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Technical Report

Guidelines for *Trypanosoma cruzi*-HIV Co-infection and other Immunosuppressive Conditions: Diagnosis, Treatment, Monitoring, and Implementation from the International Network of Care and Studies – 2023

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PRESENTATION

Since the Brazilian Ministry of Health's 1992 revision to the case definition of acquired immunodeficiency syndrome (AIDS), there has been scientific evidence showing that certain endemic infectious and parasitic processes, such as Chagas disease, visceral leishmaniasis, and paracoccidioidomycosis, can induce opportunistic behaviour in individuals with advanced Human Immunodeficiency Virus (HIV). This situation requires distinct clinical protocols within the healthcare network and epidemiological surveillance measures. These actions aim to incorporate clinical events as indications of AIDS.

At the São Luiz-MA Congress of the Brazilian Society of Tropical Medicine in 2000, recommendations were given for the co-infection of *Trypanosoma cruzi* and HIV. These included defining the reactivation of Chagas disease as a definitive AIDS condition and making notification of reactivation cases compulsory. The creation of a working group to establish specific criteria for reactivation, constitution of a national network to provide care and study co-infection and reactivation, development of a manual for conduct in co-infection and reactivation, and formation of a national hemovigilance network dedicated to co-infection were discussed. This meeting served as a reference point for all future discussions in this field.

At the meetings of the Advisory Committee on Epidemiology of the National Department of STD/AIDS and Viral Hepatitis in 2003, the re-evaluation of the definition of AIDS cases in Brazil took place. It was considered whether to include endemic diseases in the list of defining diseases. Chagas disease reactivation was ultimately added to the list and was officially recognized as a defining condition of AIDS for compulsory notification in January 2004.

This initiative had an immediate international impact beyond the Brazilian context. At a Washington-based meeting in October 2005 to discuss the Clinical Staging of HIV Infection and Case Definitions of AIDS and HIV Infection for Epidemiological Surveillance Purposes, both the World Health Organization (WHO) and the Pan American Health Organization (PAHO) advised that "national cohorts or other databases be used to assess whether additional [AIDS-defining] conditions can be included." The only extra condition proposed and accepted at the event was the reactivation of Chagas disease.

In 2005, the Brazilian Consensus on Chagas Disease was published, addressing various topics[1]. One of these was the association between Chagas disease and AIDS, which has become a significant concern for the National Epidemiological Surveillance System. As a result, during the XXI Annual Meeting of Applied Research in Chagas Disease in Uberaba-MG (October 2005), the groups responsible for AIDS and Chagas disease initiated the development of a manual[2]. *Recommendations for Diagnosis, Treatment and Follow-up of Co-infection of T. cruzi and Human Immunodeficiency Virus (HIV)* were discussed, with a focus on creating a structured approach for improved care of co-infected patients and enabling further studies on co-infection[2]. The discussion was center around greater training of human resources and a better clinical, epidemiological and therapeutic understanding of the situation. The Brazilian Network for Care and Studies in Co-infection was originally established for *T. cruzi*/HIV and subsequently expanded to cover other immunosuppressive conditions. It was officially formed during the Meeting of Applied Research in Chagas Disease and Leishmaniasis held in Uberaba, MG in 2006. One of the main aims was to identify diagnostic (**Appendix 1**) and therapeutic centers for co-infection with their agglutinations, records and case notifications (**Appendix 2**). This permits a wider case history to be attained, giving a better comprehension of various aspects of co-infection *T. cruzi*/HIV[3]. The network has now developed into an international initiative, including centers in Argentina, Chile, Bolivia and Spain. Additionally, various activities have been conducted, contributing to notable advances in this field.

The National Department of STI/AIDS and Viral Hepatitis and Technical Group for Chagas Disease/MS, under the guidance of the Health Surveillance Secretariat of the Ministry of Health, held a meeting in 2006. The expanded group of participants, mainly members of the Brazilian Network of Care and Studies in Co-infection *T. cruzi*/HIV, were tasked to finalize the technical manual, which they successfully achieved in the same year[2].

In 2016, the 2nd Brazilian Consensus on Chagas Disease was published during the XXXII Annual Meeting of Applied Research in Chagas Disease, which was held in conjunction with the 53rd Brazilian Congress of Tropical Medicine in 2017, at a gathering of the International Network for Care and Studies in Co-Infection *T. cruzi*/HIV and other immunosuppression conditions (**Appendix 3**). The 2006 update of the Technical Manual on *T. cruzi*/HIV co-infection was necessary to expand its focus beyond this specific ailment and embrace a broader perspective that incorporates other immunodeficiency conditions, instead of just limiting it to immunosuppression. This recommendation was reaffirmed during the XXXIII Annual Meeting of Applied Research in Chagas Disease held at the 54th Brazilian Congress of Tropical Medicine (2018). Furthermore, it was reinforced with the release of the Clinical Protocol and Therapeutic Guidelines (PCDT)[4] for Chagas disease. This task was assumed by the International Network for Care and Studies in Co-infection *T. cruzi*/HIV and other immunosuppressive disorders. The document was finalized in 2023 after numerous meetings at the Brazilian Society of Tropical Medicine congresses and online.

