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Chagasic Cardiomyopathy

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1. Introduction

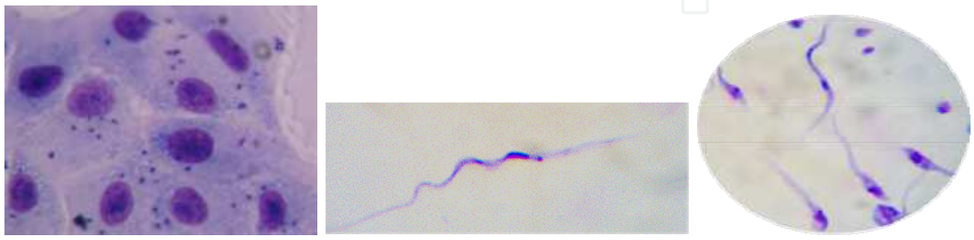
Chagas disease is a systemic parasitic infection caused by the protozoan *Trypanosoma cruzi*, which persists as an important public health problem, mainly in Latin America where triatomine vectors are located in three overlapping cycles of transmission: domestic, peridomestic, and sylvatic. Due to human migration from endemic to developed countries, in recent years Chagas disease has become a recognized global problem. This chapter reviews current literature on chagasic cardiomyopathy, its etiology, epidemiology, immunology, and diagnosis, along with etiologic and symptomatic treatment and prognosis.

2. Etiology

One-hundred years after the discovery of *Trypanosoma cruzi* (Family: *Trypanosomatidae*, Order*: Kinetoplastida*) by Carlos Chagas in Brazil, many aspects of its biology and host relationship remain unraveled. The substantial biological, biochemical, and genetic variability of this parasite, as well as the multiclonal *T. cruzi* infection character are some of the factors that have hampered its study. *T. cruzi* is considered to have a clonal structure with some overlapping events of genetic exchange occurring in the past that have brought about the six currently recognized Discrete Typing Units (DTUs) I to VI. Moreover, within each DTU biological and genetic polymorphism is present, especially in DTUs I and III. The scenario is even more complicated. Recent reports showed that multiple genotypes were obtained when isolates from a single wild mammalian reservoir host were cloned (Llewelyn et al., 2011). The authors proposed that this huge diversity is at least, partially driven by the survival in the host. Nonetheless, significant progress has been achieved with the unveiled *T. cruzi* genome and the following OMICS initiatives such as RNAomic and proteomic analyses, which seek to apply translational medicine to Chagas disease in the near future.

2.1 Life cycle

T. cruzi exhibits a complex life cycle involving four well-defined developmental stages that interplay into two hosts, the blood-sucking insect vector, and the mammalian host (humans and animals). After already-infected insects feed on the mammalian host, they eliminate in their feces the **metacyclic trypomastigotes (**parasite infective form), which penetrate the body through the bite-wound, any damaged tissue, or the mucosa from eyes, nose, or even the digestive tract and invade host cells like fibroblasts, macrophages, and epithelial cells at the inoculation site. In the cytoplasm, free-parasites are differentiated into **amastigotes** (Fig.1A), the intracellular stage, which after several replication rounds transforms back into **trypomastigotes** that rupture the host membrane cell, infecting new cells or disseminating into other organs via the bloodstream.



A B C

Fig. 1. (A) Intracellular amastigotes of *T. cruzi*-infecting Vero cells, (B) Trypomastigotes, and (C) Epimastigotes stained with Giemsa. Courtesy of Paola Lasso, and Paula Pavia, Laboratory of Molecular Parasitology, School of Sciences, Pontificia Universidad Javeriana, Bogota D.C., Colombia.

Upon feeding, insects take the **bloodstream trypomastigotes** (Fig. 1B), which once in their digestive tract differentiate into **epimastigotes**, the insect replication stage (Fig. 1C). After reaching the rectum, parasites transform into metacyclic trypomastigotes ready to infect a new mammalian host. From this cycle, it is obvious that the differential expression of parasite genes enable the parasite to accomplish the role played by each of its developmental stages. In this sense, several proteomic studies have been performed to identify molecules participating in cycle-vital processes (Ulrich et al., 2011).

3. Epidemiology and risk factors

3.1 Burden of Chagas disease

An estimated 10-million people are infected worldwide, mostly in Latin America where Chagas disease is endemic. More than 25-million people are at risk of contracting the disease. It is estimated that in 2008 Chagas disease killed >10,000 people. With a latency of 10-30 years, nearly 30% of infected patients develop life-threatening complications, mostly Chagas heart disease (CHD) (WHO, 2010). Direct and indirect costs of *T. cruzi* infection impose an overwhelming load on healthcare systems secondary to hospitalizations and medical and surgical treatments for CHD, gastrointestinal dysfunction, and meningoencephalitis in Latin America (Franco-Paredes et al., 2007). In 1995, the burden of Chagas disease in Latin America was estimated at US$8.156-billion, equivalent to 2.5% of the foreign debt of continental Latin America (Moncayo, 2003). More recent data demonstrate that, globally, Chagas disease is associated with 0.7-million disability-adjusted life years, constituting the sixth most important neglected tropical disease worldwide (Hotez et al., 2006).

3.2 Globalization of Chagas disease

Political and economic situations have stimulated the flow of migration from the 17 Latin American endemic countries to the developed ones (Schmunis & Yadon, 2010). Because of this and parasite transfer by blood contact, intrauterine transfer, laboratory accidents, and organ transplantation; CHD could potentially become a worldwide problem (WHO, 2010) and emerge as a public health issue in non-endemic countries (Field et al., 2010). Currently, in the United States it is estimated that from more than 22 million of immigrants from endemic countries there are approximately 300,000 infected individuals (Bern & Montgomery, 2009). In 15 European countries in 2005, excluding Spain, 2.9% of the 483,074 legal Latin American immigrants were estimated to be infected with *T. cruzi*. By 2008, Spain had received 1,678,711 immigrants from Latin American endemic countries; of these, 5.2% were potentially infected with *T. cruzi* and 17,390 may develop Chagas disease. Likewise, in an analysis of Chagas disease in Spain, most patients were from Bolivia (94.7%) and less from Brazil, Chile, Ecuador, Paraguay, and Honduras (Norman et al., 2010). Other countries outside Europe, where the rates of Latin American immigration are high and present an important prevalence of Chagas disease are Australia, Canada, and Japan (Schmunis & Yadon, 2010).

