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Developments in the management of Chagas cardiomyopathy

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Abstract

Over 100 years have elapsed since the discovery of Chagas disease and there is still much to learn regarding pathogenesis and treatment. Although there are antiparasitic drugs available, such as benznidazole and nifurtimox, they are not totally reliable and often toxic. A recently released negative clinical trial with benznidazole in patients with chronic Chagas cardiomyopathy further reinforces the concerns regarding its effectiveness. New drugs and new delivery systems, including those based on nanotechnology, are being sought. Although vaccine development is still in its infancy, the reality of a therapeutic vaccine remains a challenge. New ECG methods may help to recognize patients prone to developing malignant ventricular arrhythmias. The

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management of heart failure, stroke and arrhythmias also remains a challenge. Although animal experiments have suggested that stem cell based therapy may be therapeutic in the management of heart failure in Chagas cardiomyopathy, clinical trials have not been promising.

Keywords

Arrhythmias; Chagas cardiomyopathy; Chagas disease; heart failure; myocardial fibrosis; nanotechnology; oxidative stress; stem cell therapy; vaccine development

1. General considerations: transmission, epidemiology, natural history and clinical manifestations

Chagas disease, also known as American trypanosomiasis, is a zoonotic disease caused by the infection with the protozoan *Trypanosoma cruzi*. This disease was initially described in 1909 by the Brazilian physician Carlos Chagas, who named the parasite in honor of his mentor, Oswaldo Cruz. Infection is endemic in Mexico, Central and South America, where it is a serious public health problem. It remains, even today, an important cause of morbidity and mortality in endemic areas of Latin America as well as among infected individuals that have immigrated to non-endemic areas of the world. In recent years, autochthonous cases of Chagas disease have been reported in the US, especially along the southern border with Mexico.[1,2] Due to increased immigration, individuals with Chagas disease have been identified in the US, Canada, Europe, Australia and Japan.[3–5]

Chagas disease is a neglected tropical disease. The World Health Organization recently estimated that it affects 5.7 million people and many millions more are at risk.[6] According to the Global Burden of Disease Study Group, between 1990 and 2013, the estimated annual global mortality from Chagas disease decreased from 12,699 to 10,611 cases a year, with a decrease of potential years of life lost from 343,000 to 245,000 years.[7]

Human infection is acquired through parasite-laden secretions from hematophagous triatomine insects in endemic areas.[1] In many endemic areas of South America, such as in Brazil, the vector has been virtually eliminated and can be considered residual.[8] The disease can also be transmitted through blood transfusions, laboratory accidents, organ donations and vertical transmission from mother-to-child, which are reported even in non-endemic areas.[9] Oral transmission is now recognized as a major mode of transmission that cause sporadic, small human outbreaks, especially in the Amazon region of Brazil.[10]

The clinical course of Chagas disease is divided into acute and chronic phases. The acute phase is usually mildly symptomatic and often misdiagnosed as a febrile illness of childhood. Severe acute infection occurs in approximately 1% of the patients and is manifested by fever, chills, rash, liver function abnormalities, acute myocarditis, pericardial effusion, acute heart failure and/or meningoencephalitis. Clinical manifestations of acute Chagas disease acquired by the oral route are often more severe. The incidence of clinically apparent acute infection has substantially decreased since the near interruption of transmission by vectors and via blood transfusions in most Latin American countries. Blood transfusion-associated acute Chagas disease is rare due to mandatory screening of blood

donors in endemic countries. Organ donation may result in acute Chagas disease if the donor or recipient has not been appropriately screened. Acute exacerbation of infection may result in the setting of the administration of immunosuppressive drugs or as a result immunosuppressive diseases (i.e., HIV/AIDS).[4] During acute infection, trypomastigote forms of *T. cruzi* are readily observed in the bloodstream or detected by PCR.

The acute phase of the disease lasts for approximately 2–3 months, and gradually evolves into the indeterminate stage of chronic disease. In the indeterminate stage, patients have serological evidence of infection, but no clinical, radiologic, electrocardiographic or echocardiographic evidence of heart disease.[1,4] It has been demonstrated that patients in the indeterminate stage who were followed up for 5–10 years had an excellent prognosis, in that they evolved to clinical disease, mostly cardiac, at a rate of ~2% per year.[11] Overall, 20–40% of seropositive individuals develop clinically relevant Chagas heart disease. Digestive involvement, characterized by the presence of megacolon or megaesophagus, occurs in approximately 10% of the cases, but its prevalence appears to vary with the geographic origin of the patients and strain of parasite.[12,13]

Chagas cardiomyopathy is the result of cardiac remodeling and the most important clinical manifestation of Chagas disease, because of its frequency, severity and impact on morbidity and mortality (Figure 1). The chronic phase lasts throughout the lifetime of the patient and results in a shortened survival rate. It is a complex and unpredictable disease that includes a wide spectrum of manifestations, ranging from minor myocardial involvement to patients with terminal cardiac failure, with a high incidence of arrhythmias.[1,4] The clinical presentation of Chagas disease varies according to the degree of myocardial damage. The mild cardiac form of heart disease can occur without left ventricular (LV) dysfunction and is frequently characterized by the presence of some abnormalities on ECG such as premature ventricular contractions and conduction system alterations, typically right-bundle branch block with or without left anterior hemiblock [14] (Figure 2). In these patients, echocardiographic abnormalities may be observed such as segmental LV wall motion abnormalities and diastolic dysfunction. As the disease progresses, LV systolic dysfunction, dilated cardiomyopathy, heart failure, arrhythmias, thromboembolic events and sudden death may occur.[1,4] Table 1 summarizes typical and nonspecific ECG abnormalities observed in Chagas cardiomyopathy, according to the Brazilian Expert Consensus in Chagas Disease.[15]

The clinical course of Chagas cardiomyopathy is variable.[16] The prognosis of patients with Chagas cardiomyopathy appears to be worse compared with patients with idiopathic cardiomyopathy, even when adjusted for relevant risk factors.[17] Several factors related to the prognosis of Chagas disease are noted in longitudinal studies, but the value of some of these markers has yet to be validated.[16] The most consistent independent predictors of death are LV dysfunction, New York Heart Association (NYHA) functional class, and non-sustained ventricular tachycardia, which reflect a more advanced degree of myocardial damage.[18] Prediction scores of the risk of death were developed and may help to recognize high-risk Chagas disease patients.[16]

