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Strategies to enhance access to diagnosis and treatment for Chagas disease patients in Latin America

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Abstract

Introduction: Chagas disease, caused by infection with the parasite *Trypanosoma cruzi*, represents a huge public health problem in the Americas, where millions of people are affected. Despite the availability of two drugs against the infection (benznidazole and nifurtimox), multiple factors impede their effective usage: 1) gaps in patient and healthcare provider awareness; 2) lack of access to diagnosis; 3) drug toxicity and absence of treatment algorithms to address adverse effects; 4) failures in drug supply and distribution; and 5) inconsistent drug efficacy against the symptomatic chronic stage.

Areas covered: We review new approaches and technologies to enhance access to diagnosis and treatment to reduce the disease burden. We also provide an updated picture of recently published and ongoing anti-*T. cruzi* drug clinical trials. Although there has been progress improving the research and development (R&D) landscape, it is unclear whether any new treatments will emerge soon. Literature search methodologies included multiple queries to public databases and the use of own-built libraries.

Expert opinion: Besides R&D, there is a major need for continue awareness and advocacy efforts by patient associations, local and national governments and international agencies. Overall, health system strengthening is essential to ensure vector control commitments, as well as patient access to diagnosis and treatment.

Keywords: Chagas disease, comprehensive care, clinical trials, diagnosis, drug treatment, patients associations, pharmacovigilance, vector control.

Article highlights

- Chagas disease is a neglected disease caused by the parasite *Trypanosoma cruzi* (*T. cruzi*) that affects millions of people in the Americas. Traditionally stigmatizing, hidden by society and ignored by governments, thanks to researchers, patients' associations and several other actors who have raised awareness and promoted education on the disease, there has been a shift towards improved diagnosis, treatment and control in recent decades.
- There are highly sensitive and specific diagnostics for the acute and the chronic stages of the infection. However, they are not widely implemented in areas distant from reference laboratories due to logistical issues and the lack of equipped facilities and trained personnel. Thus, more practical algorithms should be used to provide point-of-care diagnostics.
- Two drugs are available to treat the infection: benznidazole and nifurtimox. Both are highly efficacious in newborns, but access to treatment generally occurs at the chronic stage when symptomatology arises. Due to their frequent adverse side effects, pharmacovigilance programs to report drugs toxicities and a better management of these in primary care establishments must be implemented to ensure tighter adherence to medication.
- Stronger involvement of public health institutions and authorities is fundamental to progress in the disease control and its adequate management. The establishment of counter-reference circuits integrated in national health services plans will be fundamental to catalyze and scale up the attention and care of Chagas disease patients in endemic and non-endemic settings.
- Some regions have made great progresses in the control of vector-related transmission, but there are yet many where vector transmission is active. Increased cooperation between vector control programs and medical programs must be put on place to maximize their impact in public health.
- The role of patients' associations must remain active and press on other stakeholders so as to keep up Chagas disease visibility. Patients' organizations are at the frontline to demand attention from the responsible authorities, ensure adequate medical care, and ask for returns on investment from R&D.
- In line with the disease awareness shift, the clinical research landscape has also changed for the better. There are several clinical studies ongoing and their results are expected to improve diagnosis and treatment policies.

1. Introduction

Chagas disease, or American trypanosomiasis, is caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) and remains one of the most deadly and intractable neglected tropical diseases (NTDs) in the Western Hemisphere [1]. Updated information from the Global Burden of Disease Study (GBD) 2016 indicates that 7,100 people die from Chagas disease annually, roughly the same number of Chagas disease-related deaths that occurred a decade previously [2]. However, these numbers may represent highly conservative estimates with further findings that as many as 200,000 people living with *T. cruzi* infection may die over the next five years [3]. The GBD 2016 also finds that 7.2 million now live with Chagas disease, while 180,000 new *T. cruzi* infections occur annually [4].

Beyond its horrific disease burden and contribution to infectious disease mortality, there is a profoundly disturbing social impact dimension to Chagas disease related to its importance as a health disparity. Today, tens of thousands of people face a death sentence from their *T. cruzi* infection due to Chagasic cardiomyopathy, which could be prevented by timely access to diagnosis and anti-parasitic treatment with one of two nitroheterocyclic drugs – benznidazole (BNZ) and nifurtimox (NFX). Fueling a growing outrage from the global health community is the finding that approximately 90% of people infected with *T. cruzi* infection now live in Latin America's three wealthiest economies: Argentina, Brazil, and Mexico. In the United States of America (USA), the richest country of the continent and main destiny of those looking for a better future, there are at least 200,000 immigrants from Latin America living with Chagas disease with limited or no access to treatment [5,6]. Furthermore, in Europe there are around 120,000 immigrants from Latin America living with Chagas diseases with a disparity of situations regarding access to care and treatment [7,8].

The overwhelming majority of Chagas disease sufferers are unable to gain access to diagnosis and treatment, not only because they are poor, but also because governmental leaders are either uninformed or uninterested. For example, it is noteworthy that the registration of BNZ for the treatment of pediatric Chagas disease in the USA has not been approved until very recently [9]. Documenting such assertions is not easy and seeking solutions to diagnosis and treatment access are not straightforward endeavors. In Mexico, for example, there are almost one million people living with Chagas disease, although even that number may represent a profound underestimate [10]. Yet, only 3,013 *T. cruzi* infection cases were registered nationally between the

years 2007 and 2011, less than 1% of the actual number of people affected with the disease [11]. A similar situation has been documented for the USA [12] and also likely holds true across the Americas. We are facing a situation where less than 1% of Chagas disease patients have access to timely and appropriate diagnosis and treatment [13,14].

Limiting access to essential medicines also has important implications for new research and development (R&D) related to therapeutic interventions. An exciting development on this front is a new orally bioavailable nitroheterocyclic drug, fexinidazole, which is also effective against human African trypanosomiasis [15]. Additional drugs are also under development, as well as there are Chagas disease vaccine (immunotherapeutic) candidates at pre-clinical stage [16]. In this respect, the Texas Children's Hospital Center for Vaccine Development, a Product Development Partnership, is exploring an approach that links therapeutic vaccination to pharmacotherapy [17]. However, any R&D successes must still face a formidable gauntlet of truncated and mostly failed global access mechanisms. Similar forces are a barrier for access to new and innovative diagnostics [18].