3.3 Chagas heart disease in endemic countries

Triatomines, the *T. cruzi* vectors, are spread from the south of the United States to the south of Argentina. The rarity of vector-borne transmission in the United States, compared with Latin America, is thought to be the result of better housing conditions and lower efficiency of North American vectors (Bern & Montgomery, 2009). In Latin America, there are more than 125 potential vectors of Chagas disease. However, species with higher vectorial capacity, with domestic habits and with the most geographical distribution belong to *Triatoma, Rhodnius,* and *Panstrongylus* genera. For these reasons, there are different targets in control programs of vectors depending on regions. Thus, the Mexico and Central America Initiative (created in 1998) is focused in the control *Rhodnius prolixus, Triatoma dimidiata, Triatoma barberi,* and *Rhodnius pallescences*; the Initiative of the Andean Countries (created in 1997) is aimed at controlling *R. prolixus, T. dimidiata* and *Triatoma maculata*; and finally, the Initiative of the Southern Cone (created in 1991) is aimed at controlling *Triatoma infestans, Triatoma brasiliensis, Triatoma sordida,* and *Panstrongylus megistus* (Guhl, 2009). The main risk factors for vector-borne transmission are related to previous exposure to poor housing conditions in Latin America (Fig. 3), such as: palm or straw roofs, dirt floors, adobe walls or walls with low quality or incomplete plastering, and the presence of animals inside the bedroom.

Fig. 2. Typical house in a Chagas disease endemic region, Departamento de Boyacá.
Courtesy of Cielo León, Grupo de Parasitología, Instituto Nacional de Salud, Colombia.

An important change has occurred in trends of Chagas disease in Latin America over recent decades. Such is recognized by several researchers, control policies of vector and blood of *T. cruzi* transmission have shown a positive effect in reducing the incidence of this disease. Thus, since the 1990s until now, an important success in the control of Chagas disease has been observed, especially in Southern Cone countries. So, in 1990, the distribution of Chagas disease in 21 countries was estimated, with more than 45,000 deaths per year and 30-million cases of human infection; while in 2006 the distribution of Chagas disease in 18 countries was estimated, with approximately 12,500 deaths per year and nearly 15-million cases of human infection (Dias et al., 2008). Success in controlling vector transmission in some countries has led to also to focus the attention to other forms of non-vector transmission. Thereby, controlling transmission by transfusion has improved and screening is now obligatory in most endemic countries. Congenital transmission has been detected as an important transmission form, mainly in Bolivia, but other endemic countries have only recently started to approach to this problem. Orally acquired human infection with *T. cruzi* has been known since the 1930s but has the interest in this transmision has increased as a result of the series of outbreaks that have occurred in the Amazon region, which have been associated with the preparation and consumption of some foods, especially in Brazil, Venezuela, and Colombia (Miles, 2010). The rural-to-urban migration movements that have occurred in Latin America since the 1970s and 1980s have changed the traditional epidemiological pattern of Chagas disease as a rural condition and transformed it into an urban infection that can be transmitted via non-vector manners (Moncayo & Silveira, 2009). Moreover, in some countries the vector infestation has occurred in urban areas where vectors have been introduced by passive transportation during migration process, for instance in Cochabamba, Bolivia (Medrano-Mercado et al., 2008), Arequipa, Peru (Bayer et al., 2009), and in Yucatán, Mexico (Guzman-Tapia et al., 2007). On the other hand, adults infected with *T. cruzi* from childhood form a transitional generation, experiencing the simultaneous impact of past infectious exposures and current cardiovascular risk factors, such as sedentary lifestyle, calorie-dense diets, hypertension, and diabetes. Other variables such as longer residence in an endemic province, residence in a rural area and poor housing conditions, male sex, and increased age have been found independent predictors of Chagas cardiomyopathy severity (Hidron et al., 2010).

3.4 Surveillance and health policy

In endemic countries, the tools to interrupt the domestic cycle of *T. cruzi* transmission, such as chemical control, housing improvement, and health education are the most useful methods to prevent Chagas disease (Moncayo & Silveira, 2009). Blood screening is vital to prevent infection through transfusion and organ transplantation and governments should implement policies to promote it (WHO, 2010). In addition, an infrastructure that assures detection and treatment of acute and chronic cases, as well as congenital infection should be developed. In non-endemic countries, screening programs in Latin American pregnant women are increasing and it has been proposed that in some non-endemic countries there is cost-effectiveness to develop it (Sicuri et al., 2011). Regarding strategies to reduce transmission by transfusion in non-endemic countries, there are two different approaches: one is the deferral of individuals at risk of Chagas disease and the second approach is to accept blood donations if specific laboratory assays are negative. This second approach is being introduced in countries where there is a substantial Latin American population, such

as the United States, Spain, and France (Castro, 2009). Also, taking into account that knowledge about Chagas disease among doctors in non-endemic countries is very limited (Verani et al., 2010), strategies to improve awareness are very important in order to enhance treatment and follow up of cases.

4. Clinical presentation

4.1 Acute phase

The symptomatic acute phase could be present at any age but it is most common in children under 10 years of age. When the infection is acquired via vector, it takes four to eight weeks for symptoms to develop**.** In this phase there is an important inflammatory response in the site of contact with bug feces and *T. cruzi* may multiply locally (cutaneous chagoma when it is in the skin). The insect prefers the thinnest skin and that is why the best known sign is Romaña's sign which consists in a unilateral conjunctivitis with periorbital edema, eyelid edema and pre-auricular adenopathy (Biolo et al., 2010). In younger children (under 4 years of age) it is common to found the following symptoms: fever, malaise, muscle pain, anorexia, anemia, sweating, hepatosplenomegaly, heart failure from myocarditis, pericardial effusion, seizures, and somnolence secondary to meningoencephalitis, the more infrequent form of presentation (Gomez et al., 2007). The acute congenital disease should be considered by the medical care system staff in endemic areas; it could be asymptomatic or may be associated to prematurity, low weight, hepatomegaly, splenomegaly, jaundice, anemia, neonatal hepatitis, meningoencephalitis, sepsis, myocarditis, fever, and less frequently megaesophagus, megacolon, megabladder. Without treatment, mortality is 5-10%, the leading causes are encephalomyelitis and heart failure (Prata, 2001). Patients with HIV could reactivate the disease and have meningoencephalitis as a first manifestation (Carod-Artal, 2006). In patients with history of solid organ or bone marrow transplants, 30% reactivate Chagas disease, the acute manifestations could be myocarditis, panniculitis, subcutaneous parasite-containing nodules, and meningoencephalitis (Bern et al., 2007).