2. Inflammation, oxidative stress and fibrosis

Infection of the myocardium elicits an intense inflammatory response which may be viewed as a "double-edge sword." Although necessary for the control of parasite proliferation, inflammation results in tissue damage leading to myocardial fibrosis and cardiac remodeling [19–22] (Figure 3). A detailed description of the immunology of Chagas disease is beyond the scope of this paper; however, the observed pro-inflammatory response includes, but is not limited to, secretion of Th1 cytokines and chemokines, eicosanoids, and endothelin-1 during *T. cruzi* infection. The cells that participate in innate and adaptive immune responses including dendritic cells, macrophages, $CD4^+$ and $CD8^+$ T cells, cytotoxic T cells and lytic antibody producing B cells are activated.[23,24] Activation of $CD4^+$ and $CD8^+$ T cells that synthesize Th1 cytokines generally correlates with myocardial damage, at least in experimental models, and may be modulated by IL-17 and T regulatory cells.[21,25,26] During acute infection, mononuclear cells produce high levels of IFN-γ,[27] TNF-α and IL-6.[28]

In chronic Chagas cardiomyopathy, T cells specific for *T. cruzi* as well as T cells that recognize cardiac-myosin are detected.[29,30] The cells infiltrating the myocardium of the patients likely express Th1 transcription factor (T-bet) and low levels of transcription factors related to Th2, Treg and Th17 cells subsets.[31] Other inflammatory mediators including IL-18, TNF-α, CCL21 and CCL2 [32,33] may be involved in induction of transforming growth factor-β (TGF-β), the development of cardiac fibrosis and cardiac myocyte hypertrophy.[21]

TGF-β is an important factor in the induction of inflammation and fibrosis. In different cell types, TGF-β contributes to tissue repair and remodeling [34] and in cell differentiation to myofibroblasts.[35] TGF-β also induces connective tissue growth factor and is critical for the preservation of the matrix by suppressing the activity of matrix metalloproteinases and inducing the production of protease inhibitors.[35] Moreover, TGF-β modulates the cardiac myocyte phenotype and triggers hypertrophic effects.[35,36] In the myocardium of Chagas disease patients, there are increased levels of TGF-β and other growth factors, such as granulocyte-macrophage-colony-stimulating factor and platelet-derived growth factor [37] that may mediate fibrosis. In a retrospective study of 54 Chagas disease patients, TGF-β1 was independently associated with all-cause mortality and hospitalization due to heart failure or cardiac arrhythmias.[38]

Recently, it was demonstrated that Galectin-3, a carbohydrate-binding protein, induced collagen deposition and cardiac fibrosis in *T. cruzi* infection.[39] Furthermore, it was suggested that Galectin-3 could contribute to the development of heart failure [36,40] and its inhibition results in reduction of cardiac fibrosis.[41] Deposits of type III and type IV collagens, laminin and fibronectin occur during subacute and chronic phases of *T. cruzi* infection.[42] Treatment with the anti-trypanosomal agents, benznidazole or MK-436 resulted in regression of the inflammatory and fibrotic lesions.[43] However, this effect may be parasite strain-dependent.[43]

Myocardial biopsies from patients with chronic Chagas cardiomyopathy have demonstrated that fibrosis is associated with activation of the Smad2 pathway, suggesting accentuated canonical TGF-β signaling. In murine Chagas disease, increased level of TGF-β in α2-macroglobulin knockout mice was associated with increased fibrosis. *T. cruzi*–infected mice treated with TGF-β inhibitors, SB-43154231 or GW78838848, showed a consistent reduction of heart fibrosis as determined by expression of fibronectin and type I collagen.[44] Others have shown that administration of ganglioside GM1 reduced arrhythmias in patients with Chagas disease [45] and fibrosis in *T. cruzi*–infected mice.[46] The antifibrotic effect was associated with a significant reduction in myocardial expression of TGF-β and in IFN-γ, TNF-α and CCL5/RANTES, all proinflammatory cytokines and chemokines. Thus, treatment with ganglioside GM1 may offer a potential therapy for modulating myocardial damage in chronic Chagas disease.

Endothelin-1 (ET-1) is a 21 amino acid peptide which has been implicated in the pathogenesis of cardiac fibrosis including that associated with Chagas disease. Mice treated with phosphoramidon, an inhibitor of endothelin converting enzyme, which mediates the synthesis of ET-1, resulted in a reduction in cardiac pathology including fibrosis when administered early in infection.[47] Additionally, *T. cruzi* infection of mice in which the ET-1 gene has been selectively knocked out resulted in a marked reduction in cardiac fibrosis.[48] In both of these models, cardiomyopathy was ameliorated and determined by cardiac imaging studies.

Verapamil is a calcium-channel blocker which has been demonstrated to reduce mortality and significantly reduce inflammation and fibrosis in infected mice treated early in infection.[49,50] This effect was not demonstrable when treatment was begun during the chronic stage. Thus, many of these experimental studies suggest that the reduction of cardiac fibrosis is dependent, in part, on the timing of therapy, mouse species and parasite strain.

The Garg laboratory has pioneered studies on the role of oxidative stress in chronic Chagas cardiomyopathy. *T. cruzi*–induced intracellular Ca^{+2} flux, required for parasite invasion,[52] elicits mitochondrial loss of membrane potential and an increase in the generation of mitochondrial reactive oxygen species (mtROS) contributing to oxidative adducts in cardiac myocytes.[53,54] The mitochondrial inefficiency in the setting of oxidative phosphorylation continues during the chronic disease phase.[55,56] Enhancing the host's capacity to scavenge ROS by chemical antioxidant or by genetic overexpression of mitochondrial superoxide dismutase led to improved mitochondrial function that was associated with a significant control of oxidative and inflammatory stress and cardiac remodeling in *T. cruzi*–infected mice.[57,58] Chagas disease patients with clinically symptomatic disease exhibited higher levels of oxidative and inflammatory stress markers and mitochondrial dysfunction than was noted in clinically asymptomatic infected individuals.[55,59] It is not mechanistically proven what triggers mitochondrial defects and why it is not arrested to prevent continuous oxidative damage. Some evidence suggest that mtROS-induced DNA adducts result in poly(ADP-ribose) polymerase 1 (PARP1) activation in infected cardiac myocytes.[60] PARP1 is a DNA repair enzyme; however, its hyperactivation results in excessive utilization of NAD^+ (substrate) and PARylation of mitochondrial proteins, thus, setting the stage for metabolic and oxidative imbalance in the heart. Other studies suggest

that PARP1/PAR result in activation of the NF-κB pathway of proinflammatory cytokines (e.g., TNF-α, IL-1β) production in infected cardiac myocytes.[60,61] Thus, it is possible that targeting PARP1 will provide dual benefits in controlling Chagas disease inflammatory and oxidative stress, to be tested in future studies.

