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REVIEW ARTICLE

Revisiting the History of Chagas Disease: "Live to tell"

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Abstract

In 1907, Carlos Chagas was designated to fight paludism in the Rio das Velhas region along the Central do Brasil railroad. During his field research, Chagas discovered a hematophagous insect (*Panstrongylus megitus*) carrying a new trypanosomatide, which he named *Trypanosoma cruzi*. On April 14th, 1909, he found the same parasite in the blood of a febrile child, submitting the announcement of his discoveries to the Brasil Médico scientific journal.

Here, we discuss the early stages in the establishment of a new human morbid entity during the first decades after its discovery with a definite influence from its discoverer, Carlos Chagas, as well the first collaborators. Moreover, we cover the importance of the Center for the Study and Prophylaxis of Chagas Disease in Bambuí (MG), unraveling the most advanced developments in research within the disease's habitat and the widening perspectives for modern research that have emerged after the 1960s and continue to improve to this day.

In this revisitation to the history of Chagas disease, we begin at Manguinhos (RJ), making our way to Lassance (MG), where the discovery took place. Then, we travel back to Rio de Janeiro in the beginning of the twentieth century and Brazilian republic until the current day, revealing milestone publications that settled Chagas disease both as a source of pride for Brazilian medicine and as a challenge with important aspects that remain to be clarified. Any similarities to our country's politics and economy in the twentieth century are not mere coincidences.

Keywords

Chagas Disease/ history; Chagas Disease/ etiology; Trypanosoma Cruzi/ Chagas, Cardiomyopathy.

Introduction

Carlos Chagas's research in the semi-arid region of Minas Gerais began in 1907 with the campaign against paludism in the Rio das Velhas river valley, aiming to save the lives of workers who worked in the expansion of the Central do Brasil railroad. The finding of a hematophagous insect (*Panstrongylus megitus*), strictly adapted to the domestic environment, with nocturnal habits and commonly known as the "barber bug" or "kissing bug" surprised Chagas, who intensified his research and found, in this insect, a new trypanosomatide named ***Trypanosoma cruzi.*** The further identification of *T. cruzi* in the blood of domestic animals and in the human blood directed Carlos Chagas to systematize a new human morbid entity.

The choice of Carlos Chagas for the noble mission of battling malaria was due in great part to his strong scientific background on the theme with the completion, in Manguinhos, of his doctoral thesis named "Hematological studies of paludism," presented in 1903. This knowledge prompted him to systematize, in 1905, the household theory of infection by paludism. In 1907, Chagas participated in the group of scientists conducting the most brilliant research at the time, being designated to the anti-paludism campaign in Minas Gerais where, in Lassance, his path became the track for one of the most fascinating discoveries in the history of Medicine. Raquel Lewinsohn[1] and Berning[2] consider that Carlos Chagas, through his example, his life, and the aspects of his discovery, is unique in the history of Medicine, thus reflecting his international recognition.

Carlos Chagas is the author of an unprecedented fact in the history of Medicine because he included the whole cycle of a disease in his discovery: the etiological agent *T. cruzi* and its life cycle, the vector insect (kissing bug), its domestic reservoirs, and the pathology.

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It is worth mentioning Oswaldo Cruz's declaration on Carlos Chagas' discovery: "The discovery of this illness constitutes the most beautiful example of the power of logic in service of science. To this day, in the domains of biological research, such a complex and brilliant discovery had never been made and, what is more, by a sole researcher."

To better understand the greatness of the events at the time, it is necessary to revisit the euphoric climate and atmosphere in Manguinhos in the beginning of the twentieth century due to successive discoveries and to the density of projects lining up as needs of a new era of science in Brazil and worldwide. To cite a few examples, a new vaccine against the plague, the pathognomonic liver injury in yellow fever patients, published works on the differentiation between smallpox and variola minor, the treatment of *lymphogranuloma venereum*, the cure for cutaneous leishmaniasis, the determination of the nuclear division process in amoeba, the discovery of the extra-erythrocyte life cycle of *Haemoproteus columbae* (responsible for pigeon malaria), the description of hundreds of new species, the study of the biology and morphological characterization of vectors of the main tropical diseases and their therapies, among other great scientific breakthroughs. This way, we can comprehend how the genius of Carlos Chagas is not an isolated factor in the tropics, but instead comes from a solid scientific background and is the product of an institution named Instituto Oswaldo Cruz, which is compared and recognized as equivalent to the institute created by Wilhelm Ostvald in the turn of the century in Munich, where the greatest names in physical chemistry in Europe made residence.

Chagas disease

We can thus systematize the essential factors for comprehending Chagas disease, whose etiological agent is *T. cruzi*. This organism uses 2 hosts in its life cycle: an invertebrate (hematophagous triatomine) and a vertebrate (mammals, including humans). Being initially an infection of wild animals, this pathology became a zoonosis because the vector insect adapted promptly to the human habitat, especially in regions with poor housing conditions such as the popular wattle and daub houses.

Chagas disease was initially considered a rural endemic and was later characterized by the occupation of the outskirts of large cities, acquiring urban aspects pressured by agriculture mechanization, which was a great determinant aspect of internal migrations. The most common transmission routes are: natural, transfusion-associated, transplacental, oral, and accidental.

T. cruzi life cycle

By analyzing the biological cycle of this parasite, we observed that it develops within the insect's gut a few hours after the ingestion of infected blood. Subsequently, the differentiation of trypomastigote life forms into epimastigotes takes place, and these multiply by binary fission. In the insect's hindgut, these life forms suffer a new differentiation process into metacyclic trypomastigotes, which will be released with the feces when the insect bites the mammal host.

From an experimental viewpoint, after the ingestion of infected blood by the vector, the full *T. cruzi* life cycle is completed within 30 to 60 days. It is known that different *T. cruzi* strains determine many differences, whether in the susceptibility of vectors or in their degrees of infectivity, parasitemia, and pathogenicity. In the host, after inoculation, trypomastigotes invade macrophages and other tissues, transforming into amastigotes and multiplying in the cell cytoplasm by binary fission. Various division cycles then take place, where parasites differentiate into trypomastigotes that in turn will be able to invade neighboring cells or even be taken by the blood stream to other tissues after cell rupture.[3]. (Figure 1)

In the acute phase of human Chagas disease, many parasites are present in the blood circulation; however, with the installation of the chronic phase, there is a tendency towards the disappearance of parasites from the peripheral blood. Carlos Chagas' discovery has, in its natural progression, very characteristic aspects: by studying the natural history of Chagas disease in Bambuí (MG), Dias observed an incidence of acute cases of 10%[3] , and Teixeira[4] observed that only 35% of patients in the acute phase presented an overt form of the disease, of which most were mildly symptomatic and went unnoticed.