4.2 Chronic phase

Once the acute phase is resolved, it begins the chronic phase. This chronic phase could be asymptomatic lifelong or progressive heart and/or gastro esophageal disease. The chronic asymptomatic or indeterminate phase lasts 10 to 30 years. For some authors its definition means epidemiological contact, positive serologic tests, normal physical examination and normal radiological, electrocardiographic and echocardiography studies. Around 30% of these have progressive disease (Higuchi et al., 2003). When Chagas becomes symptomatic, depending on the geographic zone, the disease will have different signs and symptoms. In Central America and northern South America, the heart disease is the common manifestation, but in Brazil and Southern Cone countries it coexists with digestive syndromes (Miles et al., 2003). For the purpose of this review, we will focus on Chagasic cardiomyopathy. The earliest manifestations of heart disease are electrocardiographic abnormalities as the expression of the damage of the conduction system and the symptoms that the patients experience could be related to those abnormalities: atrioventricular block, sinus bradycardia, premature ventricular contractions, atrial fibrillation, and ventricular tachycardia. In 40% of patients with mild heart disease there could be non-sustained ventricular tachycardia, as well as in 90% of patients with heart failure. Sudden death occurs

in 38% of patients with chronic disease with or without heart failure, meaning more severe heart disease. The principal cause of sudden death is the malignant ventricular arrhythmia followed by advanced atrioventricular block and cerebral emboli. Non-sustained ventricular tachycardia in Holter monitoring and in stress test, together with low ejection fraction, syncope and pre-syncope, sinus node dysfunction, history of recovery from cardiac arrest, and dyspnea NYHA class III or IV have been recognized of prognostic value in sudden death (Prata, 2001). Symptomatic heart failure occurs in some patients before there is any significant electrocardiography alteration. It could be right or left heart failure; it is very common for patients to have severe structural heart disease and not show symptoms of severe heart failure. It is also common to find severe congestive hepatic disease. Pulmonary and systemic emboli due to dilated chambers of apical aneurism are common clinical manifestations of chronic heart disease (Bern et al., 2007); they have been described in 40% of autopsies (Prata, 2001). A Brazilian study found four predictors of emboli complications: age > 48 years, primary changes in repolarization, apical aneurism, and ejection fraction < 50%, with a 4% annual incidence if all four factors where present (Sousa et al., 2008). Precordial pain is a frequent complaint of patients with Chagas disease. The incidence of this symptom is 15% but other authors report up to 30% (Marin-Neto et al., 2007). The causes of the symptom are not clear; some authors believe that this pain could be caused by microvascular disease.

4.2.1 Classification

A simple classification, published by the Brazilian Consensus on Chagas Diseases, includes functional capacity, electrocardiographic findings, function and size of left ventricle. This classification allowed defining four disease stages with the aim to orientate the patient's therapy (Table 1).

Stage	Electrocardiogram	Echocardiography	Heart failure
A	Altered	Normal	Absent
B1	Altered	Altered LVEF >45%	Absent
B2	Altered	Altered LVEF <45%	Absent
C	Altered	Altered	Compensated
D	Altered	Altered	Refractory

LVEF: left ventricular ejection fraction

Table 1. Classification of cardiac compromise in Chagas chronic cardiomyopathy

5. Pathogenesis of cardiac disease during *T. cruzi* infection

5.1 Host genetic influence

Some works approach the influence of human genetics such as Histocompability Complex Molecules (HLA) or polymorphism in cytokine promoters and their contribution to Chagas disease. So far, association with HLA class II indicated that infected individuals with and without cardiomyopathy had a higher frequency of DRB1*01, DRB1*08, and DQB1*0501 (Fernandez et al., 1998), and the DRB1*01 DQB1*0501 haplotype was more frequent in patients with Chagasic cardiomyopathy (Colorado et al., 2000). Additionally, the HLA-DRB1*1503 allele was associated with genetic susceptibility to cardiac damage (Garcia Borras et al., 2009). All these studies were conducted with small cohorts and with different

Latin American populations. Polymorphisms of cytokine promoters assess the potential pattern of cytokine hypo or hyper secretion in individuals. A study showed the association of transforming growth factor beta (TGFβ1) (Calzada et al., 2009) and lymphotoxin α (LTα) with the risk Chagasic cardiomyopathy progression (Ramasawmy et al., 2007). Tumor necrosis factor (TNFα), a pro-inflammatory agent, is the cytokine with the strongest relationship to cardiac tissue damage in Chagas. There is an association between *T. cruzi* seropositive individuals and the polymorphism in TNF-238A. Indeed, TNFα secretion is higher in non-stimulated and stimulated cells from chronic Chagasic donors (Pissetti et al., 2011) and TNFα serum levels were associated with heart failure (Talvani et al., 2004). In *T. cruzi e*xperimentally infected rats, the cardiomyopathy ameliorates in animals treated with a TNFα blocking monoclonal antibody (Perez et al., 2009).

5.2 Parasite tropisms

Geographic distribution of an organ-specific chronic disease (cardiac *versus* digestive diseases) and allocation of *T. cruzi* I and II (II to VI in the new classification), supported the hypothesis that disease outcome is linked to the *T. cruzi* genetic variations. Some studies did not show correlations among *T. cruzi* lineages and the clinical forms of Chagas disease (Zafra et al., 2011). Although, the presence of TcI was correlated with higher frequencies of electrocardiogram alterations than individuals infected with TcII, such as ventricular premature beats, first-degree atrioventricular block, sinus bradycardia, abnormal Q-waves, atrial fibrillation, and complex ventricular arrhythmias (Ramirez et al., 2010). In a mouse model infected with two different genetic populations of *T. cruzi,* both parasites were found during the acute infection in several host compartments (blood and organs). However, during chronic infection, a preferential tissue distribution with predominance of certain *T. cruzi* isolates was found (Andrade et al., 1999). Because *T. cruzi* mixed infections in triatomines are found in high rates, a similar phenomenon should take place during human infection.

5.3 Parasite invasion of the host cells

T. cruzi can reach the mammalian host cells via different mucosal tissues (i.e., conjunctiva, oral) or directly into blood (transfusion or congenital). The parasite in vivo can invade a vast range of cells such as monocyte/macrophages, dendritic cells, endothelial cells, fibroblasts, astrocytes, skeletal muscles, enteric nerves, and cardiomyocytes (Epting et al., 2010). Parasite invasion is a multistep process when several *T. cruzi* glycoproteins bind surface molecules on the host cells. Before reaching the target tissues, *T. cruzi* must interact with the endothelial cells to actively penetrate or increase endothelium vasodilatation. Parasite protease can produce inflammations that increase vascular permeability (Epting et al., 2010). Several parasite proteases and glycoprotein expressed by trypomastigotes have been associated with invasion: gp60 (penetrin) gp63, gp35/50, gp82, gp90 a parasite glycosidase, mucins and transialidase such as gp85 or Tc85. The binding of these parasite molecules to host molecules (cytokeratin 18, mucins, heparan sulfates, extracellular matrix proteins such as fibronectin and laminin, and carbohydrates with sialic acid) induce Ca++ mobilization, protein tyrosine phosphorylation and cytoskeleton reorganization in the target cells. Transialidase, glycosylphosphatidylinositol (GPI) anchors surface-bound proteins are in charge of transferring sialic acid residues from the host cell to the parasite glycoproteins. This mechanism seems to be crucial in invasion given that trypomastigotes with no expression of trans-sialidases were poorly invasive to non-phagocytic cells (Epting et al.,

2010). Also, infection by oral route involved other parasite glycoproteins such as mucin-like gp35/50 or gp82 on the surface of the trypomastigotes, resistant to protease digestion. Glycoprotein gp82 binds to the gastric mucin and allows the parasite to invade epithelial cells (Yoshida, 2008).

