Chagas disease

José A Pérez-Molina, Israel Molina

Lancet 2018; 391: 82–94
Published Online June 30, 2017
http://dx.doi.org/10.1016/S0140-6736(17)31612-4

National Referral Centre for Tropical Diseases, Infectious Diseases Department, Hospital Universitario Ramón y Cajal, Insituto Ramón y Cajal de Investgación Sanitaria, Madrid, Spain (J A Pérez-Molina PhD); **Infectious Diseases Department, Hospital Universitario Vall d'Hebron, Barcelona, Spain** (I Molina PhD); **and International Health Program of the Catalan Institute of Health, Barcelona, Spain** (I Molina)

Correspondence to:
Dr José A Pérez-Molina, National Referral Centre for Tropical Diseases, Infectious Diseases Department, Hospital Universitario Ramón y Cajal, Madrid 28034, Spain
jperezm@salud.madrid.org

Chagas disease is an anthropozoonosis from the American continent that has spread from its original boundaries through migration. It is caused by the protozoan *Trypanosoma cruzi*, which was identified in the first decade of the 20th century. Once acute infection resolves, patients can develop chronic disease, which in up to 30–40% of cases is characterised by cardiomyopathy, arrhythmias, megaviscera, and, more rarely, polyneuropathy and stroke. Even after more than a century, many challenges remain unresolved, since epidemiological control and diagnostic, therapeutic, and prognostic methods must be improved. In particular, the efficacy and tolerability profile of therapeutic agents is far from ideal. Furthermore, the population affected is older and more complex (eg, immunosuppressed patients and patients with cancer). Nevertheless, in recent years, our knowledge of Chagas disease has expanded, and the international networking needed to change the course of this deadly disease during the 21st century has begun.

Introduction

More than 100 years ago, *Trypanosoma cruzi* was identified as the causative agent of Chagas disease, yet the condition remains a major social and public health problem in Latin America and is regarded as a neglected tropical disease by WHO. According to WHO, and in common with other neglected tropical diseases, "Chagas disease is a proxy for poverty and disadvantage: it affects populations with low visibility and little political voice, causes stigma and discrimination, is relatively neglected by researchers, and has a considerable impact on morbidity and mortality".[1] In addition, stigma often precludes prompt detection and control of the disease since many patients do not want to know about the condition.[2] In the seven southernmost American countries, the disease causes the loss of about 752 000 working days because of premature deaths and US$1·2 billion in productivity.[3] The estimated annual global burden of disease is $627·46 million in health-care costs and 806 170 disability-adjusted life-years; 10% of this burden affects non-endemic countries.[4] Migration and specific modes of transmission have led to Chagas disease spreading beyond its natural geographical boundaries and becoming a global issue.[5–7] Furthermore, the typical patient profile has changed owing to increasing age and associated comorbidities.

Search strategy and selection criteria

We undertook a search of PubMed and Embase from inception to Aug 1, 2016, with no language restrictions, using the following search terms: "Chagas disease", "American trypanosomiasis", or "*Trypanosoma cruzi*", and "epidemiology or pathogenesis or symptoms or diagnosis or treatment or outcome". We selected key references and seminal papers, review articles, patient reports, and book chapters. We also reviewed abstracts from pertinent scientific meetings and publications from international organisations such as the PanAmerican Health Organization, WHO, and the Special Programme for Research and Training in Tropical Diseases (part of WHO) from Jan 1, 2010, to Aug 31, 2016.

Life cycle of *T cruzi*

T cruzi takes two forms in human beings. The trypomastigote, with a flagellum extending along the outer edge of an undulating membrane, does not divide in blood, but carries the infection throughout the body. The amastigote, which has no flagellum, multiplies within various types of cell, preferring those of mesenchymal origin (figure 1).

T cruzi is a heterogeneous species with high genetic and phenotypic diversity. It circulates between insect vectors and mammalian hosts, and has been classified into six near-clades (TcI to TcVI) known as discrete typing units.[8] This genetic diversity has been related to geographical distribution, pathogenesis, clinical features, and response to therapy.[8] *T cruzi* is transmitted in endemic areas by various species of three genera of blood-sucking triatomine insects, also known as kissing bugs (*Triatoma*, *Panstrongylus*, *Rhodnius*). All three genera are widely distributed in Latin America, from Mexico to Argentina and Chile, and inhabit both forest and drier areas.[9,10]

T cruzi can be transmitted through routes other than vectorial spread. These routes have a major role in non-endemic countries and a growing importance in endemic areas. It is estimated that the mother-to-child transmission rate is about 4·7% (range 3·9–5·6%) and that this rate could be higher in endemic countries than in non-endemic countries (5% *vs* 2·7%).[11–14] The main biological determinant for congenital transmission is maternal parasitaemia, which could be as high as 31% when *T cruzi* is detectable by PCR, although transmission is also possible when PCR is negative.[13,15] An association between some discrete typing units and lower rates of congenital transmission (eg, TcII compared with TcV in Brazil) has also been suggested.[12] On the other hand, an effective cellular immune response to *T cruzi* seems to have a relevant role in congenital infections. Sustained exposure to the vector is associated with decreased parasitaemia and congenital transmission, probably because frequent exposure to infected vectors induces a Th1 immune response that overcomes the less effective pregnancy-induced Th2 polarisation.[16]

T cruzi can also be transmitted through blood and blood products; the estimated transmission rate per

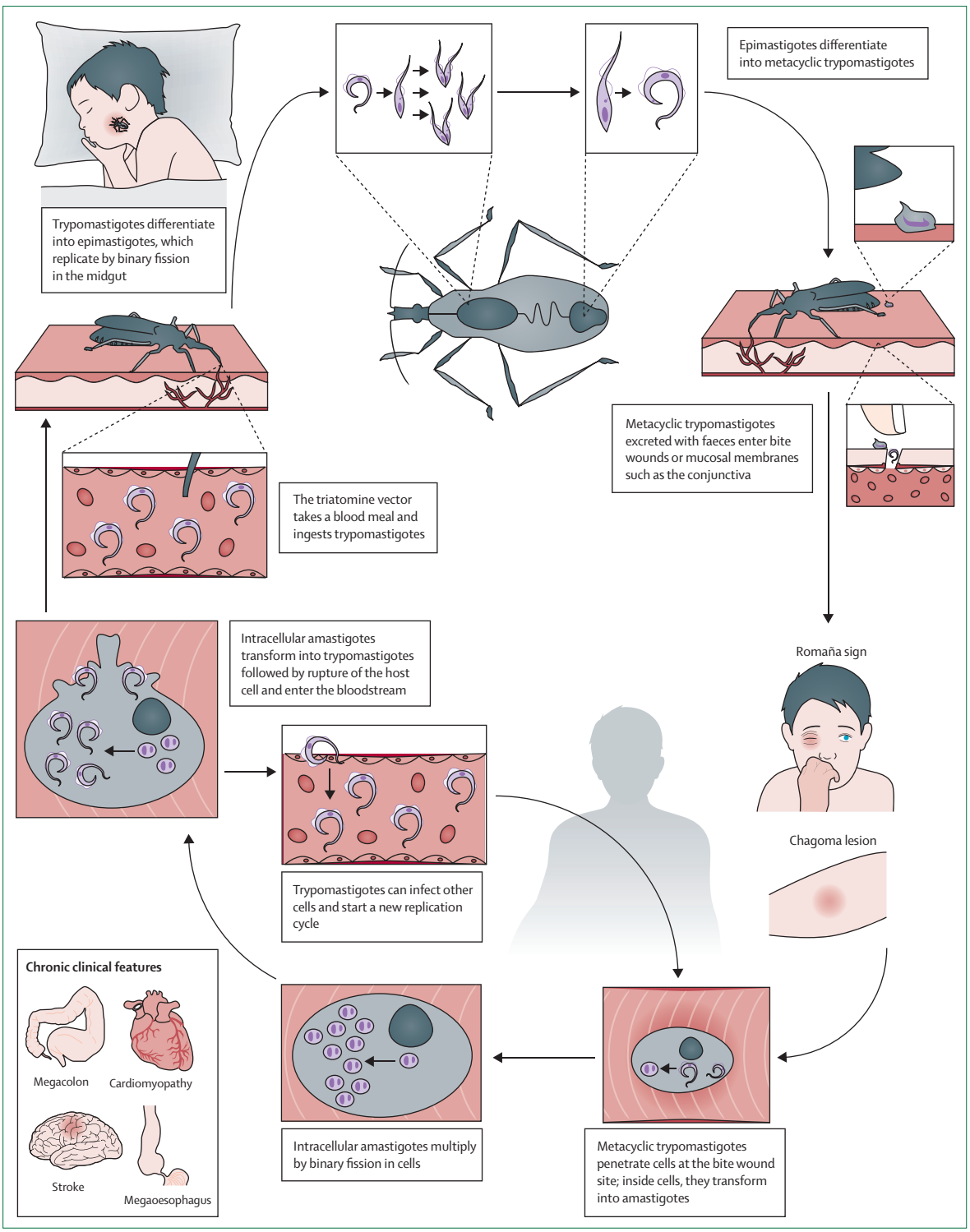

Figure 1*: Life cycle of *Trypanosoma cruzi

infected blood unit is 10–25%.[17,18] Infection rates after solid organ transplantation from an infected donor seem to be lower for kidney recipients (0–19%)[19–21] than for liver recipients (0–29%)[21,22] and heart recipients (75–100%).[21,23] Other less frequent modes of transmission include consumption of contaminated food and drink (oral transmission)[24] and laboratory accidents.[25]

	1980–85	2005	2010
Population at risk (% total)	92 895 000 (25%)	108 595 000 (20·4%)	70 199 360 (12·9%)
Number of infected people	17 395 000	7 694 500	5 742 167
Number of new cases per year	700 000	55 585	38 593
Congenital transmission	7000–49 000*	14 385	8668
Vectorial transmission	Not reported	41 200	29 925
Number of deaths per year	>45 000	12 500	12 000

*Estimation based on data provided in the report in reference 10. Data are from references 10, 29, 32, 33.

