group.

The assessment of parasympathetic activity through the HF component during RSA demonstrated that chagasic patients with preserved LV function are able to express parasympathetic activity during the RSA maneuver, with resting as the basal state (Figure 1). However, when comparing chagasic patients with controls, the first expressed significantly lower parasympathetic activity when compared to the latter ($p = 0.00002$), expressing a reduced vagal reserve (Table 2). In chagasic group during passive tilt at 70°, there was an increase in the L/H ratio in relation to rest that was slightly higher than the one observed in the controls ($p = 0.01$), with higher dispersion values as shown in Figure 2. These data show that parasympathetic dysfunction is also expressed in the slope.

According to Figure 3, chagasic patients were analyzed according to the presence of G protein-coupled antibodies (anti-M2 and anti-β1) and divided into four groups: no antibodies (n = 11), presence of anti-M2 alone (n =2) presence of anti-M2 associated with anti-β1 (n = 8) and, finally, the presence of anti-β1 alone (n = 19). We observed that patients who had only anti-m2 or anti-β1 showed lower vagal modulation during RSA. However, when associated, they promoted an increase in the vagal response, when compared to patients with no antibodies.

Regardless of the presence of electrocardiographic alterations, we found a similar response regarding the RR variability parameters observed (Table 3). Similarly, the coefficient of contingency between abnormal ECG and identification of anti-M2 antibodies was 0.1388, indicating that there is no association between variables.

Discussion

In agreement with previous studies that addressed chronic Chagas patients with preserved ventricular function, this study shows that, whereas there is still parasympathetic activity evoked by respiratory sinus arrhythmia in this group of patients, they have lower vagal reserve in relation to controls[27-29,32]. This reduction in vagal reserve was not accompanied by changes in basal heart rate of the patient. The most important and original result was the observation that this reduction was associated with the presence of protein G-coupled functionally active antibodies - particularly anti-β1 antibodies. This finding was independent from the presence of structural heart damage, suggesting that the vagal modulation can be subject to the presence antibodies.

Although subject to extensive research, the cause of this autonomic dysfunction in chronic chagasic myocarditis has yet to be clearly established. Several studies have shown that the inflammatory process is directly related to the intensity of the tissue immune response [33,34]. In turn, the association between the parasympathetic autonomic nervous system and immune regulation process was suggested over 30 years ago, when the T-lymphocyte cytotoxicity attenuation by muscarinic cholinergic stimulation was observed[35]. Afferent and efferent signaling pathways that integrate a reflex arc were described, the "neuroimmunological" or "inflammatory" reflex. Thus, the two systems (nervous and immune systems) can communicate bidirectionally using cytokines and neurotransmitters common to both systems as information mediators.

Table 1 - Electrocardiographic and echocardiographic alterations in patients with Chagas disease

Variables	Chagasic Group (n=41)	
ECG	Normal	19 (46.3%)
	AVB	5 (12.2%)
	LAHB	8 (19.4%)
	RBBB	4 (9.7%)
	RBBB + LAHB	5 (12.2%)
	AVB + RBBB + LAHB	1 (2.4%)
TTE	Normal	19 (46.3%)
	Relaxation deficit	18 (44%)
	Segmentar alteration	11 (27%)
	Mild dysfunction	2 (4.9%)

ECG: ctrocardiogram / TTE: transthoracic echocardiogram / AVB: atrioventricular block / LAHB: left anterior hemiblock/ RBBB: right bundle-branch block