In May 2020, Brazil made progress in monitoring Chagas disease by incorporating the chronic phase as an event of interest for epidemiological surveillance, through the Compulsory Notification of Cases (Ordinance No. 1,061, May 18, 2020). Earlier, only cases in the acute phase were under surveillance as per case notification and included in the National List of Diseases of Compulsory and Immediate Notification. The broadening of surveillance parameters raises the potential for nationwide identification of disease incidence across all stages and clinical presentations.

The reconstruction of these perspectives over more than three decades has widened the possibilities for patients with Chagas disease to have greater access to the care network in the Unified Health System (SUS), taking into account the existing epidemiological changes[5].

INTRODUCTION

Approximately 70 million individuals residing across 21 endemic countries in the Americas are susceptible to *T. cruzi* infection. Globally, an estimated 6-15 million people are infected, with Latin America accounting for the majority of cases. Further, in the Americas alone, more than 12,000 associated fatalities are registered each year. Chagas disease is presently recognized in several countries, including areas that do not commonly experience it, mainly due to human migration[6].

More than a century after its discovery[7], Chagas disease remains an infectious condition categorised as a neglected disease by the World Health Organization (WHO)[8].

In the initial stages of infection, Chagas disease can be cured by antitrypanosomal treatment. However, to prevent disease progression during the chronic phase, specific treatment is also necessary. Without intervention, cardiac changes may develop in up to 30% of individuals with chronic infection, and up to 10% may require treatment for digestive, neurological, or mixed complications[9].

The acute or initial phase lasts from 8 to 12 weeks, and protozoans are typically present in the peripheral blood, usually detected through direct parasitological examination using microscopy. The majority of cases are asymptomatic or exhibit symptoms of low intensity that are non-specific, such as general malaise, fever, and oedema at the site of the bite (known as inoculation chagoma), frequently appearing on the face or extremities. Severe acute illness is rare, occurring in less than 1% of cases and presenting as myocarditis or meningoencephalitis[10]. After symptoms in the acute phase have been resolved, there is a transition to the chronic phase. Direct parasitological examination reveals low sensitivity at this stage and so serological tests are required for diagnosis. Most patients remain asymptomatic during this phase and show normal electrocardiogram readings along with reactive serology[11].

Normal chest, oesophagus, and colon X-rays suggest the presence of the indeterminate clinical form of the disease. This phase usually displays a slow, sustained progression that can last for decades and has a favourable prognosis. Patients who experience clinical symptoms and changes in these complementary tests develop the cardiac and/or digestive clinical form[12].

Treatment of transplant rejection, neoplasms, autoimmune diseases, and immunodeficiency resulting from HIV infection can cause immunosuppression[13] and lead to the reactivation of Chagas disease. This can result in more severe symptoms than those experienced during the acute phase. Early diagnosis and *T. cruzi*-specific treatment can reduce morbidity and mortality. Therefore, it's necessary to enhance the level of suspicion regarding other immunosuppressive conditions that presently affect infected individuals, going beyond the solitary instance of *T. cruzi*/HIV co-infection[14].

EPIDEMIOLOGICAL CONTEXTS

• Chagas disease

Chagas disease remains a neglected tropical disease with high morbidity and mortality rates in Brazil, especially affecting males and those residing in historically endemic areas, crucial for vector transmission[15].

The anthropozoonotic nature of Chagas has led to significant changes in epidemiological patterns across the country[16,17]. Since its identification, significant progress has been made in controlling Chagas disease vectors in endemic regions. Serological surveys conducted in Brazil have demonstrated the absence of Chagas disease among children under five years old, highlighting the considerable impact of vector control. Furthermore, this control has also prevented the transmission of the disease through blood transfusions.

In 2006, the Pan American Health Organization (PAHO) awarded Brazil with the "Certification of the Interruption of the Transmission of Chagas Disease by the main domiciled vector, *Triatoma infestans*, within the scope of the Southern Cone Countries Initiative". This certification was granted due to the successful interruption of Chagas disease transmission. Challenges to controlling the disease still exist, including the danger of vector transmission from local triatomine species capable of colonization, the presence of both wild and domestic reservoirs of *T. cruzi*, regular interaction between people and these environments, remaining pockets of *T. infestans*, and the vulnerability of entomological surveillance efforts in municipalities[18].