Here we report on some of the major hurdles that currently block access to the diagnosis and treatment of Chagas disease. The problems include both scientific and socioeconomic obstacles. This paper aims to elucidate the challenges they pose and offer solutions.

2. The need of more practical and useful diagnostics

The poor access rate to Chagas disease therapeutic treatments has its roots in the clinical nature of the disease itself and its silent progression from the mostly asymptomatic acute stage into the symptomatic chronic one [1]. Unfortunately, biomarkers of disease progression and standardized tools to determine early response-to-treatment are yet unavailable, which greatly complicates the prognosis and follow-up of patients [19].

Treatment administration, as in any other disease, needs to be preceded by an adequate diagnosis. In the case of Chagas disease, when a clinical diagnosis is achieved, tissue disruptions might already be too advanced for a chemotherapeutic intervention. Therefore, parasite detection must be sought before the onset of overt symptomatology. In the acute infection stage, for instance upon congenital transmission of the parasite, parasitemia can be detected by direct microscopic observation [1]. However, this stage is short lasting and generally goes unnoticed as there are often no symptoms at all.

Approximately 30% of these infected individuals will progress to evidence either Chagasic cardiac or gastrointestinal disease. Those without clinical evidence of disease are said to be at the indeterminate stage, whereas those with cardiac or gastrointestinal involvement are at the determinate stage. The development of life-threatening heart and/or digestive tract disruptions, which can be massive and are called mega-syndromes, occurs in the long lasting chronic stage [1].

In both indeterminate and determinate Chagasic patients, parasitemia is typically low and intermittent and the diagnosis of the infection is made by means of indirect serological tests, like enzyme-linked immunosorbent assays (ELISAs). This is possible because high levels of parasite-specific immunoglobulins are produced upon *T. cruzi* infection (Figure 1). Anti-*T. cruzi* type G immunoglobulins (IgGs) levels remain above detection thresholds for many years, which is advantageous for the serological diagnosis of the infection (Figure 1). However, it turns out to be an inconvenience for a serology-based assessment of drug responses as it can take several years for them to revert after the administration of treatment [20]. Another issue to take into account in the diagnosis of the infection is the wide genetic variability of the parasite, which encompasses seven different genotypes grouped in Discrete Typing Units (DTUs) [21]. A role of the parasite and host genetics interplay has been suggested in relation to the sensibility to treatment of the distinct isolates, the pathological signatures of the infection, and also in the anti-*T. cruzi* immune response [21].

Some studies indicate that treatment interventions while patients are in the indeterminate or early determinate stages are critically important in order to prevent advanced disease progression. In contrast, from the multi-centered BENEFIT trial to evaluate BNZ efficacy it was found that patients with significant cardiac involvement progressed to advanced disease or even died despite receiving specific anti-parasitic chemotherapy [3,21]. Currently several classifications (for instance AHCC, or Kuschnir's modified) are in place to differentiate people with early-stage (grades A-B or 0-I respectively) versus late stage (grades C-D or II-III respectively) cardiac disease. Treatment of patients with grades C-D or II-III was not encouraged previously [1], a finding that appears to hold up in light of the recent BENEFIT findings. These findings highlight the importance of identifying both indeterminate patients with Chagas disease, and possibly those with AHCC grades A-B or modified Kuschnir grades 0-I too, since they might be successfully treated with antiparasitic therapy if they were captured during population-wide screening campaigns [3]. In this way, it would be possible to

identify and treat chronically infected people before they develop the symptomatology. Women at child-bearing age and newborns should receive special attention because the treatment of mothers-to-be has been shown to largely reduce the transmission rate [23–26], and the efficacy and tolerability of current drugs by infected newborns is ~100% [1]. Moreover, health economics studies evaluating Chagas disease surveillance in endemic and non-endemic settings indicate that widespread screening would be highly cost-effective [27–30]. In the two disease scenarios studied, congenital (acute infection transmitted by chronically infected mothers) and indeterminate (chronic asymptomatic stage), mass screening would save health costs even at *T. cruzi* prevalence rates as low as 0.9% or 0.05% respectively (in fact estimated in a non-endemic setting) [27,29].

Due to the very limited resources available for Chagas disease management, and echoing the answers provided by Latin American experts enquired by Picado et al. [18], efforts should focus on making widely available point-of-care (PoC) tests to diagnose congenital transmission and indeterminate chronic patients [18]. However, if we want to enable generalized Chagas disease diagnosis, there is an urgent need of more practical diagnostic reagents and kits. Availability of easy-to-use tools for the early assessment of treatment response would also be highly valuable to promote and support the administration of drugs against the infection. In this regards, there are some biomarkers under research [19], but the evaluation of anti-*T. cruzi* drug responses yet relies on the molecular amplification of the parasite DNA from periphery blood obtained at distinct times post-treatment. Unfortunately, its associated high costs and technical requirements restrict its use to the context of well-funded clinical trials [31].

2.1. Current Chagas disease diagnostics are impractical in many regions

Regarding acute stage diagnostics, classical parasitological methods (micromethod, hemoculture and xenodiagnoses) are microscopy-based and rely on finding motile trypomastigotes in blood, thus they provide both low sensitivity and specificity. Due to their poor performance, current algorithm to diagnose congenital transmission involves two micromethods (at birth and at 1-2 months of age), and a further confirmatory serological test once mother-derived IgGs have waned at infant's 8-12 months of age [32]. This has two major drawbacks: a very high loss-to-treatment risk during pediatric follow up, and the reduction of drug efficacies the longer the treatment is delayed [32]. Molecular amplification of *T. cruzi* DNA, either by conventional polymerase chain reaction (PCR) or by quantitative PCR (qPCR), has

been shown to be more sensitive and specific than classical parasitological techniques [20]. Several laboratories have worked on the standardization of the techniques so that their outcomes can be comparable and implemented in clinic-based laboratories [20]. But molecular biology laboratories are expensive to mount and maintain, plus they require highly trained personnel to run them. Therefore, despite its very good performance, molecular detection is not generally used beyond regional or national reference laboratories in endemic regions.