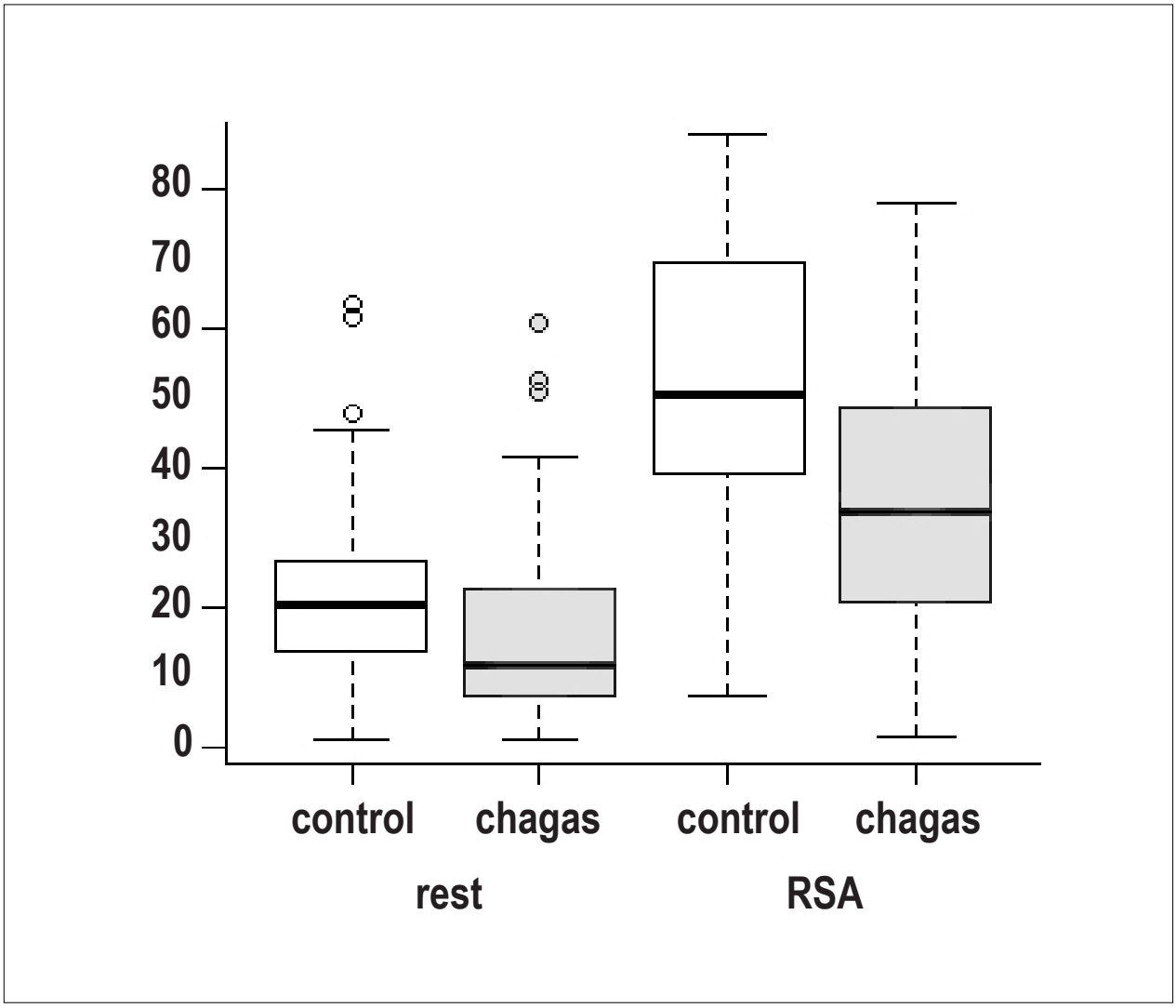

Figure 1 – *Median, interquartile range and extreme values of the spectral analysis of RRV in the domain frequency in chagasic patients and controls - HR during rest and RSA. RRV: RR variability/ HR: high rate / RSA: respiratory sinus arrhythmia*

Table 2 – RR variability data in the frequency domain in Chagasic and normal patients during respiratory sinus arrhythmia, at rest and during tilt testing (median and interquartile range)

Variables	Chagasic (IQ) (n = 41)	Controls (IQ) (n = 44)	p
Mean HR	69 bpm	72 bpm	0.64
HR – ASR (IQ)	33.9% (IQ 20.8 - 48.4%)	50.4% (IQ 38.8 - 69.2%)	0.00002
HR – rest (IQ)	11.9% (IQ 7.6 - 22.6%)	20.5% (IQ 13.7 - 26.5%)	0.16
HR – tilt test (IQ)	4.5% (IQ 1.8 - 16.5%)	9.7% (IQ 3.6 - 19.4%)	0.60
L/H ratio - RSA (IQ)	0.3 (IQ 0.2 - 0.7)	0.2 (IQ 0.1 - 0.3)	0. 0.01
L/H ratio - rest (IQ)	1.8 (IQ 0.9 - 2.9)	2.0 (IQ 1.1 - 2.8)	0. 0.88
L/H ratio - tilt test (IQ)	5.2 (IQ 2.5 - 15.0)	4.1 (IQ 2.1 - 8.2)	0 0.28

HR: heart rate / HR: high rate / RSA: respiratory sinus arrhythmia / IQ: interquartile interval; L/H ratio: low/high ratio