The disease burden in Brazil continues to be substantial. An estimated 6,180,000 individuals (4.2%) were infected with *T. cruzi* during 1980–1985, which reduced to 1,900,000 (1.0%) in 2000. In 2010, WHO estimated that 1,156,821 people (0.6%) had the *T. cruzi* infection. The scarcity of data necessitates conducting systematic reviews and meta-analyses based on the available data in Brazil, with the number of infected individuals ranging from 1.9–4.6 million (1.0-2.4% of the population)[19]. According to the 2nd Brazilian Consensus on Chagas Disease, an estimated 1,365,585–3,213,142 individuals were affected by *T. cruzi* in 2020. Of these cases, 819,350–1,927,885 were diagnosed with indeterminate, 409,676–963,943 with cardiac, and 136,559–321,314 with digestive clinical form of chronic Chagas disease[20].

In 2010, the estimated prevalence of *T. cruzi* infection among pregnant women was 1.1%. Out of these, about 35,000 were infected, leading to an average of 600 children born with congenital infection, and a transmission rate of 1.7%. Brazil's transmission rate is lower than the average of 5% observed in other countries of the Southern Cone, such as Argentina, Paraguay, and Bolivia[21].

Epidemiological surveillance data from the Notifiable Diseases Information System (SINAN) shows that acute Chagas disease has been reported in Brazil, particularly in the northern region. Oral transmission has been the most common route of transmission in the past 15 years. Between 2007 and 2021, 3,609 cases of acute Chagas disease occurred in 252 municipalities across Brazil. In 2021, there were 331 confirmed cases of Chagas disease mainly reported in the northern region, with a fatality rate of 1%. However, the burden of mortality related to the disease persisted significantly. The differences observed between regions, especially those with higher morbidity rates in the Central-West and Southeast, indicate socioeconomic inequities and variable patterns of health service access in the SUS. The significant and constant morbidity and mortality burden significantly impacts both Social Security and Social Services. To reduce the burden of morbidity and mortality, particularly in vulnerable territories, it is important to strategically integrate care, surveillance, and control actions for Chagas disease within primary healthcare. This also expands access to diagnosis and antitrypanosomal treatment[22].

Identifying individuals with Chagas disease among HIV-positive patients presents an opportunity to diagnose co-infection based on epidemiological risk and reactivation of the disease. Since 2004, Brazil has included the reactivation of Chagas disease in the list of conditions indicative of AIDS for epidemiological surveillance purposes. This is based on the definitive diagnosis of meningoencephalitis and myocarditis associated with Chagas disease. Abbreviations for technical terms will be explained upon first use. The reactivation of Chagas disease signifies an

aggravation of persistent *T. cruzi* infection, characterised by escalated parasitaemia levels akin to those witnessed in the acute phase. This is due to the immune system's inability to effectively establish mechanisms to control the infection.

This recommendation is highlighted in the Clinical Protocols and Therapeutic Guidelines[4] for both infections and provides a prime opportunity to offer targeted treatments for *T. cruzi* infection. It is important to note that reactivation of the infection can result in significant morbidity and mortality due to its impact on the central nervous system and heart.

Cases of *T. cruzi*/HIV co-infection are currently absent from reports of Chagas disease or AIDS in the SINAN database, except in instances where reactivation of *T. cruzi* infection is the defining factor. Measures to address this issue have been taken by several services and by the Ministry of Health, in the form of estimates. Thus, supposing that 1.3% of individuals with HIV/AIDS would also have a *T. cruzi* co-infection, the estimated total number of those who have *T. cruzi* and HIV co-infection would be 18,553. This calculation considers the 381,793 HIV infections that occurred from 2007 to June 2021 and the 1,045,355 AIDS cases from 1980 to June 2021[23].

It should be noted that the reactivation of Chagas disease, in addition to HIV/AIDS infection, is linked to immune suppression from pharmacological agents necessary in situations such as transplant rejection. Data from the Pan American Health Organization has estimated the prevalence of reactivation in transplants without prophylaxis with antiparasitic medication to be approximately 28% in patients with Chagas disease and immunosuppression. Specifically, the prevalence was found to be 1.8%, 23.3%, 27.3%, and 30.9% in liver, bone marrow, kidney, and heart transplantations, respectively, in comparison to HIV/AIDS infection which has a prevalence of 39.6%[24,25].

• HIV/AIDS infection

At the start of the HIV/AIDS epidemic, patients received diagnosis and treatment at a late stage, resulting in a major compromise to their cellular immunity. The diagnosis was prevalent among individuals with CD4+ T cell counts below 200 cells/mm^3, or even 100 cells/mm^3. Typically, the time elapsed between infection diagnosis and death did not exceed five years[13].