3. Vaccine development

Studies in animal models and humans have revealed the pathogenic mechanisms found during disease progression, and the features of protective immunity.[24] Parasite-specific $CD4^+$ T cells have been suggested to assist in *T. cruzi* control through secretion of Th1 cytokines (e.g., IFN-γ, IL2), amplification of the phagocytic activity of macrophages, stimulation of B-cell proliferation, and antibody production and differentiation and activation of $CD8^+$ T cells (reviewed in [24]). *T. cruzi* antigen-specific $CD8^+$ T cells contribute to parasite control, either by cytolysis of the infected cells or by secretion of Th1 cytokines (IFN-γ) that induce trypanocidal activity (reviewed in [62]). Accordingly, several antigens (e.g., GP90, cruzipain, GP82, ASP2, GP56, Tc52, CRP, TSA1, Tc24) have been tested for their ability to elicit protective immunity to *T. cruzi* in experimental animals (reviewed in [63]). A majority of the candidate antigens were selected based upon their potential to be recognized by antibodies in infected mice and have proved to be efficacious as vaccine in providing some degree of protection from *T. cruzi* infection. In parallel, efforts to enhance the protective efficacy of subunit vaccines against *T. cruzi* have included testing the use of adjuvants, e.g., saponin, CpGODN, IL-12 and GMCSF cytokines,[23,64] attenuated strain of *Salmonella* [65] or adenovirus [66] for antigen delivery; and heterologous prime-boost protocols.[67]

To prevent investigator bias in vaccine design, the Garg group developed a computational/bioinformatic algorithm for screening the *T. cruzi* sequence database for the vaccine candidates.[68] These studies identified 11 potential candidates that were submitted to rigorous analysis for eliciting immunity against *T. cruzi.*[68,69] Eventually, these were narrowed down to TcG1, TcG2 and TcG4 as the candidates that exhibited relevant characteristics as vaccine candidates. These antigens were highly conserved in clinically relevant *T. cruzi* isolates, expressed (mRNA/protein) in trypomastigotes and amastigotes (mammalian stages) of *T. cruzi*, and released during parasite differentiation in host cell cytoplasm, a characteristic required for antigen presentation for T-cell activation.[68] These antigens showed antigen-specific antibody (IgG1, IgG2a and IgG2b) and/or $CD8^+$ T-cell responses in *T. cruzi*-–infected mice and dogs; and were also recognized by antibody response in Chagas disease patients from distinct study sites (Argentina–Bolivia and Mexico–Guatemala), and expressed in diverse strains of the circulating parasites (Unpublished Data nj garg and [70]). Immunization with TcG1, TcG2 and TcG4 as a DNA vaccine provided T-cell immunity (TcG2 = TcG4 > TcG1) that was additive when the antigens were co-delivered and achieved a significant (but modest) control of challenge infection in mice and dogs.[68,69,71] The delivery of these antigens as heterologous prime/boost vaccine provided protective immunity consisting of parasite- and antigen-specific lytic antibodies and type 1 $CD8^+$ cytotoxic T lymphocytes against challenge infection and chronic disease [72–74] that was significantly better than that observed with DNA-prime/DNA-boost vaccine. [68,69]

The enhanced efficacy of a heterologous prime/boost approach for vaccine delivery could be because delivery of antigens as DNA vaccines elicits robust T-cell responses, which are critical for the development of T-cell-dependent antibody responses, and DNA immunization is also highly effective in priming antigen-specific memory B cells.[75] Delivery of vaccine candidates as recombinant proteins is generally more effective at eliciting antibody responses and may directly stimulate antigen-specific memory B cells to differentiate into antibody-secreting cells, resulting in production of high-titer, antigen-specific antibodies.[76] In a recent study, it was demonstrated that TcG2 and TcG4 DNA prime/protein boost vaccine achieved long-term protection against *T. cruzi* infection and Chagas disease, suggesting the vaccine-induced effector T cells can be long-lived and provide protection from parasitic infection. This vaccine-induced immunity waned slightly after 6 months post booster immunization, but was still sufficient to provide twofold control of invading pathogens [77] that, according to mathematical modeling, is sufficient to break the parasite transmission cycle [78] and prevent disease progression.[79] Pertinent to the theme of this review, it is important to note that many of the studies discussed highlight the importance of a preventive or therapeutic vaccine to control *T. cruzi* infection by at least decreasing parasite burden, cardiac tissue inflammation and damage or increasing survival if not providing sterile immunity. This has recently been underscored in papers by Ma et al. [80] and Pereira et al. [81].

4. Stroke and atrial fibrillation

Chagas disease is a risk factor for stroke that may result in marked functional disability and death.[82–85] The incidence of thromboembolic events in patients with Chagas disease varies widely, depending on the population studied.[86] Necropsy studies assessing patients with advanced cardiomyopathy showed high rates of cerebral infarction,[87] and an increase in the prevalence of stroke is expected as a result of aging of the *T.* cruzi-infected population in Latin America.[88]

Cardioembolism is the main cause of stroke in patients with Chagas disease.[89] Several risk factors for stroke have been reported including heart failure, LV systolic dysfunction, left atrial enlargement, apical aneurysm, mural thrombus and arrhythmias.[82,84,85,89] However, stroke may occur in patients without clinical evidence of heart disease; a careful echocardiographic study may reveal mural thrombus and apical aneurysms (Figure 4A & B).[83,90] A cross-sectional study found that the classic vascular risk factors, including hypertension, diabetes mellitus and smoking, are less common in stroke patients with Chagas disease compared with those without.[91] Recurrence of stroke has been estimated to occur in 20% of patients with Chagas disease.[83]

Atrial fibrillation often indicates advanced myocardial damage and is a predictor of mortality in patients with Chagas cardiomyopathy.[4,92,93] In addition, atrial fibrillation is an important risk factor for stroke, independent of the LV function.[82,94] Reports from a prospective hospital-based case–control study revealed that approximately 15% of patients with Chagas disease with stroke who were consecutively admitted to the hospital had atrial fibrillation. [82,85] Furthermore, a population-based cohort study showed that the presence of atrial fibrillation was a predictor of the risk of mortality from stroke in *T. cruzi*–infected

elderly patients.[94] The risk of stroke varies widely among patients with atrial fibrillation, depending on other clinical features. Increased left atrial volume has been shown to be associated with an increased risk for stroke, independent of atrial fibrillation, age and other clinical risk factors for cerebrovascular disease.[95]

Studies assessing the source of embolism in Chagas disease found that the presence of left atrial thrombi was not associated with ischemic cerebral events.[83,90] However, low left atrial appendage flow velocity has been demonstrated to be an important predictor of embolic risk in atrial fibrillation, regardless of the presence of thrombus in the left atrium.[96] The relation of LV dysfunction and stroke appears to be partially mediated by stasis of flow in the left atrial appendage.[97] Indeed, cerebral infarction in Chagas disease occurs more commonly in the advanced chronic heart disease than in asymptomatic patients in the indeterminate stage of chronic disease.[84,85,87,94]