5.4 Acute and chronic heart involvement in Chagas diseases

5.4.1 Acute myocarditis

Parasite genetic variations, the initial parasite burden, and the host immune response seem to influence the evolution of the Chagas disease. Clinical cardiac involvement is found in nearly 90% of symptomatic patients. Parasite-infected myocardiocytes with intracellular amastigotes (pseudocyst) break up and induce acute inflammation (Coura & Borges-Pereira, 2010). There is massive and diffuse infiltration with predominance of mononuclear cells (Fig. 3A) mainly CD8+ T lymphocytes (LT) up to 60% (Fig. 3B). Whether these LT are *T. cruzi* antigen specific and which chemotactic agents control this migration is unknown. Microarray studies of *T. cruzi* acute infection in mouse cardiomyocytes showed a regulation of 353 genes (111 up regulated and 242 down regulated) associated with inflammation, cytoskeleton, cell interaction, apoptosis, cell cycle, and oxidative stress. Interestingly, early genes up regulated include a vast range of chemokines, which attract mononuclear cells (Manque et al., 2011). In consequence, there is cell destruction (myocytolysis), interstitial edema, hypertrophy of myocardial fibers, and alteration of the cardiac microcirculation with platelet aggregation, production of pro-inflammatory cytokines and expression of vascular adhesion molecules by endothelial cells (Rossi et al., 2010).

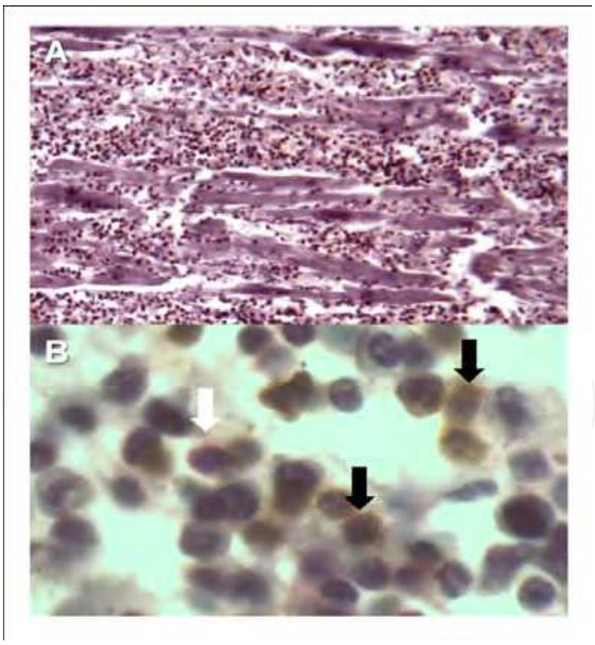


Fig. 3. Immunohistology of a heart with acute Chagas disease showing extensive cellular infiltration 10x (A), and presence of CD8+ (black arrow and brown cells) and CD4+ T cells (white arrow and red cell) with hematoxylin as contra-staining 100x (B). Courtesy of Ana M. Uribe, M.D. Pathology Department, School of Medicine, Pontificia Universidad Javeriana, Bogota D.C., Colombia.

5.4.2 Chronic myocarditis

Contrary to acute infection, in the chronic phase there is scarcity of *T. cruzi* niches; however, there is an extensive, but patchy mononuclear infiltration, with predominance of macrophages and cytotoxic CD8+ T cells (CTL). Myocarditis has a slow progression with changes in the contractile function and dilatation of the heart walls. Increase in metalloproteinase has been described in infected cardiac tissue and associated with remodeling of the extracellular matrix (Gutierrez et al., 2009). Histology analysis shows diffuse myocarditis, myocytolysis, edema, mononuclear cellular infiltration (hallmark of the delayed hypersensitivity), destruction of the conduction system with neuron loss (autonomic denervation), and extensive myocardial fibrosis. Functional studies in Chagasic cardiopathy demonstrated impaired perfusion at the coronary vessels due to microvascular changes (thrombi, inflammation, and spam) (Rossi et al., 2010).

5.5 Mechanisms of tissue damage

5.5.1 Autoimmunity

The autoimmune theory was initially based on the scarcity of parasites found in chronically infected tissue and also on the presence of antibodies and T cells that recognized parasite antigen and cross-reacted with host tissues and molecules. Antibodies against *T. cruzi* bind to human laminin, sulfo-galactosylceramides, cardiac myosin, microtubule-associated proteins, ribosomal proteins, β-adrenergic and muscarinic receptors; heart sarcolemma, blood vessel, neurons, glial cells, myocardium and skeletal muscles (Bonney & Engman, 2008). However, demonstration of the pathological consequences due to autoimmunity in *T. cruzi* infection does not have direct evidence. Most of the auto-antibodies are considered to be natural antibodies that could be induced after tissue injury and exposure of host cell molecules. Also, *T. cruzi* antigens can act as B cell polyclonal stimulators. Against the autoimmunity theory it is known that the immune-suppression exacerbates *T. cruzi* infection and specific anti-parasitic treatment ameliorates the clinical disease (Rossi et al., 2010).

5.5.2 Antigenic persistence and immune response

By using DNA techniques, the presence of *T. cruzi* in tissues during chronic infection has become clear. Antigen persistence triggers inflammation and lymphocyte infiltration. Damage mechanisms are unclear because parasite burden does not explain extensive cell loss. CD8+ T lymphocytes contribute to cytotoxicity probably via perforin and granzyme B, and TGB-β and interleukin-10 (IL-10) secreting macrophages can induce repair and fibrosis through fibroblasts. *T. cruzi* infection also alters microcirculation with the presence of platelets aggregated, microvascular spam, and secretion of vasoconstrictor agents such as tromboxane A2 (TXA2) and platelet activated factors (PAF) by macrophages or endothelin 1 (ET-1) by endothelial cells (Rossi et al., 2010).

6. Human immune response

Innate immune cells such as natural killer cells, macrophages, and dendritic cells detect invading pathogens and alert the immune system through activation cascades. The aim is to elicit innate antimicrobial and inflammatory responses and initiate adaptive immunity to control or eliminate infection. It is accepted that the establishment of chronic infection with *T. cruzi* is a consequence of the inability of the immune response to elicit sterilizing anti-

parasite immunity. Therefore, the host innate and adaptive immune response is believed to be the key determinant of the clinical outcome of the disease.

