

Chagas disease: changes in knowledge and management

François-Xavier Lescure, Guillaume Le Loup, Hector Freilij, Michel Develoux, Luc Paris, Laurent Brutus, Gilles Pialoux

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Service de Maladies Infectieuses et Tropicales, APHP Hôpital Tenon (F-X Lescure MD, G Le Loup MD, M Develoux MD, Prof G Pialoux MD), Laboratoire de Parasitologie-Mycologie, APHP Hôpital Saint Antoine (M Develoux), and Laboratoire de Parasitologie-Mycologie, APHP Hôpital La Pitié Salpêtrière (L Paris MD), Université Pierre et Marie Curie, Paris, France; UMR 216 IRD/UPD Mère et Enfant Face aux Infections Tropicales, Institut de Recherche pour le Développement/Université Paris Descartes, Paris, France (G Le Loup, L Brutus MD); and Servicio de Parasitología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina (H Freilij MD)

Correspondence to: Dr François-Xavier Lescure, Service des Maladies Infectieuses et Tropicales, APHP, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France
xavier.lescure@tnn.aphp.fr

More than 100 years after the discovery of human American trypanosomiasis by Carlos Chagas, our knowledge and management of the disease are profoundly changing. Substantial progress made by disease control programmes in most endemic areas contrasts with persisting difficulties in the Gran Chaco region in South America and the recent emergence of the disease in non-endemic areas because of population movements. In terms of pathogenesis, major discoveries have been made about the life cycle and genomics of *Trypanosoma cruzi*, and the role of the parasite itself in the chronic phase of the disease. From a clinical perspective, a growing number of arguments have challenged the notion of an indeterminate phase, and suggest new approaches to manage patients. New methods such as standardised PCR will be necessary to ensure follow-up of this chronic infection. Although drugs for treatment of Chagas disease are limited, poorly tolerated, and not very effective, treatment indications are expanding. The results of the Benznidazole Evaluation For Interrupting Trypanosomiasis (BENEFIT) trial in 2012 will also help to inform treatment. Mobilisation of financial resources to fund research on diagnosis and randomised controlled trials of treatment are international health priorities.

Introduction

The genome of the parasite *Trypanosoma cruzi* was published in 2005¹ and proteomic analysis has identified characteristics specific to the different stages of its life cycle.² Transmission of *T. cruzi* by the main vector *Triatoma infestans* was halted in 1997 in Uruguay, 1999 in Chile, 2006 in Brazil, and 2009 in Guatemala. In 2007, WHO launched an initiative for control of the disease in non-endemic regions,³ and the US Food and Drug Administration (FDA) approved the first serological screening test for blood donors.

These examples show a profound change in the understanding of the pathophysiology, diagnosis, and treatment of Chagas disease in the past few years. Last year marked the centenary of the discovery of the causative agent and vector by the Brazilian scientist Carlos Chagas. In view of recent advances in prevention and control, a new look at the epidemiology and clinical features of Chagas disease is needed.

Epidemiology

Epidemiological studies of Chagas disease are typically divided between endemic and non-endemic areas. However, increasing population movements and other routes of transmission introduce large heterogeneity in each area. In the past 25 years, major international efforts have been made to control the vector and to improve safety of blood transfusion in endemic areas. Incidence of Chagas disease has dropped from 700 000 new cases per year to 40 000, and the annual number of deaths has fallen from more than 45 000 to 12 500.⁴ However, the epidemiology of the disease has become more complex because of multiple vectors and reservoirs and the added effects of geopolitical, economic, and ecological upheavals.

In addition to Uruguay, Chile, and Brazil, *T. infestans* has been eliminated from several parts of Argentina and Paraguay.⁴ However, secondary peridomestic and even domestic vectors have emerged,^{5,6} along with resistance to insecticides.⁷ The region of Gran Chaco (southern Bolivia,

northern Argentina and western Paraguay) remains at risk for *Triatoma* spp infestation,⁴ with a high prevalence in children⁸ and cases of reinfection with *T. cruzi*.⁶ This persistent hyperendemicity seems to be caused by slow improvement of human habitats, low insecticide efficiency in tropical climates, a high initial rate of infection, inadequate vector surveillance and insecticide coverage, and lack of local political sustainability.⁹ Persistent hyperendemicity is also seen in large urban areas in parts of Bolivia.¹⁰ Safety of blood transfusion seems to be adequate, apart from in Bolivia and Chile.¹¹

The Andean region is characterised by a vector that is not only domiciliated but also sylvatic and peri-sylvatic. The region is developing new methods for chagasic risk evaluation (including risk stratification) and control activities.⁴ Coverage of screening for transfusion safety is more than 99%.¹¹

The pattern of change in central America is quite homogeneous, with substantial advances in elimination of the main vector (*Rhodnius prolixus*), well implemented national programmes, coverage of screening for transfusion safety in more than 99% of cases, and entomological and epidemiological surveillance justified by the presence of secondary vectors and boosted by community participation.⁴

In the Amazon rainforest, the epidemiology of Chagas disease is difficult to determine because of the diversity of vectors and reservoirs, major ecological upheavals (eg, deforestation), and human migration.¹² Seroprevalence of antibodies to *T. cruzi* ranges from 2% to 13% in this subregion¹² and the incidence of acute cases has increased substantially since 2005.⁴ Different epidemiological profiles include a hypoendemic sylvatic and sporadic profile,¹² an epidemic profile (a microepidemic in plant-fibre workers and outbreaks associated with oral transmission),⁴ and a domestic endemic profile.¹²

Mexico was slow to act on Chagas disease. Seroprevalence in the general population has been estimated at 1.6%, with major regional variations.¹³ For example, data for northeast Mexico suggest that prevalence has increased

in the past year.¹⁴ Rates of infection are similar across age groups, suggesting delayed mobilisation by authorities against the disease.¹⁵ There is some controversy about possible undernotification of cases¹⁶ and delays in transfusion policy.¹¹

In large South American cities, Chagas disease might be imported by migrant workers from highly endemic regions.¹⁷ In Buenos Aires, Argentina, vectors are rare and generally not infected. Nonetheless, seroprevalence among blood donors is about 4%.¹⁸ Furthermore, animal reservoirs and vectors in the city could potentially transmit *T cruzi* from the human reservoir.¹⁹ In Arequipa, Peru, seroprevalence of antibodies to *T cruzi* is about 5%.¹⁷ Migrant workers sometimes accompanied by domestic animals often living in poor housing might be the source of the urban Chagas disease cycle.¹⁷

Most other countries are non-endemic, but, because of migration patterns, the USA, Canada, Europe, Australia, and Japan are most at risk for imported Chagas disease.²⁰ In the USA, several southern states have host vectors and reservoirs for *T cruzi*.²¹ Seroprevalence of antibodies to *T cruzi* reaches 50% in some mammalian species,²¹ and exceeds 5% in domestic dogs.²² Triatomine bugs are also present, with a rate of infection sometimes exceeding 60%.²³ Only six native cases have been reported in the USA, possibly because of housing conditions that are not conducive to infestation or low vector efficiency.²¹

