

# Histopathological Analysis of the Pro-Arrhythmogenic Changes in a Suspected Chagas Disease Sudden Death

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## ABSTRACT

Sudden death is the principal cause of fatality in Chagas disease, afflicting to non-symptomatic patients younger than 50 years. For this, sudden death associated with chagasic malignant arrhythmias is underdiagnosed and their pathophysiological basis is poorly understood. In this sense, this work aimed to analyze the histopathological alterations in cardiac structures specialized in the generation/conduction of action potential in an anatomopathological case of non-diagnosed sudden death. The donor was a woman, 62 years old, which ingresses without vital signs to the emergency room of “Antonio María Pineda” hospital. The gross examination was normal, with no external evidence of structural/ischemic disease. Microscopic examination revealed nodal cell depopulation, micro vascular disturbances, chronic cardiomyopathy with mononuclear and mast cell infiltrate plus extracellular matrix reaction and profuse damage of neural structures placed in nodal region. Amastigote nest was detected. These findings suggest a complex association among parasite persistence, sinus disease, micro-ischemia foci and neural inflammation in the genesis of malignant arrhythmias of Chagas disease despite the absence of structural disease or massive necrosis. It is important to perform a protocol of examination for no explained sudden death cases in chagasic endemic countries, to avoid misdiagnosed of sudden death associated with Chagas disease.

**Keywords:** Chagas disease; Sudden death; Histopathological analysis; Arrhythmias

## INTRODUCTION

Chagas disease is a tropical illness caused by the intracellular protozoa *Trypanosoma cruzi*. The infection comprises an acute phase that often is non-symptomatic or oligosymptomatic, but in some cases courses with systemic symptoms, such as peripheral edema, pericardial tamponade, acute myocarditis, arrhythmias, and death. The chronic phase typically appears 10-30 years after infection, showing clinical manifestations in approximately one-third of infected patients. The main symptomatology during the chronic phase ranges from malignant arrhythmias, flutter/atrial fibrillation, stroke, cardiac remodeling, heart failure to disability and death.

Sudden death is the main cause of fatality in Chagas disease. Malignant ventricular arrhythmias in chagasic patients' have a worse prognosis than other cardiac etiologies [1], probably because of the multiplicity of factors implied in arrhythmogenesis in Chagas disease. In general terms, fibrotic scars are the principal source of arrhythmogenic substrates by disturbances in conduction and generation of reentry circuits [2]. In such sense, LV inferolateral scar is the main anatomical source of sustained ventricular tachycardia

reentrant circuits [3]. On the other hand, vascular disturbances and the endothelin-1 and of thromboxane A2 associated vascular spasm may generate tissular hypoxia and functional alterations in action potential conduction [4,5].

Besides, cardiac inflammation due to local immune response may predispose to arrhythmias beyond to tissue destruction and/or scar formation. Our team reported IL-2 and IL-10 as a predisponent factor for sudden death in chagasic patients in patients with minimal structural cardiac pathology [6]. Additionally, autonomous nervous system disfunction, estimated by heart rate variability parameters, strongly correlated with adiponectin, TNF and IL-6 levels, suggesting that inflammatory status may influence cardiac rhythm regulation [7]. In this same line of thought, several reports reinforce the role of autonomic dysfunction in sudden death risk in Chagasic patients. For example, impairment in baroreflex sensitivity correlates with the density and complexity of ventricle arrhythmias and adrenergic or cholinergic antibodies with G-protein coupled membrane receptor activity may be present in the sera of chagasic patients and contribute with arrhythmias appearance [8,9].

Despite advances in the comprehension of pathophysiology of

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Chagas disease, particularly in the topic of malignant arrhythmias, sudden death is a phenomenon poorly documented, and very probably underdiagnosed, especially in the last years due, among other factors, to the relative scarcity of specialized autopsies for addressing cardiac conduction structures in patients with reported sudden death. In this line of thought, the present anatomopathological report aims to describe the possible causes associated with cardiac nodal/conduction tissue in post-mortem histopathological examination in a sudden death case suspected for Chagas disease.