The acute phase of the disease has a benign character and is mildly symptomatic, requiring observation and complementary examinations for its confirmation. Its clinical course is generally favorable: mortality is low[5] (around 5%) throughout the natural history of the disease; the clinical, radiological, and electrocardiographic picture improves in a few months, signaling a return to normality when fever ceases and parasitemia is no longer observed

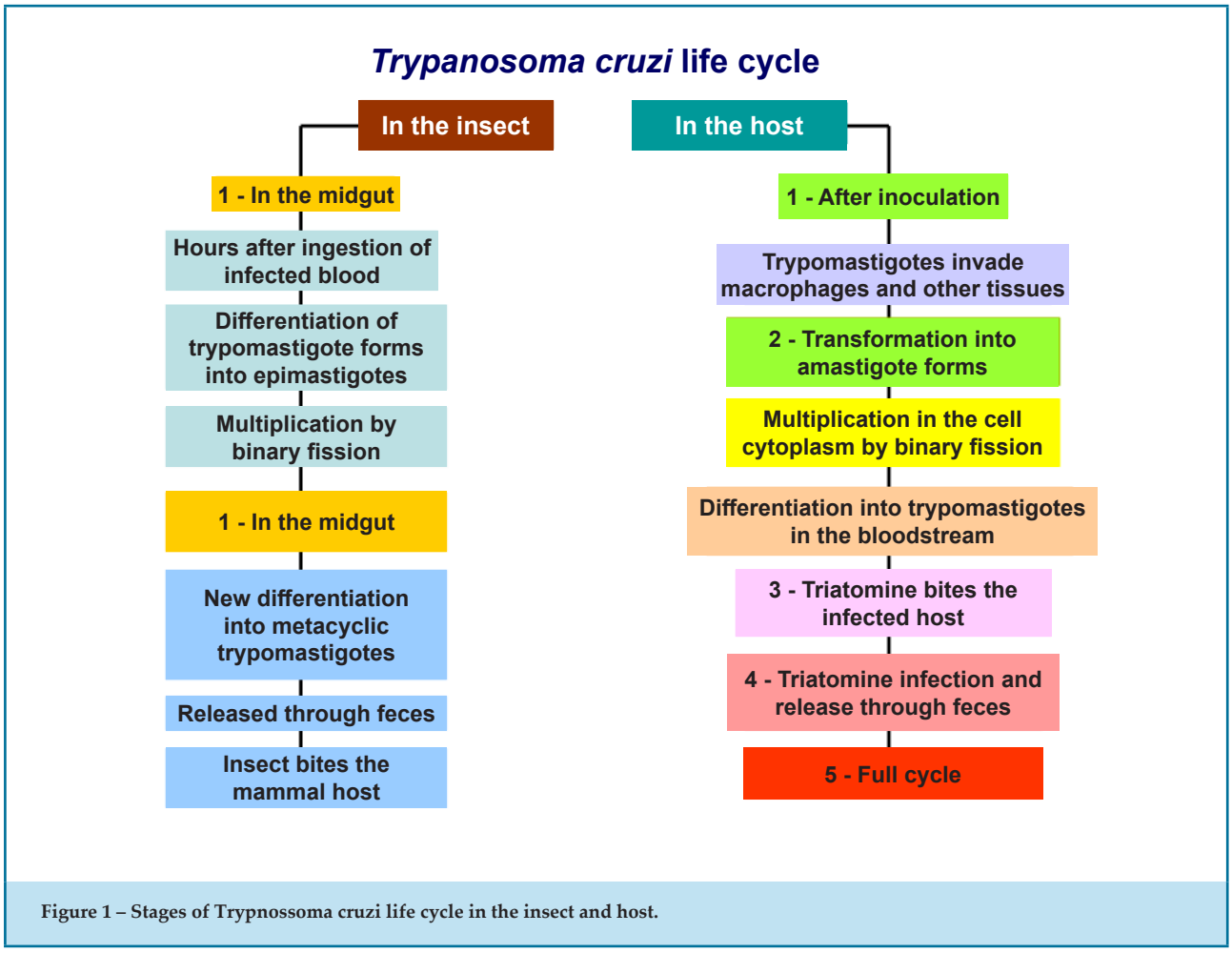

Figure 1 – Stages of Trypnossoma cruzi life cycle in the insect and host.

at direct examination. Some factors such as young age, congestive heart failure, and electrocardiographic alterations such as frequent multi-focal ventricular extrasystoles and intraventricular bundle branch blocks limit prognosis in the acute phase. An acute, diffuse, and intense myocardiopathy with high levels of parasites is the basic anatomopathological substrate found in the acute form of chagasic heart disease.[6-11]

The mortality of acute Chagas disease stems from the occurrence of acute heart disease associated or not with meningoencephalopathy due to *Schizotrypanum* infection.[12] According to Dias[3] in his anthological longitudinal study in Bambuí, more than 75% of the recorded deaths in the acute phase of the disease refer to children aged less than 3 years, with lethality varying between 1.3% and 45% of the published cases(3). In 313 cases recorded in Bambuí, mortality was 8.3% with a significant predominance in younger age groups.[3]

As trypanosomes disappear from the blood circulation, turning the direct examination of peripheral blood negative for trypanosomes, and as anti-trypanosome antibodies (IgG) begin establishing themselves in the circulation, we consider that Chagas disease is entering its chronic period.

