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Title: Congenital Chagas disease: current diagnostics, limitations and future perspectives

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Abstract

Purpose of review

Congenital transmission is an important route of *Trypanosoma cruzi* infection, both in Latin America and internationally, with considerable populations of infected women of child-bearing age residing in the United States and Europe. This review examines recent literature on congenital Chagas disease, with a focus on the changing clinical spectrum and potential new diagnostic tools.

Recent findings

Vertical transmission occurs in approximately 5 to 10% of births from *T. cruzi*-infected mothers. Historically, congenital Chagas disease was associated with high levels of neonatal morbidity and mortality. Bolivian birth cohort data from the early 1990s to the present indicate that the incidence of symptomatic neonatal disease has declined. Treatment with trypanocides is >90% effective and well tolerated in infants. Current programs face challenges from the multi-step screening algorithm, low sensitivity of microscopy and high loss to follow-up.

Summary

Congenital Chagas disease remains an important contributor to the global disease burden due to *T. cruzi*. PCR and related molecular techniques represent the most sensitive diagnostic modalities for early detection but require further optimisation for resource-limited settings. Several novel diagnostic tests show promise for the future but further validation and adaptation to field settings are needed.

Keywords

Chagas disease, congenital, *Trypanosoma cruzi*, diagnostics

Introduction

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi* and transmitted in the faeces of infected triatomine bugs, is pervasive across 21 Latin American countries, affecting 5 to 6 million individuals, with a further 70 million at risk [1]. As successful regional control initiatives reduced vectorial transmission and introduced close to universal screening of blood donations, congenital infections have become proportionally more important, now accounting for 22% of new cases in Latin America [1]. Congenital transmission also occurs outside of the endemic areas of Mexico, Central and South America. An estimated 300,000 infected persons reside in the United States, including approximately 40,000 women of childbearing age [2]. Only two cases of acute congenital Chagas disease have been reported in the United States [3, 4]. However, 60 to 315 infections are expected to occur annually, but will be undetected in the absence of screening [5]. An estimated 42,000 infected individuals reside in Spain, and in some regions, 10% of reproductive-age women are from Latin America [6, 7]. *T. cruzi*-infected infants are detected regularly in screening programs in Spain, and cases have been reported from Italy, France and Switzerland [6, 8-11].

Following *T. cruzi* exposure, infection begins with an acute phase, lasting up to three months, during which trypomastigotes are microscopically detectable in peripheral blood (Figure 1). Most individuals are asymptomatic or present with a non-specific, self-limited febrile illness [12]. Mortality in the acute phase is rare (<1% of cases), occurring most frequently in infants and immunocompromised patients, usually from severe myocarditis, pericardial effusion and/or meningoencephalitis [13, 14]. Chronic infection is initially asymptomatic, with the majority of individuals remaining clinically indeterminate for life. However, over a period of decades, 20-30% of chronic *T. cruzi*-infected patients develop irreversible, potentially fatal cardiomyopathy and/or gastrointestinal disease. The reported frequency of clinical outcomes, in particular digestive disease, varies considerably between geographical regions; this variation has long been attributed to genetic differences between major circulating parasite strains [15, 16].

Congenital Trypanosoma cruzi transmission

In the absence of treatment, women with vector-borne infection remain at risk of vertical transmission throughout their child-bearing years. Congenitally-infected women can also transmit to their children, sustaining the cycle across generations and perpetuating the infection in non-endemic countries. One major unanswered question in Chagas disease research is why vertical transmission is restricted to a small, but variable proportion of infected mothers. Reported congenital transmission rates range from virtually absent in some studies to more than 15% of births in others [17]. Some variability is attributable to differences in study design. Data from prospective and retrospective observational studies as well as case reports are often interpreted interchangeably. Completeness of detection, and therefore measured incidence, will differ based on the number, type and timing of infant specimens, sensitivity of diagnostic methods and rates of follow-up at 9 months or later [17, 18]. Nevertheless, factors shown to be associated with higher risk of congenital transmission include younger maternal age (believed to reflect more recent infection) [19, 20], maternal and neonatal immunological responses [21, 22], higher maternal parasitaemia [19, 23], twin births [19], and HIV co-infection [24, 25]. In some studies, absence of vector exposure was associated with higher risk of vertical transmission [19, 26], while others suggest the reverse [27]. Familial clustering has been reported, with mothers of one congenitally infected child being significantly more likely to transmit to that child's siblings than mothers who had not previously transmitted, a finding that could be related to the parasite strain or to maternal host factors [26].