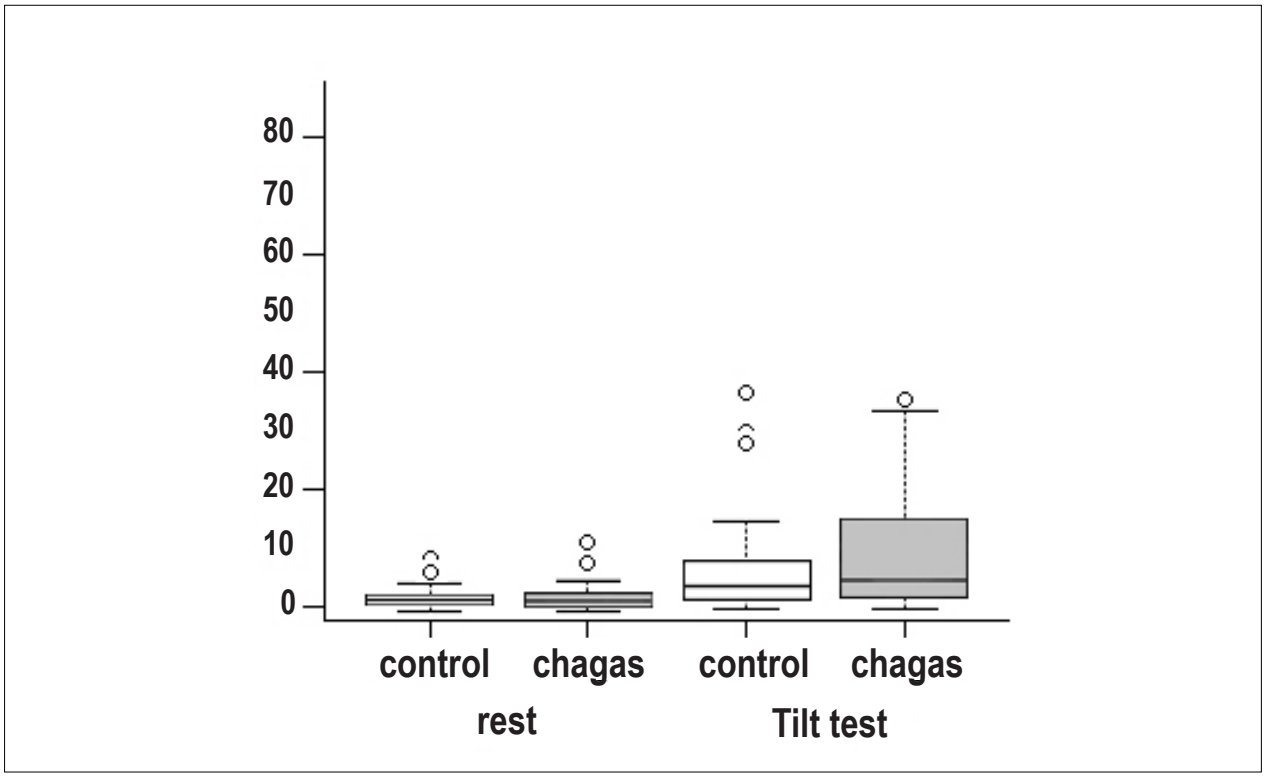

Figure 2 – *Median, interquartile range and extreme values of the spectral analysis of RRV in the domain frequency in chagasic patients and controls – L/H ratio during rest and tilt test. RRV – RR variability/ H/L ratio – high/low ratio*