Although cardioembolism is the main cause of ischemic stroke related to Chagas disease, cryptogenic and small vessel strokes can also occur, especially in patients with mild chronic heart disease.[82] The increased proportion of patients with Chagas disease who are smokers, and have diabetes, obesity, and sedentary lifestyles may increase the risk of stroke.[82,98]

5. Electrocardiology of ventricular arrhythmias

Chagas cardiomyopathy is also an arrhythmogenic condition and sudden death may be the first manifestation of the disease.[99] Chagas disease may present with ventricular premature beats, short runs of non-sustained ventricular tachycardia (VT) or with malignant VT.[100] This is most likely due to areas of necrosis and fibrosis resulting from myocardial inflammation. This is also associated with microvascular lesions or autonomic changes that regulate blood perfusion of the injured myocardium. The damage to the intercellular junctions provokes changes in electrical potential and impair conduction of the stimulus between cells, causing electrical uncoupling, slow conduction of stimuli and unidirectional block.[100] This process, associated with the fibrotic areas, forms the reentrant circuit that causes ventricular arrhythmias.

The recognition of fibrosis, either by cardiac magnetic resonance imaging (CMRI) or eletrocardiological methods, allows the recognition of patients prone to develop arrhythmic complications.[101–103] CMRI, using the delayed enhancement technique, can be useful to select patients with global or regional ventricular dysfunction, with high degree of fibrosis and at higher risk for clinical VT.[103] The presence of zones of slow conduction in the myocardium may be recognized by the presence of prolonged filtered QRS duration obtained by signal averaged ECG, which was shown to be an independent predictor of death in Chagas disease patients.[101] Since both CMRI and signal-averaged ECG are not readily available, an alternative simple, noninvasive method is a 12-lead ECG QRS scoring system, which quantifies myocardial fibrosis, correlates with the scar, evaluated by CMRI, and with LV dysfunction and with malignant VT.[102]

The role of autonomic nervous system dysfunction in the mechanisms of ventricular arrhythmias and sudden death in Chagas cardiomyopathy has been the subject of

investigations for decades,[104,105] since the classical description by Koeberle of the "cardiopatia parasympathicopriva".[106]

Chagas disease patients may have reduced vagal modulation over the sinus node, causing reduced heart rate response to physiological and pharmacological stimulus,[107–109] as well as impaired heart rate variability, measured using time and frequency domain and nonlinear methods.[110,111] These abnormalities occur early in the course of the disease, even before the development of overt heart disease or LV dysfunction, generally with mild abnormalities,[109,111] suggesting an underlying disease-specific mechanism. Vagal dysfunction appears to be more prominent in patients with LV dysfunction [112] and can be detected even in older patients with Chagas disease, but under 70 years of age.[113] Cardiac sympathetic denervation has also been detected in some patients by iodine-123 (I-123) meta-iodobenzylguanidine scintigraphic studies,[114,115] and can occur early in the course of the disease.[115]

Although it was demonstrated that autonomic dysfunction is an early and typical finding in the natural history of human Chagas disease,[105,111] the association between impaired autonomic cardiac modulation and sudden death remains elusive. Small studies have shown an association between sympathetic innervation defects (as detected by iodine-123 meta-iodobenzylguanidine scintigraphic studies) and sustained VT. [114] In addition, autonomic-driven abnormal heart rate dynamics have been shown to precede VT in Chagas disease patients.[116] No major cohort study demonstrated a prognostic value of abnormal autonomic tests or reduced heart rate variability and the clinical value of these methods is still controversial.

More robust data exist linking the presence of cardiac repolarization abnormalities and the risk of death in Chagas cardiomyopathy. Abnormal QT interval or increased QTd dispersion,[117] as well as T-wave axis deviation [118] or primary T-wave abnormalities in the 12-lead ECG,[93] were associated with an increased risk of death in cohort studies. Other methods of evaluation of repolarization variability, as T-wave amplitude variability [119] and T-wave spatial heterogeneity,[120] were independently associated with higher risk in a relatively small cohort. Microvolt T-wave alternans, evaluated by a commercial method and useful in other conditions, is abnormal in Chagas cardiomyopathy patients and may be helpful in the risk stratification of Chagas disease patients.[121]

6. Heart failure: advances in pharmacological treatment

The treatment modalities employed in the management of heart failure in patients with Chagas cardiomyopathy are similar to that employed in the management of heart failure of other etiologies.[1] Therapy relies on the inhibition of renin-angiotensin-aldosterone and sympathetic systems, including a combination of three types of drugs: diuretics, beta blockers and angiotensin-converting enzyme (ACE) inhibitors.[1] ACE inhibitors remain the first-line treatment for inhibition of the renin-angiotensin system in heart failure in the setting of Chagas disease, but angiotensin receptor blockers are reasonable alternatives in patients who cannot tolerate the side effects of ACE inhibitors. ACE inhibitors have been

shown to improve cardiac function from Chagas heart disease. Although studies were not powered to evaluate mortality.[122,123]

Beta blockers are another option in the management of Chagas heart disease. They improve clinical status and may reduce mortality.[122,124,125] However, previous studies demonstrated that the frequency of beta blocker use was lower in patients with Chagas disease when compared with other etiologies of heart failure.[17,124,125] This is likely due to concerns related to the possible risk of bradycardia and hypotension. A recommended strategy in the management of heart failure in the setting of Chagas disease is to give ACE inhibitors and diuretics at first to compensate for congestive symptoms. After the improvement of clinical status, beta blockers can be safely given at targeted doses, if necessary reducing ACE inhibitors doses.[122] Aldosterone receptor antagonists seem to be safe in Chagas cardiomyopathy and should be added in patients who are already on ACE inhibitors (or angiotensin receptor blockers) and beta blockers in symptomatic patients.[1]

Digoxin has long been used in the treatment of patients with heart failure secondary to Chagas cardiomyopathy. This drug may be added to the initial regimen in patients with severe symptoms who have not yet responded symptomatically to standard therapy.[1] Patients should not be given digoxin if they have significant sinus or atrioventricular blockade unless it has been addressed with the insertion of a permanent pacemaker. The drug should be used cautiously in patients taking other drugs that can depress sinus or atrioventricular nodal function or affect digoxin levels, especially amiodarone or beta blockers. Although treatment with digoxin had no effect on mortality in non-Chagas heart failure, the use of this drug can improve symptoms and exercise tolerance in patients with Chagas cardiomyopathy.[1,126]

There are insufficient data to make broad recommendations regarding anticoagulation in patients with Chagas cardiomyopathy. In general, anticoagulation is recommended for patients with Chagas disease who have permanent/paroxysmal atrial fibrillation, previous thromboembolic events, or the presence of a cardiac thrombus detected by echocardiography.[127] Sousa et al., prospectively studying 1043 patients with chronic Chagas disease,[128] developed a risk score for ischemic attacks. LV dysfunction was given 2 points and apical aneurism, abnormalities of the ST-T segment and age >48 years, 1 point each. Anticoagulation with warfarin was indicated in patients with 4–5 points (4.4%/year risk of stroke versus 2.0%/year risk of severe bleeding). The role of new anticoagulants and anti-platelet drugs in the prevention of thromboembolic events has yet to be determined in the setting of Chagas disease.

