

## **Sympathetic Autonomic Denervation in Heart Failure: Comparison of Chagas' Heart Disease with other dilated Cardiomyopathy**

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## ABSTRACT

*Introduction: The Chronic Chagas Cardiomyopathy (CCC) is the common cause of non-ischemic heart failure (HF) in Latin America and has a worst prognosis in relation to the others cardiomyopathies, due to high incidence of malignant arrhythmias and sudden death in young adults although the mechanism of worst prognostic is not clear. We hypothesized that cardiac sympathetic dysfunction is more severe in CCC patients explaining the incidence of malignant ventricular arrhythmias and its worst prognosis.*

*Objective: Evaluate sympathetic denervation in CCC versus non-Chagas Chronic Cardiomyopathy (non-CCC) heart failure (HF) patients.*

*Methods: 25 CCC patients (53.3±9.2 years; 17 males) and 25 non-CCC (43.3±12 years; 14 males) HF class II-IV underwent <sup>123</sup>I-MIBG planar imaging (early and late heart/mediastinum ratio (HMR) and washout cardiac rate (Wc%) to evaluate cardiac sympathetic dysfunction.*

*Results: Early and late HMR were 1.73±0.24 and 1.58±0.27, respectively, in CCC patients and 1.62±0.21 and 1.44±0.16 in non-CCC patients (p=NS). Wc% was 41.65±21.41% in CCC and 47.37±14.19% in non-CCC patients (p=0.125). There was a positive correlation between late HMR and LVEF in CCC and between early and late HMR and LVEF in non-CCC patients but no correlation between Wc% and LVEF.*

*Conclusion: These data suggested that there is sympathetic denervation in HF patients independent of its etiology and not worst in CCC than in non-CCC patients.*

**Key Words:** Chronic Chagas Cardiomyopathy; Cardiac Sympathetic Autonomic Dysfunction; <sup>123</sup>I-MIBG, Heart Failure.

## 1. INTRODUCTION

The Chronic Chagas cardiomyopathy (CCC) is the most common cause of non-ischemic heart failure (HF) in Latin America affecting approximately one-third of Chagas' disease (ChD) patients (Bocchi *et al.*, 2009; Nunes *et al.*, 2013). More recently, due to population mobility, its prevalence has increased in non-endemic areas, such as in North America, Europe and Oceania (Nunes *et al.*, 2013; Tanowitz *et al.*, 2015). The clinical manifestations have several peculiarities compared with idiopathic dilated cardiomyopathy, such as frequent malignant ventricular arrhythmias, different forms and grades of conduction disturbances (Bocchi *et al.*, 2009; Nunes *et al.*, 2013; Rassi *et al.*, 2007) and high incidence of sudden death also in young individuals and during early phases of the disease (Andrade *et al.*, 2011; Nunes *et al.*, 2013). These characteristics give to CCC its worse prognosis representing a severe public health problem by affecting individuals in their most productive age and causing high costs on treatment (Malik *et al.*, 2015; Nunes *et al.*, 2010; Rassi *et al.*, 2007).

The CCC pathogenesis involves parasite persistence in cardiac tissue and in the blood, besides immune-mediated myocardial injury and autonomic and microvascular damage (Da Cunha, 2003; Machado *et al.*, 2000; Marin-Neto *et al.*, 2013; Tanowitz *et al.*, 2015). Indeed, there are several studies showing that cardiac dysautonomia may have a pivotal role in CCC pathophysiology (Da Cunha, 2003; Marin-Neto *et al.*, 2007; Ribeiro *et al.*, 2001). Early studies have demonstrated a premature and extensive parasympathetic neuronal depopulation in the hearts of CCC patients dying from HF (Machado *et al.*, 2000; Ribeiro *et al.*, 2001). This was also clearly demonstrated by functional studies in humans showing imbalance of autonomic control of the heart rate. Later, it was proved that the sympathetic nervous are also compromised in CCC. (Marin-Neto *et al.*, 1998; Landesmann *et al.*, 2011) Mechanisms responsible for sympathetic and parasympathetic denervation in CCC have not been entirely elucidated (Andrade *et*