In relation to the current chronic stage diagnostics, conventional serological tests (like ELISAs, indirect immune-fluorescence or indirect hemagglutination assays) use serum or plasma samples that entail venous extraction and blood segregation by centrifugation, and they require a cold chain to preserve the test reagents and the samples. Moreover, due to the parasite's antigenic diversity, the advice from the World Health Organization (WHO) is to run two tests based on distinct antigenic sets and if their outcomes are not concurrent, to employ a third technique [14]. This algorithm is costly, and it requires equipment and resources that are usually not available in many laboratories of endemic regions. Furthermore, the turnaround of results to the patient can take several weeks, which involves a high risk of losing contact with the patient for treatment.

2.2. What solutions could be implemented?

Fortunately, recent technological advancements are procuring solutions to overcome the limitations mentioned above. We will outline them separately considering first those for the diagnosis of acute stage and then those for the diagnosis of chronic Chagas disease.

In recent years, isothermal amplification methods that do not require expensive equipment (such as thermocyclers or gel visualization systems) and are easier to perform than PCR assays have been developed for the molecular detection of several NTDs [20]. At present, a prototype of Loop isothermal AMPlification for *T. cruzi*-DNA (LAMP, Eiken Co., Japan) has been tested with clinical samples and shown to have a comparable performance to qPCR with blood-EDTA samples [33]. Another LAMP test developed in house by Rivero et al. [34] has also been shown to provide a comparable performance to current congenital transmission algorithm. LAMP is based on a microbiological DNA polymerase that works at a constant temperature of 65 °C for 45 minutes with a set of 4 to 6 complex primer sequences to provide a highly sensitive and

specific amplification [35]. LAMP readout is qualitative and the results can be naked eye visualized in a short time given a probe (e.g. calcein) is added to the reaction mix. If a digital fluorimeter is used (e.g. Genie III) the reading can even be semi-quantitative [33]. Notably, in Eiken's *T. cruzi*-LAMP prototype, reagents are provided dried out in the lids of the reaction tubes which allow a ready-to-use format and a much desirable room temperature storage [33]. More recently, a Recombinase Polymerase Assay (RPA), which even requires a lower amplification temperature and shorter amplification time (40 °C for 30 min) than LAMP, has been tested with samples from naturally *T. cruzi*-infected dogs [36]. This RPA has been coupled to a lateral flow strip for results reading and it was shown to provide excellent agreement with qPCR results [36]. There are RPAs for the detection of other NTDs [37,38], so it could also be very useful for Chagas disease molecular diagnosis.

For the serological detection of *T. cruzi*-specific IgGs, rapid diagnostic tests (RDTs) have been commercially developed during the last two decades [39,40]. RDTs have clear advantages over conventional serology, as they can be stored at room temperature, use a very small volume (5-25 µl) of finger pricked whole blood, have an easy-to-run and read cassette format, and provide a fast turnaround of results (less than 45 minutes) [40]. Several studies now support their implementation as they have been extensively validated against conventional tests [41–43]. For instance, a RDT is currently used for primary screening of chronic Chagas disease in Bolivia [41]. Nonetheless, following the WHO guidelines of two-tests concordance, confirmation of that RDT primary result must yet be made with a conventional serological test [14]. Such recommendations reduce the advantages of RDTs.

With the aim to fully exploit RDTs advantages and to determine whether they can substitute conventional tests, combinatory use of two RDTs has been proposed [41]. So far, in a proof-of-concept study performed in the city of Sucre (Bolivia), perfect agreement between the two RDTs used was observed, and their sensitivity and specificity in comparison with three conventional tests was 100% and 99.3%, respectively [41]. However, despite a promising performance in Bolivia [41,43], RDTs have not worked so well when they have been used in other geographical regions [44]. This might be related to the high prevalence of the disease in Bolivia, which may allow an easier detection, or to the fact that the parasite strains used to produce the RDTs antigens are those circulating in Bolivia. In any case, until more results from different epidemiological areas are available, preliminary geographical testing of the RDTs

performance has been proposed before using them in a particular region [44]. In view of the advantages they bring versus conventional tests, RDTs implementation for Chagas disease surveillance should be evaluated at larger scales.

3. Treatment of Chagas disease, and related issues

3.1. Drug regimens

Evidence about the benefits of Chagas disease treatment, together with a growing understanding of the pathogenesis of the disease, led to the paradigm that all *T. cruzi*-seropositive patients should receive treatment with anti-*T. cruzi* drugs [45]. The recently published results of the SaMi-Trop cohort study further reaffirm this statement as they demonstrate a beneficial effect of BNZ in reducing the cardiac clinical progression of chronic Chagas disease patients [46]. Therefore, anti-trypanosomatid treatment should be provided to all *T. cruzi*-infected people who do not present with advanced cardiac complications (Kuschnir grades III-IV), as by then clinical manifestations might not be improved [47]. Nonetheless, access to treatment confronts important limitations. BNZ and NFX, the only drugs available for *T. cruzi* infection, exhibit reduced efficacy during the chronic stage of the disease, and require a long period of administration which causes frequent unwanted drug-related adverse reactions (ADRs) [48–50]. Furthermore, variable drug susceptibility has been already described among distinct *T. cruzi* strains [51]. In this context, there is an urgent need for more efficacious and safer drugs or drugs' regimens, in particular for the treatment of the chronic stage of the infection.

One alternative is the reduction of the BNZ dose and/or schedule in order to improve safety and adherence to treatment; an approach that is supported by clinical and experimental data. In a pilot study Álvarez et al. [52] assessed a new scheme of BNZ administration in a small cohort of chronic Chagas disease patients treated with intermittent doses of BNZ at 5 mg/kg/day every 5 days for a total of 60 days. The study showed a satisfactory safety profile, with low rates of treatment suspension and treatment failure [52]. Furthermore, an experimental study using a mouse model of chronic *T. cruzi* infection demonstrated the effectiveness of an intermittent scheme of BNZ administered every 5 days for 40 days [53]. These findings support the intermittent administration of BNZ as a new dosage schedule, but further research to confirm its efficacy by long-term assessment of larger cohorts is needed. Another therapeutic option under investigation is the co-administration of an immunotherapeutic

(vaccine) treatment and BNZ. In this regards, enhancement of a *T. cruzi*-specific immune response has been shown to contribute to support the efficacy of reduced BNZ dosages in a mouse model of acute *T. cruzi* infection [17].