***Table 1*: Changes in prevalence, incidence, and mortality of Chagas disease, 1985–2010, in 21 endemic countries in Latin America**

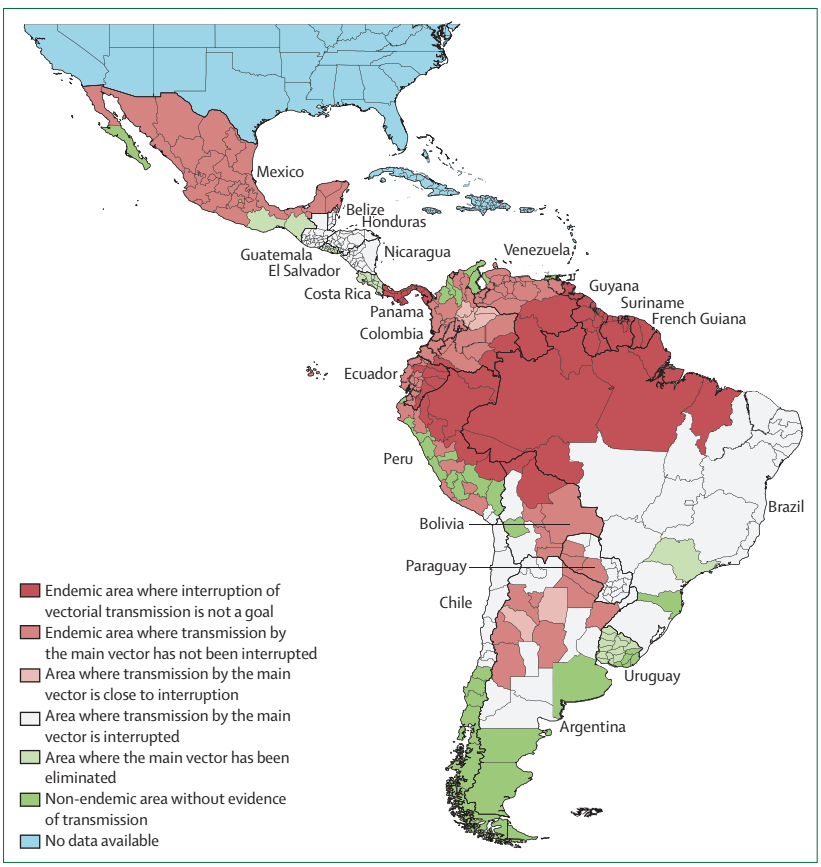

***Figure 2*: Transmission of Chagas disease by the main vector, triatominae (September, 2014)**
Adapted from the Pan American World Health Organization Chagas disease control programme.

Epidemiology

Chagas disease is endemic in 21 continental Latin American countries, from southern USA to the north of Argentina and Chile. It has traditionally been confined to poor, rural areas of Central and South America, where vectorial transmission is the main route of contagion. Residents of infested houses are continuously exposed to vector bites, and the incidence of infection by *T cruzi* is less than 0·1% to 4% per year in hyperendemic regions such as the Bolivian Chaco.[26,27] Recent internal migration from rural to urban areas, congenital transmission, and blood donation have enabled the disease to spread to previously unaffected regions, mainly large urban areas, where urban disease cycles might be established in peripheral areas.[28] The prevalence of *T cruzi* infection is highest in Bolivia (6·1 cases per 100 inhabitants), Argentina (3·6), Paraguay (2·1), Ecuador (1·4), El Salvador (1·3), and Guatemala (1·2).[29] In the USA, enzootic cycles of *T cruzi* transmission are established in selected areas of some southern states, although only a few autochthonous infections have been reported.[30,31]

Since the early 1990s, the most effective measures for Chagas disease control in Latin America have been programmes for vector control and compulsory blood bank testing.[9,10] Consequently, prevalence has decreased substantially (table 1), and vectorial transmission was interrupted in Uruguay in 1997, in Chile in 1999, and in most of Brazil in 2000 (figure 2).[10,34] Nevertheless, the frequency of transmission has increased in regions such as the Amazon basin (oral transmission)[35] and some areas of the Gran Chaco (because of resistance to pyrethroids by the vector).[36]

Chagas disease has crossed international borders and is now a global epidemic,[5] to the extent that it can be transmitted in non-endemic regions. The pooled prevalence of infection in Latin American migrants living in Europe is estimated to be 4·2% (95% CI 2·2–6·7) with the highest prevalence among migrants from Bolivia (18·1%) and Paraguay (5·5%).[6] In the USA, about 300 000 immigrants are estimated to be infected with *T cruzi*.[7] However, the estimated index of underdiagnosis is around 95%,[37] and health professionals' expertise in this area is clearly insufficient.[38]

Clinical manifestations

The clinical course of Chagas disease usually comprises an acute phase and a chronic phase (table 2). Acute infection can occur at any age, though usually during the first years of life, and is asymptomatic in most cases. Acute phase symptoms include fever, inflammation at the inoculation site (inoculation chancre), unilateral palpebral oedema (Romaña sign; when the conjunctiva is the portal of entry), lymphadenopathy, and hepatosplenomegaly. The acute phase lasts 4–8 weeks, and parasitaemia decreases substantially from 90 days onwards.[39,40] Severe acute disease occurs in less than 1–5% of patients and includes manifestations such as acute myocarditis, pericardial effusion, and meningoencephalitis (risk of mortality 0·2–0·5%).[40,41] Most congenitally infected newborn babies are asymptomatic or have mild symptoms, but a minority have severe life-threatening disease.[42] Oral transmission through food or drink contaminated with triatomine faeces seems to cause more severe disease, with higher mortality than vector-borne disease.[24,35]

The acute phase usually resolves spontaneously after which time patients remain chronically infected if untreated. Most people never develop symptoms or visceral

	Geographical distribution	Clinical signs and symptoms	Diagnosis
Acute forms including reactivation in immunosuppressed patients			
Vectorial	Endemic countries	Incubation period of 1–2 weeks. Signs of portal of entry: indurated cutaneous lesion (chagoma) or palpebral oedema (Romaña sign). Most cases are mild disease (95–99%) and unrecognised. Persistent fever, fatigue, lymphadenopathy, hepatomegaly, splenomegaly, morbilliform rash, oedema. In rare cases, myocarditis or meningoencephalitis. Anaemia, lymphocytosis, raised AST and ALT concentrations. Risk of mortality 0·2–0·5%.	Direct parasitological methods: patent parasitaemia up to 90 days. Microscopic examination of fresh blood, Giemsa-stained thin and thick blood films, or buffy coat. Concentration methods: Microhaematocrit and Strout method PCR techniques Serology is not useful
Congenital	Endemic and non-endemic countries	Incubation period: birth to several weeks. Most are asymptomatic or have mild disease. Prematurity, low birth weight, abortion, neonatal death. Fever, jaundice, oedema, hepatomegaly, splenomegaly, respiratory distress syndrome, myocarditis, meningoencephalitis. Anaemia and thrombocytopenia. Risk of mortality <2%.	Direct parasitological methods. Concentration methods: microhaematocrit, Strout method. Direct microscopy also useful. PCR: most sensitive technique Serology: after 9 months or later
Oral	Restricted areas of endemic countries (Amazon basin) and local outbreaks	Incubation period 3–22 days. Fever, vomiting, periocular oedema, dyspnoea, fever, myalgia, prostration, cough, splenomegaly, hepatomegaly, chest pain, abdominal pain, digestive haemorrhage. Risk of mortality 1–35%.	Same as vectorial
Transfusion and transplant	Endemic and non-endemic countries	Incubation period 8–160 days. Persistent fever. Clinical characteristics similar to those of vectorial cases (excluding portal of entry signs). Risk of mortality is variable and depends on the severity of baseline disease.	Same as vectorial. PCR techniques usually yield positive results days to weeks before trypomastigotes are detectable in blood. Tissue samples are needed in some circumstances.
Reactivation in HIV-infected patients	Endemic and non-endemic countries	Behaves as other opportunistic infections. Reactivation with <200 CD4 cells per µL (mostly with <100). Affects CNS (75–90%) as single or multiple space-occupying lesions or as severe necrohaemorrhagic meningoencephalitis. Cardiac involvement (10–55%): myocarditis, pericardial effusion or worsening of previous cardiomyopathy. Risk of mortality 20%.	Direct parasitological methods, as in vectorial cases. Parasite can be found in CSF, other body fluids, and tissue samples PCR: not useful for diagnosis of reactivation Serology: indicative of chronic infection and helpful in cases of suspected disease
Reactivation in other immunosuppressed patients	Endemic and non-endemic countries	Reactivation after transplantation or in patients with haematological malignancies. Clinical characteristics similar to those of patients who undergo transfusion and those with panniculitis and other skin disorders. Risk of mortality is variable and depends on the severity of baseline disease and prompt diagnosis.	Direct parasitological methods, as in vectorial cases. Parasite can be found in tissue samples PCR: increasing parasite load detected with real-time PCR in serial specimens could be indicative of a high risk of reactivation
Chronic form			
Indeterminate form	Endemic and non-endemic countries	Asymptomatic. Normal chest radiograph and 12-lead ECG.	Serology: detection of IgG PCR: low sensitivity
Cardiac and gastrointestinal form	Endemic and non-endemic countries	Cardiac manifestations: fatigue, syncope, palpitations, dizziness, stroke. Late manifestations: chest pain (atypical), dyspnoea, oedema, left ventricular dysfunction, congestive heart failure. Alterations in 12-lead ECG, echocardiography, or other heart function tests. Gastrointestinal: dysphagia, regurgitation, severe constipation (dilated oesophagus or colon). Alterations in oesophageal manometry, barium swallow, or barium enema.	Serology: detection of IgG PCR: low sensitivity

AST=aspartate aminotransferase. ALT=alanine aminotransferase. CSF=cerebrospinal fluid. ECG=electrocardiogram

***Table 2*: Clinical features and diagnosis of Chagas disease**

involvement and thus are the largest group of patients affected. This so-called indeterminate form of Chagas disease has a good prognosis and is characterised by seropositivity for *T cruzi*, absence of clinical signs and symptoms of cardiac and digestive involvement, and normal chest radiography and electrocardiography.[43] Increasingly more patients with indeterminate disease are diagnosed with subtle cardiac or digestive abnormalities, as diagnostic methods become more sensitive (eg, echocardiography, MRI, and oesophageal manometry). Nevertheless, whether such alterations are associated with poorer prognosis remains unclear.[44–46]