*et al.*, 2011; Ribeiro *et al.*, 2001; Tanowitz *et al.*, 2015). Neuronal depopulation caused by inflammatory process induced by the parasite is a likely possibility (Machado *et al.*, 2012). Also, microvascular disturbance causing myocardial ischemia may lead to nerve endings' disturbance (Marin-Neto *et al.*, 2013; Simões *et al.*, 2000). Finally, circulating antibodies against both cardiac adrenergic and cholinergic receptors, that could provoke its desensitization and/or downregulation, have been reported in patients with CCC and may actively participate in the process of myocardial denervation (Da Cunha, 2003; Tanowitz *et al.*, 2015). Ultimately, these neurogenic disturbances may play a contributing role in the CCC by triggering malignant arrhythmia and sudden death as previous described. (Gadioli *et al.*, 2016; Miranda *et al.*, 2011)

<sup>123</sup>Iodo-metaiodobenzylguanidine (<sup>123</sup>I-MIBG) imaging reproduce, with great fidelity, the cardiac sympathetic activity and constitute predictors independently of mortality, whatever the etiology of HF (Carrió *et al.*, 2010; Floras, 2009; Jacobson *et al.*, 2010). The scintigraphy late heart/mediastinum ratio (HMR) is an independent predictor of major events and mortality (Morozumi *et al.*, 1997) however there are few studies published in the literature regarding the role of cardiac autonomic dysfunction (<sup>123</sup>I-MIBG) and its prognostic role to guide therapy in CCC. (Gadioli *et al.*, 2016; Landesmann *et al.*, 2011; Miranda *et al.*, 2011)

The objective of the present study is to investigate the presence and severity of myocardial sympathetic denervation using <sup>123</sup>I-MIBG planar imaging in HF patients with CCC, in comparison with non-CCC, contributing to better understanding its pathophysiology and association to malignant arrhythmias and worst prognosis.

## 2. MATERIAL AND METHODS:

### 2.1. Subjects

This is a cross-sectional study with convenience sample of 50 patients carried out between March 2014 to February 2016. The patients were recruited from the population attended on ChD outpatient clinic and from the Heart Transplant Center of the Universidade Federal de Minas Gerais, Brazil. To be eligible for the study, patient have to diagnosis of HF included ChD etiology, based at least two positive serologic tests for antibodies against *Trypanosoma cruzi* (Lima *et al.*, 2010) together with the left ventricular systolic dysfunction or others causes of dilated cardiomyopathy; stable HF functional class II-IV; and ejection fraction  $\leq 45\%$ . All patients have been regularly followed up by several years by clinical and laboratory examination since that ChD or HF diagnosis was made.

We studied prospectively 25 patients with CCC, 17 men with an average age of  $53.3 \pm 9.2$  years and 25 with other cardiomyopathies (non-CCC), mean age of  $43.3 \pm 12$  years and 14 males. A written informed consent was obtained from all patients and the Research Ethics Committee of our institution approved the study protocol. Exclusion criteria include: patients with any chronic illness including coronary artery disease; pacemaker users; diabetes mellitus; chronic renal dysfunction; chronic obstructive pulmonary disease; and Parkinson disease. Clinical data including medical history, physical examination, resting electrocardiogram (ECG) and echocardiographic parameters were collected by the same investigator. Echocardiography (ECHO) images were acquired using a Phillips iE33® (Phillips Medical, Andover, MA, United States of America) ultrasound equipment and performed by one investigator who was blinded to the clinical evaluation of the patients. Left ventricular ejection fraction (LVEF) was calculated according to the modified Simpson's rule. (Lang *et al.*, 2015)