3.2. Drug availability

Drug access is still a huge problem in some endemic areas [13]. BNZ, generally the first line therapy for Chagas disease, is part of the WHO List of Essential Medicines [54]. It was produced during more than 40 years by Roche (Basilea, Switzerland), which transferred its production rights to Laboratório Farmacêutico de Pernambuco (LAFEPE), a Brazilian public enterprise, at the end of the twentieth century. Sadly, BNZ production and distribution by LAFEPE failed to meet expectations in terms of meeting supply and demand requirements and in 2011 an important shortage of the drug occurred. It lasted 1.5 years and left thousands of patients without treatment worldwide [13,55]. This fact led to the development of a Private-Public Partnership in Argentina involving Maprimed (for the synthesis of the drug) and ELEA (for its development and production), to promote equitable availability of BNZ [56]. Since 2012, this Argentinian Partnership has worked to guarantee the availability of the drug, distributing BNZ to the countries in the region [56]. NFX, which is mostly used as the second line treatment option, is produced and donated by the pharmaceutical company Bayer, and distributed through the Pan-American Health Organization (PAHO) Strategic Fund [57]. Recently, a NFX produced by Gador has also been registered in Argentina. Definitely, a regular, safe and accessible production of these two anti-parasitic drugs is necessary to guarantee the treatment to diagnosed patients, and the access to drugs has to be ensured in adequate quantity, quality, location and timing.

3.3. Pharmacovigilance

A major limitation of current Chagas disease treatments is the onset of Adverse Drug Reactions (ADRs), which may lead to poor medication adherence, and cause thereby therapeutic failure or ineffective treatment [48-50]. ADRs are defined as "an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product" [58]. The most commonly observed ADRs related to BNZ are headache, dermatological manifestations and gastrointestinal symptoms [49,50].

Concerning NFX, digestive symptoms are the most frequent [48]. Nevertheless, with an adequate clinical management most of the patients are able to finish treatment in the advent of ADRs [49]. Close medical follow-up, adequate monitoring of ADRs and implementation of robust pharmacovigilance systems are essential factors to avoid patient abandonment and achieve therapeutic success.

Although pharmacovigilance is crucial, it is still a neglected area. Latin-American countries are making important efforts to report ADRs, but these activities are recent and need reinforcement [59,60]. Results of an unpublished study conducted by Cortes-Serra et al. in Bolivia during 2016 indicate that 35.4% of the total patients treated for Chagas disease in fourteen healthcare centers of the department of Cochabamba suffered ADRs related to it. From all ADRs classified as moderate or severe (25% of the total ADRs registered), only about half of them (51.43%) were reported to the Bolivian Pharmacovigilance system [61]. This data illustrates the urgency of implementing policies to promote training in pharmacovigilance to all healthcare professionals, as well as strictly recommend the follow-up on drug monitoring and ADR reporting. Altogether, these features are fundamental to achieve strong and consolidated ADR reporting systems, which will improve patient safety, drug efficacy and adherence to treatment.

4. Patients' comprehensive care: reference and counter-reference circuits

With less than 1% people treated [13,14], and an economic burden of \$7.19 billion per year and \$188.8 billion per lifetime [62], Chagas disease remains neglected despite the efforts performed by several institutions focused on development and research [13]. Migratory flows have changed the epidemiology of the disease that is now emerging in some non-endemic countries [7]. During the last decades, collaboration and knowledge transfer between institutions from endemic (CEADES, Bolivia) and non-endemic countries (ISGlobal, Spain) has been strategic to build attention models for the Chagas disease patient. Such models could be scaled-up by national health systems in endemic and non-endemic countries in order to expand Chagas disease healthcare to people living in areas with limited access to health (e.g. rural areas in endemic countries) or to vulnerable populations (e.g. migrants in endemic and non-endemic countries).

4.1. The Platform for integral care of Chagas disease patients

The work made by this Platform in recent years have produced a quantitative and qualitative improvement in the healthcare provided to Chagas disease patients in Bolivia, but this improvement certainly needs to be strengthened. The experience gained with the Platform in the country has shown that the implementation of specialized centers to manage people at risk of having Chagas disease is highly effective, both based on the percentage (and number) of people diagnosed with the infection, and amongst them, those who received and completed treatment [63]. This vertical strategy has been essential to design the attention model for patients with Chagas, making the medical assistance to these people look like a normalized and necessary action [63]. Nevertheless, the sustainability of such model ultimately depends on continuously securing external funds, which greatly complicates its expansion to larger geographical levels, like national coverage by the national health system (Figure 2).

4.2. Vertical-to-horizontal healthcare model transition

In order to have a higher impact in terms of diagnosis and treatment coverage, as well as to ensure the sustainability for the model of care of Chagas disease patients already installed, it is mandatory to search for a comprehensive horizontal strategy together with local higher level entities of the public health system. In fact, based on WHO recommendations, the strategy to include the Chagas disease attention roadmap as part of the regular activities of all healthcare levels seems to be the most appropriate approach [64].

Simplifying the vertical Chagas disease model of healthcare to more realistic protocols established together with the national health institutions has allowed the improvement of healthcare access for people at risk of having the disease living in remote areas of an endemic country [63]. Researchers at ISGlobal (authors of this review) have yet unpublished data which demonstrate that the health coverage, in terms of patients diagnosed and treated in the selected area in which the project has expanded its activity, was five times higher in the three years following the horizontal comprehensive care model than the number of people covered in the five previous years with the vertical strategy (Pinazo et al., unpublished).

So far, the outcome of the vertical-to-horizontal healthcare model transition highlight that it could be worthy to replicate and/or adapt it to other regions or countries. The first step would be to coordinate with local health authorities at different levels in order to design an appropriate strategy. For the implementation of a healthcare

model, the identification of interested health workers is a key issue. Even if it is simplified, a strategy to offer a comprehensive care for people at risk of suffering from Chagas disease should include: (a) specific training of health workers on the disease management; and (b) a strategy to increase the demand of the civil society, based on promotional and educational community activities. In this regard, the establishment of referral and counter-referral circuits tailored to each epidemiological and logistic situation is highly relevant, even in nearby areas.