On the basis of the number of migrants and the prevalence rates in the countries from which they originate, the estimated number of cases of *T cruzi* infection imported into the USA in 2006 was between 30 000 and 300 000.²⁰ The prevalence of *T cruzi* antibodies in blood donors in the USA is about one in 30 000, corresponding to nearly 900 diagnosed cases of seropositivity; seven cases of *T cruzi* infection after blood transfusion have been reported.²⁴ Four cases linked to organ donation were reported in 2006.²⁵ Congenital transmission is probably underestimated: estimated seroprevalence in Latin-American pregnant women was 0.4% in 1999, but few congenital cases had been diagnosed until recently.²⁶ Further north, Canada lies outside the range of the vectors, but the seroprevalence of antibodies to *T cruzi* in Latin Americans living in Canada was estimated at 1% in 2007.²⁷

In Europe, imported cases are emerging mainly in Spain,²⁸ France,²⁹ and Switzerland,³⁰ and could potentially affect other countries depending on migration flows.^{20,31} Screening of blood donors at risk of Chagas disease showed a seroprevalence of 0.62% in Spain³² and 0.31% in France.³³ There is an increasing awareness in non-endemic areas that screening of Latin American donors, as happens in the USA, Spain, and France, should be compulsory. About 1000 imported cases were estimated to be present in Canada and between 24 000 and 38 000 in Europe, including 12 000 to 25 000 cases in Spain alone.³¹

Pathogenesis

Chagas disease occurs in two phases: an acute phase characterised by focal or diffuse inflammation mainly affecting the myocardium, and a chronic phase marked by an inflammatory fibrotic reaction that damages the cardiac muscle and conduction network and the enteric nervous system. Until recently, Chagas disease was perceived as a parasitic disease in the acute phase (myocytolysis induced by the parasite and inflammation) and as an autoimmune disease in the chronic phase. However, the disease is now regarded as a multifactorial, persistent, and variable interaction between the pathogen and host.³⁴

Autoimmunity

Five autoimmune mechanisms have been put forward: molecular mimicry between parasite and host antigens; bystander activation; release of cryptic antigens; polyclonal lymphocyte activation; and epitope spread.³⁵ Several autoantigens that cross-react with *T cruzi* antigens have been identified, including myosin, the main protein component of cardiac muscle (reacts with *T cruzi* B13 protein and cruzipain), Cha protein (reacts with *T cruzi* protein SAPA) and the β 1-adrenergic receptor (reacts with ribosomal protein P).³⁶

However, the role of autoimmunity in the pathophysiology of chronic Chagas disease remains controversial, with cases of reactivation induced by immune suppression,³⁷ detection of the same autoantibodies in both healthy individuals and patients in indeterminate disease phase or those with non-Chagas disease myocarditis, and the difficulty of demonstrating a causal relation between autoantibodies or autoreactive cells and chronic inflammation.³⁵

Parasite and pathogenesis

The detection of parasite *T cruzi* antigens and DNA by immunohistochemistry³⁸ and PCR³⁹ in damaged tissues during the chronic phase of Chagas disease has led to a change in our understanding of the pathogenesis of this phase, with an increasing focus on the parasite.

Molecular genetic analysis of the population structure of *T cruzi* has identified six main discrete typing units with specific ecological niches and geographical distribution.⁴⁰ Genetic hybridisation in *T cruzi* has been shown experimentally⁴¹ and the naturally occurring discrete typing units TcV and TcVI are heterozygous hybrids of TcII and TcIII, in accordance with the new consensual classification.⁴²

The correlation between strains and clinical manifestations and outcomes is an issue of interest,⁴³ and different strains differ in tissue tropism in experiments in mice and in patients with AIDS.⁴⁴

Surface mucins and trans-sialidases (enzymes anchored in the surface of the parasite or shed in the bloodstream) are major virulence factors, involved in adhesion to host cells, invasion, and immune evasion. Differential expression of trans-sialidase was associated with

differences in thymic lesions in a murine model infected with different strains.⁴⁵

In line with the pathogenic role of the parasite, treatment with benznidazole in murine models⁴⁶ and human beings⁴⁷ induces a shift in the phenotype of CD8 T cells seen in persistent infection to a phenotype of memory T cells with protective capacity against *T. cruzi*. Moreover, this change in the understanding of the chronic phase of the disease has led to renewed interest in vaccines that control parasitic burden.⁴⁸

Immune response: beneficial or detrimental?

The host immune response to *T. cruzi* affects the outcome of infection. During the acute phase of Chagas disease, the innate immune response is triggered by parasite mucins and DNA and involves Toll-like receptors 2 and 9 and the MyD88 and TRIF (Toll/interleukin-1 receptor domain-containing adaptor protein inducing interferon β) activation pathways. MyD88 pathways stimulate the production of cytokines (eg, interleukin 12 and tumour necrosis factor [TNF] α) and chemokines (eg, RANTES),⁴⁹ leading to a T-helper 1 response with production of interferon γ . Parasite membrane trans-sialidases are a major target of CD8 lymphocytes and are directly stimulated or stimulated by interaction with CD4 lymphocytes.⁵⁰

The host response can cause tissue damage. Tissue-resident CD8 lymphocytes express potentially pathogenic molecules such as granzymes and TIA-1. Progression from the asymptomatic form to the symptomatic form of chronic Chagas myocardopathy could involve an imbalance in T-helper 1 and T-helper 2 responses, leading to excessive production of proinflammatory cytokines.⁵¹ Variable interleukin-10 expression linked to gene polymorphisms could explain this imbalance.⁵² Polymorphism of the genes encoding transforming growth factor (TGF) β and BAT1 is an alternative mechanism.⁵³ The association between interleukin-1 receptor antagonist polymorphism and chronic chagasic cardiomyopathy has been highlighted.⁵⁴ By contrast, TNF α gene polymorphism does not seem to affect the risk of myocardopathy,⁵⁵ whereas results for HLA genes are contradictory.^{56,57} These findings open the way to adjuvant treatment with immunomodulatory drugs and to follow-up of patients on the basis of immunological markers such as TGF β .⁵⁸

Clinical manifestations and paraclinical investigations

Table 1 shows the main clinical manifestations, investigations, and differential diagnoses of Chagas disease,^{59,60} which differ according to the acute or chronic phase of the disease and patients' age and underlying immune status.⁶¹ Symptomatic acute Chagas disease occurs mainly in young children.

An imperfect nosological framework?