6.1 Innate immunity

Dendritic cells (DCs), natural killer (NK) cells, and monocytes are vital mediators of the innate immune system and promote development of adaptive immune responses. Evidence shows that *T. cruzi* may infect DCs and even proliferate inside them. Consequently, the DC antigen presentation capacity is reduced (Van Overtvelt et al., 2002). In early asymptomatic Chagas disease, higher levels of pro-inflammatory monocytes and expansion of NK cells before the adaptative immunity development has been shown (Vitelli-Avelar, 2006). The role of cytokines such as interleukin (IL)-4, IL-12, TNFα, and interferon (IFN)γ secreted by these cells can be an important element for host resistance during the early stages of infection and also in the genesis of myocarditis (Golgher et al., 2004). It has been shown that two different and independent antigenic stimuli from the parasite induce both an enhancement of IL-10 and a reduction of IL-12 secretion in DCs from Chagasic patients compared to DCs from healthy donors (Cuellar et al., 2008). Although, the innate immune system seems to have a fundamental role in Chagas disease by controlling parasite replication and spread in host tissues, it is not clear if events described here, that mediate inflammatory reaction, can be related to protection or tissue damage in the chronic phase of the disease.

6.2 Humoral immune response

A specific antibody response and B cells in animal models of Chagas disease seem to play an important role for parasite control, especially against the trypomastigotes. In spite of the large number of parasite proteins some molecules have been studied. Indeed, in our previous work, we showed that there is a consistently higher specific IgG response in chronic Chagasic patients against *T. cruzi* kinetoplastid membrane protein-11 (KMP-11), and the *T. cruzi* heat shock protein-70 (HSP-70). The recombinant KMP-11 protein recognition was focused on IgG1 sub-fraction; whereas, the lysate was on IgG3 plus IgG1 in asymptomatic and cardiopathic chronic phases, compared to acute sera from Chagasic patients (Flechas et al., 2009). These data reflect the dynamics of the humoral immune response in Chagas disease and may be an important issue given that IgG1 and IgG3 are the major complement fixing isotypes, which also mediate cooperative function with phagocytes; nevertheless, the role of these specific antibodies in controlling the infection or progressing in disease severity need to be addressed.

6.3 T cells and cytokines

Individuals undergoing chemotherapy generally show protection against viral infections controlled by T cells during lymphopenia, indicating that a small population of T cells can be protective (Turtle et al., 2009). However, reactivation of Chagas disease, defined by a demonstration of trypomastigotes on microscopic examination of blood or the identification of amastigotes on biopsy samples and/or acute clinical manifestations during the chronic phase, can occur among the immunosuppressed patients with heart transplantation (Burgos et al., 2010) or AIDS patients (Almeida et al., 2009). It may be the natural history disease demonstration that T cell response is crucial to control parasite burden and clinical manifestations in a large proportion of patients. Perhaps the most interesting question is

how adaptive immune response can contribute to most infected individuals remains asymptomatic whereas an important percentage of these patients develop severe forms of the disease. In humans, it has been shown that CD4+ T cells (Cuellar et al., 2009) and CD8+ T cells (Fiuza et al., 2009) from Chagasic patients specifically produced IFNγ after exposure to *T. cruzi* antigens. Furthermore, chronic Chagasic patients had lower levels of antigen-specific CD8+ T cells secreting IFNγ compared with non-symptomatic individuals (Laucella et al., 2004). Because *T. cruzi* is an intracellular parasite, many groups have focused on the study of CD8+ T cells. Some of them have studied specific CD8+ T cells against peptides derived from cruzipain, FL-160 (Fonseca et al., 2005), KMP-11 (Diez et al., 2006; Lasso et al., 2010), and trans-sialidases (Alvarez et al., 2008) proteins, founding similar frequency of specific CD8+ T cells for these epitopes. Nonetheless, it has been shown that patients with more severe forms of Chagas disease have more differentiated CD8+ T cells which could have lost their functional capacity (Bixby & Tarleton, 2008). One interesting aspect is the control of immune response by regulatory T cells (T_{reg}). Ex vivo, it was shown that children with asymptomatic Chagas disease display a lower frequency of natural T_{reg} CD4+ $CD25^{high}$ compared to non-infected children (Vitelli-Avelar et al., 2006). Interestingly, these cells are in increased levels in peripheral blood of late chronic asymptomatic patients (Vitelli-Avelar et al., 2005). These data suggest that T_{reg} could be important to limiting tissue damage. However, taking into account that additional molecules have been suggested to identify T_{reg}, we used a panel of antibodies for CD4, CD25, FoxP3, and CD127. Our results show higher proportion of T_{reg} in symptomatic chronic Chagasic patients compared to non-infected individuals, indicating that the frequency of T_{reg} can contribute to damage. Fig. 4 despites the CD4+ T_{reg} cells by flow citometry (Lasso et al., 2009).

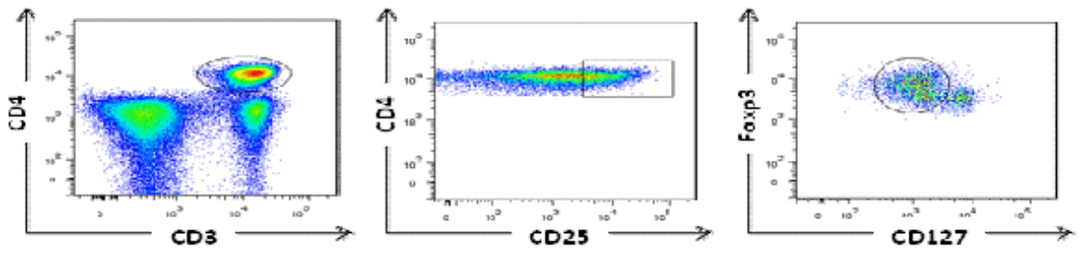


Fig. 4. Regulatory T cells from chronic Chagasic patient identified by high levels of expression of the transcription factor forkhead box transcription factor P3 (FoxP3) and low levels of CD127. Courtesy of Paola Lasso, Pontificia Universidad Javeriana, Bogota D.C., Colombia.

7. Diagnosis

The diagnosis of Chagas disease, as with other infections, is performed on the basis of clinical findings, parasite presence, serological status, and epidemiological data. Furthermore, the disease stage is also an important fact to consider. For instance, as the parasitemia dramatically decreases from acute to chronic phase, in the early phase parasite detection is achieved by parasitological conventional direct tests (see below). Nevertheless, because clinical findings in this stage can be confused with other pathologies, the epidemiological data demonstrating a connection between the patient and the parasite is of special importance (Nicholls et al., 2007). In contrast, in chronic patients, the presence of

symptoms or abnormal clinical findings usually correlates with the disease but parasite concentration is low and variable. Bearing in mind that *T. cruzi* infection is life lasting; in the chronic phase serological tests are applied to indirectly demonstrate parasite presence (Enciso et al., 2004). Indeed, the WHO recommends that to diagnose a chronic Chagasic patient; besides having clinical findings compatible with Chagas disease and history of vector contact, there must be at least two positive serological tests with different immunological principles. Finally, chronic asymptomatic patients represent a real challenge for diagnosing inasmuch as there are no clinical findings, and again parasitemia is very low and intermittent. Consequently, the epidemiological patient history is also of most importance (Gil et al., 2007).