It was determined in a specific year that reactivation of Chagas disease would be a defining situation of AIDS. Cases were managed solely based on the presence of opportunistic diseases or if CD4+ T cell count was 350 cells/mm^3 or lower. Prior to the emergence of the most powerful antiretroviral therapy (ART) in 1998, therapeutic options were limited. Nevertheless, the clinical and epidemiological aspects have transformed after the implementation of highly effective ART. Since 2013, when ART was offered to all individuals with HIV infection, regardless of disease stage and CD4+ T cell counts, with the aim of achieving the 95-95-95 target (95% of cases diagnosed, 95% of those on ART, and 95% with undetectable viral load), there has been a reduction in HIV cases at the initiation of treatment among individuals with significantly weakened immune systems.

The spread of HIV has increased the likelihood of concurrent Chagas disease. This may be due to young patients with Chagas disease migrating to areas with high HIV/AIDS prevalence, thereby contributing to this association. Furthermore, there has been a rise in the number of individuals living with HIV/AIDS in the Amazon region, leading to the region becoming endemic for Chagas disease through oral transmission. According to data from the state epidemiological bulletins and Ministry of Health released at the end of 2022, the State of Amazonas recorded 1,521 new cases of HIV infection between January and July 2022, with 394 diagnoses of AIDS. In a similar vein, the State of Pará displayed an increase (15.5%) in the incidence rate of HIV infection, as opposed to the national incidence rate, which demonstrated a decline from 2021 to 2022[26].

The incidence of immunosuppression arising from transplants, neoplasms, and autoimmune diseases has been rising. This trend is explained by the growing survival rates observed among patients with chronic or malignant illnesses, along with the encouraging progress made in the real of transplant rejection management. Therefore, healthcare professionals must evaluate Chagas disease diagnosis in all patients who undergo immunosuppression treatment, considering the epidemiological information highlighted in the 2nd Brazilian Consensus on Chagas Disease[20].

• Chagas disease and immunosuppression

A systematic review identified 291 cases of *T. cruzi*/HIV co-infection between 1980 and 2010, which may have been underestimated due to the inclusion of only indexed journal articles[27]. In the majority of these cases, clinical presentations were observed during the natural progression of both diseases. Nonetheless, reactivation of Chagas disease disrupts its natural evolution and is only observed in AIDS cases, with an imprecise frequency.

In a study conducted prospectively, encompassing pre- and post-ART periods, the rate of reactivation during extended follow-up was recorded at 20%. However, when reactivation was assessed only in cases that were monitored for several months, this percentage dipped to 9.8%[28].

Reactivation of Chagas can manifest as an intense version of meningoencephalitis, acute myocarditis, or meningoencephalitis in association with myocarditis. However, oligosymptomatic cases may also occur, featuring symptoms or signs of acute infection such as fever, asthenia, anorexia, general malaise, erythema nodosum, panniculitis, hepatosplenomegaly, and lymph node enlargement[29]. A reduction in CD4+ T cell levels must be considered as a risk factor in cases of HIV/AIDS. After opting for ART in all cases of HIV infection and rapidly initiating ART within seven days of diagnosis, it is uncommon to find people with compromised cellular immunity, who are on regular treatment and have no resistance to antiviral agents. This may reduce the number of cases of Chagas disease reactivation in patients co-infected with HIV. However, approximately 30% of patients present with CD4 T cells < 200 cells/mm^3 at the time of diagnosis, with proportions varying from 42% in Ceará to 21% in Acre[29].

CLINICAL PRESENTATIONS OF CHAGAS DISEASE RE-ACTIVATION IN *T. CRUZI*/HIV CO-INFECTION

• Central Nervous System

Chagas disease reactivation in the central nervous system (CNS) presents with unifocal or multifocal necrohemorrhagic meningoencephalitis, as the most common manifestation, in 80% of reported cases. Upon clinical examination, the symptoms and signs of intracranial hypertension, such as headache, vomiting, and sensorium disorders, are predominant and may escalate to coma. In addition, convulsions and focal motor and sensory deficits have been reported. Meningeal signs, as well as cranial nerve and spinal cord involvement, are less common but have also been observed.

Computed tomography (CT) of the skull is the primary imaging modality to diagnose CNS reactivation of Chagas disease due to its high sensitivity in detecting lesions in this area and its greater accessibility. Nonetheless, this test cannot identify the cause of Chagas disease. Upon image analysis, pseudotumoral hypodense lesions, singular or multiple, may affect the subcortical white matter of the cerebral hemisphere, brainstem, and cerebellum. These lesions may or may not induce mass effects. After intravenous contrast injection, lesions may present with or without annular reinforcement.