Roughly 30–40% of chronically infected patients can develop organ involvement 10–30 years after acute infection (mainly cardiomyopathy or megaviscera [megaoesophagus, megacolon, or both]). This evidence originates from studies done in the 1980s,[42,47,48] within a very different socioeconomic context, and they did not

account for potential confounders such as frequent loss to follow-up, concomitant comorbidities, repeated exposure to vector bites, *T cruzi* discrete typing unit, and severity of the initial episode. Updated information on the long-term natural history of the disease, however, remains scarce. Results of recent studies have shown progression to cardiac involvement of around 1·4–5·0% per year.[49–51]

Cardiac involvement is the most frequent and severe type of organ involvement, occurs in 14–45% of chronically infected patients,[10,43,47,50,52,53] and affects mainly the conduction system and myocardium. The most common initial signs are one or more of: left anterior fascicular block, right bundle branch block, and segmental left ventricular wall motion abnormalities. Late manifestations include sinus node dysfunction leading to severe bradycardia, high-degree atrioventricular blocks, non-sustained or sustained ventricular tachycardia, complex ventricular extra-systoles, progressive dilated cardiomyopathy with congestive heart failure, apical aneurysms (usually of the left ventricle), and emboli due to thrombus formation in the dilated left ventricle or aneurysm.[54] Sudden death is the main cause of death in patients with Chagas heart disease, followed by refractory heart failure and thromboembolism.[55] Chronic cardiac involvement due to Chagas disease should be differentiated from, but may be associated with, other causes of dilated cardiomyopathy.[56] Impaired left ventricular function, New York Heart Association (NYHA) class III or IV, cardiomegaly, and non-sustained ventricular tachycardia indicate a poor prognosis in patients with chronic Chagas disease.[57]

Gastrointestinal involvement is less common (10–21%) and occurs more frequently in the Southern Cone of South America.[46,47,53,58] Manifestations range from asymptomatic motility disorders to mild achalasia to severe megaoesophagus. Symptoms include dysphagia, odynophagia, oesophageal reflux, weight loss, aspiration, cough, and regurgitation. Patients with megaoesophagus may be at increased risk of oesophageal cancer, and upper gastrointestinal endoscopy might be indicated, especially in patients with new or progressive symptoms. Megacolon is characterised by persistent constipation and can lead to faecaloma, volvulus, and bowel ischaemia.[58] The sigmoid and rectum are dilated in nearly all cases of megacolon, whereas dilatation of more proximal colonic segments is rare. An increased risk of colorectal cancer has not been found in patients with megacolon. Small intestine and biliary involvement is very rare.[58] Cardiac and gastrointestinal involvement seldom present together (5–20% of patients with myocardiopathy).

Chagas disease is also a major cause of cardioembolic stroke, which is up to twice as common in Chagas heart disease as in other forms of cardiomyopathy.[59,60] The incidence of ischaemic stroke has been estimated as 2·7 events per 100 patients per year,[60] and around a third of patients who experience ischaemic stroke may have asymptomatic *T cruzi* infection.[61] Neuropathy, in the form of mild sensory polyneuropathy, can present in up to 10% of patients although may not necessarily be associated with other visceral involvement.[62]

Pathogenesis

In the acute phase of the disease, organ damage is secondary to the direct action of the parasite and the acute inflammatory response. Nests of *T cruzi* amastigotes are found in tissues (mainly cardiac, skeletal, and smooth muscle) and elsewhere (CNS, gonads, and mononuclear phagocyte system).[35,40] In this phase, highly efficient control of the parasite is the result of an intense inflammatory response with active antibody production and activation of the innate immune response (natural killer cells and macrophages) by Th1 proinflammatory cytokines such as tumour necrosis factor α and interferon γ.

The pathogenesis of chronic chagasic cardiomyopathy is not completely understood and, until recently, the consequences of the disease were considered to be autoimmune. Although several autoantigens that cross-react with *T cruzi* antigens and autoantibodies have been identified, the role of autoimmunity in pathogenesis is unknown.[63] Recent evidence has shown that tissue damage is the principal role of *T cruzi* and the chronic inflammatory response that *T cruzi* elicits.[64,65] There is a growing consensus that the balance between persistence of infection and host immune response is crucial for the establishment and progression of cardiomyopathy.[66,67] During the chronic phase, inflammation is the main determinant of progression; the other potential contributory factors are virulence of the *T cruzi* strain and tissue tropism.[66–68] An inflammatory environment predominates in the cardiac form, with the production of cytokines such as interferon γ and tumour necrosis factor α and other cytotoxic mechanisms involving CD8+ T cells, which lead to tissue damage and, ultimately, to severe cardiomyopathy; in the indeterminate clinical form, a regulatory immune response (characterised by production of interleukin 10 and interleukin 17) predominates.

Diagnosis

Parasitological diagnosis

Diagnosis of acute and congenital disease is made by direct microscopic visualisation of trypomastigotes in blood and, occasionally, other body fluids such as cerebrospinal fluid.[10] In congenital infection, diagnosis can also be based on positive serology results beyond 8 months. Parasites can be observed through a simple fresh blood examination or in Giemsa-stained thin and thick blood smears (sensitivity 34–85%)[69] (table 2). Concentration methods such as microhaematocrit and the Strout method raise the diagnostic yield to more than 95%.[69] Indirect parasitological methods include PCR, which is more sensitive than blood culture and xenodiagnosis and has proven to be very useful in the

diagnosis of mother-to-child transmission, where it is more sensitive than concentration techniques.[13,14,70]

Parasitaemia is low and intermittent in the chronic phase of the disease, thus making direct parasitological and PCR-based diagnostic methods unreliable. Diagnosis of chronic infection, therefore, relies on serological testing through detection of IgG antibodies against *T cruzi* (figure 3).[10,71] Serological techniques are based on whole parasite antigens and purified extracts (conventional tests) and on recombinant antigens and synthetic peptides (non-conventional tests). The most common tests are indirect fluorescent assay, indirect haemagglutination, and ELISA.[72] Since no single standard reference test is available, diagnosis should be based on the presence of IgG against various *T cruzi* antigens by use of at least two serological assays with different antigens.[10] Serology results can be discordant and samples can yield persistent inconclusive results (3% in a general clinical laboratory). A third assay is then indicated to clarify infection status. Techniques such as western blot can prove useful in these circumstances, especially in countries where *Leishmania* spp are endemic.[73] Given the high sensitivity and specificity of ELISA, a single test may be sufficient for screening, thus rendering serological confirmation necessary only in the event of a positive result.[71] Rapid diagnostic tests (eg, immunochromatographic assays using recombinant proteins) are not sufficiently sensitive to be used as first-line serological diagnostic tools.[74] Nevertheless, both these tests and the use of dried blood samples are a good option for patients who have difficulty accessing health-care facilities or whenever large-scale screening is needed. [74–76]

For diagnosis of chronic *T cruzi* infection, PCR has low and varying sensitivities that range from 50% to 90%.[71] The factors contributing to that variability are blood volume, methodology, genes targeted, phase of infection, presence of immunosuppression, patient's home country, and genetic diversity of *T cruzi*.[71,77] However, PCR may be helpful when serology results are inconclusive, for monitoring early detection of treatment failure, and in the management of immunosuppressed patients.

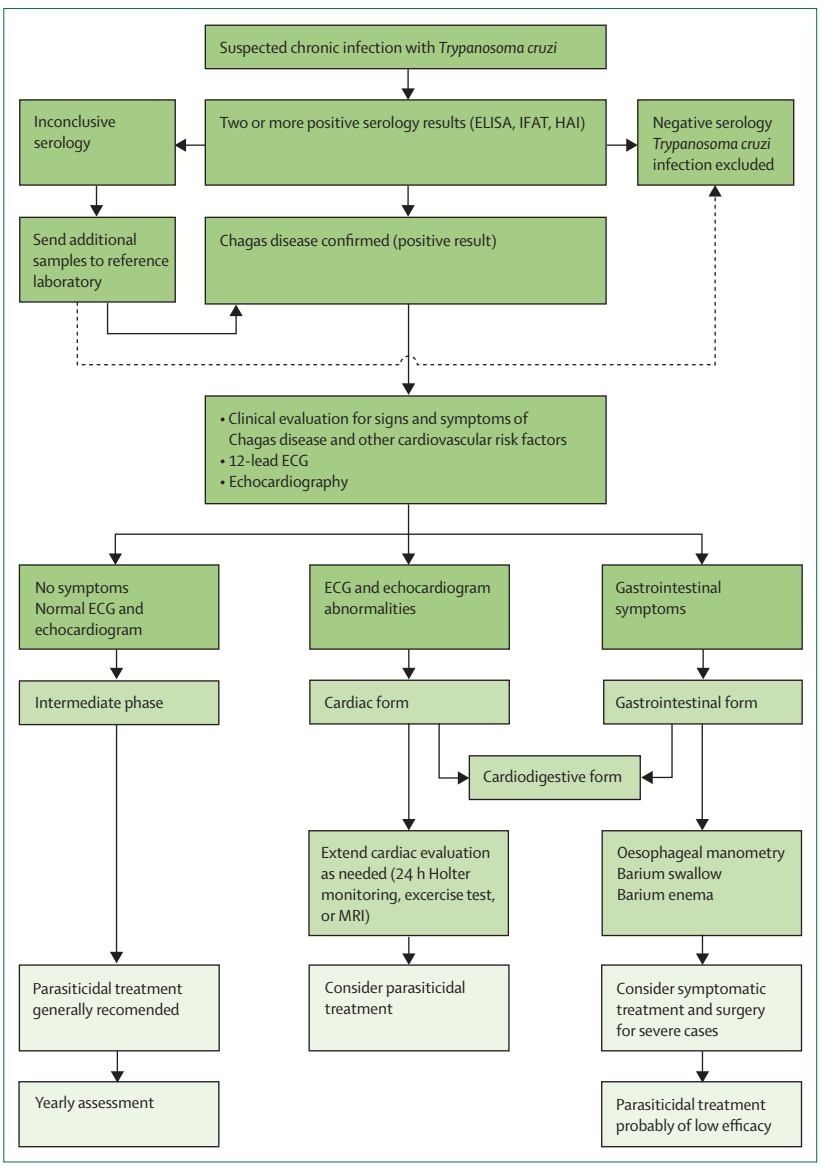

***Figure 3*: Assessment of patients with chronic Chagas disease**
IFAT=indirect fluorescent antibody test. HAI=hamagglutination inhibition. ECG=electrocardiogram. ECG=echocardiogram.