7.1 Clinical findings

7.1.1 Electrocardiogram

The most common electrocardiographic manifestations are right bundle branch block (RBBB), anterior fascicular block, premature ventricular contractions, changes in ST segment and T wave, abnormal Q waves, and low voltage of the QRS complex (Fig. 5). The combination of the RBBB and the anterior fascicular block suggest the disease (Garzón et al., 1995). The presence of frequent premature ventricular contractions, including duplets and salvos of non-sustained ventricular tachycardia are a common finding in the Holter monitoring and in the stress test. Premature ventricular contractions correlate with the severity of the ventricular function, but can also occur in patients with preserved ventricular function. Episodes of non-sustained ventricular tachycardia are observed in 40% of the patients with light to moderate ventricular contractibility alterations and in virtually all patients with heart failure, which is more frequent than in other cardiomyopathies. Sustained ventricular tachycardia is another disease marker. This arrhythmia can be produced through programmed ventricular stimulation in nearly 85% of the cases and results from intramyocardial or subepicardial reentrant phenomena, usually located on the inferoposterior and lateral wall of the left ventricle.

7.1.2 X-ray and echocardiography

In patients in the undetermined phase, the cardiac silhouette evaluated in the chest X-ray and the global systolic function in echocardiography are normal. In more advanced stages, the chest X-ray can show cardiomegaly and pulmonary congestion. The disease can cause diffuse damage of the systolic function of the left ventricle. The global systolic function of the left ventricle has prognostic implications. In a cohort of 538 patients grouped into four stages of disease progression, different survival rates were found in the five-year follow up from 98%, 91%, 45% to 13% for those with normal left ventricle function, moderately depressed function, with reversible heart failure, or irreversible heart failure, respectively (Rassi et al., 2010). Some alterations of the segmental contraction of the left ventricle can be detected. The most common is located on the posterior wall with 20% prevalence. The presence of mitral or tricuspid insufficiency is generally associated to ring expansion. The prevalence of aneurysms in the left ventricle varies in the different series, noted on an average of 8.5% in asymptomatic individuals and in patients with severe cardiac damage up to 55%. Through logistic regression analysis, the presence of an apical aneurysm in the left ventricle was an independent predictor of mural Thrombi (Albanesi-Filho et al., 1991). In another work, the finding of an aneurysm was significantly associated to a thrombus and

cerebro-vascular accident during a two-year follow up. On some instances, diminished systolic function of the right ventricle can be the only abnormality detected via echocardiography; in general, it is secondary to the severity to the damage of the left ventricle and at high levels of pulmonary pressure. With regards to diastolic function, chronic myocarditis in Chagas disease can diminish ventricular relaxation and diastolic filling. These abnormalities usually precede systolic dysfunction. Reduced compliance of the left ventricle can increase the filling pressure of the left atrium with changes in transmitral and pulmonary venous flow rates. The echocardiography study is recommended as a routine clinical evaluation method in patients with Chagas cardiopathy to determine the stage of the disease, its progression, as well as to estimate survival, dismiss the presence of aneurysms or intracavitary thrombi, and monitor response to treatment.

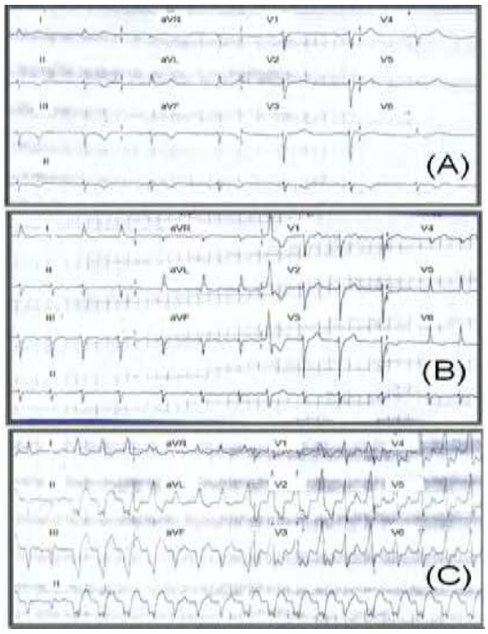


Fig. 5. ECG sequence in a 72-year-old woman diagnosed with Chagas cardiomyopathy and ejection fraction of the left ventricle at 25%. Note sinus bradycardia (A), attrial fibrillation (B), and monomorphic sustained ventricular tachycardia (C). Fundación Clínica Abood Shaio, Bogota D.C., Colombia.

In our experience at Fundación Clínica Abood Sahio (Bogotá, Colombia), from a total of 120 patients evaluated with diagnosis of Chagas cardiomyopathy, 73 women (60%) with mean age of 56.7 +/- 13 years (21-84), clinical manifestations corresponded to dyspnea (42%), palpitations (31%), chest pain (42%), presyncope (24%), syncope (27%), and aborted sudden death (2.5%). Nearly 6.7% of the cases did not present clinical manifestations. The main ECG findings were: right bundle branch block (40%), second and third degree AV block (29.2%), dysfunction of the sinus node (28.3%), ventricular tachycardia (23%), atrial fibrillation (19%), left anterior hemiblock (17.2%), atrial flutter (3.3%), and left bundle branch block (3.3%). In 31% of the cases, the chest X-ray was normal. In 15.8%, severe cardiomegaly was observed. All the patients were subjected to a color Doppler echocardiogram according to internationally recognized norms, finding a mean fraction of the left ventricle of 43.3% (SD +/- 16.5) (10-60) and of the right ventricle at 23.4% (10-40) (Fig. 6). The study was considered

normal in 33.6% of the cases. Contractility alterations were documented in 42.4%, with these being globally in 26.5% of the cases, or inferior, apical-inferior and anterior localization. Isolated compromise of the right ventricle was observed in one case (0.8%), suggesting the diagnosis of arrhythmogenic dysplasia of the right ventricle. In 24% of the cases mitral insufficiency was evidenced and 15.2% revealed tricuspid insufficiency. A total of 11 aneurysms (9.7%) were observed, 63.6% of apical localization and 36.3% of inferior localization. Some 8.8% of the patients presented intracavitary thrombi, generally related to aneurysms or global contractility alterations. Holter or electrophysiological study documented ventricular tachycardia (sustained or unsustained) in 19.4% of the cases. Additionally in 10% we observed association to sinus dysfunction and/or AV block with ventricular tachycardia. Anatomic-pathological findings obtained via biopsy or surgery in 10 Chagas patients were: a) hypertrophy and/or b) fibrosis and/or c) chronic inflammatory infiltrate. None of the cases reported parasites in the samples examined by pathology (Rosas et al., 2007).

