

Original Research

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

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ABSTRACT

Background

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.

Aims

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 living in a Chagasic endemic area.

Methods

The donor was a woman, 62-year-old, which ingressed without vital signs to the emergency room of “Antonio María Pineda” hospital, without any apparent antecedents of cardiac disease. The gross examination was normal, with no external evidence of structural/ischemic disease.

Results

Microscopic examination revealed nodal like cell depopulation, microvascular disturbances, chronic myocarditis with mononuclear and mast cell infiltrate plus extracellular matrix reaction, and profuse damage of neural structures placed in nodal region. Amastigote nest of *Trypanosoma cruzi* (*T. cruzi*) was detected.

Conclusion

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; Cardiac disease; Chagasic malignant arrhythmias.

INTRODUCTION

Chagas disease is a tropical illness caused by the intracellular protozoa *Trypanosoma cruzi* (*T. 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-year 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 a 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, left ventricular (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}

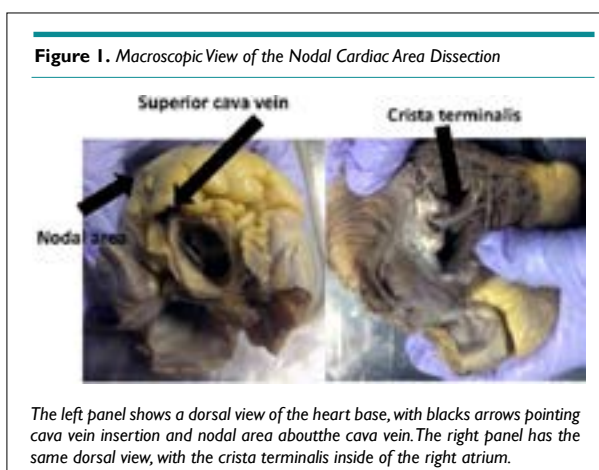
Besides, cardiac inflammation due to local immune response may predispose to arrhythmias beyond to tissue destruction and/or scar formation. Our team reported Interleukin-2 (IL-2) and IL-10 as a predisponent factor for sudden death in chagasic patients in patients with minimal structural cardiac pathology.⁶ Additionally, autonomous nervous system disfunction, estimated by heart rate variability parameters, strongly correlated with adiponectin, tumor necrosis factor (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,⁸ and adrenergic or cholinergic antibodies with G-protein coupled membrane receptor activity may be present in the sera of chagasic patients and contribute to arrhythmias appearance.⁹

Despite advances in the comprehension of pathophysiology of 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 of Chagas disease.

MATERIALS AND METHODS

Macroscopic Characterization

The entire heart pice was obtained from the anatomical museum of Lisandro Alvarado University Medical Faculty. The donor patient was a woman, 62-year-old, which ingress to the emergency service of "Antonio María Pineda" Hospital (Barquisimeto, Venezuela) without vital signs product of a sudden syncope. Non-previous clinical antecedents were available. The heart weighed 225 g and no signs of myocardial infarct/scars were detected at the gross examination.



Sinus Node Dissection and Nodal Cell Identifications

The 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 (Figure 1).^{10,11} 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

Histopathological Analysis

Myocardial tissue was surveyed for detecting histopathological damage. Thin sections (5 µm) were stained with periodic shiff acid (PAS), Masson, and routine hematoxylin and eosin (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* (*T. 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.

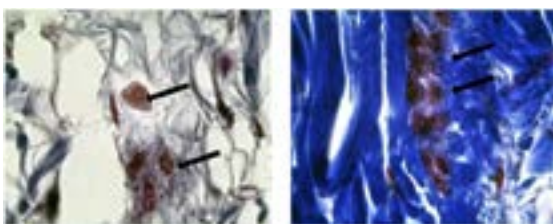
RESULTS AND DISCUSSION

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. In this case, despite relative lacking of clinical information, the antecedent of sudden death without apparent cardiac disease was the first motive that guides us to perform this study. As was referred to in the introduction, sudden death non associated with a structural disease, especially in patients of middle age, is the first cause of death in Chagas disease. In such sense, this case was a very valuable opportunity to identify a non-diagnosed case of chagasic associ-

ated sudden death (amastigote nest was identified) and perform a systematic analysis of possible structural causes associated with malignant arrhythmias.

In the first place, 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



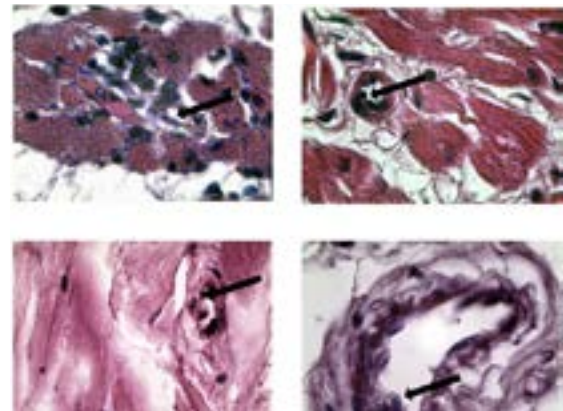
Left, Masson trichromic staining of a cluster of pyramidal cells, morphologically compatible with "P" (pale) pacemaker cells. Right, a cluster of P cells inserted in the root of the superior cava vein. In both cases, scarcity and disconnection of nodal P cells cluster are noted. Magnification 100X.

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 hyperpolarization-activated cyclic nucleotide-gated (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. Further studies with immunohistochemical markers should be performed to assess this issue in a more precise fashion.