This period begins with the so-called "undetermined" phase, where infection remains active but asymptomatic, and it could progress to a determined phase of chronic heart disease, digestive disease, mixed form, or remain indefinitely in the more benign undetermined phase. The undetermined phase may last from 10 to 20 years in most patients, who remain asymptomatic and serologically positive. No evidence of spontaneous cure has been demonstrated by serial xenodiagnoses[3,13,14] and the persistency of serological results in chronic patients.[3,14-16] Data demonstrate that the disease persists in its latent form in this period, as observed through studies with patients in the undetermined phase who died accidentally or by other causes (that not Chagas disease) showing myocarditis foci and a reduction in cardiac parasympathetic neurons.[8]

In the longitudinal study by Dias[3] in Bambuí, a follow-up of 117 patients with Chagas disease observed that, after 10–20 years of disease progression, 60.3% of patients remained in the undetermined phase; with a mean period of 27 years after the acute phase, 33.9% of patients were still in the undetermined phase.

In general, more than 70% of individuals with Chagas disease aged less than 20 years are in the undetermined chronic phase, according to various field investigations.[14,17,18] Patients in the undetermined phase who are in their third decade of life have a risk of progressing to chronic heart disease of around 2% to 5%, according to various longitudinal studies.[3,17,19,20]

Patients in the undetermined phase present only discrete focal myocarditis at microscopic examination and, according to experimental studies, the cyclic clinical course of these myocarditis foci is related with parasitic stimulus, with resolution by apoptosis of inflammatory cells and reabsorption of the excess extracellular matrix. This progression may happen throughout a long period, without greater repercussions on the patient as long as the immunopathological pattern does not change.

The trigger for progression to a determined phase such as chronic chagasic heart disease is related with multiple factors, among which we include the nature and preferential localization of injuries, the *T. cruzi* strain, and the extent of the aggression during the acute phase. For comprehending the pathogenesis of Chagas disease, controversial aspects in the literature should be analyzed, such as the direct tissue injury caused by the parasite, microvascular disease, immunological reactions, and the neurogenic theory involving the autonomic nervous system. The transition from the undetermined phase to its determination, whether it be cardiac, digestive, or nervous, constitutes a challenge in the comprehension of this true scientific gap in the history of Chagas disease.

Decades after the infection, 10%–30% of patients present one of two main clinical manifestations: heart disease associated with myocarditis and fibrosis resulting in heart failure, the formation of thrombi and strokes, or the digestive form with clinical manifestations of megacolon and/or megaesophagus, possibly associated with gastrointestinal disturbances such as regurgitation, malnutrition, and severe constipation.

The immune response results in an inflammatory process in the target tissues during the acute phase of infection by *T. cruzi* and is essential to the control of parasitism and the equilibrium of the parasite/host relationship, which is observed in most patients with Chagas disease. We have observed that, in around 30% of patients, progressive inflammation results in cardiac and/or digestive dysfunctions. In this process, we highlight the absence of parasites in the affected tissues and the apparent lack of correlation between their presence and that of inflammatory infiltrate in these tissues, which resulted in the elaboration of some theories such as:

The parasympathetic dysautonomia theory

Koberle[21], a pioneering scholar studying the autonomic nervous system, adopted in the 1950s the neuron count technique, recording a numeric reduction in parasympathetic nervous cells and thus considering chagasic heart disease to happen due to parasympathetic dysautonomia, with sympathetic predominance.

Autoimmunity

The challenge involving the transition from the undetermined to the determined cardiac phase motivated the hypothesis that "damage to the myocardium would be secondary to a delayed hypersensitivity process directed to the cardiac tissue, mediated by the lymphomononuclear inflammatory infiltrate universally associated with injury. According to this hypothesis, the autoimmune response directed to the myocardium would be caused by a cross-reaction triggered during the immune response against a *T. cruzi* antigen homologous to cardiac structures."[22]

However, Kierszenbaum[23] performed an extensive review of the autoimmunity theory, raising doubts about the relevance of autoimmune recognition in the pathogenesis of chronic manifestations of Chagas disease.

Polyclonal activation

Polyclonal B and T lymphocyte activation, observed in the acute phase of the chagasic infection, may be considered a trigger for the pathology found in the chronic phase. The proliferative activity of T cells and the intense polyclonal activation of B cells during the acute phase of an experimental infection by *T. cruzi* lead to the production of IgM and IgG antibodies with reactivity and multi-reactivity against myosin, myoglobulin, keratin, and other self-proteins. This polyclonal expansion may result from the activation of cell clones that react to a wide variety of parasite antigens or to superantigenic molecules of the parasite. This process may represent a

step in the loss of tolerance preceding an autoimmune response in chronic Chagas disease.[24]

Genetic polymorphism

Infection by *T. cruzi* with clinical manifestations of Chagas disease varying from asymptomatic patients to those with severe heart failure or of the digestive form, with the formation of megacolon or megaesophagus, already represents strong evidence of the influence of genetic factors in the susceptibility to infection by *T. cruzi*. Fernandez-Mestre and colleagues, as mentioned by Lannes-Vieira,[24] studied the genetic polymorphism of DRB1 and DQB1 molecules in Venezuelan patients with Chagas disease. They observed a decreased frequency of the DRB1*14 and DQB1*0303 alleles in comparison with uninfected individuals, suggesting an independent protective role of these molecules against chronic infection. The study of patients with or without heart disease revealed higher frequencies of DRB1*01, DRB1*08, and DQB1*0501 alleles and a lower frequency of DRB1*1501 in patients with arrhythmia and congestive heart failure. These data suggest that HLA class II genes may be associated with the development of chronic infection and chronic damage to cardiac tissue.

Microvascular alterations

Rossi and colleagues, as cited by Lannes-Vieira,[24] reported that microvascular alterations result from thromboembolic processes that play an important part in the origin of chronic chagasic heart disease. The microangiopathy characterized by platelet aggregation and occlusive thrombosis in small vessels of the epicardium and myocardium would cause myonecrosis and focal degeneration with inflammatory infiltrates and contribute to the development of apical aneurysm and cardiomyopathy.