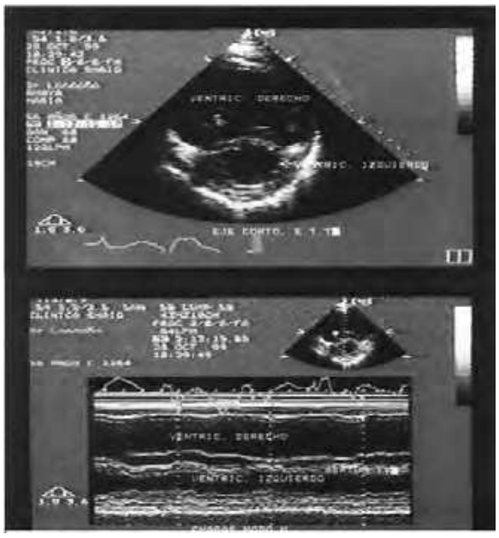


Fig. 6. Echocardiography M mode (A) and bi-dimensional (B) of 54-year-old female with a history of aborted sudden death due to ventricular tachycardia) secondary to Chagasic cardiomyopathy. Note the severe dilatation of right ventricle. Fundación Clínica Abood Shaio, Bogota D.C., Colombia.

7.2 Laboratory tests

7.2.1 Conventional parasitological tests

These can be classified into direct and indirect tests. Direct methods, employed basically in the acute phase, include parasite microscope-observation in blood fresh preparation which permits to observe parasite movement. On the contrary, thin or thick blood smears stained with Giemsa led to a better morphological identification, which is of special importance in areas where *Trypanosoma rangeli* also circulates. Importantly, parasite concentration methods like blood centrifugation, Strout method, and microhematocrit increase the probability of trypomastigote detection. Because of their great time consumption, indirect parasitological methods are generally used to diagnose patients in the chronic phase. They refer to hemoculture and xenodiagnosis (Luquetti, 2007).

7.2.2 Serological tests

There is a broad spectrum of serological tests, whose final goal is to detect anti-*T. cruzi* antibodies, usually of the IgG isotype in the chronic phase or IgM in the acute phase. The tests most used, called conventional tests for serological Chagas disease diagnosis, are the indirect immunofluorescence test (IFAT), the enzyme-linked immunoabsorbent assay (ELISA), and the indirect haemaglutination test (IHA). Generally, the antigens used are parasite lysates or mixtures of parasite recombinant proteins. Due to the huge parasite polymorphisms (Rodríguez et al., 2002), it is recommended to use isolates circulating in the specific endemic area or mixture of them.The applied method must be carefully standardized and validated in inter-laboratory international and national tests. Most of the above-mentioned tests can detect the infection in more than 95% of sera. Nevertheless, false-positive reactions can occur in *T. rangeli* or *Lesihmania*-infected patients, as well as false-negative in the case of recently-infected chronic patients or immunosuppressed patients (Gil et al., 2007; Luquetti, 2007).

7.2.3 PCR

PCR tests, because of their power of detection and specificity, constitute a complementary diagnostic method for detecting *T. cruzi* in diverse biological samples. They are of especial interest with chronic patients because of their higher sensitivity compared with conventional parasitological tests. There are several PCR tests available for detecting *T. cruzi.* Their performance varies depending on aspects like type and number of the target amplification, lack of polymorphisms among the parasite DTU-annealing primer target, sample volume, treatment and conservation, DNA extraction method, type of DNA polymerase used, and thermo-cycling program, among variables (Schijman et al., 2011). Some PCR tests show disadvantages like the amplification of polymorphic fragments or of similar-size bands in both *T. cruzi* and *T. rangeli* infections, the deviation of the test towards *T. cruzi* in mixed infections with *T. rangeli*, and the possible integration of the parasite's kDNA in the human genome (Gil et al., 2007; Pavía et al., 2003, 2007). Bearing all this in mind, the Molecular Parasitology Laboratory at Pontificia Universidad Javeriana designed and standardized the TcH2AF-R PCR, specific for *T. cruzi* (Pavia et al., 2003). This PCR amplifies the 16-255 nucleotides of the *T. cruzi* SIRE repetitive element and does not present amplification signal in *T. rangeli*. Assays on triatomine vectors experimentally and naturally infected with *T. cruzi* revealed that TcH2AF-R PCR allows identifying the parasite in all the infected specimens, with performance equal to that of S35/S36 PCR, considered among the most sensitive PCR tests for *T. cruzi* identification (Pavia et al., 2007). Likewise, in blood samples from Chagasic patients, it was observed that of 156 samples, 84 (53.8%) were positive with both TcH2AF-R and S35/S36 PCRs, while 89 (57%) were positive for indirect immunofluorescence (IIF) and enzymatic immunoassay (ELISA) (Gil et al., 2007). A study of the performance of the TcH2AF-R and S35-S36 primers in cardiac tissue of mice infected with *T. cruzi I* showed that by using both pairs of primers it is possible to detect the parasite in the acute and chronic stages of the infection, with performance above that of the micro-hematocrit and eliminate of the histopathological analysis (Barrera et al., 2008). Recently, by combining TcH2AF-R and S35/S36 PCRs, it was possible to follow up a Colombian heart transplant in a Chagasic patient, as well as the first Colombian congenital case (Pavia et al., 2009, 2011). Because of its higher sensitivity, a few real time PCR (qPCR) methods have been developed to monitor drug efficacy and Chagas disease reactivation in transplanted

Chagasic patients. However, international studies to evaluate PCR methods for parasite DNA detection in blood samples as that launched by Shijman et al., (2011) are urgently needed.

7.3 Epidemiological context

Epidemiological data, such as that shown in Table 2, seek to determine if the patient could have been in contact with the parasite.