Diagnosis of visceral involvement

Visceral complications must be assessed. Resting electrocardiography (ECG) should be performed during the initial examination even in asymptomatic patients. Cardiac ultrasonography should be used routinely, especially in patients with ECG disturbances, men over 30 years, and women over 45.[78] Additional cardiac tests such as 24 h Holter monitoring, ergometry, and cardiac MRI should be considered in symptomatic patients. Several scales have been proposed to classify the severity of Chagas cardiomyopathy.[79]

Barium swallow and enema are the most common diagnostic procedures used to assess gastrointestinal involvement in symptomatic patients. Since megacolon and megarectum can appear in almost 20% of asymptomatic patients, colon enema may be considered at screening.[80] Oesophageal manometry should be performed in patients who report related symptoms, even in the presence of a normal oesophagogram. [46,81]

Treatment

Treatment with antitrypanosomal drugs is always recommended for acute and congenital Chagas disease, reactivated infections, and chronic disease in children younger than 18 years. Since persistence of parasitosis and concomitant chronic inflammation underlie chronic chagasic cardiomyopathy, parasiticidal treatment is generally offered to patients with chronic Chagas disease in the indeterminate phase and patients with

	Treatment
Acute infection	
Vectorial and oral	Start antiparasitic treatment as soon as possible. Benznidazole 5–10 mg/kg per day for 60 days or nifurtimox 10–15 mg/kg per day for 60–90 days. In children aged <12 years, benznidazole 10 mg/kg per day for 60 days or nifurtimox 15 mg/kg per day for 60 days.
Congenital	Benznidazole 10 mg/kg per day for 60 days, or nifurtimox 15–20 mg/kg per day for 60 days.
Pregnancy	In pregnant women, consider risk–benefit ratio as with other potentially teratogenic drugs. Limited experience with benznidazole has not shown neonatal abnormalities.
Laboratory accidents	Benznidazole 5–7·5 mg/kg per day or nifurtimox 8–10 mg/kg per day for 10–14 days.
Post-transfusion or transplant from an infected donor	Benznidazole 5–7·5 mg/kg per day or nifurtimox 8–10 mg/kg per day for 60 days.
Chronic infection	
Children	Benznidazole 5–7·5 mg/kg per day for 60 days or nifurtimox 8–10 mg/kg per day for 60–90 days.
Adults	Benznidazole 5–7·5 mg/kg per day for 60 days or nifurtimox 8–10 mg/kg per day for 60–90 days. Especially indicated in women of childbearing age. Contraindicated in pregnant women. No expected benefit if long-lasting infection or moderate-advanced visceral disease.
HIV-infected individuals	Recommend antiparasitic treatment combined with cART to all patients as in non-HIV-infected patients, especially when CD4 count <200 cells per µL.
Transplant recipients	Antitrypanosomal prophylaxis to recipients infected with *Trypanosoma cruzi* before transplantation is not generally recommended; consider pretransplant treatment in potential heart transplant recipients or in living donors infected with *T cruzi*. Benznidazole 5 mg/kg per day is preferred to nifurtimox 8–10 mg/kg per day for 60 days, with close monitoring for drug toxicity. Careful post-transplant parasitological monitoring necessary to exclude possible donor-derived infection (seropositive donor and seronegative recipient) or reactivation (seropositive recipient).
Patients receiving immunosuppressive drugs	Treat all patients considering the additional benefit of preventing future reactivations. Parasitological monitoring is recommended and should be particularly close in patients with no previous trypanocidal treatment.
Reactivation	
HIV-infected	Start antiparasitic treatment as soon as possible. Benznidazole 5–7·5 mg/kg per day or nifurtimox 8–10 mg/kg per day for 60 days. Consider higher doses for CNS involvement (benznidazole 15 mg/kg per day). Early initiation of cART (no reports of *T cruzi* IRIS). Secondary prophylaxis with benznidazole 5 mg/kg per day three times per week or 200 mg daily until CD4 count >200–250 cells per µL for 6 months and undetectable HIV viral load.
Transplant recipients	Benznidazole 5–7·5 mg/kg per day for 60 days or nifurtimox 8–10 mg/kg per day for 90 days, although longer courses have been recommended. Close monitoring for toxicity.
Treatment failure	
Immunocompetent	Treatment failure is usually seen as a positive PCR result. Consider retreatment if indications for original treatment remain unchanged or monitor closely. Antiparasitic treatment could include the same or a different drug for 60–90 days. Combination therapies and longer drug courses can be considered.
Immunosuppressed	Initiate retreatment with the same or a different drug for 60–90 days. Combination therapies and longer drug courses could be considered.

cART=combination antiretroviral therapy. IRIS=immune reconstitution inflammatory syndrome.

***Table 3*: Treatment of Chagas disease according to disease phase**

mild-to-moderate disease (table 3).[10,79,82] However, opinions differ about the impact of aetiological treatment in this phase. Results of a systematic review and meta-analysis[83] showed that treatment with benznidazole had little benefit, and that the observed effect could be marginal compared with placebo or no treatment in the chronic phase. In the BENEFIT trial, treatment with benznidazole did not significantly reduce cardiac clinical impairment in patients with moderate-to-severe cardiomyopathy.[84] On the other hand, treatment of women of childbearing age has proven effective in interrupting vertical transmission.[13,85] Treatment should be individualised for patients older than 50 years and for patients with comorbidities.

Only two drugs, benznidazole and nifurtimox, are licensed for the treatment of Chagas disease. Both have been the mainstay of parasiticidal treatment for almost 50 years, although their safety and efficacy profile is far from ideal. Furthermore, since the effectiveness of treatment seems to decrease with time from primary infection, early detection and intervention are crucial. Nifurtimox was the first drug used and is administered orally in three to four doses for 60–90 days (table 3).[10,79,82] Cure rates in the chronic indeterminate phase range from 86% in children younger than 14 years to 7–8% in adults.[86,87] The frequency of adverse effects with nifurtimox is 43·0–97·5%; the most common are anorexia, weight loss, neurological disorders (irritability, insomnia, disorientation, mood changes, paresthesias, and peripheral neuropathy), digestive manifestations such as nausea and vomiting, and, occasionally, fever and rash. Treatment is discontinued in 14·5–75·0% of cases.[86,88]

Benznidazole is generally preferred over nifurtimox because of its better tolerability profile, tissue penetration, and, possibly, efficacy. It is administered orally in two or three doses usually for 60 days (table 3). Higher doses of up to 15 mg/kg are recommended in cases of meningoencephalitis.[10] According to some reports, 30 days of treatment can be useful for chronically infected adults.[50] Benznidazole has considerable activity during the acute and early phases of *T cruzi* infection: serological cure is achieved in up to 100% of patients with congenital disease[89,90] treated during the first year of life and in 76% of patients with acute disease.[91] In the chronic phase, cure rates are much lower: 60–93% in children aged up to 13 years[92–94] and 2–40% in adults with late chronic disease, although these values improve with longer follow-up.[50,91,95] The most common adverse effects involve hypersensitivity, mainly in the form of skin rash (29–50%), digestive intolerance (5–15%), and general symptoms such as anorexia, asthenia, headache, and sleeping disorders (40%). Neuropathy and depression of bone marrow are considered rare. Treatment is discontinued in 9–29% of cases, even though these reactions are reversible and severe in less than 1% of cases.[52,84,96,97] Neither elevated serum drug levels nor high daily doses (>300 mg) have been associated with an increased frequency of adverse events.[52,98]

The symptoms of mild toxic drug reactions can be treated with antihistamines, corticosteroids, or both, although antitrypanosomal treatment should be

interrupted when toxicity is severe. Nifurtimox seems to be safe as a secondary treatment after interruption of benznidazole because of adverse effects.[99] The addition of corticosteroids to prevent adverse effects does not appear to benefit patients taking nifurtimox,[100] and discouraging results have been found in patients taking benznidazole.[101]

Novel therapeutic approaches

Since the introduction of benznidazole and nifurtimox, only allopurinol and the triazoles (ergosterol biosynthesis inhibitors) have been studied in clinical trials, observational studies, and case reports. Monotherapy with ravuconazole or posaconazole has not proven to be efficacious for the treatment of chronic *T cruzi* infection,[102,103] and the combination of posaconazole and benznidazole did not provide any further efficacy or safety advantages over benznidazole monotherapy.[104] Recent evidence suggests that current benznidazole regimens can be optimised by administration of intermittent dosing schedules,[105] dose reduction schedules,[106,107] or combination therapies.[108]

An important obstacle for drug development is the poor translation of in-vivo data to human disease. Available animal models have limited predictive value for the preclinical evaluation of novel therapies, thus partly explaining the clinical failures observed with novel triazoles.[109] Animal models that can predict more confidently the efficacy of new drug candidates in clinical trials must be standardised.[110]

Medical follow-up

The clinical course of Chagas cardiomyopathy is difficult to predict. Some patients with ECG or echocardiographic evidence of disease remain asymptomatic for life, whereas others show a progressive course with severe arrhythmias or heart failure. Some patients die suddenly without previous symptoms. Nevertheless, for patients with more advanced disease (NYHA class III or IV, left ventricular dysfunction, cardiomegaly, or ventricular arrhythmias), the risk of mortality is clearly increased and can reach 85% at 10 years.[111] Treatment with benznidazole does not seem to reduce the clinical impairment of patients with moderate-to-severe cardiomyopathy,[84] since there is no evidence that antiparasitic treatment affects the progression of gastrointestinal involvement. [79]

The current criterion for cure is reversion to negative results in conventional serological tests. In adult patients in the chronic phase, this might take 10–20 years.[10,91] Although research in this area is very active,[112–114] there are no reliable early surrogate laboratory markers for cure or for progression to cardiomyopathy, especially in patients in the indeterminate phase. Some of the markers studied include immunological molecules, host antigens, cytokine production, lymphocytic phenotype, metabolic molecules, and specific serological techniques. Although there are candidates that, alone or in combination, could satisfy some criteria for use in clinical practice,[112] none has been validated for routine clinical use. Some of the difficulties faced in the validation of those surrogate markers for cure are the lack of a consensus definition for early therapeutic response, the likelihood of reinfection in endemic areas, the different biological behaviour between acute and chronic infection, the potential role of parasite genotype, and the need for long follow-up to establish cure with current methods needed for validating new methods. Because PCR techniques have low sensitivity for establishing cure and do not show association with visceral involvement or clinical outcomes after treatment,[84] multiple medical visits and complementary tests are ordered for patients who might not need them. Patients should be followed up for early detection of clinical progression and implementation of prompt therapy to treat visceral complications regardless of whether they have been treated. Long-term follow-up not only provides advantages in terms of control of Chagas disease, but also enables us to act against other risk factors (both cardiovascular and lifestyle) that are as important as the disease itself. Typical recommendations include a clinical interview and physical examination at least once a year, annual ECG, and echocardiography every 2–3 years depending on symptoms and disease severity. Serological testing can be performed once a year, since it guides cure criteria, and PCR can be used to monitor treatment failure.[79,115]