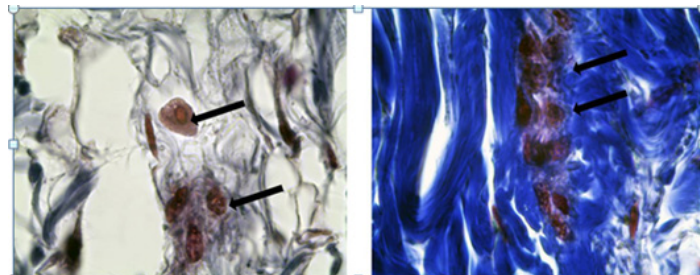
## CASE STUDY

### Macroscopic characterization

Entire heart pice was obtained from the anatomical museum of Lisandro Alvarado University Medical Faculty. The donor patient was a woman, 62 years old, which ingress to emergency service of “Antonio María Pineda” hospital (Barquisimeto-Venezuela) without vital signs product of a sudden syncope. The heart weighed 225 gms and no signs of myocardial infarct/scars were detected at the gross examination.

### Sinus node dissection and nodal cell identifications

Sinus node was dissected following previous reports. Briefly, dissection was performed with a longitudinal incision between superior and inferior cava venous insertion at right atria (2 × 1 cm), with crista marginalis and sinus node artery (branch of the right coronary artery) as the reference point [10,11] (Figure 1). For identification of sinus node region, attending previous histological descriptions, nodal resident cells were described. Pacemaker cells (P cells), showed star/pyramidal morphology and organization in clusters. T type cells were defined as transitional and immature myoid cells and, finally, fibroblast-like cells with long bipolar extensions [12].



**Figure 1:** Macroscopic view of the nodal cardiac area dissection. The left panel shows a dorsal view of the heart base, with black arrows pointing cava vein insertion and nodal area in reference with cava vein. The right panel has the same dorsal view, with the crista terminalis inside of the right atrium.

### Histopathological analysis

Myocardial tissue was surveyed for detecting histopathological damage. Thin sections (5 µm) were stained with Periodic Schiff Acid (PAS), Masson and routine H & E staining. Samples were taken from the nodal area, right atrium, and right ventricle. To explore differential causes of sudden death, foci of coagulative necrosis suggestive of myocardial ischemia, as well as an intracoronary thrombus and viral inclusion bodies, were discarded. Following the Dallas criteria, myocarditis requires an inflammatory infiltrate and associated myocyte necrosis or damage not characteristic of an ischemic event. Borderline myocarditis requires a less intense inflammatory infiltrate and no light microscopic evidence of myocyte destruction [13]. Additionally, inflammatory cells were

counted by the 40X power field, and the presence of inclusions compatible with *Trypanosoma cruzi* was carefully analyzed with 40-100X power magnification. Masson Trichromic staining was performed for evaluating extracellular matrix reaction and/or muscular fibers replacement by connective tissue.

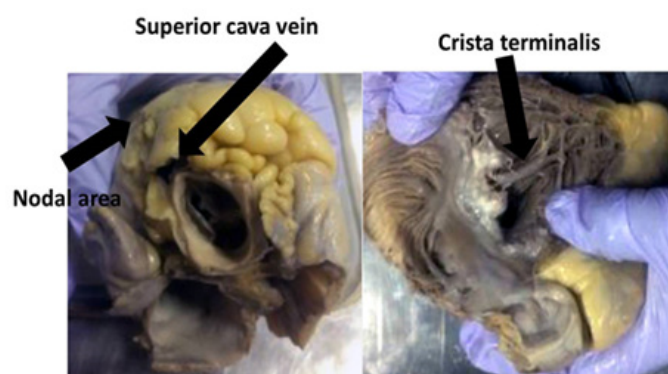
Some especial structures were particularly studied. Myocardial vessel integrity was evaluated considering endothelium integrity and presence of inflammatory cells and or exudate in the intravascular space or the perivascular area. Intramural ganglia were identified, particularly in peri-nodal area, and structural integrity and presence of parasite/inflammatory cells were assessed.