The participation of cytokines

Experimental models have demonstrated that inflammatory cells such as macrophages and T cells, as well as the cytokines produced by them, play important roles in the protective response and immunopathogenesis of parasitic infections. Studies show that cytokines, just as other inflammatory mediators (prostaglandins, thromboxanes, leukotrienes, platelet aggregation factors) play an important role in regulating the immune response during infection by *T. cruzi* and are involved both in infection resistance and mechanisms related with the progression of Chagas disease.[24]

Participation of cell adhesion molecules

D'Avila Reis and colleagues, Higuchi and colleagues, and Tostes and colleagues, as mentioned by Lannes-Vieira,[24] described a predominance of T CD8+ cells in the myocardium of patients in the chronic phase of Chagas disease; however, the molecular mechanisms determining the prevalence of CD8+ cells in this cardiac tissue remain undiscovered. Most inflammatory cells in the myocardium of patients with chronic Chagas disease express cell adhesion molecules such as LFA-1 (leukocyte function-associated antigen-1; CD11a/CD18), ICAM-1 ligand (intercellular adhesion molecule-1; CD54), CD44 ligand of fibronectin and hyaluronic acid and VLA-4 (Very late antigen-4; CD49d/CD29, $\alpha 4\beta 1$) VCAM-1 ligand (vascular cell adhesion molecule-1;CD106) and fibronectin. This way, D'Avila Reis and colleagues suggest that these molecules would contribute to the progression of the inflammatory reaction by mediating the adhesion of lymphocytes to the endothelium of cardiac tissue vessels activated by cytokines and by being important in cell infiltration and localization at inflammation sites.

Participation of chemokines

Plasmatic chemokine concentrations have been correlated with disease worsening in patients with heart failure. Elevated plasma levels of the CCL2/MCP-1 chemokine, but not of CCL3/MIP1α, were directly correlated with heart damage in patients with chagasic heart disease in a study by Talvani and colleagues, as cited by Lannes-Vieira.[24(

Apoptosis

Lopes and colleagues, as cited by Lannes-Vieira,[24] showed in their study that programmed cell death by apoptosis in immune cells (including B and T cells) occurs during infection by *T. cruzi*. Experimental infection models showed a significant loss of T CD4+ cells through an increase in the expression of Fas (CD95) and Fas-ligand (CD95L), with subsequent induction of apoptosis by activation-induced cell death.

Some factors are already consolidated, and we can state that "inflammation persists during the whole chronic phase of Chagas disease, continuously triggering new fibrosis foci."[25] Repeated cycles of parasitic infection and hypersensitivity and autoimmunity phenomena

seem to be responsible for perpetuating this picture.[26-28] Fibrosis, within the natural course of Chagas disease, represents a new anatomical element that, to a specific extent, may be capable of disrupting the balance of the chagasic heart, possibly triggering heart failure.[25]

Chronic chagasic heart disease has a slow and variable progression and generally appears in the fourth decade of life, when the first signs of heart failure become apparent.[9,29,30]. Once established, chronic chagasic heart disease presents itself as a progressive and severe myocardial pathology, exhibiting a varied picture of isolated or combined arrhythmias that has not yet been verified in any other heart disease. This heart pathology has been thoroughly investigated using necropsy material and findings were correlated with clinical and electrocardiographic manifestations, especially thromboembolic phenomena. The rare presence of parasites in sections, as opposed to what is observed in the acute phase, raised discussions about the histopathological diagnosis and interpretation of pathogenesis.

The digestive form

Clinical and epidemiological evidence, reliable serological studies, and a positive serological test for *T. c ruzi* lead to the diagnosis of the digestive form of Chagas disease. Anatomical studies contributed decisively to the recognition of the digestive form of Chagas disease, and the conclusive acceptance of a chagasic etiology for megaesophagus and megacolon came with studies by Köberle.[31,32] and anatomopathological findings of degenerative injuries of the myenteric plexus in autopsied cases of megaesophagus; these injuries were found not only in dilated segments, but in the whole digestive tract.

The main function of the myenteric plexus in the digestive tract wall is to coordinate motility in its different segments.

Köberle performed his research with autopsies of patients with Chagas disease, naturally infected animals, and through the experimental infection of laboratory animals. In the acute phase of the infection, he observed parasitism of the muscular layer of the digestive tract and an inflammatory process involving the myenteric plexus. In the chronic phase, he observed that denervation was irregular, with variable distribution and intensity.

In the neuron count, performed in the lower third of the esophagus, the reduction in neuron numbers was shown to be very variable. Denervation was a constant in patients with Chagas disease, but it was less intense in cases where the esophagus appeared normal. He concluded, after comparing cases with and without megaesophagus, that the progression of chagasic esophageal disorder to a typical megaesophagus occurs when denervation reaches a threshold, which was estimated in 90%.

The nervous form

The chronic nervous form of Chagas disease is still not unanimous to this day, even though it was recognized by its discoverer Carlos Chagas and despite the classic study by Köberle in 1967.[33]

Studies have shown cortical atrophy with or without hydrocephaly, a decreased neuron population and activation of glial cells such as astrocytes and microglia, but no histopathological alterations or even parasitism (as reviewed by Alencar, 1982). Differently from other models, the study by Silva[34] using C3H/He mice infected with the Colombian *T. cruzi* strain showed the presence, even if rare, of the parasite in the chronic phase. However, the absence of inflammation suggests the resolution of inflammatory processes found in the central nervous system during the acute phase of the experimental infection, since all animals survived acute infection.

Specific treatment of Chagas disease

Great variability is seen regarding the types of cases and cure control employed in different studies. Generally, we consider adequate the results for the acute phase and cases of recent infection, mainly among children, who not only tolerate better long-term treatments but also present high cure rates. This was demonstrated by randomized field studies with benznidazole in Brazil (Andrade et al., 1996)[35] and Argentina (Sosa Estani et al., 1998).[36] The mean estimated parasitological cure rate is around 60% for acute cases and recent infections. Results are considered poor for chronic infections, in the Brazilian experience; in the Southern Cone, results were considered superior, probably owing to a different parasite strain (Silva et al. 1974; Cerisola et al., 1977).[37,38]

The clinical progression of Chagas disease after specific treatment is controversial and study results are inconclusive due to differences in cases, assessment methods, follow-up periods, and data interpretation.

Macêdo and Silveira[39,] aiming to assess the electrocardiographic progression of heart disease, studied 171 adults with chronic Chagas disease who

were treated with nifurtimox or benznidazole with a follow-up of 7 years and observed electrocardiographic improvements in 6.7% of the cases against 8.8% among untreated patients, with no significant difference between groups. Ianni et al. (1993)[40] evaluated 33 adults in the undetermined phase for 8 years and observed electrocardiographic progression in 13.3% of cases treated with benznidazole (n=15) and 0% of patients who received placebo (n=18); since this was a small number of cases, definitive conclusions could not be reached.