Treatment of chagasic cardiomyopathy and digestive involvement

The haemodynamic and neurohormonal responses in Chagas cardiomyopathy are similar to those of other cardiomyopathies. Medical therapy should be started with the same regimens as in heart failure of other causes. However, since patients with Chagas disease frequently have low blood pressure and a high incidence of bradyarrhythmias, they might be unable to tolerate target doses of angiotensin-converting enzyme inhibitors and beta-adrenergic blockers.[116] Amiodarone may improve survival in patients who are at high risk of sudden arrhythmic death syndrome and can be recommended as the treatment of choice for patients with sustained ventricular tachycardia and for those with non-sustained ventricular tachycardia with myocardial dysfunction.[117] Implantation of a pacemaker is the recommended treatment for severe bradyarrhythmias and advanced conduction abnormalities. The roles of cardiac resynchronisation therapy and implantation of a cardioverter-defibrillator are not well established in Chagas heart disease, although these approaches could improve NYHA functional class, left ventricular ejection fraction, and survival. [118,119]

There is no specific management for mild digestive symptoms such as dysphagia or constipation. For severe cases (megasyndrome), endoscopic or surgical management is essential.[120]

Prevention

Prevention of infection requires control of vectorial transmission and screening of blood and organs for donation. Travellers should avoid sleeping in hovels or mud dwellings potentially infested with triatomines, use insect repellent and bednets, and avoid potentially contaminated fruit or cane juices such as those from street vendors. In the laboratory, personnel should use protective equipment suitable for risk group 2 organisms.[121] No vaccine is available for prevention of transmission of *T cruzi*. To avert the serious consequences of chronic infection, early diagnosis is crucial in both endemic and non-endemic areas where screening should be offered to all Latin American migrants (excluding those from the Caribbean region), especially in women of childbearing age, who might have been exposed to the vector or contaminated blood products. Screening should likewise be offered to children whose mothers were born in endemic areas and to all family members of an index case.[122] Especially for highly prevalent populations, the screening of Chagas disease turns out to be cost-effective.[123]

Pregnant and lactating women

Pregnant women potentially exposed to the parasite should be screened for *T cruzi* infection. For a woman with a new diagnosis, the appropriate protocol for assessment of visceral involvement and treatment after delivery should be followed. The infection itself does not justify caesarean section. [124]

Although there is no definite evidence of teratogenicity,[125] treatment with benznidazole or nifurtimox is not recommended during pregnancy because of the lack of data on fetal safety. Parasiticidal treatment has been associated with chromosomal aberrations in children[126,127] and binding of reactive metabolites to fetal proteins in rats.[128] In cases of acute infection or reactivation, the risk–benefit ratio should be evaluated as with other potentially teratogenic drugs. If benznidazole is taken inadvertently, termination of pregnancy is not indicated. Chronic infection should be treated after delivery to decrease the risk of mother-to-child transmission in future pregnancies.[13,85]

Discontinuing or interrupting breastfeeding in mothers with chronic Chagas disease is not recommended. Data on the transmission of *T cruzi* through lactation in human beings are scarce, and available reports are subject to substanial limitations.[129] If the mother has fissured or bleeding nipples, temporary discontinuation of breastfeeding may be recommended, although thermal treatment of expressed milk (pasteurisation or microwaving) before feeding the infant can be a safe alternative.[130] Similarly, in cases of acute infection or reactivations, breastfeeding may pose a risk for the infant. To avoid any possibility of transmission, human milk banks should exclude mothers with Chagas disease as donors. Treatment with benznidazole or nifurtimox during lactation is commonly discouraged, mainly because parasiticidal treatment is not immediately necessary to the mother with chronic infection. Recent information has shown that benznidazole seems to be safe in this situation,[131] as may be nifurtimox.[132]

Immunosuppressed patients

Organ and bone marrow recipients never exposed to *T cruzi* can be infected through graft tissue, bone marrow, or blood products. Acute infection has a prolonged incubation period (mean ~112 days) and severe and sometimes atypical clinical manifestations such as long-lasting fever, panniculitis, and meningoencephalitis.[22,133] Diagnosis relies on the detection of circulating trypomastigotes using parasitological or molecular tests (table 2). Treatment with benznidazole should be initiated as soon as possible to improve prognosis.[22,133] Although screening for *T cruzi* is now part of transplant programmes, the shortage of suitable organs for donation has encouraged the use of organs from donors infected with the parasite. Though useful for some recipients, this practice is not risk free.[22,134]

Early diagnosis of chronic infection in immunosuppressed individuals is important and, ideally, should be confirmed before immunosuppressive treatment is administered. *T cruzi* can behave as an opportunistic parasite, where reactivation is the most life-threatening disorder.[133,135] Reactivation can be confirmed with two approaches—namely, microscopy, which reveals trypomastigotes in blood and other body fluids, and histology, which reveals inflammatory signs around tissue amastigotes. In general, positive findings in blood by PCR are not automatically indicative of reactivation, since they are also seen during the indeterminate chronic phase. The risk of reactivation varies according to the organ transplanted and the degree of immunosuppression: 20–50% in heart recipients,[136] 8–37% for kidney recipients,[19,137] 19% for liver recipients,[137] and around 27% for patients undergoing bone-marrow transplantation.[138,139]

In cancer, reactivations have been reported mainly in a few patients with haematological malignancies, whose cellular immunity is compromised, and usually result in fatal neurological complications.[115] In patients co-infected with HIV and *T cruzi*, reactivation typically occurs in those who do not take antiretrovirals, with CD4 counts below 200 cells per µL or previous opportunistic infections.[135] In patients with systemic autoimmune diseases, reactivation seems to be rare and has been associated with immunosuppressive drugs such as mycophenolate mofetil, azathioprine, and high-dose cyclosporin.[140]

Future challenges and opportunities

Prevention of new infections and end organ disease is a major challenge, which can be addressed by making diagnosis and treatment readily available, especially in children, young adults, and women of childbearing age. Thus, the frequency of secondary transmission would

decrease, and the effectiveness of current drugs, which are far from ideal, would be maximised. Such measures must be undertaken within a global strategy that includes improving the socioeconomic conditions of the less fortunate populations in endemic countries, maintaining of programmes for vectorial control, and increasing awareness of the disease in non-endemic countries. This strategy should go hand in hand with measures to facilitate access to the health system and drugs for most vulnerable populations, thereby empowering the primary health level and favouring social participation. [141]

Prospective cohort studies based on standardised methods are needed to establish the epidemiology of Chagas disease, both in endemic and non-endemic areas, while accounting for other competing risk factors for cardiovascular disease. The data obtained will ensure more accurate prognosis and identify individuals most at risk and able to undergo more proactive measures. Multidisciplinary groups comprising professionals caring for patients with Chagas disease (such as infectious disease specialists, cardiologists, gastroenterologists, surgeons, psychologists, and social workers) are increasingly needed in an ageing population with increasing comorbidities. Measures to ensure long-term follow-up, which is very often interrupted, should be implemented. These challenges are especially pertinent for migrants who have to face many barriers to accessing diagnosis and treatment (legal and bureaucratic challenges, illegal employment, lack of knowledge about the disease, and the health-care resources of the host country). Active searching for patients is often the only way to bring them into the health-care system. [142]

Investigation of reliable prognostic factors for visceral involvement in asymptomatic patients is also a priority. The identification of low-risk patients would reduce unnecessary complementary tests and medical visits. Similarly, the lack of early surrogate markers of cure remains a major obstacle to clinical management (very long follow-up and many unnecessary tests) and increases concern during follow-up (uncertainty of cure). Furthermore, the lack of early markers of cure hinders the development of new treatments owing to the need for an unfeasibly long follow-up period to determine efficacy.

Effective, better-tolerated, low-cost drugs are needed and can only be developed if data can be translated rapidly from the laboratory to clinical practice. To do that, we should improve the drug development process to identify new targets for drug action and the translation of data from the laboratory to clinical research. Better animal models are needed that reflect more accurately the conditions of chronic infection in human beings. More stable international collaborations, ideally public–private partnerships, should be established to face the great challenges for drug discovery and clinical research, and to ensure that Chagas disease is no longer regarded as a neglected tropical disease.

Contributors

JAP-M and IM performed the scientific literature search and reviewed the most relevant articles. JAP-M wrote the first draft of the manuscript and prepared the tables and figures. IM reviewed and edited the full report. Both authors have seen and approved the final text.

Declaration of interests

We declare no competing interests.

Acknowledgments

Funding was provided by Ministerio de Economía y Competitividad; Instituto de Salud Carlos III, Subdirección General de Redes; Network Biomedical Research on Tropical Diseases (RICET) (ref RD16/0027/0020).

References

1 WHO. First WHO report on neglected tropical diseases: working to overcome the global impact of neglected tropical diseases. WHO/HTM/NTD/2010. Geneva: World Health Organization, 2010.

2 Ventura-Garcia L, Roura M, Pell C, et al. Socio-cultural aspects of Chagas disease: a systematic review of qualitative research. *PLoS Negl Trop Dis* 2013; **7:** e2410.

3 Conteh L, Engels T, Molyneux DH. Socioeconomic aspects of neglected tropical diseases. *Lancet* 2010; **375:** 239–47.

4 Lee BY, Bacon KM, Bottazzi ME, Hotez PJ. Global economic burden of Chagas disease: a computational simulation model. *Lancet Infect Dis* 2013; **13:** 342–48.

5 Schmunis GA, Schmunis GA, Yadon ZE. Chagas disease: a Latin American health problem becoming a world health problem. *Acta Trop* 2010; **115:** 14–21.