### Ethical statement

Heart samples from human donors and data collecting were approved by the ethical committee of Lisandro Alvarado medical faculty, according to the Helsinki protocol for human ethics.

## RESULTS AND DISCUSSION

One of the main characteristics of chronic Chagas disease is nodal dysfunction. To address this, the histological morphology of sinus node cells in a suspected case of Chagas disease was analyzed. Pyramidal shaped cells were identified as alongside nodal region dissected (Figure 2). Remarkably, cells were grouped in small (4-10 cells) and widely spread clusters, surrounded by connective tissue and without contact with internodal tracts, which plausibly may affect the diffusion of the action potential in the atrium.

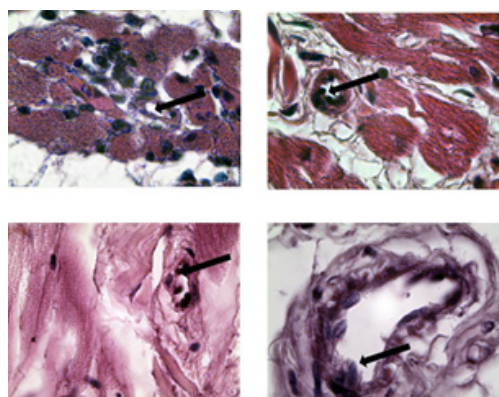


**Figure 2:** Histological pattern of nodal like cells in a post-mortem diagnosed Chagas disease. Left, Masson trichromic staining of a cluster of pyramidal cells, morphologically compatible with “P” (pale) pacemaker cells. Right, a cluster of P cells inserted in the root of the superior cava vein. In both cases, scarcity and disconnection of nodal P cells cluster are noted. Magnification 100X.

Atrial Fibrillation (AF) is the most common supraventricular arrhythmia in chagasic patients. Some authors estimates than 5% of electrocardiographic tracings of patients with chronic Chagas disease [14]. AF can lead to anatomical and electrophysiological remodeling in both atria, including the region of the sinoatrial node. Changes including atrial fibrosis, altered calcium channel metabolism and transformed gene expression have been demonstrated in patients with AF and sinus node dysfunction [15]. It is a topic with particular importance, due to the development of atrial fibrillation is a predictor and an important risk factor for stroke, independent of LV function [16]. Besides, in experimental models, our team has demonstrated overexpression of HCN channel protein in acute Chagas disease, reinforcing the idea that cardiac remodeling may affect sinus node functionality and influences on cardiac arrhythmias [17]. Although the present study is only base on morphological evidence of sinus node damage is, to our best

knowledge, one of the few attempts to describe the damage of nodal cells in a chagasic related sudden death anatomopathological report.

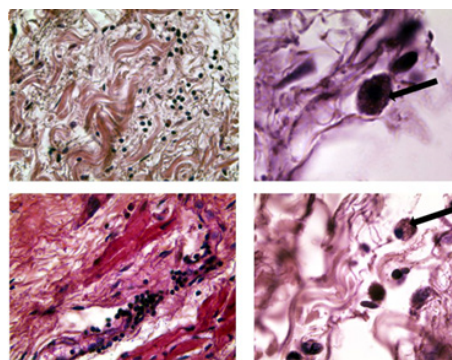
A remarkable vascular affection was detected during the histopathological examination. Perivascular inflammatory infiltrate was registered in muscular coronary vessels alongside working myocardium, closely related to cardiac fiber degeneration (Figure 3). Additionally, images of mononuclear cells compatible with the inflammatory rolling process and consequent endothelial reaction. These microscopical changes may be involved in the increased sudden death risk in chagasic patients. In experimental infection models, platelet aggregates, forming transient occlusive thrombi, were detected in small epicardial and intramyocardial vessels, direct evidence of microcirculatory disease associated with necrosis focus [4]. The release of vasoconstrictor substances, such as thromboxane A<sub>2</sub> (TXA<sub>2</sub>) and Platelet-Activating Factor (PAF) by macrophages, which are the predominant inflammatory cells, was proposed to cause transient ischemia and myocytolytic necrosis [18]. Although the micro-ischemic focus is not able itself of causing extensive and life-threatening myocardial necrosis, it is a plausible attribute to this phenomenon pro-arrhythmogenic properties. Structural myocardial abnormalities, such as foci of inflammation, areas of fibrosis, ventricular dilation, and akinetic or dyskinetic areas, generate a unidirectional block and slow conduction in circumscribed ventricular regions, essential for the appearance of reentrant ventricular arrhythmias, which are the main triggering factor of sudden death in chronic Chagas' heart disease [19]. In this sense, slow conduction generated by micro-ischemic focus may be one important mechanism associated with the appearance of malignant and sudden death.