Magnetic resonance imaging (MRI) and computed tomography are comparable in assessing brain involvement in reactivated Chagas disease. Nevertheless, MRI exhibits superior sensitivity to detect lesions in the cerebellum and brainstem. Lesions found on imaging tests are also indicative of potential cases of toxoplasmosis. In cerebral toxoplasmosis, preferential lesion locations include the thalamus and basal ganglia. However, it is important to also consider other neurological conditions prevalent among patients living with AIDS, such as primary CNS lymphoma, progressive multifocal leukoencephalopathy, tuberculosis and cryptococcosis. Additionally, associations with other microorganisms causing meningoencephalitis have been observed. The diagnosis of CNS reactivation in Chagas disease may also be confused with that of stroke or other encephalopathies seen in patients living with AIDS[30].

Cerebrospinal fluid (CSF) examination is considered the most reliable method for diagnosing chagasic aetiology of lesions that are identified in the CNS using imaging tests. This is because *T. cruzi* is frequently found in its trypomastigote form. It is important to note that technical terms will be explained the first time they are used. Cellular and biochemical data are not specific enough, showing only mild pleocytosis (< 100 cells per mm^3) and an absolute predominance of lymphomononuclear cells. Hyperproteinorrhea can be of small to moderate intensity but may sometimes exceed 1 g/dL, and glycorrhea can be normal or low. In cases involving the CNS, *T. cruzi* is commonly present in the peripheral blood, and parasitaemia should be investigated whenever possible. Molecular biology examinations have been used in isolated cases, which use the Polymerase Chain Reaction (PCR) technique in CSF and have proven positive, despite the absence of parasites in direct examinations. In the past, the lack of standardization and the absence of this technique in the routine of most services limited its use. However, following the approval of commercial kits by regulatory and control agencies, such as the National Health Surveillance Agency (ANVISA) in Brazil, and the widespread use of PCR techniques, molecular biology has emerged as a diagnostic method. It monitors variations in parasitic load and complements or replaces conventional parasitological methods. Nevertheless, these tests are currently unavailable for use in the laboratory networks of the SUS.

The diagnosis of chagasic aetiology can be confirmed by detecting amastigote forms of *T. cruzi* in brain tissue obtained through biopsy, surgical specimens, or necropsy[31].

Patients experiencing Chagas disease reactivation in the nervous system should receive antitrypanosomal treatment for acute infection immediately after diagnosis. The recommended medication is benznidazole, at dosages and timeframes stated in this manual. This approach markedly diminishes mortality when early treatment is administered. In the absence of such treatment, the mortality rate is close to 100% when the CNS is impacted.

• Heart

Reactivation of Chagas disease in the heart is observed in around 30–40% of cases. The precise magnitude of reactivation in the heart cannot easily be determined as the indications are comparable to those illustrated by most immunocompetent patients encountering persistent chagasic heart disease; this leads to heart reactivation typically being underestimated in cases of minor involvement. Consequently, isolated detection of heart reactivation is infrequent[32].

The acute myocarditis presents clinically as reactivation in the heart. This condition is described by various degrees of heart failure symptoms or signs, including tachycardia, dyspnoea, cyanosis, oedema, jugular stasis, pulmonary congestion, hepatomegaly, arrhythmias, and blockages that can be severe in some cases. On occasion, only myocarditis or electrocardiographic alterations are identified via myocardial histopathology examination. Therefore, distinguishing between the reactivation of Chagas disease and the decompensation of chronic Chagas heart disease poses diagnostic challenges. There are also other potential differential diagnoses, including myocarditis as a result of the HIV infection itself, toxoplasmosis, cytomegalovirus, and other viral infections. In addition, potential associations with microorganisms that cause myocarditis must also be considered.

The complementary evaluation included all the tests used in the cardiological evaluation of Chagas heart disease, as presented in the 2nd Brazilian Consensus on Chagas Disease[20]. Nevertheless, these evaluations are non-discriminatory for the identification of reactivation. Deterioration of the clinical presentation or exacerbation of the ancillary tests in comparison to those formerly known, along with positive parasitaemia, indicates reactivation. Endomyocardial biopsy is crucial for diagnosing reactivation of *T. cruzi* when nests of amastigote forms are detected in associated material. However, in the case of immunocompetent individuals with chronic chagasic heart disease[33], limited quantities of amastigote forms can also be present in the myocardium. It is important to note that a negative biopsy result does not entirely exclude the possibility of reactivation due to limitations in tissue sample size and number. Similar to diagnosing reactivation in the CNS, applying real-time PCR techniques to biopsy myocardial tissue samples can enhance the diagnostic sensitivity for reactivation in the heart[34].