6 Requena-Mendez A, Aldasoro E, de Lazzari E, et al. Prevalence of Chagas disease in Latin-American migrants living in Europe: a systematic review and meta-analysis. *PLoS Negl Trop Dis* 2015; **9:** e0003540.

7 Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. *Clin Infect Dis* 2009; **49:** e52–54.

8 Zingales B, Miles MA, Campbell DA, et al. The revised *Trypanosoma cruzi* subspecific nomenclature: rationale, epidemiological relevance and research applications. *Infect Genet Evol* 2012; **12:** 240–53.

9 Yamagata Y, Nakagawa J. Control of Chagas disease. *Adv Parasitol* 2006; **61:** 129–65.

10 WHO. Control of Chagas disease: second report of the WHO expert Committee. WHO technical report series, 905. Geneva: World Health Organization, 2002.

11 Howard EJ, Xiong X, Carlier Y, Sosa-Estani S, Buekens P. Frequency of the congenital transmission of *Trypanosoma cruzi*: a systematic review and meta-analysis. *BJOG* 2014; **121:** 22–33.

12 Luquetti AO, Tavares SBDN, Siriano LDR, et al. Congenital transmission of *Trypanosoma cruzi* in central Brazil. A study of 1,211 individuals born to infected mothers. *Mem Inst Oswaldo Cruz* 2015; **110:** 369–76.

13 Murcia L, Carrilero B, Munoz-Davila MJ, Thomas MC, López MC, Segovia M. Risk factors and primary prevention of congenital Chagas disease in a nonendemic country. *Clin Infect Dis* 2013; **56:** 496–502.

14 Bern C, Verastegui M, Gilman RH, et al. Congenital *Trypanosoma cruzi* transmission in Santa Cruz, Bolivia. *Clin Infect Dis* 2009; **49:** 1667–74.

15 Bua J, Volta BJ, Velazquez EB, Ruiz AM, Rissio AMD, Cardoni RL. Vertical transmission of *Trypanosoma cruzi* infection: quantification of parasite burden in mothers and their children by parasite DNA amplification. *Trans R Soc Trop Med Hyg* 2012; **106:** 623–28.

16 Kaplinski M, Jois M, Galdos-Cardenas G, et al. Sustained domestic vector exposure is associated with increased Chagas cardiomyopathy risk but decreased parasitemia and congenital transmission risk among young women in Bolivia. *Clin Infect Dis* 2015; **61:** 918–26.

17 Bern C, Montgomery SP, Katz L, Caglioti S, Stramer SL. Chagas disease and the US blood supply. *Curr Opin Infect Dis* 2008; **21**: 476–82.

18 Cancino-Faure B, Fisa R, Riera C, Bula I, Girona-Llobera E, Jimenez-Marco T. Evidence of meaningful levels of *Trypanosoma cruzi* in platelet concentrates from seropositive blood donors. *Transfusion* 2015; **55:** 1249–55.

19 Riarte A, Luna C, Sabatiello R, et al. Chagas' disease in patients with kidney transplants: 7 years of experience 1989–1996. *Clin Infect Dis* 1999; **29**: 561–67.

20 Cicora F, Escurra V, Silguero S, González IM, Roberti JE. Use of kidneys from *Trypanosoma Cruzi*-infected donors in naive transplant recipients without prophylactic therapy. *Transplantation* 2014; **97:** e3–4.

21 Huprikar S, Bosserman E, Patel G, et al. Donor-derived *Trypanosoma cruzi* infection in solid organ recipients in the United States, 2001–2011. *Am J Transplant* 2013; **13:** 2418–25.

22 Chin-Hong PV, Schwartz BS, Bern C, et al. Screening and treatment of Chagas disease in organ transplant recipients in the United States: recommendations from the Chagas in Transplant Working Group. *Am J Transplant* 2011; **11:** 672–80.

23 Kun H, Moore A, Mascola L, et al. Transmission of *Trypanosoma cruzi* by heart transplantation. *Clin Infect Dis* 2009; **48:** 1534–40.

24 Alarcón de Noya B, Díaz Bello Z, Colmenares C, et al. Large urban outbreak of orally acquired acute Chagas disease at a school in Caracas, Venezuela. *J Infect Dis* 2010; **201:** 1308–15.

25 Herwaldt BL. Laboratory-acquired parasitic infections from accidental exposures. *Clin Microbiol Rev* 2001; **14:** 659–88.

26 Feliciangeli MD, Campbell-Lendrum D, Martinez C, Gonzalez D, Coleman P, Davies C. Chagas disease control in Venezuela: lessons for the Andean region and beyond. *Trends Parasitol* 2003; **19:** 44–49.

27 Samuels AM, Clark EH, Galdos-Cardenas G, et al. Epidemiology of and impact of insecticide spraying on Chagas disease in communities in the Bolivian Chaco. *PLoS Negl Trop Dis* 2013; **7**: e2358.

28 Bowman NM, Kawai V, Levy MZ, et al. Chagas disease transmission in periurban communities of Arequipa, Peru. *Clin Infect Dis* 2008; **46:** 1822–28.

29 WHO. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. *Wkly Epidemiol Rec* 2015; **90:** 33–43.

30 Bern C, Kjos S, Yabsley MJ, Montgomery SP. *Trypanosoma cruzi* and Chagas' disease in the United States. *Clin Microbiol Rev* 2011; **24:** 655–81.

31 Hernandez S, Flores CA, Viana GM, Sanchez DR, Traina MI, Meymandi SK. Brief Report: Autochthonous Transmission of *Trypanosoma cruzi* in Southern California. *Open Forum Infect Dis* 2016; **3**: ofw227.

32 Moncayo A, Silveira AC. Current epidemiological trends for Chagas disease in Latin America and future challenges in epidemiology, surveillance and health policy. *Mem Inst Oswaldo Cruz* 2009; **104:** 17–30.

33 Organizacion Panamericana de la Salud. Estimación cuantitativa de la enfermedad de Chagas en las Américas. OP5/HDM/CD/425-0G 2006. http://ops-uruguay.bvsalud.org/pdf/chagas19.pdf (accessed May 29, 2017).

34 Salvatella R, Irabedra P, Castellanos LG. Interruption of vector transmission by native vectors and the art of the possible. *Mem Inst Oswaldo Cruz* 2014; **109:** 122–30.

35 Shikanai-Yasuda MA, Carvalho NB. Oral transmission of Chagas disease. *Clin Infect Dis* 2012; **54:** 845–52.

36 Gurevitz JM, Gaspe MS, Enriquez GF, Provecho YM, Kitron U, Gürtler RE. Intensified surveillance and insecticide-based control of the Chagas disease vector *Triatoma infestans* in the Argentinean Chaco. *PLoS Negl Trop Dis* 2013; **7:** e2158.

37 Basile L, Jansa JM, Carlier Y, et al. Chagas disease in European countries: the challenge of a surveillance system. *Euro Surveill* 2011; **16:** pii 19968.

38 Stimpert KK, Montgomery SP. Physician awareness of Chagas disease, USA. *Emerging Infect Dis* 2010; **16**: 871–72.

39 Wegner DH, Rohwedder RW. The effect of nifurtimox in acute Chagas' infection. *Arzneimittelforschung* 1972; **22:** 1624–35.

40 Laranja FS, Dias E, Nobrega G, Miranda A. Chagas' disease. A clinical, epidemiologic, and pathologic study. *Circulation* 1956; **14:** 1035–60.

41 Pinto AY, Valente SA, Valente VDC, Ferreira Junior AG, Coura JR. Acute phase of Chagas disease in the Brazilian Amazon region: study of 233 cases from Pará, Amapá and Maranhão observed between 1988 and 2005. *Rev Soc Bras Med Trop* 2008; **41:** 602–14 (in Portuguese).

42 Bittencourt AL, Sadigursky M, Barbosa HS. Congenital Chagas' disease. Study of 29 cases. *Rev Inst Med Trop Sao Paulo* 1975; **17:** 146–59 (in Portuguese).

43 Dias JC. The indeterminate form of human chronic Chagas' disease. A clinical epidemiological review. *Rev Soc Bras Med Trop* 1989; **22:** 147–56.

44 Viotti RJ, Vigliano C, Laucella S, et al. Value of echocardiography for diagnosis and prognosis of chronic Chagas disease cardiomyopathy without heart failure. *Heart* 2004; **90:** 655–60.

45 Rochitte CE, Oliveira PF, Andrade JM, et al. Myocardial delayed enhancement by magnetic resonance imaging in patients with Chagas' disease. *J Am College Cardiol* 2005; **46:** 1553–58.

46 Pérez-Ayala A, Pérez-Molina JA, Norman FF, Monge-Maillo B, Faro MV, López-Vélez R. Gastro-intestinal Chagas disease in migrants to Spain: prevalence and methods for early diagnosis. *Ann Trop Med Parasitol* 2011; **105:** 25–29.

47 Coura JR, de Abreu LL, Pereira JB, Willcox HP. Morbidity in Chagas' disease. IV. Longitudinal study of 10 years in Pains and Iguatama, Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz* 1985; **80**: 73–80 (in Portuguese).

48 Espinosa R, Carrasco HA, Belandria F, et al. Life expectancy analysis in patients with Chagas' disease: prognosis after one decade (1973–1983). *Int J Cardiol* 1985; **8:** 45–56.

49 Sabino EC, Ribeiro AL, Salemi VMC, et al. Ten-year incidence of Chagas cardiomyopathy among asymptomatic *Trypanosoma cruzi*-seropositive former blood donors. *Circulation* 2013; **127:** 1105–15.

50 Viotti R, Vigliano C, Lococo B, et al. Long-term cardiac outcomes of treating chronic Chagas disease with benznidazole versus no treatment: a nonrandomized trial. *Ann Intern Med* 2006; **144:** 724–34.

51 Machado-de-Assis GF, Diniz GA, Montoya RA, et al. A serological, parasitological and clinical evaluation of untreated Chagas disease patients and those treated with benznidazole before and thirteen years after intervention. *Mem Inst Oswaldo Cruz* 2013; **108:** 873–80.

52 Pérez-Ayala A, Pérez-Molina JA, Norman FF, et al. Chagas disease in Latin American migrants: a Spanish challenge. *Clin Microbiol Infect* 2011; **17:** 1108–13.