Heart transplantation can be used for selected patients with refractory end-stage heart failure [127] and may be the only option for some refractory patients.[129] This approach has been successful at some centers. However, the problems with this approach include high cost, donor availability and accessibility especially in rural areas. Additionally, there is the risk of re-activation of the infection as a result of the administration of immunosuppressive drugs. [130] Recurrent life-threatening ventricular arrhythmia that is refractory to all currently available treatments is a less common indication for cardiac transplantation in patients with Chagas cardiomyopathy. Monitoring of the reactivation of infection with *T. cruzi* after heart

transplantation should be performed routinely.[127] Biventricular pacing could be an effective alternative for selected patients,[131,132] but should be restricted to patients who presented with spontaneous or pacemaker-induced left bundle branch block, since those with right-bundle branch block do not benefit with the implantation of the pacemaker.[133] Multidisciplinary approach of heart failure patients, including cardiac rehabilitation and pharmaceutical care, may be helpful in improving symptoms and quality of life in Chagas disease patients.[134,135]

7. Heart failure: stem cell therapy

As stem cell therapy has been used increasingly often for other cardiomyopathies (for review see [136,137]), a number of studies have now applied variations of this strategy in rodent models of chronic Chagas cardiomyopathy and a small number of clinical trials have begun. One strategy for therapy is to use the entire population of bone marrow mononuclear cells (BMMCs), which contains hematopoietic progenitors and also mesenchymal stem cells (MSCs), which are capable of differentiating into multiple cell types including cardiac myocytes and endothelial cells.[138]

An initial report, in a mouse model of Chagas cardiomyopathy,[139] demonstrated that intravenous BMMC injection significantly reduced cardiac inflammation and fibrosis for up to 6 months after treatment. This study also used enhanced green fluorescent protein-tagged donor cells to show that at least some BMMCs had migrated to and were retained within the heart. In a follow-up study using cardiac MRI, tail vein injection of BMMCs was shown to prevent and even reverse the right ventricular (RV) dilatation induced by both acute (1 month) and chronic (up to 6 months) *T. cruzi*-infection.[140] Subsequently, it was reported that fibrosis and inflammation were reversed in chronically infected mouse heart 2 months after mononuclear cell injection.[141] Moreover, microarray analysis in this study revealed that the extensive gene expression changes previously identified in chronic Chagas cardiomyopathy [142] were largely (about 85%) restored to control values by cell therapy. Because the proportion of MSCs in the BMMC population is very low (<0.02%; [143]) and these cells are both anti-inflammatory and capable of differentiating into myocytes, purified MSCs have been evaluated for efficacy in cell therapy.

Jasmin and colleagues [144,145] demonstrated that tail vein injected MSCs were as effective as BMMCs in reducing RV dilation and restoring expression patterns of selected genes. Tracking of nanoparticle-labeled cells revealed significant homing to the heart at early times after injection in infected mice, although improvement of cardiac function was attributed to paracrine action because newly differentiated labeled myocytes were not found in the heart. The success achieved with BMMC and MSC therapy in rodent model of Chagas cardiomyopathy led to clinical trials evaluating safety and efficacy of BMMC cell therapy in these patients.[144,146,147] Both trials involved patients with heart failure due to chronic Chagas cardiomyopathy, with severely compromised left ventricular ejection fraction (LVEF) and NYHA class III or IV. Bone marrow was aspirated and BMMCs enriched through gradient centrifugation were injected in coronary arteries. After 25 days, patients received G-CSF injections for 5 days (based on studies demonstrating that this factor

decreased fibrosis and inflammation: [148]). Results of the initial trial were demonstrated safety of the procedure and small but significant increase in LVEF and 6-min walking.

Efficacy of BMMCs in patients with chronic Chagas cardiomyopathy was tested in a more extensive phase 2/3 study,[147] with data from 183 patients analyzed. The results showed that both groups, BMMCs and placebo, improved LVEF at 6 and 12 months follow-up, but there was no significant difference between the two groups. This was also observed in secondary endpoints such as the 6-min walking distance, and NYHA class. It was concluded that the procedure was safe but that injection of autologous BMMCs in patients with chronic Chagas cardiomyopathy did not provide additional benefit when compared to standard drug therapy.

Substantial progress has thus been made toward realizing therapeutic efficacy for chronic Chagas cardiomyopathy in rodent models and is beginning to clarify mechanisms underlying efficacy. However, significant therapeutic benefit of stem cell therapy has not been demonstrated in the few clinical trials that have been undertaken testing cell-based approaches in Chagas disease patients. In order to optimize translation of the success in small animal studies to clinical practice, several new strategies could be applied.

a. Intermediate-sized models, such as dogs, should be used to confirm rodent results whenever possible.[146]

b. Novel stem cell types could be used in the studies. Most studies of cell therapy in chronic Chagas cardiomyopathy have used BMMCs or MSCs, but there are multiple other sources of MSCs, including adipose tissue, dental pulp, umbilical cord and other tissues. It is notable that early studies by Guarita-Souza et al. [149] injected a mixture of skeletal myoblasts and mesenchymal bone marrow–derived cells that had been co-cultured for 2 weeks into the LV of chronically infected rats. At 1 month after injection, end-systolic and diastolic volumes were decreased and ejection fraction was increased. This study raises the possibility that cells with greater differentiation potential might show promise. One new avenue worth pursuing is reprogramming to reverse fate of differentiated blood cells or fibroblasts by a cocktail of only a few transcription factors,[150] thereby providing cells that might replace damaged myocytes.

c. Manipulations of treatments used to vary such parameters as route of delivery and co-therapy with adjunctive treatment. In small animal model studies, stem cells have been delivered through injection into tail vein, local injection into the myocardium, and even intraperitoneally. In patient trials, the route of delivery is generally though catheterization for local placement within the cardiac circulation.

d. Severity of disease at time of treatment and possible presence of comorbidities should be carefully evaluated in human patients who qualify for the trials on the basis of disease severity.