The indeterminate phase of Chagas disease is defined by a combination of seropositivity for *T. cruzi*, a normal

chest radiograph and electrocardiogram, a normal barium swallow and enema, and the absence of clinical signs and symptoms.⁵⁹ However, some patients have various lesions. In a study of 505 patients with indeterminate-phase Chagas disease, 13.8% had segmental cardiac lesions on two-dimensional echography.⁶² Tissue doppler echocardiography can show abnormal contractility, especially of the interventricular septum, in patients with normal echocardiography. Delayed-enhancement MRI shows areas of cardiac fibrosis in about 20% of patients with indeterminate-phase disease,⁶³ and the extent of fibrosis relates to the severity of cardiac manifestations in symptomatic patients. Dysautonomy and left ventricular diastolic dysfunction have also been reported.^{64,65} Minor abnormalities of ventricular contractility in some patients with indeterminate-phase disease are predictive of deteriorating ventricular function.⁶⁶ The indeterminate phase of the disease has also been linked to cases of sudden death.^{67,68}

In about two-thirds of patients with the indeterminate phase, the disease does not progress, but in the remaining third it becomes symptomatic.^{69,70} In patients with symptomatic disease, two-thirds develop a cardiac form and a third develop a gastrointestinal form. Progression from the indeterminate phase to a symptomatic form can take years or even decades.⁷¹

These data challenge the past case management of the so-called indeterminate phase of Chagas disease. No determinants of progression from the asymptomatic form to a symptomatic form have been identified.

New neurological manifestations

Chronic Chagas disease is an independent risk factor for vascular ischaemic events.⁶⁸ Stroke is more common in Chagas cardiopathy than in other forms of cardiomyopathy.⁷² In a recent study, ventricular ejection fraction of less than 35% and increased left atrial volume were independent risk factors for stroke.⁷³ Ischaemic events can occur at all stages of chronic Chagas disease and can sometimes lead to diagnosis.⁶⁸

Congenital Chagas disease

Risk of congenital transmission seems to be increased by multiple pregnancies,⁷⁴ high maternal parasitaemia during the acute phase,⁷⁵ and a deficient adaptive immune response of the mother or child, indicated by low interferon- γ production.⁷⁶ Risk of congenital transmission seems to be independent of parasite genotype⁷⁷ and similar in endemic and non-endemic settings, at around 5%.¹⁰ Congenital transmission can occur long after a woman has moved away from an endemic region. Only 10–30% of infected newborn babies have symptoms and about 10% die within 2 days if left untreated.⁷⁵ The severity of congenital infection correlates with neonatal and maternal parasitaemia, and with maternal reinfection.⁷⁸

	Clinical manifestations	Diagnostic procedures	Diagnosis	Differential diagnosis
Acute Chagas disease	Incubation: vectorial transmission 5–14 days, oral transmission 5 days, transfusion 30–110 days; painless unilateral bpalpebral oedema (Romaña's sign), conjunctivitis, dacryoadenitis, lymphadenopathy; erythematous indurated skin lesion (chagoma); malaise, prolonged fever, hepatomegaly, splenomegaly, morbilliform rash, diarrhoea; heart failure; meningoencephalitis	Complete blood count: microcytic or normocytic anaemia, lymphocytosis, raised AST and ALT concentrations, hypergammaglobulinaemia, proteinuria; ECG: sinus tachycardia, T-wave abnormalities, conduction disorders, low QRS voltage; echocardiography: ventricular dysfunction, pericarditis	Direct parasitological methods: identification of the parasite in Giemsa-stained thick and thin blood film and by concentration methods (microhaematocrit, Strout's method); PCR; indirect parasitological methods: xenodiagnosis (endemic zone), blood culture	Cytomegalovirus infection, infectious mononucleosis, primary HIV infection, toxoplasmosis, brucellosis, leishmaniasis, typhoid fever, endocarditis
Chronic Chagas disease: cardiac form	Early manifestations: malaise, palpitations, syncope, (sometimes initial manifestation), abdominal pain (right upper quadrant), jugular venous distension, peripheral oedema, stroke; late manifestations: atypical chest pain, syncopal episodes, sudden cardiac death, dyspnoea with exertion or at rest, orthopnoea, fatigue, heart murmurs, stroke	ECG (early): right bundle branch block, fascicular block, premature beats, non-sustained ventricular tachycardia, atrioventricular blocks; ECG (late): atrial fibrillation or atrial flutter, low QRS voltage; echocardiography (early): wall motion dysfunction, segmental thinning of the wall; echocardiography (late): dilated cardiomyopathy, tricuspid and mitral regurgitation, systolic regurgitation, apical aneurysm; Holter monitoring: arrhythmias and conduction disorders; stress testing; thrombi on transoesophageal echocardiogram; electrophysiological studies; others: MRI (myocardial fibrosis, wall impairment), doppler angiography, scintigraphy	Serology	Other dilated cardiomyopathy: ischaemic, idiopathic, infectious, metabolic, isolated familial, granulomatous disorders, toxic
Chronic Chagas disease: gastrointestinal form	Oesophageal disease: dysphagia, odynophagia, regurgitation, cough, weight loss, aspiration pneumonia; colonic disease: chronic abdominal pain, constipation, faecaloma, occlusion (volvulus), peritonitis; small intestine disease: abdominal pain, bacterial overgrowth syndrome, malabsorption, pseudo-obstruction	Barium oesophagogram: dilated oesophagus; nanometry: impaired peristalsis, non-relaxing lower oesophageal sphincter; endoscopy: mucosal lesions; barium enema: megacolon (sigmoid and rectum)	Serology	Achalasia: idiopathic, stomach cancer, lymphoma, eosinophilic gastroenteritis, neurodegenerative disorders; constipation: colonic tumours, metabolic disorders, Hirschsprung's disease, amyloidosis, systemic sclerosis, dermatomyositis
Chagas disease and HIV/AIDS	Meningoencephalitis: fever, seizures, nuchal rigidity, paresis and paralysis, myelitis, heart failure, peritonitis	Cerebrospinal fluid: pleocytosis, lymphocytosis (100 per μ L); increased protein, decreased glucose, parasites; CT scan, MRI: white matter involvement, unique or multiple haemorrhagic foci with ring enhancement with or without mass effect	Reactivation diagnosed by direct parasitological methods or histology	Toxoplasmosis, lymphoma, progressive multifocal leucoencephalopathy, tuberculous meningitis, cryptococcosis
Chagas disease and transplantation	Transmission with transplantation of kidney (frequency: 20–35%), liver, and haemopoietic cells; reactivation after transplantation: heart (frequency: 20–75%), kidney (9–18%), liver, and haemopoietic cells; clinical manifestations: nodules and erythematous plaques on skin; myocarditis, meningoencephalitis	ECG, chest radiograph, echocardiography, cerebral CT scan	Reactivation diagnosed by direct parasitological methods; histology (endomyocardial biopsy; skin biopsy)	Other infections of transplant recipients
Congenital Chagas disease	Prematurity, low birthweight, hepatomegaly, splenomegaly, jaundice, oedema, respiratory distress syndrome, meningoencephalitis	ECG, chest radiograph, echocardiography, cerebral CT scan	Direct parasitological methods; PCR; serology (>9 months)	Toxoplasmosis, rubella, parvovirus B19, cytomegalovirus, HIV, syphilis, herpes, hepatitis

AST=aspartate transaminase. ALT=alanine aminotransferase. ECG=electrocardiogram.