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 (Figure 3). 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 ves-

sels, direct evidence of microcirculatory disease associated with necrosis focus.⁴ The release of vasoconstrictor substances, such as thromboxane A2 (TXA2) 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.

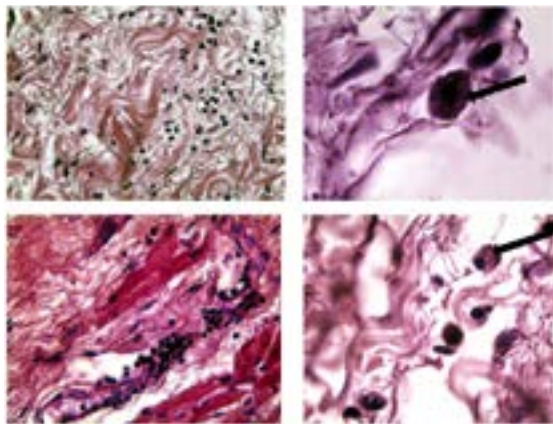
Figure 3. Vascular Morphological Alterations of Myocardial Vessels



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. Arteriolar vessel with inflammatory cells lining endothelial layer. Lower right. Endothelial reactivity (black arrow) in the intima of a medium coronary artery. Magnification 100x.

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 congenital heart disease (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.

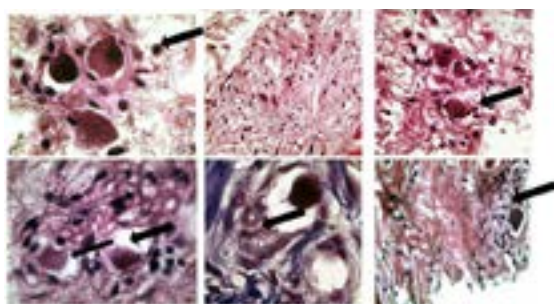
Figure 4. Inflammatory Pattern Alongside Cardiac Tissue of Sudden Death Is Probably Associated With Chagas Disease



Upper and lower left. Mononuclear infiltrates are 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 (Figura 5), Inflammation showed a multi-focal pattern, with the focus observed with >10 cells/40X fields. Remarkably, mastocyte like cells (Figure 5, arrows) were seen in myocardial interstitium slides micrograph.

Figure 5. Intracardiac Ganglia Degeneration of Sudden Death is 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. Right. Degenerative changes in intramural cardiac ganglia are linked to peri-neuronal inflammatory infiltrate. In a higher magnification (bottom, left, and center) mononuclear cells infiltrate inside of cardiac ganglia, generating neuronal death. Finally, at lower magnification, massive infiltration can be detailed, with matrix disorganization in the proximal region of cardiac ganglia. Black arrows. Magnification 40X

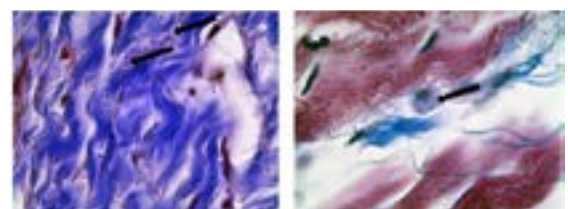
Inflammatory markers mediators may be involved in arrhythmogenesis and sudden death in chagasic patients. Several works postulate the association of inflammatory markers and sudden death. C-reactive protein (CRP), a universal and unspecific inflammatory marker, was reported as a predictor of sudden death in otherwise healthy patients.²¹ It is particularly true regarding supra-ventricular 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- α , 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 heart²⁴ and have a plethora of functions reflected in the secretion of histamine, TNF, proteases, lysosomal enzymes (β -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,²⁵ 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,²⁶ and mast cell density and fibrosis in the tongue muscles correlated with cardiac fibrosis in human autopsies of chagasic patients.²⁷ This profibrotic 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 anatomicopathological studies, the parasite load observed was proportionally higher in heart tissues from patients with the cardiac form,²⁸ although other reports of sudden death failed to find parasite nest in cardiac tissue.²⁹

Figure 6. Intracellular Inclusions Compatible with Amastigotes of *Trypanosoma Cruzi* in Peri-Nodal Area



Left, Masson trichomic staining of connective tissue. Arrows points rounded intracellular inclusions. Right, intracellular matrix positive to Masson trichomic staining, it can be appreciated a macrophage with cytoplasmic inclusions. Magnification 100x.

In this study, 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 cardiomyopa-

thy 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 1) unexpected sudden death normally afflicts younger patients (30-50-years-old) otherwise non-symptomatic³⁰ 2) Heart may be grossly normal or only slightly altered,¹⁹ making very difficult the identification of patients under sudden death risk. Taken together, these data strongly suggest the association of sudden death in Chagasic patients with non-evident structural changes in otherwise healthy patients. These changes, in fact, often are chronic and associated with long-lasting pathophysiology, making very necessary the instauration of serological controls in potentially exposed population and a complete cardiological valorization and following to positive

CONCLUSION

In conclusion, we 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. We concluded that nodal depopulation, microvascular changes, neural degeneration, and amastigote nest presence may be unapparent changes potentially associated with malignant arrhythmias. Due to the scarcity 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 of sudden death associated with chagas disease and, additionally, the most exhaustive correlation among pre-mortem clinical data and post-mortem findings should be performed in further studies in sudden death cases. Last but not least, this study was the opportunity to revise inconjunct the different mechanisms of sudden death and improve the comprehension of malignant arrhythmias pathophysiology.

ACKNOWLEDGMENT

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DISCLOSURE

None.

PERMISSIONS

None.

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