53 Salvador F, Treviño B, Sulleiro E, et al. *Trypanosoma cruzi* infection in a non-endemic country: epidemiological and clinical profile. *Clin Microbiol Infect* 2014; **20:** 706–12.

54 Rassi A, Little WC. Chagas' heart disease. *Clin Cardiol* 2000; **23:** 883–89.

55 Rassi A Jr, Rassi SG, Rassi A. Sudden death in Chagas' disease. *Arq Bras Cardiol* 2001; **76:** 75–96.

56 Hidron AI, Gilman RH, Justiniano J, et al. Chagas cardiomyopathy in the context of the chronic disease transition. *PLoS Negl Trop Dis* 2010; **4:** e688.

57 Rassi A, Rassi SG. Predictors of mortality in chronic Chagas disease: a systematic review of observational studies. *Circulation* 2007; **115:** 1101–08.

58 de Oliveira RB, Troncon LE, Dantas RO, Menghelli UG. Gastrointestinal manifestations of Chagas' disease. *Am J Gastroenterol* 1998; **93**: 884–9.

59 Cardoso RN, Macedo FYB, Garcia MN, et al. Chagas cardiomyopathy is associated with higher incidence of stroke: a meta-analysis of observational studies. *J Card Fail* 2014; **20:** 931–38.

60 Nunes MCP, Barbosa MM, Ribeiro ALP, Barbosa FBL, Rocha MOC. Ischemic cerebrovascular events in patients with Chagas cardiomyopathy: a prospective follow-up study. *J Neurol Sci* 2009; **278:** 96–101.

61 Carod-Artal FJ. Policy implications of the changing epidemiology of Chagas disease and stroke. *Stroke* 2013; **44:** 2356–60.

62 Sica RE, Gonzalez Cappa SM, Sanz OP, Mirkin G. Peripheral nervous system involvement in human and experimental chronic American trypanosomiasis. *Bull Soc Pathol Exot* 1995; **88:** 156–63.

63 Bonney KM, Engman DM. Chagas heart disease pathogenesis: one mechanism or many? *Curr Mol Med* 2008; **8:** 510–18.

64 Tarleton RL. Parasite persistence in the aetiology of Chagas disease. *Int J Parasitol* 2001; **31:** 550–54.

65 Burgos JM, Diez M, Vigliano C, et al. Molecular identification of *Trypanosoma cruzi* discrete typing units in end-stage chronic Chagas heart disease and reactivation after heart transplantation. *Clin Infect Dis* 2010; **51:** 485–95.

66 Dutra WO, Menezes CAS, Magalhães LMD, Gollob KJ. Immunoregulatory networks in human Chagas disease. *Parasite Immunol* 2014; **36:** 377–87.

67 Machado FS, Dutra WO, Esper L, et al. Current understanding of immunity to *Trypanosoma cruzi* infection and pathogenesis of Chagas disease. *Semin Immunopathol* 2012; **34:** 753–70.

68 Poveda C, Fresno M, Gironès N, et al. Cytokine profiling in Chagas disease: towards understanding the association with infecting *Trypanosoma cruzi* discrete typing units (a BENEFIT TRIAL sub-study). *PLoS One* 2014; **9:** e91154.

69 Brener ZE, Andrade ZA, Barral-Neto M. *Trypanosoma cruzi* e Doença de Chagas. 2nd edn. Rio de Janeiro: Guanabara Koogan, 2000.

70 Bua J, Volta BJ, Perrone AE, et al. How to improve the early diagnosis of *Trypanosoma cruzi* infection: relationship between validated conventional diagnosis and quantitative DNA amplification in congenitally infected children. *PLoS Negl Trop Dis* 2013; **7:** e2476.

71 Brasil PEAA, De Castro L, Hasslocher-Moreno AM, Sangenis LHC, Braga JU. ELISA versus PCR for diagnosis of chronic Chagas disease: systematic review and meta-analysis. *BMC Infect Dis* 2010; **10:** 337.

72 Flores-Chávez M, Cruz I, Rodríguez M, et al. Comparison of conventional and non-conventional serological tests for the diagnosis of imported Chagas disease in Spain. *Enferm Infecc Microbiol Clin* 2010; **28**: 284–93 (in Spanish).

73 Riera C, Verges M, Iniesta L, et al. Identification of a Western blot pattern for the specific diagnosis of *Trypanosoma cruzi* infection in human sera. *Am J Trop Med Hyg* 2012; **86:** 412–16.

74 Sanchez-Camargo CL, Albajar-Vinas P, Wilkins PP, et al. Comparative evaluation of 11 commercialized rapid diagnostic tests for detecting *Trypanosoma cruzi* antibodies in serum banks in areas of endemicity and nonendemicity. *J Clin Microbiol* 2014; **52:** 2506–12.

75 Chappuis F, Mauris A, Holst M, et al. Validation of a rapid immunochromatographic assay for diagnosis of *Trypanosoma cruzi* infection among Latin-American migrants in Geneva, Switzerland. *J Clin Microbiol* 2010; **48:** 2948–52.

76 Holguín A, Norman FF, Martín L, et al. Dried blood as an alternative to plasma or serum for *Trypanosoma cruzi* IgG detection in screening programs. *Clin Vaccine Immunol* 2013; **20:** 1197–202.

77 Schijman AG, Bisio M, Orellana L, et al. International study to evaluate PCR methods for detection of *Trypanosoma cruzi* DNA in blood samples from Chagas disease patients. *PLoS Negl Trop Dis* 2011; **5:** e931.

78 Sánchez-Montalvá A, Salvador F, Rodríguez-Palomares J, et al. Chagas cardiomyopathy: usefulness of EKG and echocardiogram in a non-endemic country. *PLoS One* 2016; **11:** e0157597.

79 Bern C, Montgomery SP, Herwaldt BL, et al. Evaluation and treatment of chagas disease in the United States: a systematic review. *JAMA* 2007; **298:** 2171–81.

80 Salvador F, Mego M, Sanchez-Montalva A, et al. Assessment of rectocolonic morphology and function in patients with Chagas disease in Barcelona (Spain). *Am J Trop Med Hyg* 2015; **92:** 898–902.

81 Sánchez-Montalvá A, Moris M, Mego M, et al. High resolution esophageal manometry in patients with Chagas disease: a cross-sectional evaluation. *PLoS Negl Trop Dis* 2016; **10:** e0004416.

82 Andrade JP, Marin-Neto JA, Paola AAV, et al. I Latin American guidelines for the diagnosis and treatment of Chagas' heart disease. *Arq Bras Cardiol* 2011; **97:** 1–48 (in Portuguese).

83 Pérez-Molina JA, Pérez-Ayala A, Moreno S, Fernandez-Gonzalez MC, Zamora J, López-Vélez R. Use of benznidazole to treat chronic Chagas' disease: a systematic review with a meta-analysis. *J Antimicrob Chemother* 2009; **64:** 1139–47.

84 Morillo CA, Marin-Neto JA, Avezum A, et al. Randomized trial of benznidazole for Chronic Chagas' cardiomyopathy. *N Engl J Med* 2015; **373:** 1295–306.

85 Fabbro DL, Danesi E, Olivera V, et al. Trypanocide treatment of women infected with *Trypanosoma cruzi* and its effect on preventing congenital Chagas. *PLoS Negl Trop Dis* 2014; **8:** e3312.

86 Rodriques Coura J, de Castro SL. A critical review on Chagas disease chemotherapy. *Mem Inst Oswaldo Cruz* 2002; **97:** 3–24.

87 Streiger ML, del Barco ML, Fabbro DL, Arias ED, Amicone NA. Longitudinal study and specific chemotherapy in children with chronic Chagas' disease, residing in a low endemicity area of Argentina. *Rev Soc Bras Med Trop* 2004; **37:** 365–75 (in Portuguese).

88 Jackson Y, Alirol E, Getaz L, Wolff H, Combescure C, Chappuis F. Tolerance and safety of nifurtimox in patients with chronic Chagas disease. *Clin Infect Dis* 2010; **51:** e69–75.

89 Russomando G, de Tomassone MM, de Guillen I, et al. Treatment of congenital Chagas' disease diagnosed and followed up by the polymerase chain reaction. *Am J Trop Med Hyg* 1998; **59:** 487–91.

90 Schijman AG, Altcheh J, Burgos JM, et al. Aetiological treatment of congenital Chagas' disease diagnosed and monitored by the polymerase chain reaction. *J Antimicrob Chemother* 2003; **52:** 441–49.

91 Cancado JR. Criteria of Chagas disease cure. *Mem Inst Oswaldo Cruz* 1999; **94:** 331–35.

92 Sosa-Estani S, Segura EL, Ruiz AM, Velazquez E, Porcel BM, Yampotis C. Efficacy of chemotherapy with benznidazole in children in the indeterminate phase of Chagas' disease. *Am J Trop Med Hyg* 1998; **59:** 526–29.

93 de Andrade AL, Zicker F, de Oliveira RM, et al. Randomised trial of efficacy of benznidazole in treatment of early *Trypanosoma cruzi* infection. *Lancet* 1996; **348:** 1407–13.

94 Yun O, Lima MA, Ellman T, et al. Feasibility, drug safety, and effectiveness of etiological treatment programs for Chagas disease in Honduras, Guatemala, and Bolivia: 10-year experience of Médecins Sans Frontières. *PLoS Negl Trop Dis* 2009; **3:** e488.

95 Fabbro DL, Streiger ML, Arias ED, Bizai ML, del Barco M, Amicone NA. Trypanocide treatment among adults with chronic Chagas disease living in Santa Fe city (Argentina), over a mean follow-up of 21 years: parasitological, serological and clinical evolution. *Rev Soc Bras Med Trop* 2007; **40:** 1–10.

96 Pinazo MJ, Muñoz J, Posada E, et al. Tolerance of benznidazole in treatment of Chagas' disease in adults. *Antimicrob Agents Chemother* 2010; **54:** 4896–99.

97 Molina I, Salvador F, Sanchez-Montalva A, et al. Toxic profile of benznidazole in patients with chronic Chagas disease: risk factors and comparison of the product from two different manufacturers. *Antimicrob Agents Chemother* 2015; **59:** 6125–31.

98 Pinazo MJ, Guerrero L, Posada E, Rodriguez E, Soy D, Gascon J. Benznidazole-related adverse drug reactions and their relationship to serum drug concentrations in patients with chronic Chagas disease. *Antimicrob Agents Chemother* 2012; **57:** 390–95.