It is worth noting that, among 120 cases (adults and children) studied by Miranda el al.,[41] progression of the electrocardiogram was observed in 10.5% of patients treated with benznidazole against 63.6% of the placebo group. Patients were monitored for 10 to 16 years, but the combination of results from adults and children and the interpretation of electrocardiograms hampered result analyses.

Carlos Chagas, the man of the twentieth century

It is also vital to know other faces of the man Carlos Chagas and his marriage with Ms. Iris Lobo, a bond that strengthened his fighting spirit and full dedication to research and where he found the support and comprehension indispensable to the life of a researcher in the beginning of the twentieth century.

Iris Lobo was the daughter of a Minas Gerais state senator, Fernando Lobo Leite Pereira, and met Carlos Chagas in one of these weekend social gatherings, sealing a mutual complicity that would remain for their whole lives. In the beginning, their union was not easy due to the objection by the senator's family; his wife alleged that Chagas's family had "Black blood." This was not enough to keep them apart, and in face of Iris' persistence and a friendly interference by Miguel Couto (who was married to Carlos' mother's first cousin), the senator did not resist and authorized the wedding, which happened in July 1904. Iris' personality was well defined by her son, Carlos Chagas;[42] "In moments of glory, she knew how to dim herself, but in terrible moments such as when Carlos Chagas was attacked by the papers at Rio de Janeiro as a public health director or in the episode at the Academy of Medicine, where malevolent and ignorant detractors tried to disparage his work, she stood up to his defense as a hero from a Greek tragedy, not allowing for even a moment that his partner's spirit be invaded with disheartenment."

Chagas disease post-Lassance

In Lassance (MG) after his discovery, Chagas continued the studies of this new human morbid entity and, within his anatomopathological studies, it was up to his dedicated colleague Gaspar Vianna to prove the existence of leishmaniform bodies in the myocardium fibers of experimental animals and human beings. This unlocked the perspective for describing the cardiac form of the disease, which was magnificently systematized by Chagas and Villela in 1922 with vital contribution by the Boullite electrocardiograph, which was difficult to manipulate and significantly advanced for the time. The cardiac form would only be modified in 1942 by Laranja, Dias, Nóbrega, and Miranda based on exceptional works performed at the Center for the Study and Prophylaxis of Chagas Disease in Bambuí (MG) under guidance of the Oswaldo Cruz Institute. Among their changes, we highlight the consolidation of the chronic cardiac form (determined phase) and its connection to the acute infection by *T. cruzi* according to the reproduction of chronic infections in dogs. [43] After this phase, the cardiac form of Chagas disease is defined as progressive, preferentially affecting men aged between 20 and 50 years, with frequent clinical manifestations of disturbances in the formation and conduction of cardiac stimuli and congestive heart failure; anatomically, this form becomes characterized by an extensive inflammatory infiltrate, usually followed by circumscribed injuries in the parietal endocardium, and by the slow development of ischemic myocardial alterations.[44]

The clinical, electrocardiographic, and prophylaxis-related contribution of Chagas disease studies at Bambuí reach international repercussion and Brazil starts to adopt a prophylaxis model where "Casa de Oswaldo" (the Oswaldo Cruz Institute) is fully invested in fighting Chagas disease, penetrating "Cafuas" (wattle and daub houses) in the region, exterminating triatomines, proposing the construction of decent housing, examining and cataloguing the sick population in a beautiful process of state-coordinated change in established values and health promotion through the correction of perverse social inequalities, especially in the countryside.

The late Dr. Francisco Laranja, in a personal communication to researchers from Rio de Janeiro at his house in Leblon (RJ) in 1981, reported significant information on the historical Center for the Study and Prophylaxis of Chagas Disease at Bambuí, enumerating the contributions by this institution to the knowledge on Chagas disease:

-The introduction of clinical and serological criteria for diagnosing chronic infection;

-The introduction of the electrocardiograph as an instrument for assessing a public health problem;

-The first demonstration of the link between the acute phase and chronic heart disease through longitudinal studies of progressing cases;

-The demonstration of the undetermined chronic phase and its clinical and epidemiological importance;

-The experimental reproduction of the chronic heart disease in dogs and of the megaesophagus in Rhesus monkeys;

-The norms for applying insecticides (benzene hexachloride, BHC) in the domestic environment and the demonstration of its efficacy in controlling vector-borne transmission of the infection, enabling the elimination of new cases in a densely infected area;

-The first demonstrations of *T. cruzi* transmission through blood transfusions.

Starting at Bambuí, a new phase in the study of Chagas disease is inaugurated, highlighting great challenges that would encompass decades and establish solid pillars in the history of this new clinical entity, such as the specific treatment of the acute phase, prophylaxis in endemic areas through the use of insecticides, and improvements in housing conditions.

The progress of Brazilian Science, as reflected by the following studies and their undisputable contributions

Chronic chagasic heart disease stands out as the object of many studies, classified as cross-sectional cohort,[45,46] prospective,[47,48] case-control,[49] and community-based intervention studies.[50] Studies on the pathogenesis of Chagas disease demonstrate the discovery, in humans

Bambuí's Center for Study and Prophylaxis of Chagas' disease

- ⇨ **Introduction of clinical and serological criteria for diagnosing chronic infection**
- ⇨ **Introduction of the electrocardiograph as an instrument for assessing a public health problem**
- ⇨ **The first demonstration of a link between the acute phase and chronic heart disease through longitudinal studies of progressing cases**
- ⇨ **Demonstration of the chronic undetermined form, of its clinical and epidemiological importance**
- ⇨ **Experimental reproduction of chronic heart disease in dogs and of the megaesophagus in the Rhesus monkey**
- ⇨ **Norms for the application of insecticides (BHC) in the domestic environment and demonstration of its efficacy in controlling vector-borne transmission, allowing the prevention of new cases in a densely infected area**
- ⇨ **First demonstrations of T. cruzi transmission through blood transfusions**

Chart 1 – Notable contributions of Bambuí's Center for Study and Prophylaxis of Chagas' disease

or experimental models during the acute phase, of focal inflammatory injuries, severe myocarditis, cardiac myocyte necrosis, and amastigote nests in the heart, as observed by Kosma[51] and Tafuri.[52]

Systematized studies analyzing autonomic function in Chagas disease were performed at Ribeirão Preto Medical School, coordinated by Professor Dalmo Amorim. Following these studies, various faces of autonomic cardiac control appeared in the literature, presented by independent groups in Brazil (Ribeirão Preto and Brasília), Argentina (Córdoba), and Venezuela (Mérida). In general, studies performed in Ribeirão Preto by Amorim et al.,[53] and Marin Neto[54] and in Brasília by Junqueira et al.[55] reflect the functional impairment of the parasympathetic system, while studies from Córdoba (Argentina) performed by Iosa[56] and Palmero et al.,[57] have shown to be compatible with cardiovascular sympathetic dysfunction.