4.3. Requirements for the expansion of the Chagas disease healthcare model

Referral and counter-referral circuits, in terms of patients and samples for diagnosis or any other test, should include different healthcare levels to cover different levels of complexity in terms of care. The specialized centers in which the model of care has been rehearsed in Bolivia are key towards organizing these circuits, as well as to act as reference centers for complicated cases and to accompany the doubts and the continuing education of health professionals from primary care centers. This is particularly important in health systems where there is often a fast renewal of healthcare personnel. Another crucial point to make sustainable a comprehensive horizontal strategy against Chagas disease is to promote an inter-sectorial collaboration with vector control authorities (promoters of house refurbishment, disinfestation programs,...) and the educational system itself. Vector control interventions are fundamental to halt vector-dependent transmission and enable an enhanced drug control of the cases. Whereas educational activities to widen the population knowledge and perception of the disease, its impact, and treatment possibilities are crucial, because producing changes in beliefs, attitudes, and behaviors on both medical staff and patients still stands as one of the major challenges that must be faced when dealing with Chagas disease. All these measures have a role to play in order to consolidate the successful management of the disease.

On the other hand, lessons learned from the primary healthcare network have shown that it is mandatory to ensure the supervision of the circuits and surveillance the quality of the process, tasks that should be carried out by the national health system responsible personnel in duty. Political engagement at this point is mandatory to contribute to a better control of the disease as a Public Health problem. In this context, it is important to respect the capability of local health institutions, agreeing with them timelines and a progressive increase of the number of people diagnosed and treated, in

order to answer adequately to people's demands. It must be noted that external factors like the poor availability of drugs for Chagas disease treatment have a sourly negative impact on any planning.

5. Patients treatment in relationship with vector control

Access to diagnosis and treatment of Chagas disease in areas with active vector transmission requires a multidisciplinary approach. It involves the participation of players from several areas of expertise and different government sectors, from vector control authorities to health service providers, including primary healthcare [65].

Following the advice from the PAHO, intergovernmental regional initiatives were created at the end of the twentieth century and beginning of the twenty first century to establish supranational levels of action to bolster and monitor the implementation of activities to prevent, control, diagnose and treat Chagas disease in Latin America. These initiatives were started by Southern Cone countries in 1991, and then followed by Andean countries and Central American countries in 1997 and Amazonian countries in 2004 [66,67]. Despite these efforts, it is evident that in the region there was, and still is, a breach between the programs for vector control and the areas that are responsible for providing universal health care (including diagnosis and etiological treatment) [68].

It is important to consider that although policies related to the primary prevention of Chagas disease are defined at the national level, primary healthcare attention for the patient is the responsibility of the provincial/departmental or municipal/local entities [69]. Often times, these final effectors of national policies have little or no relation/communication with the national entity and are sometimes even unaware of the policies themselves. For example, suggestion of treatment of the infection by *T. cruzi* for postpartum women and their newborns, and the mandatory treatment for women of childbearing age and children under the age of one with Chagas disease that are in the national or supranational norms [32], make no specific mention of which protocols need to be applied with respect to vector surveillance in the houses of those patients living in endemic areas with active vector transmission. However, recent public health interventions have shown that activities related to access to diagnosis or treatment of the disease are usually implemented and promoted by those responsible for vector control programs [70]. Thus, without consideration of the need of appropriate structures and circuits (i.e. access to an adequate laboratory for diagnosis or presence of

anti-trypanosomal drugs), which are not usually present in rural areas, one of the main actions needed is to establish and/or strengthen the link between vector control programs and the health system providers [70]. Thereafter, following a stepwise approach, another action to accomplish would be to provide technical recommendations to establish criteria for categorizing the risk of vector transmission status in an endemic area. With that information available, it could be possible to explicitly detail under which conditions Chagas disease diagnosis and treatment has to be made mandatory in the area for the entire population, or segments of it (i.e. women at childbearing age, newborns). Finally, a most desired third action would definitely be to integrate the procedures for disease diagnosis and treatment within the health system as a transversal program.

Nowadays there are already a few experiences that have been able to coordinate vector control actions together with diagnosis and treatment in a successful manner [71, 72]. These could serve as proof-of-concept strategies for integral interventions to build on, improve and replicate. As an example, the experience of Fundación Mundo Sano, with direct and uninterrupted action in the field since 2002, is worth mentioning. Its integral program includes vector control and sanitary improvement of rural houses in the Department of General Taboada (Santiago del Estero, Argentina). In 2015, after 13 years of vector surveillance and control, Mundo Sano was able to install two doctors' offices dedicated to the diagnosis and treatment of Chagas disease together with local institutions, one in Añatuya City and another one in Colonia Dora. Since then, they have been struggling with the problems of having a mono-disease health service in a community that did not demand treatment. Despite the difficulties, after almost three years of implementation, the amount of people diagnosed and treated begun to increase. The work from these two Chagas disease specific offices was integrated to the local hospital of Añatuya in 2018, thus reaching a higher amount of *T. cruzi*-infected individuals derived from obstetrics, gynecology and cardiology services in addition to those attending the offices from spontaneous demand. In the same line, the Platform for the Integral Care of Chagas disease Patients was settled in Bolivia in 2009. Initially in the province of Cochabamba in the center of the country, the Platform now works also in municipalities of the provinces of Chuquisaca and Tarija [63]. Each experience, proposal or pilot study must be evaluated objectively in order to improve and multiply its impact, avoiding a future with a new inequality: one were those affected by the

disease will have access to health depending on whether they live in an endemic or non-endemic area.

6. The role of patients' associations

Similarly to what happens with other NTDs programs to promote scaling up of healthcare interventions, strategies towards the control of Chagas disease often confront with structural weaknesses of the health systems in which they aim to be integrated [73–75]. Therefore, more effective strategies to strengthen those health systems are required to ensure a sustainable integration of the required health innovations within them. Importantly, these strategies need to incorporate the patients' perspective in order to overcome key access barriers [13,14,75]. Barriers that nowadays still hinder the access to diagnosis and treatment, barriers impeding a better health care.