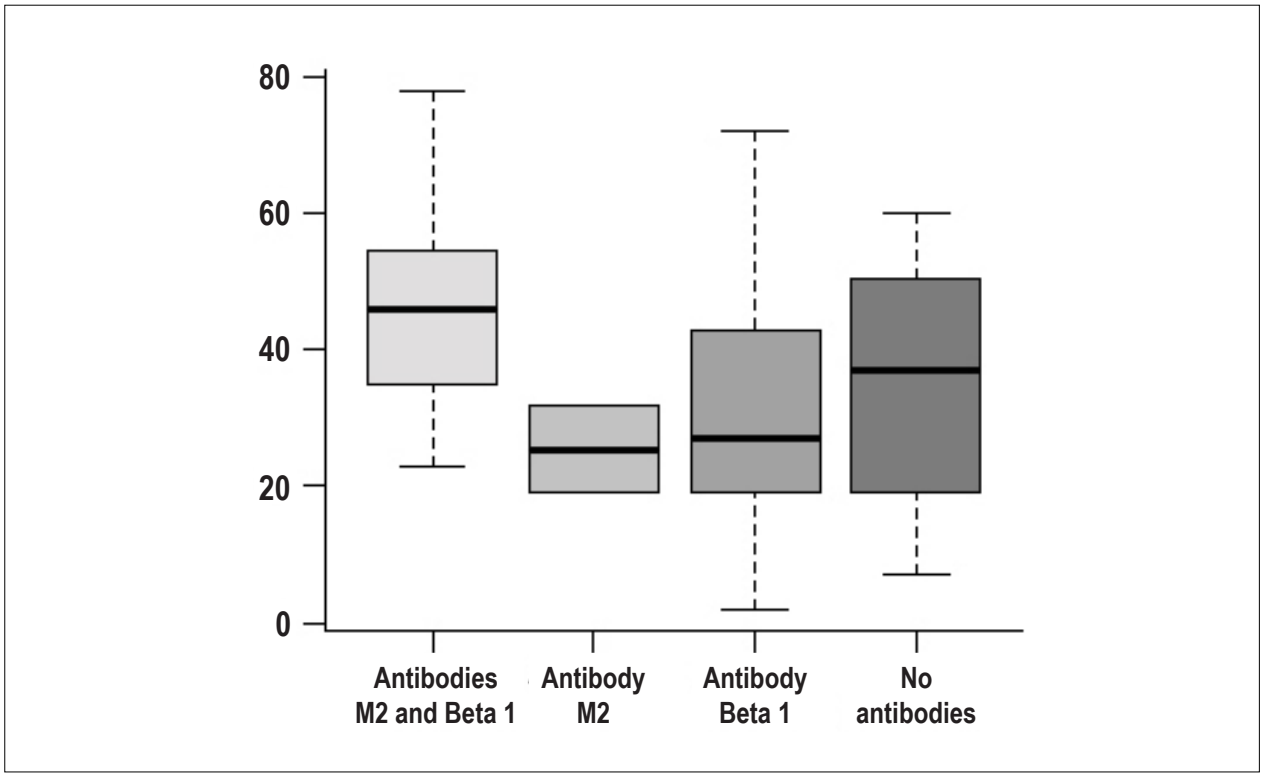

Figure 3 – *Median, interquartile range and extreme values of the spectral analysis of RRV in the domain frequency in chagasic patients according to the type of antibody: HF in RSA / RRV: RR variability/ HR: high rate / RSA: respiratory sinus arrhythmia*

Table 3 – RR variability data in the frequency domain of Chagasic patients with normal and altered ECG during respiratory sinus arrhythmia, at rest and during tilt testing (median and interquartile range)

	Chagas Normal ECG (n = 19)	Chagas Altered ECG (n = 22)	p
HR - RSA	40.5% (IQ 21.2 - 49.8)	33.0% (IQ 21.6 - 37.4)	0.59
HR - rest	13.3%(IQ 7.9 - 22.8)	11.45%(IQ 7.3 - 21.9)	0.74
HR – tilt test	3.9% (IQ 1.5 - 8.7)	10.8% (IQ 3.1 - 22.9)	0.14
L/H ratio - RSA	0.3 (IQ 0.1 - 0.5)	0.5 (IQ 0.2 - 1.2)	0.3167
L/H ratio - rest	1.6 (IQ 1.3 - 3.3)	1.95 (IQ 0.8 - 2.8)	0.32
L/H ratio – tilt test	5.3 (IQ 4.3 - 19.9)	3.2 (IQ 1.4 - 7.5)	0.29

HF: high rate / RSA: respiratory sinus arrhythmia / IQ: interquartile range / L/H ratio: low-high ratio

The efferent pathway of the Central Nervous System (CNS) acts on the immune system (IS) through the parasympathetic nervous system, the so-called anti-inflammatory cholinergic pathway. The parasympathetic nervous system innervates organs of the IS and its mediator acetylcholine acts on immune cells, particularly macrophages, via activation of acetylcholine receptor[36,37]. In fact, the contribution of immune regulation in dysautonomia has been hypothesized even in early studies of this disease due to inappropriate bradycardia present in patients with Chagas disease[38]. One of the possible mechanisms is the cross-reactivity (molecular mimicry)[13].