8. Parasite persistence and specific treatment

The role of the persistence of a low-grade *T. cruzi* infection in the development of chronic heart disease had been a matter of controversy for decades.[151–154] The presence of both

tissue parasitism and parasitemia in chronic Chagas disease has been established and supported by the transmission of the disease by chronically infected persons by blood and placental route, [155,156] as well as the reactivation of the disease due to immunosuppression after organ transplantation, HIV infection or autoimmune diseases. [157] There is substantial evidence that parasite persistence is important in the progression of chronic Chagas disease. In a landmark study, Zhang and Tarleton [158] showed, in a murine model, that parasite persistence correlates with the presence of disease in the heart and the clearance of parasites from tissues was associated with amelioration of inflammation. Subsequently, Schijman et al. demonstrated that the extent of inflammation, fibrosis and severity of the disease was associated with persistence of parasite DNA in cardiac lesions obtained from Chagas disease patients.[159] These findings were confirmed by Benvenuti et al. [160], who analyzed heart biopsies from 29 individuals for *T. cruzi* DNA and found 25 positive by PCR, as well as a significant positive association between myocardial parasite persistence and high-grade myocarditis. In an article from the NIH-sponsored REDS II cohort, Sabino et al. [161] studied 499 *T. cruzi* seropositive blood donors and 101 patients with clinically diagnosed Chagas cardiomyopathy. Rates of PCR detection of *T. cruzi* DNA in blood were significantly higher in those diagnosed with Chagas cardiomyopathy (75.2%) compared with seropositive donors without cardiomyopathy (51.3%). Presence of parasitemia significantly correlated with known markers of disease progression, such as QRS prolongation, reduced LV ejection fraction and higher levels of troponin and NTpro-BNP.[161]

The importance of the parasite persistence in the development of Chagas cardiomyopathy is also supported by a large non-randomized clinical trial reported by Viotti et al. [162], showing that benznidazole treatment, compared with no treatment, was associated with reduced progression of Chagas disease and increased negative seroconversion for patients with chronic disease.

The results from various studies suggested that specific antiparasitic therapy could potentially halt the progression of the disease. [162,163] In fact, most experts recommend the anti-trypanosomal treatment for most of patients with chronic *T. cruzi* infection, with or without cardiomyopathy, especially in those below 50 years of age.[152,163–165] However, there are many concerns related to the toxicity of the currently available drugs, the absence of evidence of benefit in those older than 50 years of age and the absence of a randomized clinical trial that documented the beneficial effect of the specific treatment.[127,166] There are only two drugs currently available for the treatment of Chagas disease, nifurtimox and benznidazole, both associated with significant toxicity, especially in adults. A recent meta-analysis on trypanocidal drugs for chronic asymptomatic Chagas disease included 13 studies involving 4229 participants and showed that, although trypanocidal drugs reduce parasite-related outcomes, the effect on clinical outcomes remains elusive.[167] It was expected that the results of a multicenter, randomized, double-blinded, placebo-controlled trial would demonstrate a beneficial effect of benznidazole on cardiac outcomes in patients with chronic infection (BENEFIT study).[168] However, the recently published results of that study revealed that although there was a significant reduction in parasitemia, benznidazole therapy did not halt the progression of cardiac disease.[169]

Treatment of Chagas disease is mandatory for all acute infections, caused by vector transmission, oral transmission, congenital infection, laboratory accidents or organ transplantation associated with reactivation due to immunosuppression and for children with chronic infection up to 12 or up 18 years of age.[127]

Posaconazole, a triazole antifungal drug with high trypanocidal activity, was a promising antiparasitic drug, but a randomized clinical trial recently published showed that most patients treated with posaconazole recur with detectable parasitemia during a follow-up period of 10 months.[170] Another randomized clinical trial evaluating the association of posaconazole and benznidazole, the STOP-CHAGAS trial (NCT01377480), is currently underway. The antiparasitic effect of the anti-arrhythmic drug amiodarone, demonstrated in experimental studies, was not confirmed in the clinical setting.[171]

9. Other developments in drug therapy

Considering the uncertainties regarding the effectiveness and the toxicity of currently available drugs, there is a renewed interest in the discovery and development of new drugs for treatment of chronic Chagas disease.[172,173] A major goal of drug therapy is the delivery of drug not only to the bloodstream but also to the intracellular site and nanotechnology offers the possibility of enhancing the drug delivery to intracellular sites. There are several drug strategies that show promise with respect to limiting the severe vascular/cardiac consequences of chronic inflammatory response to the chronic infection. These drug strategies are based on the reported efficacy of nitric oxide (NO) and its related bioactive forms,[174–176] curcumin [177] and selenium. [178,179] Two of these (NO and curcumin) also hold promise with respect to limiting infectivity. A major limitation in translating the promising preclinical studies into actual therapies that can be deployed and administered in a cost- and time-effective manner is the issue of drug delivery with respect to enhancing bioavailability, rate and duration of delivery, mode of delivery and site-specific targeting to tissues.

Nanoparticle platforms have been developed that show promise with respect to overcoming many of the drug delivery limitations. One such platform is based on a hybrid composition in which the high porosity of a silane-derived hydrogel is “plugged” using chitosan to form a glass-like hydrogen bonding network.[180] NO and N_2O_3, a potent nitrosating agent, can be generated from nitrite within this glassy matrix. The release of NO or any other incorporated therapeutics does not occur when the nanoparticles are either dry or in a nonaqueous medium. The introduction of water triggers the release. Release rates can be easily controlled by tuning the composition of the nanoparticle. These nanoparticles can deliver therapeutic payloads through topical,[181] mucosal, intravenous [182] and intraperitoneal administration routes.[182] Mucosal delivery of myristic acid containing NO releasing nanoparticles produces sustained systemic NO-related vascular consequences seemingly comparable to the sustained response to NO administered intravenously (IV) releasing nanoparticles (unpublished results).

The NO releasing nanoparticles have been shown to be highly effective as a very broad spectrum antimicrobial agent. [133,183–185] Additionally, and most significantly, is that

these nanoparticles, when administered IV in animal models, have been shown to be anti-inflammatory [182] with respect to vascular dysfunction and the inflammatory cascades induced by several triggers including acellular hemoglobin, acute hemorrhage,[186] and lipopolysaccharide induced endotoxemia. These biocompatible nontoxic NO-releasing nanoparticles were shown to normalize the cytokine profiles, restore/increase tissue perfusion, deactivate platelets, inhibit leukocyte activation and normalize shock-activated macrophage populations (unpublished results). Similar anti-inflammatory properties were observed for IV infused nanoparticles containing a curcumin–selenium complex (unpublished results) and for curcumin-loaded nanoparticles applied topically to mouse knees in an induced osteoarthritis model (unpublished results). The relevant finding with respect to Chagas disease is that the nanoparticles traversed the skin and are then localized in fat pads where drug is stored and slowly released. The implication is that these nanoparticles can be used topically to create a large reservoir of curcumin (or curcumin–selenium complex) in fatty tissues that will provide sustained therapeutic levels not achievable by oral administration.