## 2.2. Study Protocol

Patients then underwent resting planar imaging with  $^{123}\text{I}$ -MIBG to assess cardiac innervation. An activity of 111MBq/3mCi of  $^{123}\text{I}$ -MIBG (IPEN/CNEN) was slowly administered intravenous and a 10min/frame anterior thorax image (128x128) was acquired beginning 15min after tracer injection using a dual-head gamma-camera Hawkeye® (GE Healthcare, Milwaukee, United States of America) were performed with a 20% energy window centered at the 159keV photopeak of  $^{123}\text{I}$  and with low-energy high-resolution collimators (LEHR). Early (15 minutes) and late (3 hours) post-injection planar images were processed to determine the early and late heart/mediastinum ratio (HMR). A whole-heart Region of Interest (ROI) and a square mediastinum ROI (7x7 pixels) was drawn in the heart and upper mediastinum, respectively. HMR was calculated as the ratio of the counts per pixel in the two ROIs.  $^{123}\text{I}$ -MIBG washout cardiac rate were also calculated in percentage (Wc%), and was calculated as:  $(\text{early heart} - \text{early mediastinum}) - (\text{late heart} - \text{late mediastinum}) / (\text{early heart} - \text{early mediastinum}) \times 100\%$ , without decay. (Morozumi *et al.*, 1997) The effective dose to the patient resulting from an administration of activity of 111MBq/3mCi of  $^{123}\text{I}$ -MIBG is about 4,8mSv, comparable among cardiac perfusion study using  $^{99\text{m}}\text{Tc}$ -isonitrite. (Perkins, 1994) Inter-observer concordance was 98% between two nuclear medicine physicians, blinded to the clinical evaluation of the patients, when analyzed all images. A Wc% values  $>27\%$  were considered abnormal as well as  $\text{HMR} \leq 1.8$ . (Patel and Iskandrian, 2002)

## 3. STATISTICS ANALYSIS

For this analysis, the sample of 50 patients was calculated to detect a difference of 15% in the HMR variation between the two groups, with  $\alpha$  error of 5% and  $\beta$  of 80% (CI=95%). Descriptive statistics of continuous variable were expressed by the mean, standard deviation or by the median and the categorical variables by absolute values or percentage. The presence or absence of normal distribution was performed by Shapiro-Wilk test. The evaluation of HMR was controlled by age and LVEF, using covariance analysis based on 1 factor model. The linear relationship between the variables was evaluated by the Pearson correlation coefficient. The Chi-Square test, Fisher exact test and Student "t" test were used in the comparisons between the groups, regarding of characterization of the individuals. The level of significance of 0.05 was considered significant. The analysis was performed using the statistical software SPSS 17.0 (SPSS Inc., Illinois, United States of America). (Montgomery, 1991; Johnson and Bhattacharyya, 1986)

## 4. RESULTS

Table 1 summarizes the study subjects' demographic, clinical and echocardiographic characteristics. Patients were under active treatment with angiotensin-converting enzyme inhibitors (n=43),  $\beta$ -blockers (n=46) without difference between groups but more non-CCC patients have used diuretics (n=43) – (p=0.020). About 70% of patients maintained heart rate levels above 80bpm despite therapeutics in both groups (p=1.000). No patients included in the study were treated with tricyclic antidepressant agents.

**Table 1: Summary of Patient Characteristics**

Characteristics	CCC	non-CCC	p
Male sex (%)*	68	56	0.382
Age (years) †	53.3±9.2	43.3±12	0.003
HR>80bpm *	69.2	66.7	1.000
NYHA II-IV (%)*	62.5	84.0	0.088
LVEF % (ECHO) †	30.6±7.8	25.9±8.0	0.047
ACEi (%)*	91.3	88	1.000
β-blockers (%)*	91.3	100	0.224
Diuretics (%)*	78.3	100	0.020

CCC= Chronic Chagas cardiomyopathy; non-CCC= non-Chronic Chagas Cardiomyopathy;

HR= heart rate; NYHA=New York Heart Association. Data expressed by \* percentages or † mean±SD; HR=bpm; ACEi= angiotensin-converting enzyme inhibitors.

The cardiac autonomic dysfunction assessed by  $^{123}\text{I}$ -MIBG is shown in Table 2. All patients presented abnormal  $^{123}\text{I}$ -MIBG uptake (Figure 1). The mean of early HMR to CCC patients was  $1.73\pm 0.24$  and for non-CCC was  $1.62\pm 0.21$  ( $p=0.260$ ). Late HMR was  $1.58\pm 0.27$  and  $1.44\pm 0.16$  ( $p=0.052$ ), respectively. The CCC patients showed higher values for late uptake compared to the non-CCC. CCC late HMR was 9.7% higher than non-CCC but without significance when adjusted for age and LVEF (figure 2). There was no significant difference between groups for Wc% values ( $p=0.125$ ).