Once diagnosing Chagas disease reactivation in the heart is confirmed, immediate specific treatment for *T. cruzi* infection is necessary, similar to the approach for CNS reactivation. Considering the difficulties in establishing a definitive diagnosis and the limitations of studies following these cases of co-infection and reactivation in the heart, the results of this treatment cannot be stated in relation to the evolution of heart disease, as is already known for reactivation in the CNS. Similar to the treatment during the acute phase in immunocompetent patients with reactivation in the heart and nervous system, administering specific treatment is crucial for managing the clinical condition of patients and preventing fatality[35].

• Other clinical presentations

Rare cases of reactivated peritonitis, cervicitis, pericarditis, pleuritis, and erythema nodosum have been documented in literature. Diagnosis is based on the detection of parasites in the lesions at these sites, as this does not happen naturally during the chronic phase of Chagas disease. Prompt treatment for *T. cruzi* infections should be given straight away after diagnosis, as is recommended for the nervous system and heart.

• *T. cruzi*/HIV co-infection in pregnant women

Pregnancy causes immunosuppression as a protective measure for the conceptus, through the development of a Th2 response. A study comparing the immune response of mothers of *T. cruzi*-infected children to that of mothers of uninfected children revealed low levels of IFNγ and TNFα and high levels of IL10 when undergoing antigenic stimulation. However, infected mothers with detectable parasitaemia exhibited an increase in IFNγ and TNFα levels compared to their uninfected counterparts[36].

In pregnant women co-infected with *T. cruzi*, the rate of congenital transmission exceeds 50% of all reported cases. High parasitaemia in these mothers may be linked to the reactivation of Chagas disease, resulting in severe cases of meningoencephalitis, myocarditis, and disseminated disease in new-borns and high mortality rates[37]. Co-infected pregnant women should receive high-risk prenatal care in addition to the general care observed in these services. It is recommended that they undergo a direct search for *T. cruzi* in the peripheral blood, especially during the period of infectious disease clinics, primarily when fever is present[38].

The objective is to identify an increase in parasitaemia, with or without clinical reactivation. Elevated parasitaemia in pregnant women is associated with greater risk of vertical transmission of the parasite, as well as increased morbidity and mortality in the foetus or new-born. Upon identifying high parasitaemia or clinical reactivation, the employment of benznidazole as an antiparasitic treatment during pregnancy remains a controversial discussion. The drug is contraindicated for pregnant women due to the potential teratogenic effect it may have on the fetus[39]. Nonetheless, a report has outlined a case where a pregnant woman with CNS reactivation recovered after receiving medication and did not encounter any issues with the newborn. Other reports have documented accidental exposure of pregnant women to benznidazole without foetal harm. Therefore, in these cases, the risk *vs.* benefit must be assessed as conducting a clinical trial on pregnant women presents several ethical and safety limitations for the foetus. Generally, the doses and treatment times were the same during the acute phase. While breastfeeding is recommended for mothers with chronic Chagas disease, it is advisable to consider suspension in cases of *T. cruzi*/HIV co-infection due to the added risk of transmitting HIV infection to the infant[40].

The newborn exposed to Chagas disease must undergo direct testing for *T. cruzi* in either the umbilical cord or peripheral blood to assess the potential for vertical transmission. Appropriate testing methods include direct testing, thick drop, microhematocrit, and Strout or Quantitative Buffy Coat (QBC). For newborns, microhematocrit should be preferred due to the small amount of blood required for examination. The use of PCR as a diagnostic tool warrants evaluation as it is a sensitive and prompt method compared to other indirect parasitological methods. It is advisable to perform PCR on the peripheral blood of the neonate to prevent contamination from maternal blood during the collection process[41].

Serology should be conducted in infants at 9 months of age via IgG antibody testing, as maternal antibodies are no longer in circulation at this point. In the case that a positive result is obtained and other forms of transmission are eliminated, vertical transmission can be diagnosed[42]. If Chagas disease is confirmed in a new-born, specific treatment consisting of benznidazole at a dose of 10 mg/kg/day for 60 days should be administered[43]. No follow-up trials have been undertaken regarding the progression of Chagas disease in neonates. However, by comparing with the treatment of the acute phase and including cases concerning children, a parasitological cure is predicted in almost 100% of instances, except when the infecting strain manifests resistance to the administered medication. It is essential to adhere to the current guidelines[44] while assessing the vertical transmission of HIV infection.