In recent years, several associations of Chagas disease patients have emerged and joined in a Federation of Associations whose purposes are: (i) to increase the visibility of the disease at all levels as well as of its overwhelming impact in the patients' health status; and (ii) to promote a more active role of the people affected by the disease in the decision-making processes. But these associations face many difficulties (dispersion of objectives, multiplicity of voices with different requirements, little real mobilization in many countries, and lack of resources) to achieve their objective of having an effective role in the health policies makings of their countries.

The WHO global strategy on integral health services centered on people could be a way through which these associations can play an important practical role [77]. In fact one of the goals of such program is to empower individuals, families and communities by including in it some well-known activities led by patients such as community education, groups of patients for mutual support, expert patients to engage and help others, etc. [77]. In addition, this WHO program goes beyond a single disease and has the virtue of encompassing other actors with decisive roles in health systems performance, like governmental policy-makers, municipalities, donors, and providers.

Overall, the strategy is thus envisaged to allow the community and patients to actively participate in the decision-making process, together with other involved actors, in issues regarding their health. By doing so, they also play a role on the way the health system is organized, for instance ensuring that community and primary healthcare are prioritized. Therefore, patients associations should look after the implementation and

compliance with this WHO strategy as it underlies the activities they are already taking on.

7. The R&D agenda: clinical trials for new drugs or new strategies of treatment with current drugs

7.1. Results from recently completed trials

Upon the questioned efficacy of currently available therapies (BNZ and NFX) against the chronic symptomatic stage of the disease, clinical testing of new drugs and/or of new regimens of BNZ and NFX is being pursued. Nonetheless, some major obstacles are in the way to getting more adequate treatments, including the poor understanding of the infection pathophysiology leading to the disease clinical progression, and the lack of biomarkers to determine prognosis and cure (see review by Pinazo et al., [19]). Particularly the latter hinders the follow-up of treated patients both in the daily clinic and in clinical trials. At present, an absence of molecular amplification of *T. cruzi* DNA from periphery blood is used as surrogate of “treatment success”. But this approach is far from optimal, especially at the chronic stage of the disease when the parasitemia is low and intermittent. A negative result obtained at a determined time point does not exclude the possibility that a positive one will be obtained in the next visit. A false-negative PCR, i.e., a negative PCR result (“treatment success” event) in the patient follow up is then less informative than a positive determination, as this one will indeed define a case of treatment failure.

Despite this limitation, several clinical trials have been completed in the last years, and there are several more currently ongoing. This advance represents a major shift in the research landscape of this historically forgotten NTD. The vast majority of the performed trials have been Phase 2 randomized, multi-centric, double-blinded, efficacy and safety studies to evaluate oral dosage schemes for the treatment of adult patients at indeterminate (asymptomatic) chronic stage (see Table 1). Amongst them, CHAGASAZOL [78], STOP CHAGAS [79] and E1224 [31] have evaluated for the first time two triazole anti-fungal drugs (posaconazole (POS), and the ravuconazole precursor E1224) for their anti-*T. cruzi* properties. In addition, the BENEFIT trial that evaluated the use of BNZ for chronic stage treatment must also be highlighted as it has been the largest Phase 3 Chagas disease trial performed so far [47].

CHAGASAZOL and E1224 trials entailed a direct comparison of POS and E1224 monotherapy at a high and a low dose to a BNZ standard dosage group (5

mg/kg/day orally divided in two daily doses for 60 days) [31,78]. In addition to monotherapy branches, STOP CHAGAS trial also included a study group that received POS and BNZ as combined therapy [79]. In the latter, treatment success was defined as a negative PCR value at the day 180 follow-up visit [79], whereas parasitological cure in CHAGASAZOL was determined by a negative PCR result at 12 months follow-up [78]. The most stringent success criteria was that of E1224 trial, which involved serial negative PCR results (3 negative PCR results from 3 samples collected over 7 days) at 65 days after end of treatment and a further negative PCR outcome at 12 months follow-up [31].

Although both POS and E1224 drugs showed good safety and efficacy profiles at end of treatment in the three studies, the suppressive effect on parasite clearance after 12 months was much reduced in comparison to BNZ, which showed early and sustained efficacy until 12 months of follow-up (Table 1). Thus, neither POS nor E1224 were as good as BNZ and could not substitute it as monotherapies.

In the BENEFIT trial, the treatment response was evaluated in a sub-group of 1,896 subjects who had PCR results at baseline (60.5% of them were positive) by PCR conversion at the end of treatment, and at two and five years post-treatment [47]. Being a large multi-national study, the PCR conversion rates varied geographically with Bolivia and Argentina showing the best results, though it must be noticed that such conversion did not correlate with clinical outcome [47]. BENEFIT was devised to study whether BNZ administration to patients at the chronic stage of the disease would widely have a clinical benefit for them [22,47]. Sadly, its conclusions, far from reassuring the role of BNZ turned out to be a setback, as they suggested not-to treat patients with advanced cardiac disease [47]. As it has been criticized elsewhere, this devastating conclusion drains from the fact that many subjects with advanced cardiac involvement were enrolled in the study [3,22]. Despite its negative outcome due to a rather questionable study protocol design [22], the BENEFIT trial involved ample cooperation between multiple study sites opening the door to the performance of multi-national Phase 3 Chagas disease trials in South America [47].

7.2. Ongoing clinical trials of anti-*T. cruzi* drugs

There are now some active and/or ongoing clinical trials. Some test alternative BNZ dosing regimens to reduce exposure, improve tolerability and maintain efficacy as described elsewhere [52], based also on the description of BNZ pharmacokinetics (PK)

in Chagas disease adult patients [80]. In others the evaluation of NFX has gained prominence under the promotion of Bayer, NFX producer. The use of fexinidazole, a nitroimidazole drug like BNZ that is being trialed for human African trypanosomiasis and leishmaniasis (respectively caused by *T. cruzi* closely related Kinetoplastid parasites *T. brucei gambiense* and *T. brucei rhodensiense*, and by *Leishmania* spp.), and the antiarrhythmic drug amiodarone are also being clinically assessed for the first time against *T. cruzi* based on their anti-parasitic capacities [81,82]. These ongoing trials range from smaller Phase 1 to larger Phase 2 and 3 efficacy studies. There are: (1) a Phase 1 safety, tolerability and bioavailability assessment of new NFX tablets and another Phase 1 study to determine NFX PK in relation to dietary habits; (2) a Phase 2 study to evaluate different BNZ regimens (MULTIBENZ); (3) another Phase 2 study to evaluate reduced and intermittent BNZ regimens, either given alone or in combination with E1224 (BENDITA); (4) two more Phase 2 studies evaluating fexinidazole to respectively determine dosing regimens and the minimal efficacious and safety dose; (5) a Phase 2-3 trial to compare safety and efficacy of NFX and BNZ (EQUITY); (6) a Phase 3 study to assess amiodarone, a commonly used antiarrhythmic with selective anti-*T. cruzi* properties, administered over 6 months to individuals with mild-to-moderate Chagas cardiomyopathy (ATTACH); (7) a Phase 3 study of a pediatric formulation of NFX (CHICO); and (8) a very recently added Phase 3 study to evaluate a short dose of BNZ in child-bearing age women (BETTY) (Table 2).