**Figure 3:** Vascular morphological alterations of myocardial vessels. Upper left. Myocardial arteriolar vessel. Extensive mononuclear perivascular infiltrate, intravascular fibrinous exudate (black arrow) and degenerative changes in the surrounding muscular fibers. Upper right and lower left.

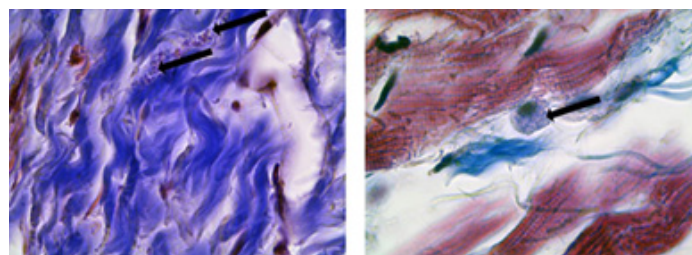
Wide neural depopulation and nervous fiber degeneration were observed in the histopathological study of the nodal and perinodal area. Mononuclear lining inflammatory infiltrates, neuronal loss and cytoplasmatic degenerative changes and nervous fiber disorganization and interfibrillar infiltrate were the main histological alterations reported (Figure 4). The coexistence of denervated and hyperinnervated areas in the diseased myocardium could result in increased electrophysiological heterogeneity during sympathetic activation and may lead to ventricular arrhythmia and sudden cardiac death [20]. Additionally, abnormal heart rate kinetics and sympathetic innervation defects have been shown to precede ventricular tachycardia in ChD patients. Moreover, autoimmune disturbances may be linked with the generation of autoantibodies against adrenergic and muscarinic cardiac receptors

[9], adding pro-arrhythmogenic substrates to merely structural alterations in cardiac neural structures. All of these pieces of evidence allow establishing the importance of the cardiac nervous system in the pathophysiology of sudden death in Chagas disease.



**Figure 4:** Inflammatory pattern alongside cardiac tissue of sudden death probably associated with Chagas disease. Upper and lower left. Mononuclear infiltrates associated with muscular fiber degeneration and extracellular matrix reaction. Magnification 10 and 40X respectively. Upper and lower right. Plasmocytic infiltrates detail, rounded cells with acidophilic cytoplasm and granules. Magnification 100X.

Inflammatory infiltrate was a common finding on the pieces of myocardium studied. Mononuclear cells were the principal inflammatory cells detected in the myocardium, often associated with intensive intercellular matrix reaction. Inflammation showed a multi-focal pattern, with the focus observed with >10 cells/40X fields. Remarkably, mastocyte like cells were seen in myocardial interstitium slides micrograph (Figure 5).



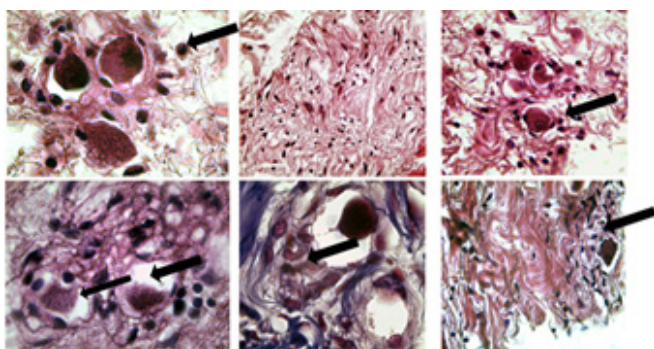
**Figure 5:** Intracardiac ganglia degeneration of sudden death probably associated with Chagas disease. Upper: At right, mononuclear infiltrate (black arrow) in the vicinity of intracardiac ganglia placed on the nodal region. Center. Degeneration and inflammatory infiltrate in an atrial nervous plexus.