Table 1: Clinical manifestations and diagnosis of Chagas disease

Pregnant women at risk must be screened for *T. cruzi* infection, and the neonate's venous or umbilical cord blood must be tested for the parasite. Direct methods (microhaematocrit) and PCR are highly sensitive during the first months of life. Serological tests based on two different techniques must be done at least 8 months after birth (the time taken to clear maternal antibodies).⁷⁹

Transplantation

Chagas disease can be transmitted during solid organ or bone marrow grafting and by blood transfusions during

the transplant procedure. It can also be reactivated by the accompanying immunosuppression. In view of the shortage of available organs, use of the kidneys, liver, and bone marrow of infected donors is not formally contraindicated in endemic areas, but remains quite controversial because many experts are against the use of such organs.⁸⁰ Hearts of infected people should not be used. Episodes of graft rejection, cancer, and use of mycophenolate mofetil for immunosuppression increase risk of reactivation after heart transplantation.⁸¹ Reactivation is diagnosed by use of direct parasitological

examination of blood or body fluids (microhaematocrit or Strout's method) or organ biopsy (especially endomyocardial biopsy, brain granuloma, and skin) or on the basis of compatible clinical events. Quantitative PCR could theoretically detect an increase in parasitaemia several weeks before the onset of clinical manifestations.

Three therapeutic strategies for management of Chagas disease during transplantation have been described: systematic recipient prophylaxis; pre-emptive therapy before the onset of clinical manifestations if parasitaemia increases; and treatment of reactivation or acute manifestations.⁸² PCR may facilitate use of pre-emptive therapy. Treatment is generally effective when started sufficiently early, with parasite clearance and clinical cure after 7–14 days.⁸³ The optimum duration of treatment with benznidazole or nifurtimox is controversial because of the paucity of clinical trials.

Patients with HIV infection

Transmission of Chagas disease has been associated with intravenous drug use in individuals with HIV infection. Reactivation of Chagas disease in patients with HIV usually occurs when the CD4-cell count is below 200 cells per μL or 50 cells per μL ⁸⁴ and commonly involves the CNS and, to a lesser extent, the heart. The cause of this cerebral tropism is unknown, but *T. cruzi* type 1 was recently implicated.⁴⁴ Parasitaemia is more common and higher in individuals with HIV infection than in uninfected individuals.⁸⁵

Monofocal or multifocal necrohaemorrhagic meningo-encephalitis is the main clinical consequence of reactivation and can be confused or coexist with cerebral toxoplasmosis. In the specific context of HIV infection, differential diagnoses include primary CNS non-Hodgkin lymphoma, progressive multifocal leukoencephalopathy, cryptococcosis, and tuberculous meningitis. Systematic serological screening is recommended for individuals with HIV infection who have lived in or visited regions endemic for Chagas disease.

Mortality from Chagas disease in patients with HIV infection is high,⁸⁶ but antiparasitic treatment is effective if started early and continued for 30–60 days. Negative direct examination indicates the end of reactivation but does not signify parasite eradication. Secondary reactivation can occur, possibly warranting half-dose secondary prophylaxis,^{87,88} at least until the CD4 lymphocyte count reaches 200 cells per μL .

Laboratory diagnosis

Diagnosis of Chagas disease during the acute phase is based on direct observation of the parasites in blood. Strout's method or microhaematocrit are the reference methods, and PCR could probably override blood cultures and xenodiagnosis in the future.⁸⁹ In the chronic phase, individual diagnosis is based on serological testing but the results are sometimes difficult to interpret. WHO therefore recommends use

of at least two techniques, concomitantly positive, to establish a diagnosis of Chagas disease.³ The different serological techniques are based on native or recombinant antigens.

Table 2 compares the most commonly used standardised techniques for diagnosis of Chagas disease: indirect haemagglutination, indirect immunofluorescence, and ELISA. The promising immunoblot is awaiting standardisation, as is radioimmunoprecipitation assay.¹¹⁰ Recent data for PCR^{32,103–109} are presented because this method, once standardised, would be useful to monitor the chronic phase of the disease. Because of methodological shortcomings, identification of the best test among all studied methods is difficult. No panel of reference samples validated by the international community exists, tests undertaken in contexts of different prevalence are hard to compare, and interobserver and intraobserver reliability (eg, κ , correlation coefficient, coefficient of variation) is rarely assessed.

Data for the effectiveness of indirect haemagglutination are quite heterogeneous,^{90–93} but those for indirect immunofluorescence more favourable.^{91,93} However, these techniques are highly operator-dependent, and few studies have examined their reproducibility. Rapid tests are very interesting in the context of large-scale screening, but their sensitivity is too low for wide acceptance as first-line serological diagnostic tools.^{10,98–102} Because techniques such as radioimmunoprecipitation assay and immunoblot are not yet standardised, conventional and recombinant ELISAs seem the best methods to fulfil the criteria of sensitivity, reproducibility, and predictive values.^{90–97} In the case of PCR, studies of methods devised in individual laboratories showed low sensitivity.^{32,103–106} Optimum indications for PCR, once validated and standardised, could be diagnosis and follow-up of the acute phase including the congenital form;⁸⁰ monitoring to detect reactivation before clinical onset in immunodeficient patients (either qualitatively by conversion of previous consistently negative PCR or quantitatively by increasing parasitic load—eg, in graft recipients); early assessment of the response to treatment by quantitative ultrasensitive PCR;^{70,106} and genotyping.¹⁰⁴ Post-treatment follow-up is based on a decline or loss of specific IgG antibodies against *T. cruzi*. Seronegative conversion occurs within a few months in young children but can take 10–12 years in adults, or might never occur at all. PCR monitoring, if possible with a quantitative and ultrasensitive method, could be a valuable future approach for assessing the response to treatment.^{76,106} There is a need for further research on early criteria of cure by means of molecular and proteomic studies.²

Prognosis

Cardiac complications usually occur in 20–30% of cases of Chagas disease and are the main prognostic factor.⁵⁹ Most fatalities are caused by sudden death (50%) or heart failure (37%).¹¹¹ Prognostic factors for the progression of