All Phase 2 or above trials in Table 2 but CHICO involve the evaluation of drugs in chronically infected adult patients, and the measurement of treatment success will rely on molecular detection of the parasite DNA. In contrast, in CHICO, designed to assess NFX performance in Chagas disease infant population, the way to measure cure is seroconversion a year after treatment because this is much easier to timely occur in children than in adults and its readout is less ambiguous than the PCR output. All these studies are currently in the recruiting stage, except FEXI NCT02498782 and CHICO which are active but not recruiting, and BETTY that is not yet recruiting (<https://clinicaltrials.gov>). Regarding the latter, benefits of treating *T. cruzi*-infected women before pregnancy have been described by several smaller studies [23–26]. Hopefully BETTY's outcome will serve to enforce treatment administration to all child-bearing age women as soon as possible.

8. Conclusion

Increasing the awareness on the prevalence and health impact of Chagas disease among patients, health practitioners and political health authorities may eventually translate to enhanced population-based diagnostic screening and treatment interventions. The tools needed to facilitate this activity are being tested currently in the field and incorporate more practical and point-of-care diagnostics, new drugs regimens, and the standardization of daily clinical care routines. In addition, efforts to obtain biomarkers of disease prognosis and early assessment of treatment are also being pursued and will probably yield results soon.

Upon failure of azoles, the majority of presently ongoing Chagas disease clinical trials either evaluate alternative regimens of current drugs or other chemical entities distinct from azoles (e.g., fexinidazole, amiodarone) with the aim to identify dosages with lower toxicity and at least equal efficacies compared to present chemotherapy with either benznidazole or nifurtimox. There is certain optimism on that a new treatment for Chagas disease can be scheduled in the next few years. Further complementing the new anti-parasitic drugs is a new therapeutic biologic, a vaccine, now advancing to the clinic. However, access to these new drugs and innovations will require enhanced policies and advocacy activities. In parallel, it will be essential to shape and disseminate pharmacovigilance protocols so that the drugs performance can be closely monitored, and patients' and healthcare providers' confidence on these therapies is promoted and maintained.

The functional deployment of counter-reference circuits fully integrated in the national health services will be required to scale up the attention to Chagas disease patients. In this regards, improved coordination between vector control authorities and sanitary authorities needs strengthening in those regions with active vector transmission of the infection. Widespread access to diagnosis and treatment will not yield the desired outcome unless chances to get re-infected are minimized or eliminated. This approach also requires maintaining blood screening and programs to stop congenital transmission. An improved maternal diagnosis and treatment protocol would in itself reduce congenital transmission.

Despite recently achieved advancements, Chagas disease management and control is still a huge challenge. Therefore, to succeed in this matter it will be paramount the continued involvement of key actors, including patients associations, health authorities at regional and national levels, governmental and non-governmental institutions, basic and clinical researchers, and of course financial partners.

9. Expert opinion

A huge drawback in the management of Chagas disease has traditionally been the lack of awareness of the disease and its impact by both health professionals and the patients themselves, as well as by governmental institutions with the power to dictate health policies. However, Chagas disease awareness is gradually increasing, so that efforts to combat this neglected tropical disease now need to shift in order to address its often insidious onset and silent clinical progression. These features currently complicate the access to timely diagnosis and treatment, which is also ballasted by the very limited resources available for research and development of improved treatment and disease prevention methodologies.

After so many years of neglecting Chagas disease, new efforts at R&D have the potential for high returns on investment. However, the R&D needs for Chagas disease are pervasive and span requirements for both basic and applied research, as well as new drugs, diagnostics and vaccines. With the ultimate goal of controlling the disease in endemic and non-endemic regions, the consequences of widening our understanding on Chagas disease and the pathogen that causes it will have an impact on the lives of millions of people. For instance, research on parasite-host interactions is needed to fully comprehend the pathogenic processes that lead to the life-threatening symptomatology characteristic of chronic *T. cruzi* infections. Furthermore, considering that in the absence of treatment ~30% of those chronically infected will develop cardiac and/or digestive tract disruptions, studies on both parasite and host genomics (and other omics) are required to determine the key factors leading to the development of pathogenesis. Deeper understanding of the parasite biology and of its interactions with the host is fundamental for the discovery of safer drugs or vaccines. Another challenge is the dearth of public policies and advocacy that so far has mostly failed to attract requisite funding.

Currently the most urgent needs include an expansion in clinical studies to test an enlarged portfolio of new drugs, together with improved biomarkers to monitor disease progress. There is also urgency for inexpensive and accessible point-of-care diagnostics, especially for mass screenings as well as for the early assessment of treatment responses. Their availability will widen access to treatment because they would introduce a more accurate picture of the disease epidemiology, as well as the ability to acknowledge cure upon treatment.

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Declaration of interest

J Alonso-Padilla holds a Juan de la Cierva – Incorporación contract from the Spanish Science Ministry and is member of the NHEPACHA network. J Gascon is Director of the Chagas Initiative at ISGLOBAL and Head of the Tropical Medicine Service at Hospital Clinic (Barcelona, Spain). J Gascon currently coordinates NHEPACHA network and is member of the scientific committees of CEADES (Bolivia) and ISGLOBAL. M J Pinazo and S Sosa-Estani are members of the NHEPACHA scientific network. M E Botazzi and P J Hotez are patent-holders and lead investigators of a program for the development of a therapeutic Chagas disease vaccine. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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Table and Figure Legends

Table 1. List of the most relevant anti-*T. cruzi* clinical trials recently completed.