A newly developed platform incorporates paramagnetic nanocrystalline centers (derived from gadolinium oxide) into the hybrid hydrogel nanoparticles described above. These paramagnetic nanoparticles can be rapidly and efficiently localized subsequent to intravenous administration using a powerful external magnetic field placed at the target site. This platform would allow for targeted delivery of high levels of NO, nitrosothiols, curcumin, curcumin–selenium to the heart. Similarly, this paramagnetic platform could deliver siRNAs designed to repair or rebuild damaged myocardium. Nanoparticle technology will very likely open up new therapeutic options for the treatment of many aspects of Chagas disease.

Expert commentary

Chagas disease is an important cause of heart disease not only in endemic areas of Latin America but also among those that have emigrated from endemic areas to non-endemic areas of North America, and Europe where physicians are now encountering what is for them a “new disease.” Chagas disease persists as a disease related to poverty, both in endemic and non-endemic countries, affecting people who came from rural areas with poor housing conditions. The access of those millions of infected persons to health care and drugs remains one the most difficult challenges of this neglected disease.

Chagas disease is a complex disease with a very long natural history and a wide spectrum of clinical manifestations, ranging from asymptomatic patients without apparent cardiomyopathy to severe cardiac and digestive manifestations. Although several aspects of the disease had been elucidated in the last decades, important questions remain unsolved. While several studies have documented mechanisms involved in the development of inflammation and fibrosis, as well as the role of the oxidative stress, the translational aspects of these studies have not been fully achieved. A better understanding of the role of parasite persistence in the severity of the manifestations of the chronically infected patients is an important area of research for future studies.

There only two antiparasitic drugs commercially available for clinical use that have problems with both efficacy and toxicity. The first phase of the BENEFIT trial which aimed to evaluate the effectiveness of benznidazole in chronic Chagas disease, has finished the required follow-up period and the results have been disappointing. Thus, new and safer effective drugs are needed. It is possible that new strategies can be developed from innovative approaches, as effective vaccines or the use of nanotechnology for targeted delivery of antiparasitic drugs.

In the clinical setting, there are many challenges related to the paucity of specific studies addressing questions about the specificity of the care of patients with Chagas cardiomyopathy. Further research is needed on the use of diagnostic methods and for guiding treatment of the various clinical syndromes seen with Chagas disease. Promising new treatments, such as stem cell therapy, have not fulfilled our expectations, and research to understand why stem therapy results have been different in mice and humans should lead to improved stem cell therapeutics for this infection.

Five-year view

Since research on Chagas disease has now intensified, we expect to see, in the next 5-year span, more data on the diagnostic and prognostic values of several clinical tests, especially of ECG-derived and blood biomarkers. The initial results of the BENEFIT trial while disappointing may open the possibility of new clinical trials, perhaps employing a combination of drugs.

The role of mitochondrial oxidative stress and inflammation in pathogen control versus causing host injury can only be delineated with future longitudinal studies in humans, or large animal models, and will guide the promise of antioxidant and anti-inflammatory therapies in controlling progressive Chagas cardiomyopathy.

There remains a dearth of data on the immune response in human Chagas disease, and these data are needed for further development and testing of prophylactic and therapeutic efficacy of currently developed vaccine formulations as well as guide the development of next generation vaccines.

The promising results of mononuclear and mesenchymal stem cells in the rodent model of Chagas cardiomyopathy, in our opinion, warrant continued optimization in this model. Such studies should focus on selection of most effective and clinically applicable stem cell type, development of adjunctive therapy to improve cardiac recovery and determination of mechanisms responsible for functional improvement. Other large animal models will likely be useful for fine-tuning the translational studies to the clinic.

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Key issues

- Chagas disease remains an important cause of morbidity and mortality in endemic areas of Latin America and among infected individuals that have immigrated to non-endemic areas of the world.
- Myocardial fibrosis is a major pathophysiological process in Chagas cardiomyopathy. While the mechanistic basis is not entirely known, induction of TGF-β, Galectin-3, ET-1 and other proinflammatory cytokines and chemokines appears to be important.
- Clinical recognition of fibrosis, either by cardiac magnetic resonance imaging or eletrocardiological methods, may allow the recognition of patients prone to develop arrhythmic complications.
- Clinical management of stroke, atrial fibrillation and heart failure is still challenging and clinical studies in Chagas cardiomyopathy are needed.
- Current research in vaccine development for prevention and/or therapy is only in its infancy but there is hope that this can be achieved.
- Although stem-cell-based therapy showed promising results in animal experiments, clinical trials did not demonstrate clinical benefit in patients.
- Specific antiparasitic therapy could potentially halt the progression of the disease; however, the toxicity and limited effectiveness of current available drugs, benznidazole and nifurtimox, are obstacles to their widespread use.
- There is a renewed interest in the discovery and development of new drugs and methods for treatment of chronic Chagas disease; nanoparticle platforms have been developed that show promise with respect to improving drug delivery with respect to enhancing bioavailability, rate and duration of delivery, mode of delivery, and site-specific targeting to tissues.

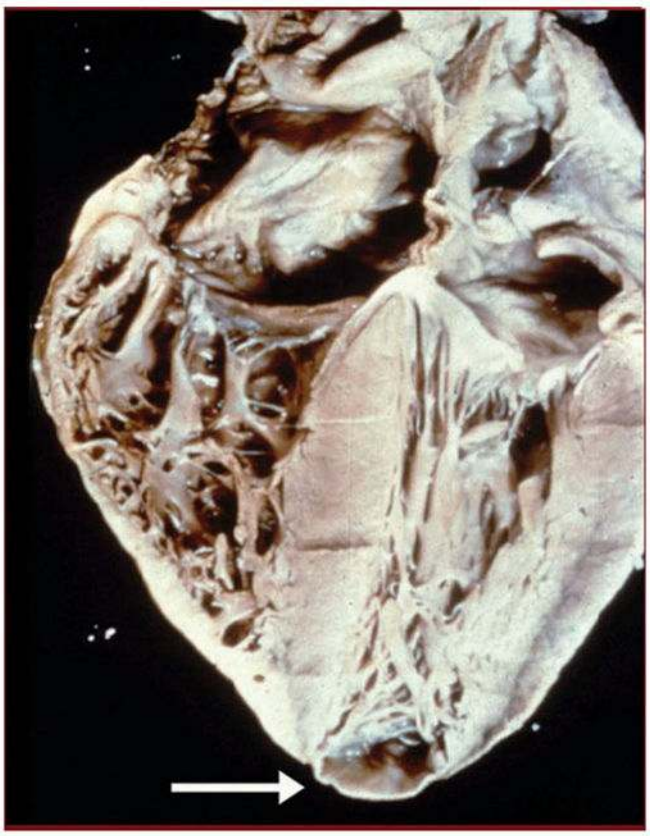

Figure 1. Heart of a patient who died of Chagas cardiomyopathy

There is cardiac enlargement and a prominent apical aneurysm (arrow). Courtesy of Armed Forces Institute of Pathology, United States.