	Population	Country	Number of samples	Standard method	Reference criteria definition	Manufacturer	Sensitivity, specificity	Positive predictive value, negative predictive value*	Reproducibility	Comments
IHA										
Ferreira et al (2001) ⁹⁰	Blood donors, selected patients	Argentina, Brazil	290	Four tests: IHA, IIF, conventional ELISA, recombinant ELISA	Three tests	..	100%, 86.8%	80%, 100%	Not done	..
Duarte et al (2006) ⁹¹	Selected patients	Brazil	414	Five tests: IHA, IIF, ELISA, PCR, chemoluminescence	Two tests	Biolab Mérieux (Rio de Janeiro, Brazil)	97.5%, 98%	96.9%, 98.8%	κ=1.0	Prevalence 39%
Gadelha et al (2003) ⁹²	Selected specimens from laboratories	Brazil	287	Three tests: IHA, conventional ELISA, recombinant ELISA	Two tests	Biolab Mérieux (Rio de Janeiro, Brazil)	52.7%, 100%	100%, 91%	Not done	Prevalence unknown
Pirard et al (2005) ⁹³	Blood donors	Bolivia	400	Six tests: one IHA, one IIF, two conventional ELISAs, two recombinant ELISAs	Latent class analysis (mathematical model)	BioMérieux (Paris, France)	97.5%, 93%	83.2%, 97.4%	κ=0.73	Prevalence 43.3%
IIF										
Ferreira et al (2001) ⁹⁰	Blood donors, selected patients	Argentina, Brazil	290	Four tests: IHA, IIF, conventional ELISA, recombinant ELISA	Three tests	..	100%, 85.2%	78%, 100%	Reproducibility	..
Duarte et al (2006) ⁹¹	Selected patients	Brazil	414	Five tests: IHA, IIF, ELISA, PCR, chemoluminescence	Two tests	Biolab Mérieux (Rio de Janeiro, Brazil)	100%, 98%	96.9%, 100%	κ index 0.908	Prevalence 40%
Pirard et al (2005) ⁹³	Blood donors	Bolivia	400	Six tests: one IHA, one IIF, two conventional ELISAs, two recombinant ELISAs	Latent class analysis (mathematical model)	BioMérieux (Paris, France)	100%, 96.3%	94.3%, 100%	Not done	Prevalence 40%
Conventional ELISA										
Malan et al (2006) ⁹⁴	Selected patients, selected population	Brazil, USA	220	Four tests: one IIF, three ELISAs	Two positive tests	USA: PANBIO, the FDA-cleared Hemagen Chagas' kit (Columbia) and the IVD Research Chagas (T cruzi) serum microwell ELISA (Biotech Trading Partners)	Cellabs: 100%, 100%; IVD Research: 100%, 100%; MarDx Diagnostics: 100%, 93%	..	Coefficient of variation 2.4–13.5%	Prevalence unknown
Pirard et al (2005) ⁹³	Blood donors	Bolivia	400	Six tests: one IHA, one IIF, two conventional ELISAs, two recombinant ELISAs	Latent class analysis (mathematical model)	INDIECH (Buenos Aires, Argentina); Gull (Salt Lake City, UT, USA)	INDIECH: 100%, 97%; Gull: 98.6%, 96.6%	INDIECH: 95.7%, 100%; Gull: 95.1%, 99%	Not done	Prevalence 40%
Oelemann et al (1998) ⁹⁵	Blood donors	Brazil	1065	IIF, in-house ELISA, three ELISAs	Two or more positive tests (IIF and in house ELISA)	Abbott Lab (São Paulo, Brazil), BIOELISACRUZI kit (Biolab Mérieux, Rio de Janeiro, Brazil), and the BIOZIMA Chagas kit (Polychaco, Buenos Aires, Argentina)	Abbott Lab: 99%, 96.8%; Biolab Mérieux: 98.6%, 99.8%; Polychaco: 100%, 94.7%	Abbott Lab: 77.5%, 99.9%; Biolab Mérieux: 98.2%, 99.8%; Polychaco: 67.7%, 100%	Not done	Prevalence 10%
Gadelha et al (2003) ⁹²	Selected specimens from laboratories	Brazil	287	Three tests: IHA, conventional ELISA, recombinant ELISA	Two tests	Meridian Diagnostics (OH, USA)	98.2%, 100%	100%, 98.6%	Not done	Prevalence unknown
Duarte et al (2006) ⁹¹	Selected patients	Brazil	414	Five tests: IHA, IIF, ELISA, PCR, chemoluminescence	Two tests	Biolab Mérieux (Rio de Janeiro, Brazil)	98.2%, 96.4%	94.8%, 98.7%	Not done	Prevalence 40%

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	Population	Country	Number of samples	Standard method	Reference criteria definition	Manufacturer	Sensitivity, specificity	Positive predictive value, negative predictive value*	Reproducibility	Comments
(Continued from previous page)										
Recombinant ELISA										
Pirard et al (2005) ⁹³	Blood donors	Bolivia	400	Six tests: one IHA, one IIF, two conventional ELISAs, two recombinant ELISAs	Latent class analysis (mathematical model)	Chagatest, Wiener Laboratories, (Rosario, Argentina); BIOSChile (Santiago, Chile)	Wiener: 98.9%, 98.9%; BIOSChile: 99.3%, 95.3%	Wiener: 98.4%, 99.3%; BIOSChile: 93.4%, 99.5%	Not done	Prevalence 40%
Gorlin et al (2008) ⁹⁶	Blood donors and selected patients	USA, Bolivia, Colombia, Guatemala, Mexico, Nicaragua	42 007	IHA, IIF, ELISA, RIPA	Two positive tests	ORTHO-Clinical Diagnostics, (Raritan, NJ, USA)	99.9%, 100%	100%, 99.99%	Coefficient of variation interobserver 2.5–3.7%; coefficient of variation intraobserver 5.8–8%	Blood donors in the USA 0.03%
Tobler et al (2007) ⁹⁷	Blood donors and selected patients	USA, Bolivia, Ecuador, Brazil, Venezuela, Argentina, Nicaragua	10 192 (blood donors); 180 (selected patients)	ELISA (Hemagen diagnosis) or Stat Pak (Chembio) plus IIF, IHA, ELISAs if necessary	Two positive tests	Ortho-Clinical Diagnostics (Rochester, NY, USA)	97.7%, 100%	100%, 99.9%	Not done	Prevalence in blood donors in the USA 0.03%
Gadelha et al (2003) ⁹²	Selected specimens from laboratories	Brazil	287	Three tests: IHA, conventional ELISA, recombinant ELISA	Two tests	Biomanguinhos/FIOCRUZ (Brazil)	98.2%, 100%	100%, 98.6%	Not done	Prevalence unknown
Ferreira et al (2001) ⁹⁰	Selected patients, selected population	Argentina, Brazil	290	IHA, IIF, ELISA, TcF ELISA (<i>T cruzi</i> fusion protein)	Three positive tests (IHA, IIF, ELISA)	Biolab Mérieux (Roskilde, Denmark)	100%, 94.7%	..	Coefficient of variation intratest 1.37%; coefficient of variation intertest 1.78%	..
Rapid test										
Luquetti et al (2003) ⁸⁸		Argentina, Brazil, Bolivia, Honduras, Venezuela	702	Three tests: IHA plus ELISA plus IIF in-house	Three positive tests	Chembio Diagnostic (Medford, NY, USA)	99.3%, 96.9%	..	Not done	Prevalence unknown
Ponce et al (2005) ⁹⁹		Honduras, Nicaragua, Ecuador	3575 (blood donors); 2423 (patients)	One test: ELISA (Wiener plus in-house)	One positive ELISA	Chembio Diagnostics (Medford, NY, USA)	99.8%, 100%	..	Not done	Prevalence 0.3–4.5% in blood donors
Brutus et al (2008) ¹⁰		Bolivia	460	Two tests: IHA (Polychaco) plus ELISA (Wiener)	Two positive tests	InBios (Seattle, WA, USA)	92.7%, 99.0%	97.9%, 96.4%	Not done	Prevalence 33.9%
Roddy et al (2008) ¹⁰⁰		Bolivia	1913	Two tests: IHA (Polychaco) plus ELISA (Wiener)	Two positive tests	Chembio Diagnostics (Medford, NY, USA)	93.4%, 99.0%	86.3%, 99.6%	Interobserver reliability	Prevalence 6.3%
Sosa-Estani (2008) ¹⁰¹		Argentina, Bolivia, Honduras, Mexico	2495	One test: ELISA (Wiener)	One positive ELISA	Chembio Diagnostics (Medford, NY, USA)	94.6%, 99.0%	..	$\kappa=0.83-0.93$	Prevalence 0.8–28.8%