The analysis of this dysautonomia proceeds with a group from Rio de Janeiro, which has developed a series of studies[58-60] reporting, for the first time, the presence of functionally active autoantibodies that reacted against muscarinic cholinergic receptors in patients with Chagas disease and different degrees of cardiac impairment (asymptomatic patients with a normal electrocardiogram/echocardiogram, asymptomatic patients with a normal electrocardiogram and alterations in the echocardiogram, symptomatic patients with alterations in the electrocardiogram/echocardiogram, but no important functional abnormalities, and finally severe symptomatic patients with cardiac conduction and mechanic abnormalities). This group showed that the presence of functional antibodies does not depend on the degree of cardiac impairment.

Animal experiments have shown that antibodies interact with cellular constituents, influencing metabolism and cardiac contractility. The interruption of the beta-adrenergic pathway may affect contraction strength and myocardial relaxation. Leite[61] demonstrated that the serum of chronic chagasic patients reduced, in a dose-dependent manner, contraction amplitude in the atrial myocardium of rabbits. Sera of patients without myocardial dysfunction did not significantly alter any of the measured parameters.

Other studies on autoantibodies as the expression of dysautonomia have followed, such as that by Savio-Galimberti and colleagues,[62] who observed antibodies with adrenergic properties compromising cardiac muscle function in isolated heart muscle. Another interesting study was published by Hernandez and colleagues,[63] who showed antibodies, this time with muscarinic properties, decreasing L-type calcium current via the non-competitive activation of the M2 muscarinic receptor. The impairment of this current by antibodies with adrenergic or muscarinic properties could contribute, at least in part, to the myocardial dysfunction seen in the chronic phase in patients with Chagas disease.

As studies progressed, Gimenez and colleagues[64] were able to show cardiac impairment as a consequence of the autoimmune process generated by antibodies with adrenergic or cholinergic properties. They immunized mice with a plasmid coding for the M2 and beta-1 adrenergic receptors. The authors found antibodies not only against the second loop of M2 and beta-1 receptors, but also against the third intracellular loop of the M2 receptor. Through binding assays, they could observe a twofold increment in the expression of M2 receptors, a decrease in beta-1 receptors, and signs of autonomic dysregulation in immunized animals. Other relevant findings observed in these animals were myofibrillar disarray and fibrosis as a consequence of continued exposure to antibodies. The research group in Rio de Janeiro advanced in their studies on dysautonomia and De Carvalho and colleagues,[65] in a pioneering study, indicated that a humoral component could be involved in the genesis of arrhythmias in chagasic cardiomyopathy. The authors described that sera from rabbits infected with *T. cruzi* generated electrocardiographic disturbances in isolated rabbit hearts. Subsequently, the same group confirmed this hypothesis, now showing that antibodies from patients with chronic Chagas disease who had complex arrhythmias decreased the heart rate and caused atrioventricular block in isolated rabbit hearts.[66] Still in Rio de Janeiro, Costa and colleagues[58] studied the sera of 58 patients with chronic chagasic heart disease and described that some of them, with beta-adrenergic properties, blocked conduction via communicating junctions in cultures of neonatal rat cardiomyocytes, suggesting one more mechanism through which these antibodies might contribute to the occurrence of arrhythmias. Medei and colleagues[67] showed, for the first time, that patients with chronic chagasic heart disease who had antibodies with muscarinic properties presented increased QT interval dispersion when compared to patients with chronic disease who did not have them. These antibodies with muscarinic properties, when perfused through isolated hearts under controlled heart rates, increased QT intervals.

Considering that the parasympathetic nervous system only has a discrete effect on coronary circulation, the

imbalance between the sympathetic and parasympathetic nervous system with a predominance of sympathetic action on coronary vessels can contribute with microcirculatory constrictions, which would cause possible spastic events and aneurysms.[68] Approaches of the anatomical and functional aspects of the coronary circulation and microcirculation in humans and experimental models infected with *T. cruzi* demonstrated no obstructive injuries capable of inducing myocardial ischemia. The perfusion disturbances observed by these studies are related with microvascular alterations, mostly in patients with chronic Chagas disease with normal coronary arteries at angiographic examination. This would explain regional contractile perfusion dysfunctions that resemble those observed in ischemic heart disease.

Investigations in this direction continued and, in 1997, Carrasco et al.,[69] managed to show sympathetic system impairment, with a significant reduction of this component in the sympathovagal balance of patients with chronic Chagas disease, relating it with an increase in myocardial contractile dysfunction.

In 2003, Cunha et al.,[70] demonstrated a decrease in heart rate variability expressed through daytime and nighttime SDANN (standard deviation of the mean of all recorded 5-minute intervals [ms]), daytime and nighttime SDNN (standard deviation of all recorded P-P cycles [ms]), daytime SDNN I (mean standard deviation of all recorded 5-minute intervals [ms]), in addition to an increase in daytime and nighttime RMSSD (root mean square of successive differences [ms]) and nighttime pNN50 (proportion [%] of variations of more than 50 ms between normal successive cycles), suggesting that dysautonomia may occur early in Chagas disease.

The complexity of *T. cruzi*'s biological cycle suggests that infection control involves a combination of adaptive immunological responses by the host that operate in various levels of the immunological system.

Some authors[71] indicate that the immunization of mice with a recombinant *T. cruzi* protein (TcP2β) promotes intense and specific antibody production against the C-terminal portion of the 13-residue epitope (*R13 peptide: EEEDDDMGFGLFD*) and has a simultaneous β1-adrenergic stimulating activity. Other animals subjected to the same immunization did not provide similar results. These data showed that the R13 epitope could induce an antibody that recognized the second extracellular loop of β1 receptors and could induce ventricular arrhythmias when passively transferred to mice.