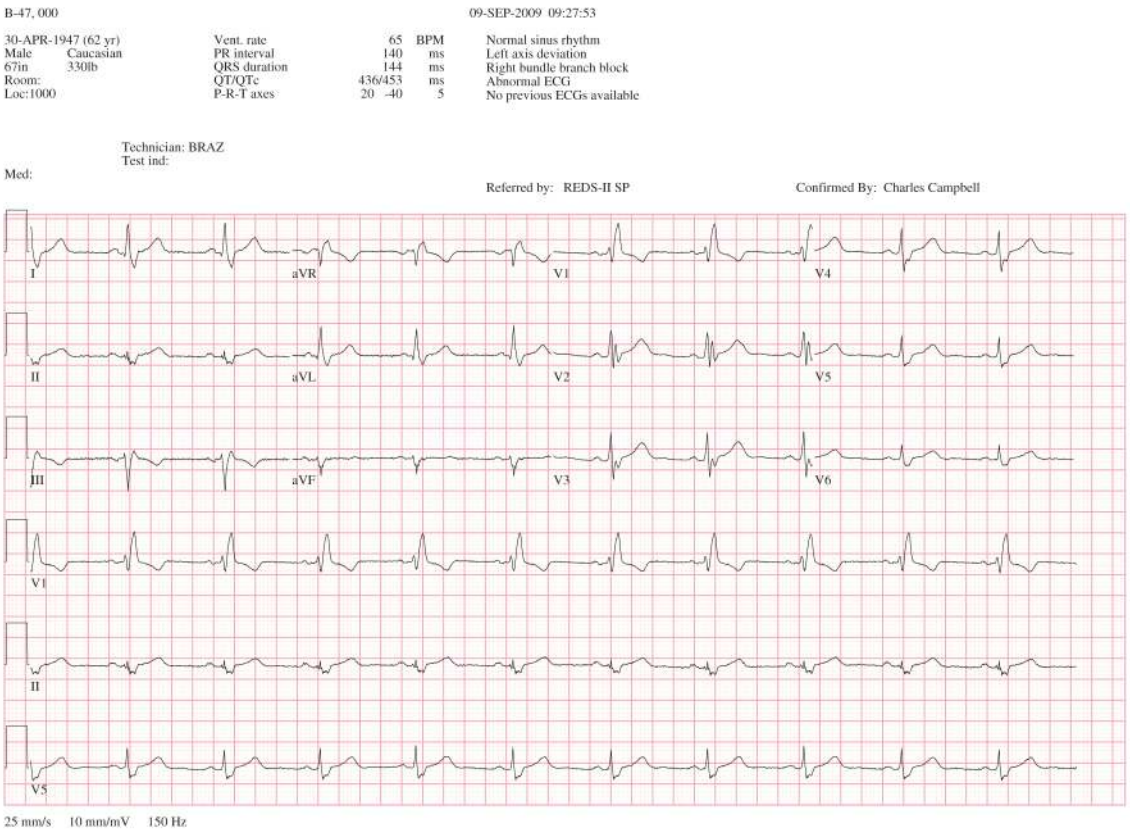

Figure 2.

This is a 12-lead ECG of a patient with severe Chagas cardiomyopathy, showing right bundle branch block and left anterior hemiblock.

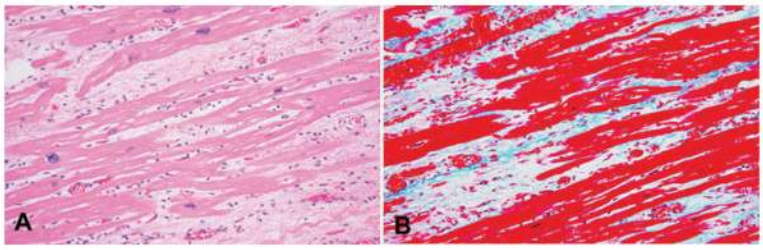

Figure 3. Histopathology of the heart from a patient who died while awaiting cardiac transplant

(A) H&E showing bands of fibrous tissue. (B) Trichrome staining showing the bands of fibrous tissue.

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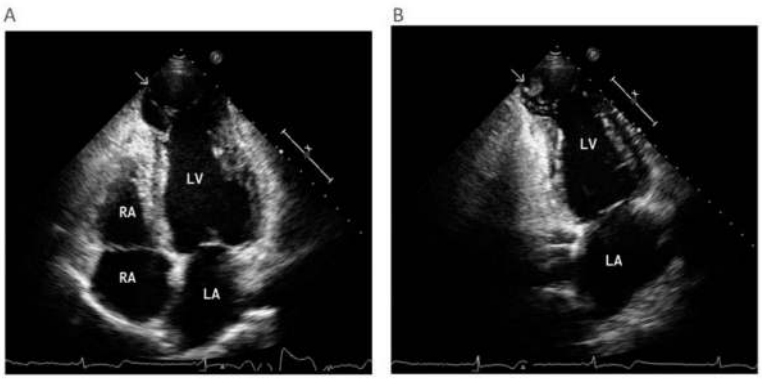

Figure 4. Echocardiography of a patient with chronic chagas cardiomyopathy

(A) Two-dimensional echocardiography at the apical four-chamber view of the left ventricle demonstrating a dilated apical aneurysm. There is a distinct limit between the normal contractile myocardium and the dyskinetic apex (arrow) with bulging motion during systole. (B) Two-chamber apical view showing an apical thrombus (arrow). There is a mass attached to the left ventricular apical aneurysm with a margin distinct from the underlying wall, which is dyskinetic.

LA: Left atrium, LV: Left ventricle; RA Right atrium; RV Right ventricle.

Table 1

Typical and nonspecific ECG abnormalities in Chagas cardiomyopathy, according to the Brazilian Expert Consensus in Chagas Disease.

Typical ECG changes for Chagas disease
– Right bundle branch block, associated or not with left anterior fascicular block
– Frequent ventricular premature beats (>1 by ECG), polymorphous or repetitive
– Nonsustained ventricular tachycardia
– Second and third degree atrioventricular block
– Sinus bradycardia with heart rate ≥40 bpm
– Sinus node dysfunction
– Left bundle branch block
– Atrial fibrillation
– Electrical inactive segment
– Primary alterations of ST-T wave
Nonspecific ECG changes observed in Chagas disease
– Sinus bradycardia with heart rate ≥40 bpm
– Low limb voltage
– Nonspecific ST-T changes
– Incomplete right bundle branch block
– Left anterior fascicular block
– Isolated ventricular premature beats
– First degree atrioventricular block