(Continues on next page)

cardiac involvement have recently been identified, and comprise age over 50 years, systolic diameter more than 40 mm, intraventricular conduction disorders, sustained ventricular tachycardia,¹¹² and ventricular arrhythmia on

exercise testing.¹¹³ Treatment with benznidazole was the only identified protective factor.¹¹⁴

The prognosis of the indeterminate phase of Chagas disease is highly variable. One study reported that survival

Population	Country	Number of samples	Standard method	Reference criteria definition	Manufacturer	Sensitivity, specificity	Positive predictive value, negative predictive value*	Reproducibility	Comments
(Continued from previous page)									
Chippaux et al (2009) ¹⁰²	Bolivia	995 (non-selected population); 1489 (pregnant women)	One test: ELISA 3 (Wiener)	One positive test	Chembio Diagnostics (Medford, NY, USA)	Pregnant women: 97.0%, 96.3%; general population: 92.1%, 99.2%	Pregnant women from Carapari: 93.0%, 93.7%; Pregnant women from Santa Cruz: 97.0%, 98.9%; general population from Carapari: 99.1%, 93.0%	Not done	Prevalence pregnant women: 70.5% in Carapari, 23.5% in Santa Cruz; general population: 48.4% in Carapari
PCR									
Gomes et al (1999) ¹⁰³	Brazil	113	Two tests: IIF (Biolab) plus ELISA (Abbott) with or without haemocultures	Two positive tests	In-house PCR kDNA 330 bp	83.5%, 52.4%	..	Not done	Prevalence unknown
Meira et al (2002) ¹⁰⁴	Brazil	184	Conventional serology (IIF, IHA, ELISA)	Two or three positive tests	In-house PCR kDNA 330 bp primers 121/122	Not done	Not done	Not done	81.5% with positive PCR in patients with chronic disease
Castro et al (2002) ¹⁰⁵	Brazil	69	Conventional serology (IIF, IHA, ELISA)	Three positive tests	In-house PCR kDNA 330 bp primers 121/122	Not done	Not done	Not done	86.7% with positive PCR in patients with chronic disease (after analysis of three consecutive samples)
Galvão et al (2003) ¹⁰⁶	Brazil	127	Conventional serology (IIF, IHA, ELISA)	Three positive tests	In-house PCR kDNA 330 bp primers 121/122	Not done	Not done	Not done	84.3% with positive PCR in infected children
Gil et al (2007) ¹⁰⁷	Colombia	156	Conventional serology (IIF, ELISA)	Two positive tests	In-house PCR nDNA 234 bp: primers H2A	88.7%, 92.5%	..	Not done	..
Fitzwater et al (2008) ¹⁰⁸	Bolivia	526	Two tests: in-house IIF plus ELISA 1 (Biomérieux)	Two positive tests	In-house PCR kDNA 330 bp primers 121/122	Whole blood: 40.0%, 100%; clot: 60.1%, 100%	Whole blood: 100%, 80.4%; clot: 100%, 86.0%	Not done	Prevalence 28.9%
Altchek et al (2003) ¹⁰⁹	Argentina	152	Three tests: IHA (Polychaco), ELISA 1 (Wiener) plus PPA (Bayer)	Three positive tests or presence of parasites by micro-haematocrit	In-house PCR kDNA 330 bp primers 121/122 plus quantitative PCR	79.5%, 98.6%	79.0%, 98.6%	Not done	Prevalence 6.2%
All investigations included patients with the chronic phase of Chagas disease apart from reference 109, in which patients with acute or chronic disease were included. IHA=indirect haemagglutination. IIF=indirect immunofluorescence. FDA=Food and Drug Administration. RIPA=radio immunoprecipitation assay. PPA=particle agglutination test. *Calculated with prevalence results reported by the investigators.									
Table 2: Performance of different techniques for diagnosis of the chronic phase of Chagas disease									

in patients with the indeterminate phase of Chagas disease was generally similar to that in the non-infected population.¹¹⁴ However, a more recent study reported that, every year, about 3% of patients with the indeterminate phase develop cardiac disorders.¹¹⁵

Prognostic factors for vital outcome have been examined in large retrospective series.^{63,111,116} Several factors are independently associated with death, including dyspnoea with New York Heart Association

class III–IV, cardiomegaly, left ventricular dysfunction, and congestive heart failure.¹¹⁷ Disease progression is directly and independently associated with mortality. A recently validated prognostic score for vital outcome is shown in panel 1.^{111,112}

Viotti and colleagues¹¹⁸ showed a positive effect of specific treatment on vital outcome in a non-randomised study. The Benznidazole Evaluation For Interrupting Trypanosomiasis (BENEFIT) randomised trial is designed

Panel 1: Prognosis^{111,112}**Score for the progression of cardiac involvement**

- Age older than 50 years; 2 points
- Systolic diameter more than 40 mm; 3 points
- Intraventricular conduction disorders; 2 points
- Sustained ventricular tachycardia; 3 points
- Benznidazole treatment; -2 points

Risk of progression is

- 3.6% for a score of 0
- 6.9% for a score between 1 and 3
- 16% for a score between 4 and 6
- 52.5% for a score above 7

Prognostic score for mortality from Chagas disease

- New York Heart Association III-IV; 5 points
- Cardiomegaly; 5 points
- Wall motion disorders; 3 points
- Non-sustained ventricular tachycardia; 3 points
- Broadened QRS complex; 2 points
- Male sex; 2 points

Risk of death is

- 2% at 5 years and 10% at 10 years for a score between 0 and 6 points
- 18% at 5 years and 44% at 10 years for a score between 7 and 11 points
- 63% at 5 years and 84% at 10 years for a score between 12 and 20 points

to assess the effect of benznidazole on survival or chagasic clinical events and results are expected in 2012.¹¹⁹ Prognostic scores for progression and mortality do not take into account cardiac involvement assessed by means of advanced radiological techniques¹²⁰ or invasive methods.¹²¹ The prognosis for immunodeficient patients is unknown.