DIAGNOSIS OF CHAGAS DISEASE IN *T. CRUZI*/HIV CO-INFECTION

• Parasitological diagnosis

Due to the significant parasitaemia linked to the acute phase of Chagas disease, traditional microscopy remains the benchmark technique for detecting this stage of the illness, as well as for reactivation cases in individuals co-infected with HIV or other immunosuppression. The parasite can be readily detected through direct research in peripheral blood, cerebrospinal fluid, or other bodily fluids, utilizing direct fresh research techniques in the buffy coat (QBC), microhematocrit, or Strout, with greater sensitivity attributed to tests based on the concentration of the biological material under analysis. These tests display high sensitivity in the diagnosis of other hemoparasitic diseases[45].

It is important to note that despite negative results from direct studies on the parasite, there remains a possibility of Chagas disease reactivation which warrants repeated studies. It is also worth noting that the positivity of xenodiagnosis, blood culture, and PCR should not be considered as definitive evidence of reactivation, as parasitaemia can be demonstrated through indirect parasitological methods during the chronic phase of the disease in immunocompetent cases. Quantification of *T. cruzi* load through indirect methods can potentially aid in diagnosing reactivation. Therefore, it is important to emphasise the significance of quantitative PCR (qPCR), a complementary method exhibiting superior performance in result interpretation compared to other indirect methods such as xenodiagnosis and blood culture[46].

• Serological diagnosis

Parasitological tests are not particularly sensitive in diagnosing patients with *T. cruzi*/HIV co-infection without reactivation due to low parasitaemia during the chronic phase of Chagas disease (refer to **Appendix 4**). Consequently, the diagnosis was made using serological tests. Performing two tests using different methodological principles, (indirect hemagglutination, immunofluorescence, immunochromatography, or ELISA), or the same provided that the antigenic matrices are different (crude or semi-purified, recombinant, and chimeric recombinant antigens), as is recommended in immunocompetent patients. It is advisable to assess individuals affected by HIV or experiencing immunosuppression, who may be at risk in endemic regions, via serological tests to diagnose Chagas disease. It is worth noting that these tests may be unresponsive in the presence of immunosuppression[47]. Consequently, in suspected cases of Chagas disease occurrence, repeated serological testing through different methods is crucial. The PCR should be carried out whenever viable.

• Molecular diagnosis

Molecular techniques are recommended for detecting and tracking parasitaemia in individuals with chronic Chagas disease who are immunosuppressed. Such techniques serve as a valuable

tool for post-treatment monitoring of *T. cruzi* and diagnosing congenital disease. Additionally, they aid in early detection of contamination in cases of accidental infection[48].

Under conditions of immunosuppression, it is possible to detect reactivation of Chagas disease before symptoms or signs manifest through molecular methods during the prospective monitoring of affected individuals. Therefore, it is crucial to have these methods readily available for sequential and periodic screening of parasitaemia and other indications of reactivation. Molecular methods have high sensitivity and specificity and provide a short turn-around time without the need for exposure to parasites. Qualitative molecular methods must be excluded for reactivation diagnosis. The presence of parasitic DNA in peripheral blood and tissues during the chronic stage of Chagas disease can be demonstrated through this method in immunocompetent cases. The advent of qPCR, however, enables early detection and monitoring of elevated parasite load in peripheral blood and tissues.

Endomyocardial biopsy samples can help systematically and sequentially monitor heart transplant patients, enabling early detection of *T. cruzi* infection reactivation and targeted treatment. In addition, proactive blood sample monitoring in infected recipients has identified parasite circulation. To this aim, quantitative qPCR tests have shown more advantages than direct parasitological tests as they allow for early detection of increased parasitaemia. In recipients without Chagas disease, *T. cruzi* DNA was detected by PCR two months before traditional methods were used to identify the parasite[49].

Longitudinal and cross-sectional studies indicate that the sequential rise in parasitaemia detected by qPCR suggests reactivation. Reactivity thresholds that distinguish between increased parasitaemia and levels indicative of reactivation have not been established, resulting in varying information in the literature. Also, it should be noted that DNA copies do not express live parasites despite some suggestions to the contrary[50].

Reactivation may be linked to a combination of parasite strains from various discrete typing units (DTUs) found in peripheral blood samples of immunocompromised patients, including *T. cruzi*/HIV co-infection cases, in addition to the rise in parasitaemia. The genetic diversity of *T. cruzi* during reactivation is believed to be affected by immunosuppression[51]. Early diagnosis of Chagas disease reactivation in co-infected patients is strongly recommended, along with the initiation of antiparasitic treatment and ART. In addition, when molecular methods are employed to identify the parasite genotype in biological samples from those who have experienced reactivation, prevalence of specific *T. cruzi* strains is identified, indicating a unique neurotropism within different DTUs[52,53].

While genotyping algorithms are applied to characterise parasite isolates from blood samples, additional validation is required for various other sample types in Chagas disease reactivation cases. Hence, the definitive way of diagnosing Chagas disease reactivation in immunosuppressed patients remains identification of parasites by direct microscopy of blood, other biological fluids, or tissues[54].