Current evidence for the influence of parasite genetics is anecdotal [15]. *T. cruzi* is highly diverse and currently recognised as a complex of seven major lineages (TcI-TcVI and TcBat), with discrete but overlapping distributions [16, 28, 29]. In general, TcI, TcII, TcV and TcVI are responsible for the majority of human infections; TcI is the principal cause of Chagas disease in Mexico, Central America and northern South America [30-32], while TcII, TcV and TcVI are more frequently isolated from domestic transmission cycles in southern South America [33]. The majority of congenital genotyping studies have been conducted in southern endemic regions, particularly Argentina, Bolivia and Chile, and in general, congenital infections mirror the distribution of TcII/V/VI genotypes detected among local chronic adult populations [15]. A retrospective analysis suggested that the rate of congenital transmission in southern Brazil (presumed TcV predominant) was higher than in central Brazil (presumed TcII predominant) [34]. A recent multi-centre study in Argentina, Honduras and Mexico reported a predominance of non-TcI maternal infections associated with congenital transmission [35]. To date, there is a paucity of studies from northern South and Central America. Additional data are warranted from regions of domestic TcI transmission to determine whether rates of congenital Chagas disease do indeed differ by parasite lineage.

Clinical manifestations of congenital Chagas disease

Historically, congenital Chagas disease was associated with frequent morbidity, ranging from low birthweight to meningoencephalitis, myocarditis, anaemia, thrombocytopenia and respiratory distress syndrome, leading to high mortality rates, both *in utero* and during the neonatal period [20, 36]. In a comparison of congenitally infected infants born in 1992-1994 with those born in 1999-2001, the prevalence of low birthweight, Apgar scores <7 and prematurity fell from 50% to 18%, 40% to 5% and 30% to 10%, respectively, while mortality fell from >40% to <5% [20]. In the same two birth cohorts, the frequency of low birthweight, low Apgar scores, mortality and other clinical signs also fell significantly among uninfected infants, regardless of maternal infection status, suggesting that improvements in prognosis may have been related, at least in part, to advances in prenatal care and nutrition [20]. In a later Bolivian cohort of births from 2010-2014, 22% of *T. cruzi*-infected singletons had low birthweight compared to 8% of infants of uninfected mothers [18]. Twenty-nine percent of infected infants had one or more manifestations consistent with congenital Chagas disease; no severe morbidity or mortality due to *T. cruzi* infection was observed. In *T. cruzi*-infected infants born between 2007 and 2016 to Bolivian women residing in Spain, the rate of symptomatic disease was 18.8% [6]. Symptomatic infants have significantly higher parasite loads, compared to their asymptomatic counterparts [18, 37, 38]. Congenital infection is generally assumed to carry similar long-term risk of cardiac disease as vector-borne cases, but direct data to support this assumption are lacking.

Congenital Chagas disease screening and diagnosis

Early diagnosis of congenital Chagas disease is impeded by many biological and operational constraints. Because the majority of infected infants are asymptomatic or have non-specific signs, they are unlikely to be identified without laboratory screening for *T. cruzi* infection. Comprehensive detection relies on a complex, multistep algorithm, beginning with maternal serological screening, followed by direct parasitological detection in the infant in the first months of life ("micromethod") [39], and IgG serology after 9 months of age (once maternal antibodies disappear) (Table 1 and Figure 2).

The micromethod consists of concentration of infant blood in capillary tubes by centrifugation followed by microscopic examination of the buffy coat [39]. Although the most widely used test for early detection in Latin America, micromethod fails to detect more than half of infections [18, 40, 41]. This technique is

susceptible to differences in specimen quality and operator proficiency, with sensitivity declining with delayed sample processing; trypomastigotes become less motile over time and therefore harder to observe. However, the micromethod has the important advantage of affording unequivocal diagnosis, enabling the initiation of immediate treatment before mother and child leave the hospital. In addition, the high parasitemia associated with symptomatic congenital Chagas disease results in substantially higher sensitivity of micromethod in this subset of babies compared to asymptomatic infected infants [18].

PCR has emerged as a promising, highly sensitive diagnostic test, particularly important in cases with parasite burdens below the threshold of microscopic detection. However, multiple infant specimens are required to maximize sensitivity [18]. A number of PCR assays are now available to detect congenital *T. cruzi* infections, including quantitative PCR [40] and a recent loop-mediated isothermal amplification (LAMP) protocol [51]. Molecular techniques require considerable equipment, expertise and rigorous quality control, which are difficult to implement and sustain in resource-limited settings. The LAMP assay is intended to mitigate some of the infrastructure requirements, but to date remains experimental and awaiting further validation. In addition, false-positive PCR results have been reported among a small proportion (<1%) of uninfected infants, due to transplacental transfer and transient persistence of maternal parasite DNA [18, 40].