Levitus and colleagues[72] demonstrated that cloned parasitic peptides (JL5) reacted with sera from patients with Chagas disease. The cloned peptide was identified as the C-terminal portion of the parasite's ribosomal P protein, which developed cross-reactivity with the host's ribosomal P protein. This is due to a homologous amino acid sequence covering almost 90% of the parasite and host proteins.

Some authors[73] have shown that a cloned parasitic peptide (*λgt 11*) named *JL5* reacted with sera from patients infected by *T. cruzi* and this peptide was identified as the C-terminal portion of *T. cruzi's* ribosomal P protein. Many studies indicate that *JL5* has epitopes that promote cross-reactivity with the host's ribosomal P protein. Most amino acids in this peptide are homologous to the C-terminal portion of the human ribosomal P protein.

In patients with Chagas disease, the allosteric nature of the interaction between IgGs and the muscarinic receptor site was confirmed by observing that these patients' sera, and not those of healthy donors, increased agonist activity, inducing bradiarrhythmias. This effect disappears in the presence of gallamine, an allosteric antagonist. The main strength of this study was the characterization of the action of antibodies in the serum of patients with Chagas disease with the allosteric interaction occurring in the second extracellular loop of M2 acetylcholine receptors and of their ability to exert an agonist and signal-transducing effect.[72,73]

In order to establish a cytokine profile for the undetermined and chronic (cardiac) phases of Chagas disease, Vitelli-Avelar and colleagues[74] developed methods for calculating the mean percentage of cytokine appearance in leukocytes, establishing a threshold that defined low and high levels of cytokine production and, from there, constructing diagrams that characterized each phase. A low frequency of cytokines, in the undetermined phase, is observed by a reduced frequency of T CD4+ inflammatory cells.

IFNγ levels allowed a distinction between patients in the chronic (cardiac) and undetermined phases of the disease. *IFNγ* levels were significantly higher in the chronic phase, just as IL-10 and CD8+ levels were significantly higher in the cardiac form of the chronic phase when compared to the control group. In this study, the control group did not present a specific cytokine profile when compared to patients in the undetermined and chronic (cardiac) phases.

We can relate the pathogenesis of acute phase injuries (especially in the initial phase) with the presence of

intracellular parasites. The analysis chronic phase pathogenesis is reason for intense controversy, since this phase presents delayed injuries and for a long time it was believed that the presence of parasites was rare. In 1993, Bocchi et al.[75] observed that the most common finding after cardiac transplants in patients with Chagas disease was the reactivation of infection by T. cruzi, and Higuchi et al.,[76] still in 1993, demonstrated a significant correlation between the presence of T. cruzi and moderate to severe inflammatory infiltrate in cardiac biopsy sections and necropsy examinations of patients with Chagas disease. Later, in 1996, Belloti et al.[77] also sought to investigate the presence of parasites in the hearts of patients with chronic Chagas disease, frequently finding these organisms and relating them with the severity of the myocardial inflammatory process, thus strengthening the idea of an important role for the parasite in the pathophysiology of the chronic phase and unlocking a true perspective for specific treatment of the chronic phase of Chagas disease.

Aiming to treat chronic chagasic cardiomyopathy and based on previous studies suggesting that autologous bone marrow stem cell transplant improved cardiac function in patients with chronic chagasic cardiomyopathy, researchers performed a study using cell therapy in chronic chagasic heart disease as an arm of a multi-center randomized cell therapy study in cardiomyopathies. [78]

The study evaluated patients aged between 18 and 75 years with chronic chagasic cardiomyopathy, functional class II–IV (New York Heart Association, NYHA), ejection fraction < 35%, who received optimized clinical therapy. They were randomized for intracoronary injection of bone marrow stem cells or placebo. The primary endpoints were differences in ejection fraction 6 months and 1 year after treatment in both groups. This study followed up 234 patients between July 2005 and October 2009.

Their conclusion was that the intracoronary injection of bone marrow stem cells did not improve left ventricular function or quality of life in patients with chronic chagasic cardiomyopathy.

Within this perspective and attending to the needs of researchers of chronic chagasic heart disease, in 2015 the Benefit study[79] searched for an answer to the role of benznidazole in chronic chagasic cardiomyopathy; it was conducted as a prospective, multi-center, randomized study involving 2854 patients with chagasic cardiomyopathy who received benznidazole or placebo for 80 days; participants were followed up for a mean period of 5.4 years.

The study demonstrated that therapy with benznidazole in patients with established chronic chagasic heart disease significantly increased conversion rates to negative polymerase chain reaction (PCR) results but did not significantly reduce clinical deterioration during the 5.4-year follow-up period.

Challenges still remain: among them, the search for comprehending autoimmunity based on numerous studies that have been published and stimulated since the demonstration, by Higuchi et al.,[76] of the presence of the parasite in hearts of patients with chronic Chagas disease. The logical foundation relies on the parasite presence stimulating a strong immune response, which certainly causes damage and inflammation in the affected cardiac tissues. A molecular mimicry response between parasitic and host molecules may result in cross-reaction with self-molecules and consequently in autoimmunity with autoantibodies and self-reactive cells. Although there is still controversy, autoimmunity may be related with the progression of chronic chagasic heart disease.[80)].

In this line of thought, Cunha et al.,[81] demonstrated with 24 h Holter monitoring that, during a period of mostly parasympathetic activation (02:00–06:00h), a direct and significant correlation between anti-M2 antibodies and SDANN was observed. In the stress test, they found a direct correlation between anti-β1 antibodies and double product.

Among other improvements in the search for comprehending the pathological substrate for chronic chagasic heart disease, we highlight the research on the inheritance and fixation of *T. cruzi* kDNA minicircles in the genome of patients with Chagas disease and their family members, developed by Aragão.[82]

In this study, the lateral and vertical transfer of *T. cruzi* DNA minicircles (kDNA) was investigated in 26 people from 4 families, and active infection was observed in 5 people with nuclear *T. cruzi* DNA; evidence of kDNA integration was documented in all these 5 cases. This work explicitly reports the occurrence of kDNA transfer from *T. cruzi* to humans and expands the knowledge on the inheritance of mutations generated by the integration of minicircle sequences in most host chromosomes. Evidently, these findings are still premature and controversial, but they uncover new perspectives in the pathophysiology and vertical transmission of Chagas disease.