In this study, we observed that functionally active G-protein coupled circulating antibodies against cardiac receptors (anti-m2 and anti-1), when combined, act positively in parasympathetic modulation, a fact demonstrated by increase in the high frequency component the RRV analysis, supposedly due to an interaction between them. However, the isolated action of both promotes lower vagal modulation, particularly in patients with anti-m2 alone. These data allow us to infer an association between these antibodies and vagal modulation. In earlier studies, our group demonstrated that serum from chronic Chagasic patients with muscarinic action was able to reduce the HR and/or generate atrioventricular conduction disturbances in isolated rabbit hearts[13-18,21]. Recently, Ribeiro et al. showed a correlation between high levels of anti-m2 detected by ELISA and a decrease in the high frequency component at the RRV analysis ($r = -0.32$, $p = 0.023$)[20]. The authors suggest that this vagal dysfunction is caused by the inhibitory effect of anti-m2, based on the desensitization phenomenon.

Another finding in this study is the maintenance of basal heart rate in patients with Chagas disease despite a reduction in vagal reserve. This HR maintenance could not be justified by the lack of desensitization of m2-muscarinic receptors. In other words, the agonist antibodies against cardiac muscarinic receptors alter the vagal modulation (RRV index), but not the vagal tone. In previous studies, we showed that antibodies present in the serum of chronic chagasic patients against m2-muscarinic receptors did not induce receptor desensitization in isolated rabbit hearts[13,14].

The lack of desensitization of the m2 muscarinic receptors is not particularly surprising, as both ligands (acetylcholine and IgG) possibly interact in different locations in the receptors situated on the surface of the sinus node cells.

The mean age in our control group was lower than that found in chagasic patients. This effect of age on autonomic dysfunction in Chagas' disease is yet to be clarified. Resende et al., comparing healthy elderly individuals, young adults and elderly chagasic patients showed no difference in RRV between the elderly in the presence of sympathetic and parasympathetic stimuli. One reason suggested by the authors would be that the senescence alterations would promote a distinct autonomic response, masking the alterations induced by Chagas disease [39].

Most available studies, performed in several independent centers, show that intracardiac nervous system lesions are practically constant, especially the parasympathetic system aggression[39,40]. On the other hand, we know that the abnormal ECG shows infarction. So it is plausible to suppose that the autonomic dysfunction (RRV rates) could be secondary to the structural cardiac injury. In this study, we show that the abnormal ECG was not associated with RRV impairment, making this hypothesis less likely. Villar et al.[25] did not find, among patients with ECG alterations, differences in the HF component at rest. Moreover, Rocha et al.[32] showed that the autonomic modulation (RRV index) is independent from the cardiac ventricular dysfunction.

The presence of functionally active circulating antibodies against G-protein coupled cardiac receptors (anti-m2 and anti-β1) in patients with Chagas' disease is an already established finding. In previous studies, our group and others reported that these antibodies are independent from ventricular dysfunction[13,21,40]. The search for a possible correlation between the presence of functionally active circulating antibodies against cardiac receptors coupled to G protein (anti-m2 and anti-β1) and electrocardiographic alterations was performed using the contingency coefficient. The obtained value of 0.1388 makes it unlikely that the first event justifies the second.

The evaluation of the low/high ratio during the tilt test allows the observation of the sympathetic modulation on the vagus system. Although in absolute terms there is less parasympathetic action during the orthostatic stimulus, as there is often a concomitant involvement of the sympathetic component of the SNA in Chagas' disease, the association between them may not be altered

as they are both decreased[40]. In this study, it was slightly higher in chagasic patients than in controls, a fact possibly explained by the lower vagal reserve (represented by HF that comprises the ratio denominator). *Clinical relevance*: Autonomic dysfunction in Chagas' disease can be involved in the injury-repair mechanism of the heart in different clinical situations. Little is known on the stimulation effects of the anti-inflammatory cholinergic pathway on the modulation of cardiac arrhythmias and sudden death in Chagas' disease. The results from this study can provide important information on the interaction of the autonomic nervous system and its relationship with the immune system through the cholinergic anti-inflammatory pathway, opening a line of research that will establish the pharmacological modulation of vagal activity as a therapeutic target in different models of myocardial injury-repair – with an effect on cardiac arrhythmias and sudden death. However, to date, it has been very much a theoretical hypothesis than a clinical phenomenon, and still needs to be demonstrated.

Conclusion

The lower vagal reserve in Chagas patients with preserved function was associated with the presence of functionally active G-protein coupled antibodies, but not with the presence of structural heart lesion.

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Potential Conflict of Interest

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

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Study Association

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