The principal shortcoming of conventional IgG serological assays is the approximately 9 month delay in diagnosis, with resulting low rates of follow-up. Depending on the sensitivity of the test, maternal IgG falls below detectable levels in infant serum from 6 to 10 months after birth [18, 40]. Programmatic participant losses for the 9-month specimen exceed 80% in endemic regions [42, 43]. Thus, alternative assays have focused on identification of earlier serological markers. IgM Western immunoblots and IgG ELISAs have both been developed to react to shed acute phase antigen (SAPA), indicative of an acute infection. The IgM trypomastigote secreted-excreted antigens (TESA) -blot detects SAPA within the first three months of infection and has higher sensitivity than micromethod but lower sensitivity than PCR [18, 40]. The IgG SAPA-ELISA utilizes recombinant antigens but otherwise relies on pre-existing equipment used for routine serological diagnoses [48, 49]. However, in the first 3 months of life, the maternal SAPA-ELISA optical density values must be subtracted from the infant values to account for transferred IgG; after 3 months of age, infant SAPA-ELISA results are said to be interpretable without reference to the maternal results [48]. The IgM TESA-blot suffers from issues of reproducibility and subjectivity; strips must be produced in-house, requiring high quality control of standardization between batches and laboratories [18]. The IgG SAPA-ELISA is reported to be more field-friendly, but rigorous data are not yet available in the published literature. Finally, a novel experimental assay based on concentration and detection of parasite antigens in neonatal urine using hydrogel nanoparticles may represent a viable, non-invasive early alternative, if it can be adapted for use in endemic regions and produced for an affordable cost [52].

Congenital Chagas disease treatment

Treatment with trypanocidal compounds during early infancy is very well tolerated and effectiveness is reported to be >90% in the first year of life [6, 53, 54]. The standard recommended regimen of benznidazole, the first-line drug, is 5 to 7.5 mg/kg/day in two divided doses for 60 days. Observational data from Bolivia suggest that at least for neonates, 30 days may be equally effective [55].

Benznidazole was approved by the US Food and Drug Administration in August 2017 and became commercially available in the United States as of May 14, 2018. The approval is for treatment of Chagas disease in children ages 2 to 12 years; use for other age groups is off-label. The drug is provided in 12.5

and 100 mg tablets; infants are treated by dissolving the drug in water prior to administration. The appropriate weight of the drug can be separated and macerated by a compounding pharmacy in envelopes for each dose. Prescriptions require submission of a completed Fast Access order form, available at http://www.benznidazoletablets.com/ or by contacting Foundation Care: Phone: 877-303-7181; Fax: 877-620-2849; Email: FastAccess@Exeltis.com. Nifurtimox is not approved by the FDA but can be obtained from the Centers for Disease Control and Prevention (CDC) and used under an investigational protocol. Consultations and drug requests should be addressed to the Division of Parasitic Diseases Public Inquiries line (404-718-4745; email parasites@cdc.gov), the CDC Drug Service (404-639-3670), and, for emergencies after business hours, on weekends, and on federal holidays, through the CDC Emergency Operations Center (770-488-7100).

Women treated prior to pregnancy are significantly less likely to transmit to their infants [6, 56-58]. Benzinidazole or nifurtimox administration during pregnancy is contraindicated due to lack of safety data. Although current prescribing information recommends against maternal treatment during lactation, a study from Argentina suggests that a minimal amount of drug passes in breast milk and that this restriction may not be necessary [59]. The risk of *T. cruzi* transmission through breast milk is low, and breastfeeding of an uninfected infant by an infected mother is not contraindicated, except in cases of nipple bleeding, or acute or reactivated infection [60].

Conclusions

Congenital *T. cruzi* transmission remains an important source of new infections, both in Latin America and wherever Latin American women reside. Screening currently requires a multistep diagnostic algorithm, leading to high loss to follow-up and low rates of detection. The ideal congenital Chagas disease diagnostic test would have high sensitivity in a single specimen, preferably at birth, and yield definitive results within a few hours, before mother and baby have left the health care facility. This would obviate the need for 9 months of monitoring and expedite timely initiation of treatment. This urgent need is widely recognized in the Chagas disease research community, with Latin American experts ranking a point-of-care test for congenital infection as the top diagnostic priority [61]. However, until such a test is developed, efforts should be focused on incorporating *T. cruzi* infection screening into routine prenatal care and well baby visits for populations at risk. In non-endemic settings, medical educational programs to raise awareness and knowledge of congenital Chagas disease are needed, especially for obstetricians and pediatricians [2].

Key points

- Congenital transmission is an important route of *Trypanosoma cruzi* infection, occurring in 5 to 10% of births from infected mothers.
- Approximately 20-30% of infected infants are born with low birth weight or other clinical signs, but most are asymptomatic, requiring specific laboratory screening for diagnosis.
- Trypanocidal treatment of infants is highly effective and well tolerated, making early detection an important priority.
- Current screening programs are impeded by the multi-step screening algorithm, low sensitivity of microscopy and high loss to follow-up.
- PCR is the most sensitive diagnostic test for early detection, and several novel experimental assays have been developed, but adaptation to primary care settings is needed.

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Figure legends

Figure 1. The trypomastigote form of *Trypanosoma cruzi* in a peripheral blood smear stained with Giemsa. Photo credit: Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention. https://www.cdc.gov/dpdx/trypanosomiasisamerican/index.html

Figure 2. Optimal diagnostic algorithm for congenital Chagas disease, based on currently available assays.