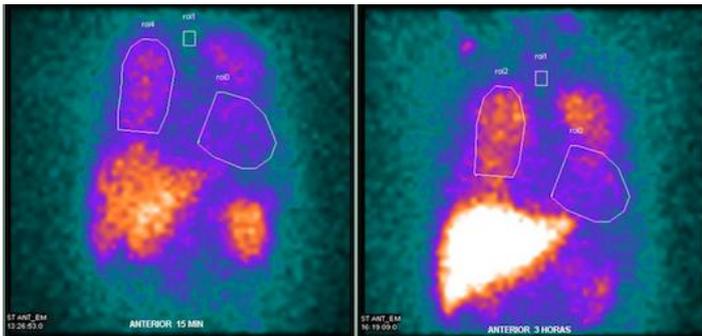
**Table 2: Planar  $^{123}\text{I}$ -MIBG imaging analysis**

$^{123}\text{I}$ -MIBG image parameters	CCC	non-CCC	p
Early HMR	$1.73\pm 0.24$	$1.62\pm 0.21$	0.260
Late HMR	$1.58\pm 0.27$	$1.44\pm 0.16$	0.052
Wc (%)	$41.6\pm 21.41$	$47.37\pm 14.19$	0.125

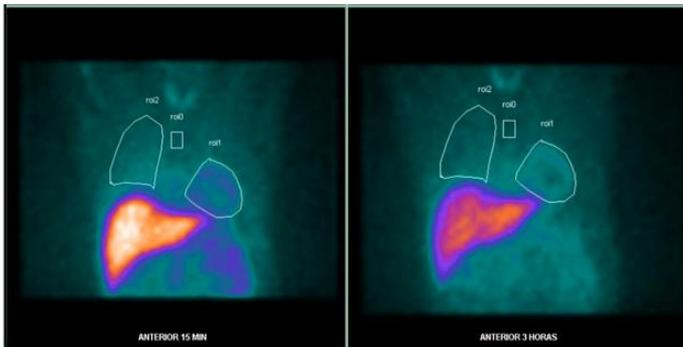
CCC = Chronic Chagas cardiomyopathy; non-CCC = non-chronic Chagas Cardiomyopathy; Early HMR=Early heart/mediastinum ratio; Late HMR=late heart/mediastinum ratio; Wc%=washout cardiac rate. Results adjusted considering age and left ventricular ejection fraction.

**Figure 1:** <sup>123</sup>I-MIBG CCC and non-CCC scintigraphic patterns on planar images

(A)

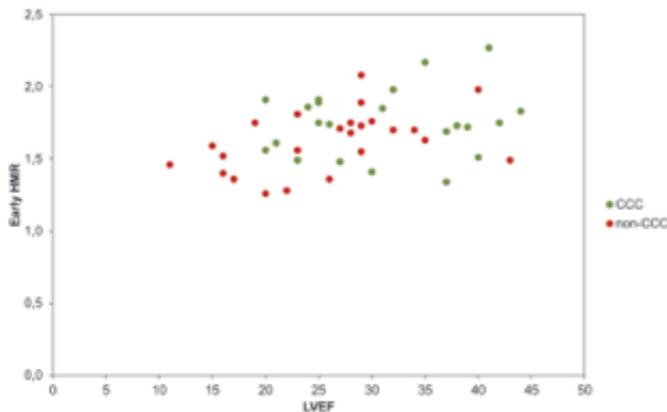


(B)



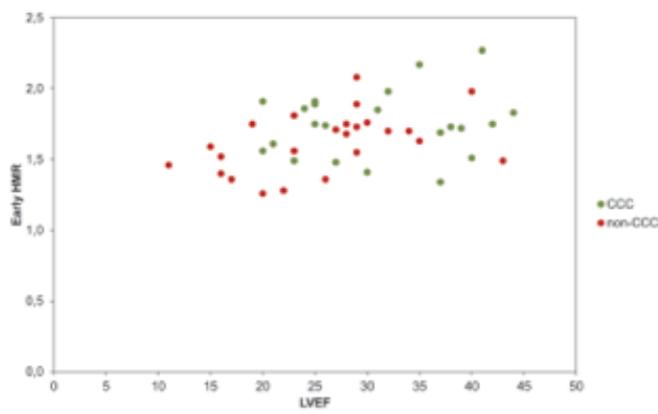
Anterior thorax <sup>123</sup>I-MIBG images at 15min and anterior thorax images at 180min in: (A) CCC and (B) non-CCC patients.