Inflammatory markers mediators may be involved in arrhythmogenesis and sudden death in chagasic patients. Several works postulate the association of inflammatory markers and sudden death. C-Reactive Protein (CRP), a universal and unspecific inflammatory marker, was reported as a predictor of sudden death in otherwise healthy patients [21]. It is particularly true regarding supraventricular arrhythmias, which also may be associated with sudden death. there was a vast literature linking AF and the inflammatory biomarkers, not only CRP but also hs-CRP, fibrinogen, IL-1, IL-2, IL-6, IL-8, TNF- $\alpha$ , serum amyloid A and monocyte chemoattractant protein [22]. In the case of ventricular arrhythmias, as we stated before, our team found that IL-10 and IL-2 were the most predictive variable for sudden death risk in chronic chagasic patients [6]. We found in this report an important extension of mononuclear infiltrate that, interestingly, not always was related to fiber necrosis, which leads to speculate the possibility of pro-arrhythmogenic stimuli beyond structural inflammatory damage.

On the other hand, mast cells were observed in the microscopic

slides. Mast cells principally survey the microenvironment and respond to stimuli via expression of Pattern Recognition Receptors (PRRs) that detect Pathogen and Damage-Associated Molecular Patterns (PAMPs and DAMPs) [23]. These cells are located in sites throughout the body, including the heart [24] and have a plethora of functions reflected in the secretion of histamine, Tumor Necrosis Factor (TNF), proteases, lysosomal enzymes ( $\beta$ -hexosaminidase), biogenic amines (histamine, serotonin, dopamine), cytokines (TNF, interleukin (IL)-4, IL-5), and growth factors (Stem Cell Factor (SCF) and basic Fibroblast Growth Factor (bFGF) among others [25]. Mast cells have been involved in cardiac fibrosis remodeling in response to ischemia, although this still matters of debate. Notwithstanding, the presence of mast cells in the context of profuse interstitial matrix reaction and vascular alterations could be following this proposal. Interestingly, in an *ex vivo* model, mast cells coming from chagasic hearts were associated with profuse interstitial fibrosis and mast cell density and fibrosis in the tongue muscles correlated with cardiac fibrosis in human autopsies of chagasic patients [26,27]. This pro-fibrotic property may favor arrhythmogenic focus, in conjunction with ischemic changes related to microvascular alterations and, in consequence, be involved in chagasic sudden death.

Finally, scarce images compatible with intracellular amastigotes were found in the slice observed (Figure 6). In anatomopathological studies, the parasite load observed was proportionally higher in heart tissues from patients with the cardiac form, although other reports of sudden death failed to find parasite nest in cardiac tissue [28,29].



**Figure 6:** Intracellular inclusions compatible with amastigotes of *Trypanosoma cruzi* in peri-nodal area. Left, Masson trichromatic staining of connective tissue. Arrows points rounded intracellular inclusions. Right, intracellular matrix positive to Masson trichromatic staining, it can be appreciated a macrophage with cytoplasmic inclusions. Magnification 100X.

In conclusion, these results show the marked complexity of tissular pro-arrhythmogenic substrates that may generate sudden death in Chagas disease. We observed chronic myocarditis without evidence of thrombotic disease which afflicted microvasculature and nervous nodal tracts. Nodal-like cells were also scarce and widely spread by the nodal region. Chronic chagasic cardiomyopathy mixes arrhythmogenic mechanisms normally observed in different kinds of cardiac pathologies, such as arrhythmogenic, ischemic and structural cardiomyopathy. Additionally, it is necessary to keep in mind the fact that unexpected sudden death normally afflicts younger patients (30-50 years old) otherwise non-symptomatic [30]. Heart may be grossly normal or only slighted altered [19], making very difficult the identification of patients under sudden death risk.