Another study in the same direction was presented by Morini[83] and characterized by the demonstration of a protein associated with the *T. cruzi* kinetoplast, named *TcKAP7*, which had low molecular weight, a basic

nature, and a role in DNA charge neutralization and condensation. By using immunofluorescence, researchers observed that the *TcKAP7* protein was located around kinetoplast poles, suggesting it may be involved in late stages of minicircle replication.

Concerns around Chagas disease considering the comprehension of its pathophysiology in the chronic phase, the still unanswered questions regarding its undetermined phase, and particularly an efficient therapy for this phase of the disease are fully justified when observing the astounding numbers, still in the twenty-first century, of 6 million infected people in Latin America and the globalization of Chagas disease, showing thousands of infected people in the USA, Japan, Australia, and Spain.[84]

According to the World Health Organization (WHO), until 1990 there were 11 million infected people and more than 300 000 cases had been observed in the USA. An expressive increase in oral transmission and in congenital transmission route valorization is noted.[85-87]

The global incidence of new human infections by *T. cruzi* has increased in approximately 67%. Only in Brazil, the estimate accounts for 4.6 million infected people.[88,89]

In the sense of facing all these problems related with Chagas disease and searching for the ideal parameters for solving this challenging problem, in 2014 the London Declaration[90] on Neglected Tropical Diseases was signed. It proposed, for 2020, measures aiming at interrupting the main forms of Chagas disease transmission globally, stimulating antiparasitic treatment, the improvement of vigilance systems in affected countries, and easily accessible care to infected patients.

A study performed in 2018 in Yucatan[91] questioned the objectives of the London Declaration for 2020 regarding their sufficiency in controlling Chagas disease. This study showed that reductions in domestic vector-borne transmission, congenital transmission, and transmission through blood transfusion may successfully reduce human infection rates (up to 82% in a year), reaching the 2020 objectives, but would still result in 0.5 new acute cases per 2000 people in 5 years.

Progress has been made considering basic research on *T. cruzi*, especially in the genetic characterization of parasite groups, evidencing their genomic sequence. Zingales,[92](in a review on the subject, mentions that *T. cruzi* is divided into 7 discrete typing units (DTUs), among which are Tcl (*Trypanossoma cruzi* I), TcVI (*Trypanosoma cruzi* VI), and Tcbat (bat *Trypanosoma cruzi*); the latter is restricted to bats. This review emphasized that the interaction between the parasite and host genetics should have an important role in defining the pathogenesis of Chagas disease, the anti-*T. cruzi* immunological response, and the chemotherapy response, and it should be considered in future investigations.

The *TcI*, *TcVI*, and *TcBat* DTUs have a yet unknown geographical distribution. *TcI* is the most spread around the geographical distribution of this parasite, including Brazilian biomes.[93]

Recently, Vermelho et al.,[94] discussed the urgent need for intensifying research on new efficient drugs for treating Chagas disease, reminding that since the 1960s few advances in this area have outdone the only 2 drugs currently in use, benznidazole and nifurtimox. They mention that today, few drugs are going through pre-clinical tests and highlight only 3 classes of compounds that have shown high cure rates in rat infection models: nitroimidazole, the oxaborole DNDi-6148, and proteasome inhibitors (GNF6702).

Considering the educational aspects of Chagas disease, Sanmartino et al.,[95] observed that, since the first publication more than 110 years ago, the need for comprehending the complex relationship between Chagas disease and social and environmental aspects beyond the biomedical and epidemiological aspects still remains.

Scenarios in this matter, both rural and Latin American, urban and global, make it clear that education about Chagas disease should include all possible contexts: the location of vectors in Latin America in rural, peri-urban, and urban areas using formal and informal educational environments. The authors stress the requirement for a whole health approach that overcomes the biomedical aspect, including the multi-dimensionality of this matter and providing an educational dialogic perspective, finally allowing individuals and communities to analyze, decide, and lead preventive and promotion actions contextualized to their health.

Conclusion

In our revisitation to the history of Chagas disease, our observations allow us to state that research is progressing and representing the respectability of the Brazilian scientific community; on the other hand, we have noticed the need for improvements in assistance and social, hospital, and preventive care that can cope

Important landmarks in the history of Chagas disease

Anos	
1909	Discovery of Chagas disease (Carlos Chagas)
1922	Description of the cardiac form (Chagas & Villela)
1942	Modifications of the cardiac form (Laranja and colleagues)
1943	Center for Studies in Bambuí - MG
1968–1985	Studies of the central autonomic nervous system
1993–1996	Parasite in the chronic form (Higuchi, Bocchi, Belloti)
2012	Cellular therapy in chronic Chagas heart disease
2013–2015	Genome and demonstration of the TcKAP7 protein London Declaration
2015	Benefit - Benznidazol therapy for chronic Chagas heart disease
2018	Modeling scenarios for the Yucatan peninsula
2020	New drugs and educational aspects

Chart 2 – Summary of important landmarks Chagas' disease knowledge.

with the strong demand for assistance by people with Chagas disease.

By communicating his Discovery to the Brazilian society, Carlos Chagas, with his deep sensitivity, revealed the precarious conditions of life in the countryside, where people lived under inhuman conditions and fell victim to numerous devastating diseases such as the recently announced morbid entity. All the political turmoil that ensued originated a deep discussion that extrapolated the science world and gave rise to a fruitful political and social debate, which in turn contributed to a change in consciousness and preparation of the society for facing the massive social problem ingrained in the rural environment and that later would reach large cities through internal migration movements.

Today, in the modern world and under undisputable influence of technology, challenges to the control, care, and comprehension of Chagas disease remain and occupy a more complex position that maintains Carlos Chagas' discovery (one of the most beautiful pages of Brazilian medicine) in the list of diseases neglected by the public sector.

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Author contributions

Conception and design of the research: Cunha AB, Cunha DM. Acquisition of data: Cunha DM, Cunha AB. Analysis and interpretation of the data: Cunha AB, Cunha DM. Writing of the manuscript: Cunha AB. Critical revision of the manuscript for intellectual content: Cunha AB.

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