**Figure 2:** Early (A) and late (B) HMR values in CCC and non-CCC patients

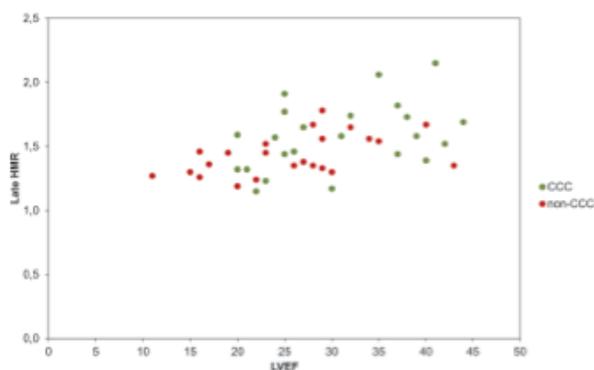


Early HMR=Early heart/mediastinum ratio;  
 Late HMR=Late Heart/mediastinum ratio;  
 CCC=Chronic Chagas Cardiomyopathy;  
 non-CCC= non-Chronic Chagas Cardiomyopathy.

The mean value of LVEF on echocardiogram was  $30.6 \pm 7.8\%$  for the CCC and  $25.9 \pm 8\%$  for the non-CCC patients ( $p=0.047$ ). There was a positive correlation between: late HMR and LVEF in CCC ( $r=0.42$ ,  $p = 0.045$ ); early HMR and LVEF in non-CCC ( $r=0.46$ ,  $p=0.023$ ); late HMR and LVEF in non-CCC ( $r=0.49$ ,  $p = 0.015$ ); but no correlation between Wc% and LVEF in both groups (Figure 3, 4).

**Figure 3: Relationship between early  $^{123}\text{I}$ -MIBG and LVEF in CCC and non-CCC patients**

Early HMR=Early heart/mediastinum ratio; CCC= Chronic Chagas Cardiomyopathy; non-CCC= non-Chronic Chagas Cardiomyopathy.

**Figure 4: Relationship between late  $^{123}\text{I}$ -MIBG and LVEF in CCC and non-CCC patients**

Late HMR=Late heart/mediastinum ratio; CCC= Chronic Chagas Cardiomyopathy; non-CCC= non-Chronic Chagas Cardiomyopathy.

## 5. DISCUSSION

This study investigated the presence and severity of cardiac sympathetic denervation in CCC patients with left ventricular dysfunction (mean value LVEF = 30%), by using  $^{123}\text{I}$ -MIBG scintigraphy, once this is the only noninvasive method with enough sensitivity to evaluate the “in vivo” sympathetic neurotransmission. (Jacobson *et al.*, 2010; Floras, 2009; Nakata *et al.*, 2013)  $^{123}\text{I}$ -MIBG is an analogue of norepinephrine selective to presynaptic sympathetic termination that is uptaken by type 1 mechanism. It is not metabolized when present in the intracellular environment, allowing the scintigraphic images to be acquired. (Jacobson *et al.*, 2010; Morozumi *et al.*, 1997; Raffael and Wieland, 2010)

The  $^{123}\text{I}$ -MIBG uptake by cardiac sympathetic endings is estimated by the ratio between radioactive counts of the Region of Interest (ROI) which delimits the myocardium and upper mediastinum (HMR). Thereby, the early (15min) and late (180min), HMR means the density and integrity of receptors and presynaptic fibers, while the Wc% means the catecholamines turnover relation to grade of sympathetic drive. (Morozumi *et al.*, 1997; Carrió *et al.*, 2010) These parameters are considered to

be strongly reproducible, with good interobserver agreement (95-98%). (Patel and Iskandrian, 2002; Currie *et al.*, 2011) It could also be verified in this study in which 98% of interobserver agreement was obtained.