## CONCLUSION

In this report was performed a specific histopathological study of

specialized cardiac structures more susceptible to be altered in an anatomopathological case of sudden death non-initially diagnosed as Chagas disease, which pretends to be completed further with histochemical markers survey. However, due to the decreasing of anatomopathological studies in sudden death cases, it is important to set the basis for performing protocols to examine anatomical structures involved in the genesis of malignant arrhythmias and avoid unregistered sudden death associated to Chagas disease and. Finally, this study was the opportunity to revise in conjunct the different mechanisms of sudden death and improve the comprehension of malignant arrhythmias pathophysiology.

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## REFERENCES

1. Martinelli F M, De Siqueira SF, Moreira H, Fagundes A, Pedrosa A, Nishioka SD, et al. Probability of occurrence of life-threatening ventricular arrhythmias in Chagas' disease versus non-Chagas' disease. *Pacing Clin Electrophysiol.* 2000; 23: 1944-1946.
2. Milei J, Pesce R, Valero E, Muratore C, Beigelman R, Ferrans VJ. Electrophysiologic-structural correlations in chagasic aneurysms causing malignant arrhythmias. *Int J Cardiol.* 1991; 32: 65-73.
3. Sarabanda AV, Sosa E, Simoes MV, Figueiredo GL, Pintya AO, Marin-Neto JA. Ventricular tachycardia in Chagas' disease: A comparison of clinical, angiographic, electrophysiologic and myocardial perfusion disturbances between patients presenting with either sustained or nonsustained forms. *Int J Cardiol.* 2005; 102 :9-19.
4. Rossi MA, Tanowitz HB, Malvestio LM, Celes MR, Campos EC, Blefari V, et al. Coronary microvascular disease in chronic Chagas cardiomyopathy including an overview on history, pathology, and other proposed pathogenic mechanisms. *PLoS Negl Trop Dis.* 2010; 4.
5. Prado CM, Jelicks LA, Weiss LM, Factor SM, Tanowitz HB, Rossi MA. The vasculature in Chagas disease. *Adv Parasitol.* 2011; 76 :83-99.
6. Rodriguez-Angulo H, Marques J, Mendoza I, Villegas M, Mijares A, Girones N, et al. Differential cytokine profiling in Chagasic patients according to their arrhythmogenic-status. *BMC Infect Dis.* 2017; 17 :221.
7. Barbosa-Ferreira JM, Mady C, Ianni BM, Lopes HF, Ramires FJ, Salemi VM, et al. Dysregulation of autonomic nervous system in chagas' heart disease is associated with altered adipocytokines levels. *PLoS One.* 2015;10 :131-447.
8. Santos AM, Scanavacca MI, Darrieux F, Ianni B, Melo SL, Pisani C, et al. Baroreflex sensitivity and its association with arrhythmic events in Chagas disease. *Arq Bras Cardiol.* 2014;102: 579-587.
9. Medei EH, Nascimento JH, Pedrosa RC, Carvalho AC. Role of autoantibodies in the physiopathology of Chagas' disease. *Arq Bras Cardiol.* 2008; 91: 257-262.
10. Waller BF, Gering LE, Branyas NA, Slack JD. Anatomy, histology, and pathology of the cardiac conduction system-Part IV. *Clin Cardiol.* 1993; 16: 507-511.
11. Unudurthi SD, Wolf RM, Hund TJ. Role of sinoatrial node architecture in maintaining a balanced source-sink relationship and synchronous cardiac pacemaking. *Front Physiol.* 2014; 5: 446.
12. Balbi T, Ghimenton C, Pasquinelli G, Foroni L, Grillini M, Pierini G. Advancement in the examination of the human cardiac sinus node: An unexpected architecture and a novel cell type could interest