Treatment

Only two randomised controlled trials on antitrypanosomal treatment for Chagas disease have been published^{122,123} and only one is in progress.¹¹⁹ No studies have compared antitrypanosomal treatments with one another, and there are no standardised endpoints with which to judge response to treatment. As a result, the precise aims of treatment are controversial. Despite several reviews and partial meta-analyses,¹²⁴⁻¹²⁶ firm conclusions about the effectiveness of antitrypanosomal drugs are not possible because of the heterogeneity of study populations, designs, endpoints, treatment schedules, and durations of follow-up. Moreover, in some studies many patients were lost to follow-up, and studies with negative results were not adequately powered.

The objective of antitrypanosomal treatment is to eradicate the parasite, but there are no early criteria of parasitological cure. The one accepted criterion is obtaining a negative serological test and that might not be possible until several years after the end of treatment. Furthermore, despite recent advances in knowledge of immune response,⁴⁷ parasitic endpoints in most studies are based on serological techniques (indirect haemagglutination,

indirect immunofluorescence, and ELISA), sometimes on xenodiagnosics, and rarely on PCR, making the results difficult to compare across studies.¹²⁷

Electrocardiographic criteria¹²⁸ and sonographic criteria¹²⁹ for Chagas heart disease were only recently standardised. Various classifications are used to express the severity of cardiac involvement, including Kuschnir's modified classification,¹³⁰ the Brazilian consensus classification,¹³¹ the modified Andes classification,¹³² and the American Heart Association staging system.¹²⁹

The benefits of antitrypanosomal treatments are most clear-cut in children in the indeterminate phase.^{127,133} A meta-analysis of paediatric studies showed that benznidazole reduced the incidence of abnormalities on electrocardiogram (odds ratio [OR] 0.41, 95% CI 0.09-1.85) and increased the rate of seroreversion compared with placebo (OR 10.9, 6.07-19.6).¹²⁴ 4-15% of patients had adverse events in those studies that reported them.^{123,133} For adults in the chronic phase of Chagas disease, there are no clinical trials of sufficient methodological quality to assess the efficacy of any drug.¹²⁵ Only one randomised trial has been designed for this purpose, but follow-up lasted only 1 year.¹³⁴

Experimental studies^{135,136} and large observational studies have shown some positive effects of treatment in adults,¹¹⁵ but these findings need to be substantiated in randomised trials. One large non-randomised trial showed a 76% reduction in disease progression after 30 days of benznidazole treatment and an increased rate of seroreversion (OR 2.1, 1.06-4.06) compared with no treatment.¹¹⁸ Most studies with negative results^{137,138} had methodological biases or inadequate statistical power. These results have broadened the indications of treatment.¹³⁹ Therefore, the results of the BENEFIT trial are much anticipated.¹¹⁹

Treatment is formally indicated for patients with acute or congenital forms of the disease or reactivation, and for children.^{59,117,139} In adults aged 19-50 years without advanced heart disease, antitrypanosomal treatment should generally be prescribed.¹³⁹ Beyond the age of 50 years, treatment is more controversial, but we think it should be given routinely in the same clinical forms, in view of the increased frequency of comorbidities. Most recent experimental studies in mice show a benefit of treatment,^{46,135,136} and treatment should be considered for all patients with Chagas disease.¹⁴⁰ Whatever the evolution of symptomatic disease (pending the results of BENEFIT), the importance of side-effects¹⁴¹ should not be disproportionately taken into account when considering whether to treat patients and should not prevent treatment in most cases.¹⁴²

Currently, the two drugs recommended for Chagas disease are benznidazole and nifurtimox.⁵⁹ Other drugs have also been tested. Posaconazole (20 mg/kg per day) was at least as effective as benznidazole in a murine model.¹⁴³ Other triazole derivatives (ergosterol biosynthesis inhibitors) such as ravuconazole¹⁴⁴ are in