We found the value of  $1.58 \pm 0.27$  for late HMR in CCC patients. It is lower than those described for normal patients (Currie *et al.*, 2011; Patel and Iskandrian, 2002) and than in CCC patients with mild left ventricular dysfunction ( $HMR \leq 2.19$ ). (Landesmann *et al.*, 2011) It is also lower than that described for HF patients with worst prognosis ( $HMR < 1.74$ ) – (Jacobson *et al.*, 2010; Nakata *et al.*, 2013; Carrió *et al.*, 2010), confirming the presence of sympathetic autonomic dysfunction in our CCC patients. Our late HMR value for CCC patients is quite near to the cutoff point ( $HMR < 1.60$ ) adopted by Jacobson (2010), which could stratify HF patients to the occurrence of cardiac events (sensitivity: 84% and specificity: 60%) and by Gadioli (2016) who found a strong correlation between late HMR value ( $1.68 \pm 0.19$ ) and severe ventricular arrhythmias. We must also consider that our values are overestimated due to the regular use of  $\beta$ -blockers. (Stefanelli *et al.*, 2013)

Although it has been possible to identify cardiac sympathetic dysfunction in CCC patients as severe as described by others authors (Landesmann *et al.*, 2011; Gadioli *et al.*, 2016), we could not prove that it is worst than in non-CCC patients, even when corrected by age and LVEF ( $p=0.052$ ). It is possible that a sustained sympathetic hyperactivity observed in HF, regardless of its etiology, did not allowed us to identify difference between groups. The release of catecholamines in the early stages of HF, arising from increased synthesis and release in sympathetic synaptic clefts, would compensate the reduction of myocardial contractility. However, in the long term, this excess of circulating catecholamines would result in depletion of the neurotransmitter deposits, causing loss of neurons and down-regulation of norepinephrine receptors. (Ogita *et al.*, 2001; Floras, 2009; Carrió *et al.*, 2010; Imamura *et al.*, 1995; Mann *et al.*, 2005; Triposkiadis *et al.*, 2009)

Even though, the sympathetic dysfunction might be relevant in CCC for adequate prognosis and therapy, there are several conceptual obstacles challenging its applicability such as: the subtleness and variability of the intensity of cardiac denervation; the lack of correlation between parasympathetic denervation and the extent of myocardial dysfunction; (Andrade *et al.*, 2011; Marin-Neto *et al.*, 2013) its occurrence in early stages of ChD; (Landesmann *et al.*, 2011) and the sustained presence of inflammation (Machado *et al.*, 2012) which seems to contribute to the increase of the morbidity and mortality of these patients, in despite of the high level of serum catecholamines, that highlights the importance of using the  $^{123}\text{I}$ -MIBG in ChD. (Ungerer *et al.*, 1993)

We also found a positive correlation between late HMR and LVEF (ECHO) in CCC patients ( $r=0.42$ ) as well as in non-CCC ( $r=0.49$ ) which suggests that late HMR has a prognostic value similar to LVEF as previously described by others authors (Jacobson *et al.*, 2010; Carrió, 2010) although Carrió (2010) identified this correlation only for values of  $HMR < 1.20$ .

The Wc% values were high in both groups studied as described in others HF patients (Jacobson *et al.*, 2010; Currie *et al.*, 2011) but without correlation to LVEF. It might be justified by the large dispersion of values around the mean in our data. (Ogita *et al.*, 2001; Currie *et al.*, 2011)

### 5.1. Limitations of this study

There are numerous limitations in this study. Firstly, it is a cross-sectional study, non-randomized study. As patients belong to functional class II-IV (NYHA), it is not possible to generalize the findings to all patients with ChD. Furthermore, the use of  $\beta$ -blockers was kept, which raises the HMR values and underestimates the severity of neuronal sympathetic involvement. (Stefanelli *et al.*, 2013)

The scintigraphic parameters values have important limitations. A strong one is the use of different protocols for images analysis as decay factor and deconvolution to correct septal penetration. (Chen *et al.*, 2006) There are also the lack of reference values and the diversity of the population studied in works published to date which are mostly non-randomized and thereby hinder the incorporation of these parameters to the cardiology guidelines. (Jacobson *et al.*, 2010; Patel and Iskandrian, 2002) We believe that future works should pay particular attention to the parameters' methodology to increase the internal validity of these studies.

## 6. CONCLUSION

This study suggested that there is severe sympathetic denervation in HF patients, independent of its etiology, and that it is not worse in CCC patients, possibly because in this study they are in advanced HF status.

## 7. CONFLIT OF INTEREST

The authors declares that there is no conflict of interest

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