- the forensic science. *Am J Forensic Med Pathol.* 2011; 32: 112-118.
13. Aretz HT, Billingham ME, Edwards WD, Factor SM, Fallon JT, Fenoglio JJ, et al. A histopathologic definition and classification. *Am J Cardiovasc Pathol.* 1987; 1: 3-14.
  14. Nunes MCP, Beaton A, Acquatella H, Bern C, Bolger AF, Echeverria LE, et al. Chagas cardiomyopathy: an update of current clinical knowledge and management: A scientific statement from the American Heart Association. *Circulation.* 2018; 138:169-209.
  15. Jackson LR, Rathakrishnan B, Campbell K, Thomas KL, Piccini JP, Bahnson T, et al. Sinus node dysfunction and atrial fibrillation: A reversible phenomenon? *Pacing Clin Electrophysiol.* 2017;40 :442-450.
  16. Ribeiro AL, Marcolino MS, Prineas RJ, Lima CMF. Electrocardiographic abnormalities in elderly Chagas disease patients: 10-years follow-up of the Bambuí cohort study of aging. *J Am Heart Assoc.* 2014;3:632.
  17. Rodriguez Angulo HO, Diana Colombet DC, Juan Marques JM, Ivan Mendoza IM, Rafael Bonfante RBC, Nuria Girones NG, et al. Electrocardiographical and tissular evidence of HCN1/4 channels subunits over expression in acute chagasic myocarditis model. *EP Europace.* 2018;20 :147
  18. Rossi MA, Carobrez SG. Experimental *Trypanosoma cruzi* cardiomyopathy in BALB/c mice: histochemical evidence of hypoxic changes in the myocardium. *Br J Exp Pathol.* 1985; 66: 155-60.
  19. Rassi A, Rassi SG, Rassi A. Sudden death in Chagas' disease. *Arq Bras Cardiol.* 2001; 76 :75-96.
  20. Chen LS, Zhou S, Fishbein MC, Chen PS. New perspectives on the role of the autonomic nervous system in the genesis of arrhythmias. *J Cardiovasc Electrophysiol.* 2007; 18: 123-127.
  21. Albert CM, Ma J, Rifai N, Stampfer MJ, Ridker PM. A prospective study of C-reactive protein, homocysteine, and plasma lipid levels as predictors of sudden cardiac death. *Circulation.* 2002;105: 2595-2599.
  22. Patel P, Dokainish H, Tsai P, Lakkis N. Update on the association of inflammation and atrial fibrillation. *J Cardiovasc Electrophysiol.* 2010; 21: 1064-1070.
  23. Halova I, Ronnberg E, Draberova L, Vliagoftis H, Nilsson GP, Draber P. Changing the threshold-Signals and mechanisms of mast cell priming. *Immunol Rev.* 2018; 282:73-86.
  24. Sperr WR, Bankl HC, Mundigler G, Klappacher G, Grossschmidt K, Agis H, et al. The human cardiac mast cell: Localization, isolation, phenotype, and functional characterization. *Blood.* 1994;84: 3876-3884.
  25. Legere SA, Haidl ID, Legare JF, Marshall JS. Mast cells in cardiac fibrosis: New insights suggest opportunities for intervention. *Front Immunol.* 2019; 10: 580.
  26. Postan M, Correa R, Ferrans VJ, Tarleton RL. In vitro culture of cardiac mast cells from mice experimentally infected with *Trypanosoma cruzi*. *Int Arch Allergy Immunol.* 1994; 105: 251-257.
  27. Roldao JA, Beghini M, Ramalho LS, Porto CS, Rodrigues DB, Teixeira VP, et al. Comparison between the collagen intensity and mast cell density in the lingual muscles and myocardium of autopsied chronic chagasic and nonchagasic patients. *Parasitol Res.* 2012; 111: 647-654.
  28. Marcon GE, de Albuquerque DM, Batista AM, Andrade PD, Almeida EA, Guariento ME, et al. *Trypanosoma cruzi*: Parasite persistence in tissues in chronic chagasic Brazilian patients. *Mem Inst Oswaldo Cruz.* 2011;106: 85-91.
  29. Satoh F, Tachibana H, Hasegawa I, Osawa M. Sudden death caused by chronic Chagas disease in a non-endemic country: Autopsy report. *Pathol Int.* 2010; 60:235-240.
  30. Bestetti RB, Freitas OC, Muccillo G, Oliveira JS. Clinical and morphological characteristics associated with sudden cardiac death in patients with Chagas disease. *Eur Heart J.* 1993; 14: 1610-1614.