99 Perez-Molina JA, Sojo-Dorado J, Norman FF, et al. Nifurtimox therapy for Chagas disease does not cause hypersensitivity reactions in patients with such previous adverse reactions during benznidazole treatment. *Acta Trop* 2013; **127:** 101–4.

100 Rassi A, Amato Neto V, de Siqueira AF, et al. Treatment of chronic Chagas' disease with an association of nifurtimox and corticoid. *Rev Soc Bras Med Trop* 2002; **35:** 547–50 (in Portuguese).

101 Górgolas M, Robles I, Cabello A, et al. The use of steroids to prevent cutaneous reactions to benznidazole in patients with Chagas disease. *Pathog Glob Health* 2013; **107:** 157–60.

102 Molina I, Gómez i Prat J, Salvador F, et al. Randomized trial of posaconazole and benznidazole for chronic Chagas' disease. *N Engl J Med* 2014; **370:** 1899–908.

103 Torrico F, Gascon J, Ribeiro I. E1224 – Results of proof of concept clinical trial in patients with chronic indeterminate Chagas disease. ASTMH 62nd Annual Meeting; Washington DC; Nov 13–17, 2013.

104 Morillo CA, Waskin H, Sosa-Estani S, et al. Benznidazole and posaconazole in eliminating parasites in asymptomatic *T. cruzi* carriers: the STOP-CHAGAS Trial. *J Am Coll Cardiol* 2017; **69:** 939–47.

105 Alvarez MG, Hernández Y, Bertocchi G, et al. New scheme of intermittent benznidazole administration in patients chronically infected with *Trypanosoma cruzi*: a pilot short-term follow-up study with adult patients. *Antimicrob Agents Chemother* 2015; **60:** 833–37.

106 Soy D, Aldasoro E, Guerrero L, et al. Population pharmacokinetics of benznidazole in adult patients with Chagas disease. *Antimicrob Agents Chemother* 2015; **59:** 3342–49.

107 Altcheh J, Moscatelli G, Mastrantonio G, et al. Population pharmacokinetic study of benznidazole in pediatric Chagas disease suggests efficacy despite lower plasma concentrations than in adults. *PLoS Negl Trop Dis* 2014; **8:** e2907.

108 Bustamante JM, Craft JM, Crowe BD, Ketchie SA, Tarleton RL. New, combined, and reduced dosing treatment protocols cure *Trypanosoma cruzi* infection in mice. *J Infect Dis* 2013; **209:** 150–62.

109 Francisco AF, Lewis MD, Jayawardhana S, Taylor MC, Chatelain E, Kelly JM. Limited ability of posaconazole to cure both acute and chronic *Trypanosoma cruzi*infections revealed by highly sensitive in vivo imaging. *Antimicrob Agents Chemother* 2015; **59:** 4653–61.

110 Chatelain E, Konar N. Translational challenges of animal models in Chagas disease drug development: a review. *Drug Des Devel Ther* 2015; **9:** 4807–23.

111 Rassi A, Little WC, Xavier SS, et al. Development and validation of a risk score for predicting death in Chagas' heart disease. *N Engl J Med* 2006; **355:** 799–808.

112 Pinazo M-J, Thomas MC, Bua J, et al. Biological markers for evaluating therapeutic efficacy in Chagas disease, a systematic review. *Expert Rev Anti Infect Ther* 2014; **12**: 479–96.

113 Vallejo A, Monge-Maillo B, Gutiérrez C, Norman FF, Lopez-Velez R, Perez-Molina JA. Changes in the immune response after treatment with benznidazole versus no treatment in patients with chronic indeterminate Chagas disease. *Acta Trop* 2016; **164:** 117–24.

114 Okamoto EE, Sherbuk JE, Clark EH, et al. Biomarkers in *Trypanosoma cruzi*-infected and uninfected individuals with varying severity of cardiomyopathy in Santa Cruz, Bolivia. *PLoS Negl Trop Dis* 2014; **8:** e3227.

115 Perez-Molina JA, Perez AM, Norman FF, Monge-Maillo B, Lopez-Velez R. Old and new challenges in Chagas disease. *Lancet Infect Dis* 2015; **15:** 1347–56.

116 Braga JCV, Reis F, Aras R, et al. Clinical and therapeutics aspects of heart failure due to Chagas disease. *Arq Bras Cardiol* 2006; **86**: 297–302 (in Portuguese).

117 Scanavacca MI, Sosa EA, Lee JH, Bellotti G, Pileggi F. Empiric therapy with amiodarone in patients with chronic Chagas cardiomyopathy and sustained ventricular tachycardia. *Arq Bras Cardiol* 1990; **54**: 367–71 (in Portuguese).

118 Gali WL, Sarabanda AV, Baggio JM, et al. Implantable cardioverter-defibrillators for treatment of sustained ventricular arrhythmias in patients with Chagas' heart disease: comparison with a control group treated with amiodarone alone. *Europace* 2014; **16**: 674–80.

119 Pereira FTM, Rocha EA, Monteriro MDPM, et al. Long-term follow-up of patients with chronic chagas disease and implantable cardioverter-defibrillator. *Pacing Clin Electrophysiol* 2014; **37:** 751–56.

120 Pinazo M-J, Cañas E, Elizalde J-I, et al. Diagnosis, management and treatment of chronic Chagas' gastrointestinal disease in areas where *Trypanosoma cruzi* infection is not endemic. *Gastroenterol Hepatol* 2010; **33:** 191–200.

121 Centers for Disease Control and Prevention, National Institutes of Health. Biosafety in microbiological and biomedical laboratories. 5th edn. Bethesda: US Department of Health and Human Services, 2010.

122 Zulantay I, Apt W, Ramos D, et al. The epidemiological relevance of family study in Chagas disease. *PLoS Negl Trop Dis* 2013; **7:** e1959.

123 Imaz-Iglesia I, Miguel LG-S, Ayala-Morillas LE, et al. Economic evaluation of Chagas disease screening in Spain. *Acta Trop* 2015; **148:** 77–88.

124 González-Tomé MI, Rivera Cuello M, Camaño Gutierrez I, et al. Recommendations for the diagnosis, treatment and follow-up of the pregnant woman and child with Chagas disease. Sociedad Española de Infectología Pediátrica. Sociedad de Enfermedades Infecciosas y Microbiología Clínica. Sociedad Española de Ginecología y Obstetricia. *Enferm Infecc Microbiol Clin* 2013; **31:** 535–42 (in Spanish).

125 Lorke D. Embryotoxicity studies of nifurtimox in rats and mice and study of fertility and general reproductive performance. *Arzneimittelforschung* 1972; **22:** 1603–7.

126 Gorla NB, Ledesma OS, Barbieri GP, Larripa IB. Assessment of cytogenetic damage in chagasic children treated with benznidazole. *Mutat Res* 1988; **206:** 217–20.

127 Gorla NB, Ledesma OS, Barbieri GP, Larripa IB. Thirteenfold increase of chromosomal aberrations non-randomly distributed in chagasic children treated with nifurtimox. *Mutat Res* 1989; **224:** 263–7.

128 de Toranzo EG, Masana M, Castro JA. Administration of benznidazole, a chemotherapeutic agent against Chagas disease, to pregnant rats. Covalent binding of reactive metabolites to fetal and maternal proteins. *Arch Int Pharmacodyn Ther* 1984; **272:** 17–23.

129 Norman FF, Lopez-Velez R. Chagas disease and breast-feeding. *Emerg Infect Dis* 2013; **19:** 1561–66.

130 Ferreira CS, Martinho PC, Amato Neto V, Cruz RR. Pasteurization of human milk to prevent transmission of Chagas disease. *Rev Inst Med Trop Sao Paulo* 2001; **43:** 161–62.

131 García-Bournissen F, Moroni S, Marson ME, et al. Limited infant exposure to benznidazole through breast milk during maternal treatment for Chagas disease. *Arch Dis Child* 2015; **100:** 90–94.

132 Garcia-Bournissen F, Altcheh J, Panchaud A, Ito S. Is use of nifurtimox for the treatment of Chagas disease compatible with breast feeding? A population pharmacokinetics analysis. *Arch Dis Child* 2010; **95:** 224–28.

133 Bern C. Chagas disease in the immunosuppressed host. *Curr Opin Infect Dis* 2012; **25:** 450–7.

134 Salvador F, Len O, Molina I, et al. Safety of liver transplantation with chagas disease-seropositive donors for seronegative recipients. *Liver Transpl* 2011; **17:** 1304–08.

135 Perez-Molina JA. Management of *Trypanosoma cruzi* coinfection in HIV-positive individuals outside endemic areas. *Curr Opin Infect Dis* 2014; **27:** 9–15.

136 Bestetti RB, Theodoropoulos TAD. A systematic review of studies on heart transplantation for patients with end-stage Chagas' heart disease. *J Card Fail* 2009; **15:** 249–55.

137 Chagas' Disease Argentine Collaborative Transplant Consortium, Casadei D. Chagas' disease and solid organ transplantation. *Transplant Proc* 2010; **42:** 3354–59.

138 Altclas J, Sinagra A, Dictar M, et al. Chagas disease in bone marrow transplantation: an approach to preemptive therapy. *Bone Marrow Transplant* 2005; **36:** 123–29.

139 Guiang KMU, Cantey P, Montgomery SP, et al. Reactivation of Chagas disease in a bone marrow transplant patient: case report and review of screening and management. *Transpl Infect Dis* 2013; **15:** e264–67.

140 Burgos LG, Ortiz BDM, Canese A, Ojeda A, Melo M. Reactivation of Chagas disease by immunosuppressive therapy in a patient with systemic lupus erythematosus: report of an exceptional case. *Am J Dermatopathol* 2012; **34:** e84–89.

141 Sartor P, Colaianni I, Cardinal MV, Bua J, Freilij H, Gürtler RE. Improving access to Chagas disease diagnosis and etiologic treatment in remote rural communities of the Argentine Chaco through strengthened primary health care and broad social participation. *PLoS Negl Trop Dis* 2017; **11:** e0005336.

142 Navarro M, Berens-Riha N, Hohnerlein S, et al. Cross-sectional, descriptive study of Chagas disease among citizens of Bolivian origin living in Munich, Germany. *BMJ Open* 2017; **7:** e013960.