Epidemiological data	Information included
Born in endemic areas	Housing conditions like thatched roof, dirt floors, adobe walls, etc. Presence of domestic animals. Rural, peri-domestic, or domestic dwellings Time of living in relationship to already performed vector control campaigns in the area (important in congenital transmission)
Living in endemic areas	
Visits to endemic areas	
Vector knowledge	Awareness of vectors circulating in the specific area
Chagasic relatives	Parents, siblings, or any family member infected
Work activity	Important in accidental transmission in both endemic and non-endemic areas
History of blood transfusions	Amount and place
History of organ transplant	Medical and epidemiological history of the donor

Table 2. Epidemiological data supporting risk of *T. cruzi* infection

8. Treatment

8.1 Symptomatic

In the absence of random clinical studies in patients with Chagas disease and heart failure, traditionally the recommendations have been extrapolated from the management guides for heart failure from other causes (Jessup et al., 2009). However, it should be noted that in the physiopathology of the Chagas disease there are clinical and therapeutic peculiarities with important implications. For example, high doses of diuretics are necessary in advanced stages of the disease due to predominance of the systemic congestion manifestations over signs of pulmonary congestion. In patients with Chagas cardiopathy, conduction disturbances are also frequent, which may be aggravated by the use of Digoxin, Amiodarone, and specially Beta-blockers (Marin-Neto et al., 2010). Cardiac re-synchronization is a treatment alternative for patients with heart failure, especially in the presence of left bundle branch block. However, its usefulness in patients with right bundle branch block common in Chagas disease has not been shown as patients with another type of pathology in the presence of this conduction alteration. Other palliative procedures like dynamic cardiomyoplasty and partial left ventriluculotomy are contraindicated because of unsatisfactory results. Heart transplant is an option indicated in patients selected in final stages of cardiac insufficiency. In these cases, it must be highlighted that the immunosuppressant therapy indicated to avoid transplant rejection may induce reactivation of the *T. cruzi* infection (Campos et al., 2008). Under certain circumstances, reducing the dosage of immunosuppression is recommended, as well as starting etiological treatment in

cases of reactivation (Fiorelli et al., 2005). The potential benefit of transplanting stem cells in patients with Chagasic cardiopathy is under evaluation (Tura et al., 2007). Because of the high frequency of thrombus-embolic phenomena, anticoagulation is indicated in patients with atrial fibrillation, in the prior embolism, in the presence of aneurysms or thrombi, and in cases of heart failure in advanced stages even in the absence of random controlled studies that prove its efficacy. Some observational data suggest that Amiodarone can improve survival in patients with Chagas disease with risk of sudden death due to malignant arrhythmia (Garguichevich et al., 1995). For this reason, Amiodarone is usually recommended in patients with sustained ventricular tachycardia and in cases of unsustained ventricular tachycardia associated to ventricular systolic dysfunction (Leite et al., 2003). Patients with sustained ventricular tachycardia with hemodynamic instability and in cases of aborted sudden death, the implant of a cardio-defibrillator is recommended (Rassi et al., 2009). Radiofrequency ablation is an alternative in patients with ventricular tachycardia (D´Avila et al., 2002); however, its impact on survival and recurrence of the arrhythmia is yet to be established. The finding of severe bradyarrhythmias like those observed in the complete AV block and in the sinus dysfunction must be treated by implanting a definitive pacemaker as in other cardiac conditions (Epstein et al., 2008). The benefit of the pacemaker implant in patients with Chagasic cardiopathy is mainly based on reports of case series.

8.2 Etiological

The only medications currently used with Chagas disease due to ethical and efficiency reasons are Nifurtimox and Benznidazole (Bern et al., 2007). Based on the literature review, the recommendations of the antitrypanocidal therapy vary according to the phase and form of the Chagas disease, the patient's age, and the severity of the disease. The pharmacological therapy is recommended in all acute and congenital cases, in infection by reactivation, in patients up to 18 years of age, and in children. For adults between 19 and 50 years of age and without advanced cardiopathy, the treatment can be offered (Bern et al., 2007). In individuals above 50 years of age, risk of toxicity from the drug may be higher than in young adults and the treatment is considered optional. Once the diagnosis has been confirmed through corresponding serological tests, patients must be evaluated with a clinical history and a careful physical exam. Additionally, in all cases, an electrocardiogram should be performed. With asymptomatic individuals without electrocardiographic alterations, the prognosis tends to be favorable and it is recommended that these patients be monitored every 12 to 24 months. Patients with electrocardiographic changes consistent with the disease's cardiovascular compromise should be evaluated via thoracic X-ray and echocardiogram that permit defining the ventricular size and function, as well as other types of structural alterations and via 24-h electrocardiographic monitoring or Holter test to detect arrhythmias.

9. Prognosis

The prognosis of some diseases like Chagas has not been easy to establish because of the great differences in their clinical course among the affected countries. Results like the survey by Maguire et al., (1987) showed that from 20-59 years of age, the risk was strongly related to electrocardiography status. Indeed, patients with ventricular conduction defects have

higher mortality rates than infected patients without electrocardiographic abnormalities. Also, it was observed that abnormal diastolic function is related to severe myocardial damage (Rocha et al., 2009). Another survey found that there are six prognostic factors of disease development: NYHA class III or IV, cardiomegaly on chest radiography, segmental or global wall motion abnormalities on echocardiography, non-sustained ventricular tachycardia on Holter monitoring, low QRS voltage on electrocardiography, and male sex (Rassi et al., 2006). Recent studies have found that there are four echocardiographic variables associated with the disease outcome: left ventricular ejection fraction, right ventricular function, E/E′ ratio, and left atrium volume (Rocha et al., 2009). Finally, the prognosis of the patient will rest on the good care and follow up of the caregivers. Chagas disease is no longer a disease of the poor; it is now a disease of any country with important socioeconomic impact.

10. Conclusions

Prediction markers for disease development, and progression, immunotherapy and vaccine strategies, new anti-*T. cruzi* drugs, and world-standardized PCR tests, are urgently required to improve early diagnosis and treatment of this worldwide health problem. Government, health organizations, and scientists all over the world need to come together to construct policies and strategies to prevent and control this silent but devastating disease in endemic and non endemic countries.

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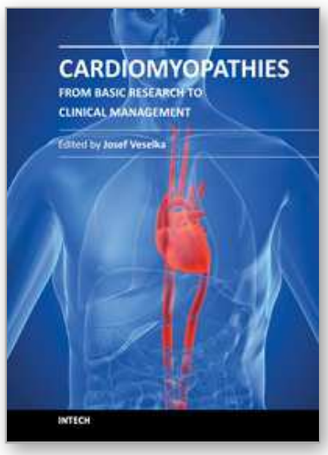

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Cardiomyopathy means "heart (cardio) muscle (myo) disease (pathy)". Currently, cardiomyopathies are defined as myocardial disorders in which the heart muscle is structurally and/or functionally abnormal in the absence of a coronary artery disease, hypertension, valvular heart disease or congenital heart disease sufficient to cause the observed myocardial abnormalities. This book provides a comprehensive, state-of-the-art review of the current knowledge of cardiomyopathies. Instead of following the classic interdisciplinary division, the entire cardiovascular system is presented as a functional unity, and the contributors explore pathophysiological mechanisms from different perspectives, including genetics, molecular biology, electrophysiology, invasive and non-invasive cardiology, imaging methods and surgery. In order to provide a balanced medical view, this book was edited by a clinical cardiologist.

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