Table 2. List of currently ongoing clinical trials of anti-*T. cruzi* drugs.

Figure 1: Timescale scheme of Chagas disease diagnosis and clinical status progression.

Figure 2: Comparison of the respective characteristics and requirements (in boxes) of the vertical and horizontal strategies to implement Chagas disease healthcare.

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Table 1. List of the most relevant anti-*T. cruzi* clinical trials recently completed.

Name	NCT number ¹	Drug tested	No. Patients	Outcome ²	Ref.
CHAGASAZOL	01162967	POS	78	>80% (POS) 38.4% (BNZ)	[78]
STOP CHAGAS	01377480	POS POS-BNZ	120	>86% (POS) 14.3% (BNZ) 20% (POS+BNZ)	[79]
E1224	01489228	E1224	231	≥81% (E1224) 18% (BNZ)	[31]
BENEFIT	00123916	BNZ	2854 (1896) ³	44.6% (BNZ) ⁴	[47]

¹Clinical trial identification number (check it out at <https://clinicaltrials.gov>).

²Study outcome shown as treatment failure measured by PCR; STOP CHAGAS entry also includes the failure rate in the POS+BNZ group.

^{3,4}Number in parenthesis stands for the number of patients with a PCR result at baseline of which 60.5% were positive. The outcome shown indicates the % of initially PCR positive patients who were reported PCR positive again at two years.

Table 2. List of currently ongoing clinical trials of anti-*T. cruzi* drugs.

Name	NCT number ¹	Drug tested	Study type	No. Patients (estimated)	Promoter
-	03350295	NFX	Phase 1	48	Bayer
-	03334838	NFX	Phase 1	36	Bayer
MULTIBENZ	03191162	BNZ	Phase 2	240	Hospital Vall d'Hebron (Spain)
BENDITA	03378661	BNZ and E1224	Phase 2	210	DNDi
-	02498782	FEXI	Phase 2	140	DNDi
-	03587766			45	
EQUITY	02369978	NFX and BNZ	Phase 2-3	500	Autonomous University of Bucaramanga (Colombia)
ATTACH	03193749	AMD	Phase 3	200	Fundación Cardioinfantil - Instituto de Cardiología de Bogotá (Colombia)
CHICO	02625974	NFX	Phase 3	330	Bayer
BETTY	03672487	BNZ	Phase 3	600	Tulane University (USA)

¹Clinical trial identification number (check it out at <https://clinicaltrials.gov>).

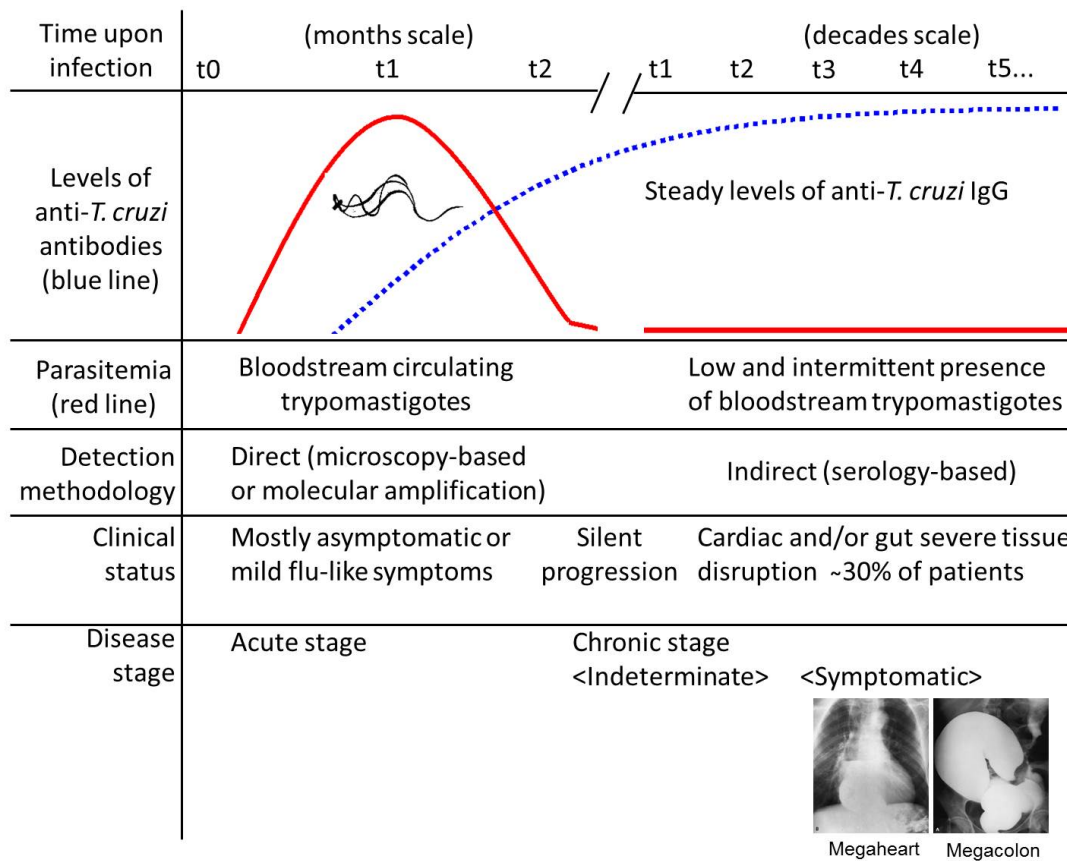


Figure 1

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**Specialized centres:
Vertical strategy**

- Protocolized attention: high complexity
- 2nd level healthcare centres
- Limited human resources
- Urban and rural areas
- Prototype (Project)
- Highly effective
- Activities included: prevention, diagnosis, treatment, IEC, training and RESEARCH
- External funds are needed

vs

**Chagas national network:
Horizontal strategy**

- Simplified protocolized attention
- **All healthcare levels integrated**
→ including primary healthcare
- Human resources of National Health System
- Urban and **rural areas**
- Model accepted by the National Health System
- Activities included: prevention, diagnosis, treatment, IEC, and training
- Depends on national funds

Figure 2

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