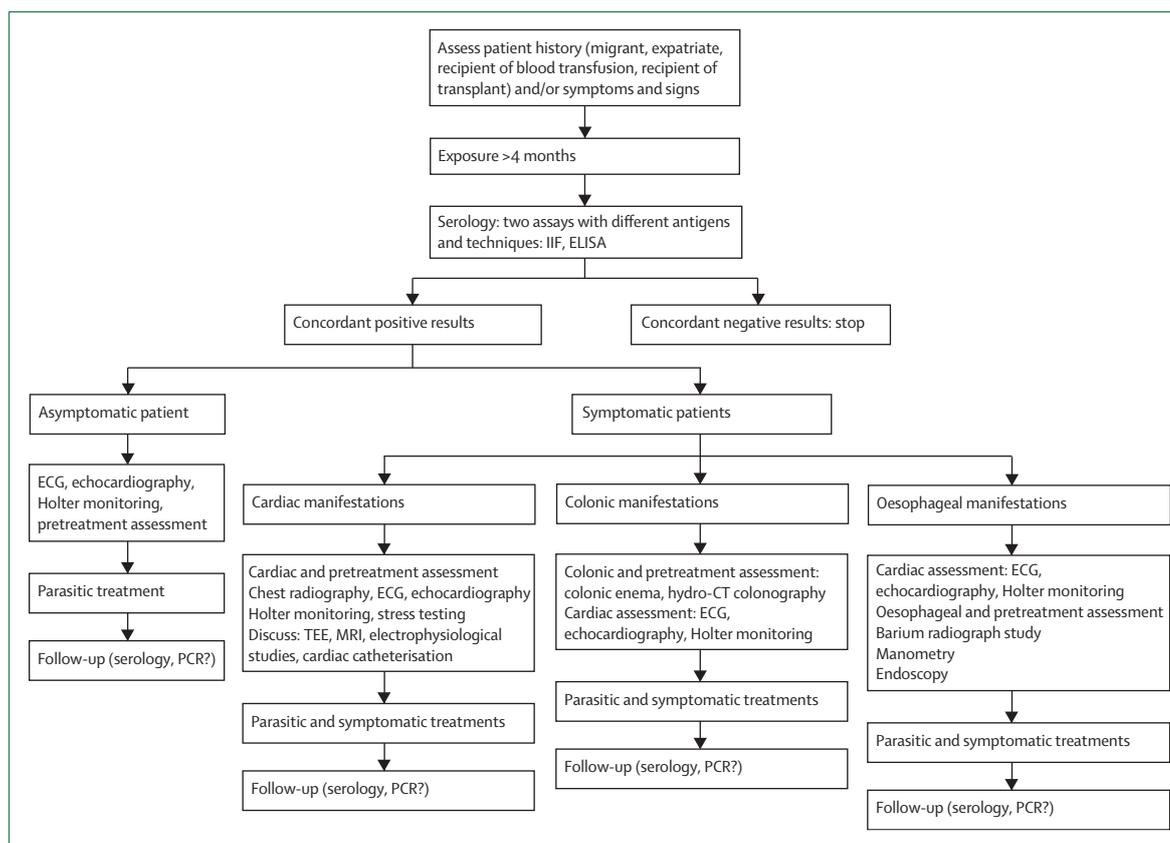


Figure: Management of the immunocompetent patient during the chronic stage of Chagas disease

Pretreatment assessment: complete blood count, hepatic enzymes, bilirubin, serum creatinine. During treatment: physical examination and complete blood count, hepatic enzymes, bilirubin, serum creatinine every 2 weeks. IIF= indirect immunofluorescence. ECG=electrocardiogram. TEE=transoesophageal echocardiography.

development, along with inhibitors of cruzipain, a key parasite protease.¹⁴⁵

Factors affecting the response to treatment are poorly documented. Young age and short duration of infection seem to be associated with good response. More recently, some investigators have suggested that the parasite lineage might affect response to antitrypanosomal treatment.^{146,147}

The figure shows a treatment algorithm for the chronic phase of Chagas disease. In view of current therapeutic practices in other infections, especially those caused by kinetoplastids, several outstanding questions regarding treatment of Chagas disease include the value of drug combinations such as those currently tested in African trypanosomiasis,¹⁴⁸ the value of serial treatments, which were recently shown to be beneficial in a murine model,⁴⁶ and the place of immunomodulatory therapy, in view of recent data about the role of interferon.^{46,149} Development of a vaccine against Chagas disease has seen little success,⁴⁹ despite the potential benefits for individuals and for public health of this strategy for disease control.

Public health challenges and priorities

Major successes in disease control have been obtained in endemic regions, as shown by the respective declarations

by Chile, Uruguay, and Brazil¹⁵⁰ that *T cruzi* transmission by the vector *T infestans* has been eliminated. These results might be explained by focus on vector control and safety of blood transfusions, strong political involvement, mobilisation of human and financial resources within vertical programmes, international cooperation between countries and with international institutions, and community participation.^{150–152} These programmes have been highly beneficial in financial terms.¹⁵⁰

However, despite these efforts, Chagas disease is still the main parasitic disease of Latin America with a burden of 670 000 disability-adjusted life-years, and the annual cost of morbidity and death from the disease in endemic countries was estimated to be more than US\$8 billion in 2000. Panel 2 lists priorities for management of Chagas disease. Regarding access to care, management of Chagas disease needs to be integrated into the primary health-care system at the municipal level in isolated rural populations. Access to care also requires the availability of antiparasitic drugs (especially paediatric forms) and sustained access to specialised cardiology and gastrointestinal care. However, the potential instability of decentralised health-care policies and human and financial rationing of the

Panel 2: Priorities for future management of Chagas disease

- Increased vector control, surveillance of reinfestation or emergence of vectors, and monitoring of drug resistance in countries where domestic transmission is persisting
- Diagnosis and follow-up of patients, with standardised methods for detection of *T. cruzi*, and monitoring of disease progression and response to treatment
- Drug availability and treatment strategies (duration, combination of drugs, re-treatment in case of initial failure)
- Transfusion safety in terms of screening coverage (Bolivia, Mexico) and quality of screening methods in several countries¹¹
- Access to care¹⁵³ and sustainability
- Design of control and decentralisation policies according to the geographical and epidemiological context of the setting¹⁵²

poorest municipalities can threaten the sustainability of Chagas programmes. These must be based on the maintenance of Chagas disease as a national and regional public health priority.

The support of international institutions, and especially of WHO, through the global WHO network launched in 2007, the agreement between WHO and Bayer (manufacturer of nifurtimox), and the Drugs for Neglected Diseases Initiative agreement with the Brazilian manufacturer of benznidazole (LAFEPE) are important factors of sustainability. The cost-effectiveness of strategies linking vector control to case management has been shown¹⁵⁴ but financial mobilisation must be ensured at national and international levels.

Among the non-endemic regions, Chagas disease is a public health issue in the USA and Spain but not in other countries, where only a few cases have been reported. The launch of the WHO initiative in non-endemic regions in 2007 will help to mobilise governmental and medical stakeholders. Apart from sharing the challenges of diagnosis, treatment, and access to care (since access to diagnosis and treatment is hindered by the frequent illegal immigrant status of patients) with endemic countries, non-endemic countries have also specific issues to address.³¹

In the USA, screening of blood donors for *T. cruzi* infection has been widely implemented since 2007 but is not obligatory. However, between 75% and 90% of blood banks screen either all blood donors or only those with risk factors. In 2000, the US Centers for Disease Control and Prevention recommended screening for Chagas disease before bone marrow donation but not before solid organ donation. Prevention of congenital transmission of the disease is largely neglected. In Europe, transfusion screening policies have already been implemented, but screening of populations at highest risk of infection (particularly Bolivian immigrants) has begun in Spain, France, and

Search strategy and selection criteria

Data for this Review were obtained by searching PubMed and Web of Science for articles published in English, Spanish, or Portuguese from 1980 to March, 2010. We used the key words "Chagas disease", "pathophysiology", "diagnosis", "treatment", "vector control", "transplantation", "HIV/AIDS", "congenital", "health policy", "programs", "clinical trial", and "transfusion", as well as the names of countries in endemic and non-endemic regions. We focused on articles published since 2001, apart from articles on therapeutic trials. Each article was then assessed for its methodological quality and the relevance of its results.

Switzerland and must be extended throughout Europe. A surveillance system for Chagas disease should be implemented. Another challenge in non-endemic regions is the specific training and coordination of infectious disease specialists, gynaecologists, obstetricians, paediatricians, cardiologists, and gastroenterologists within reference centres, to improve prevention (eg, in travellers and pregnant women) and care.

Finally, as an international health priority, worldwide mobilisation of resources in endemic and non-endemic countries should promote research and randomised controlled trials that focus on diagnosis and treatment. These studies should also respect ethical principles of research in low-income countries.

Contributors

GP and F-XL conceived the Review. F-XL and GLL undertook article selection, designed the study, and wrote the paper. GP, LB, HF, MD, and LP critically revised the paper.

Conflicts of interest

We declare that we have no conflicts of interest.

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