Although PCR is commonly used in-house, international efforts have been made to standardise and validate the detection and measurement of *T. cruzi* in blood samples from patients with Chagas disease. A PAHO-supported multicenter study established a consensus for quantifying parasite load using qPCR, which was validated using samples from Chagas disease cases in different endemic countries[55]. Currently, the reproducibility of molecular methods poses a challenge and restricts their use to groups with significant expertise in molecular biology and diagnosis. As a consequence, commercial kits need to be developed and validated. However, there has been improvement in this scenario lately, with the emergence of validated kits for qPCR-based molecular diagnosis of Chagas disease. These kits can be used for research purposes or in vitro diagnosis of human samples[56]. Upon registration by ANVISA, these kits can be integrated into the SUS and made accessible at Central Public Health Laboratories (LACENs). Nevertheless, despite advancements, the advantages of molecular tests still require verification through multicenter clinical trials formulated for the diverse reactivation scenarios of Chagas disease in immunosuppressed individuals.

ANATOMOPATHOLOGICAL PRESENTATION OF *T. CRUZI*/HIV CO-INFECTION AND REACTIVATION

Anatomopathological presentations are commonly observed during the natural progression of *T. cruzi*/HIV co-infection. Differences have been noted when Chagas disease reactivates these conditions.

• Central Nervous System

Morphological data have been derived from biopsies, surgical specimens, and necropsies. Macroscopic observations consist of necrosis, typically accompanied by foci of recent haemorrhage. This finding is preferably observed in the white matter of the cerebral hemispheres and can exist in singular or multiple forms. Under microscopic examination, it is noted that inflammation is more pronounced in the brain as compared to the leptomeninges. Predominantly mononuclear exudate and amastigote forms of *T. cruzi* are observed inside macrophages and microglial cells at varying intensities. The presence of these forms is also observed in areas of necrosis and haemorrhage.

• Heart

Cardiac involvement lacks a specific macroscopic anatomopathological substrate that resembles chronic chagasic heart disease. Upon histological examination, the main characteristics comprise a leukocyte exudate, with a mononuclear predominance, associated with parasitism of myocardial fibres by amastigote forms of *T. cruzi*, typically exhibiting high intensity.

The lesions can be diffuse or restricted to heart chambers (atria or ventricles) or segmentary. Alongside acute myocarditis, there is also observed a lesser involvement of the epicardium or endocardium, with the possible detection of trypomastigote forms of *T. cruzi* in the pericardial fluid. Typically, there are indications of prevailing chronic chagasic heart disease, like fibrosis and myocarditis. These indicators are generally present[57,58].

• Other anatomopathological presentations

Trypomastigote forms of *T. cruzi* have been detected in ascites and pleural fluid, as well as in the skin, cervix, duodenum, and stomach. These findings are considered indicative of Chagas disease reactivation.

PREDICTIVE FACTORS FOR CHAGAS DISEASE REACTIVATION IN *T. CRUZI*/HIV CO-INFECTION

High *T. cruzi* parasitaemia, as detected by both direct and indirect quantitative parasitological methods, is the primary predictor of reactivation. In a prospective study, high parasitaemia, identified by quantitative xenodiagnosis with over 20% positive nymphs, was observed as a predictive factor for reactivation in up to 50% of *T. cruzi*/HIV co-infection cases over a five-year follow-up period.

During the early stages of the HIV epidemic, individuals with the infection were administered ART solely after exhibiting clinical symptoms of the disease. Chagas disease reactivation was solely noticed in instances of AIDS diagnosis, usually accompanied by CD4+ T lymphocyte counts lower than 200 cells/mm, highlighting the role of immunosuppression in reactivation. A reduction in CD4+ T cells, a decrease in CD4+/CD8+ ratio, and an increase in viral load can act as cofactors in parasitaemia growth. Co-infection can lead to correlation between parasite copy number and viral load as well as parasite copy number and CD4/CD8 ratio reduction. A study involving 241 patients with co-infection and 60 with reactivation has illustrated that low CD4+ T cell levels at co-infection diagnosis were linked to reactivation. Furthermore, the risk of death by reactivation is associated with low CD4+ T cell levels and male sex. Nevertheless, there is no confirmation of a connection between viral load and reactivation. It is noteworthy that none of the analysed patients were undergoing ART[59].

Therefore, the rise in parasitaemia, associated with a reduction in CD4+ T-cell levels below 200 cells/mm^3, suggests the need for monitoring to detect clinical reactivation of Chagas disease.

For other immunosuppressive conditions, previous research has reported an association between the use of corticosteroids and other immunosuppressive drugs in the treatment of autoimmune diseases with increased parasitaemia and a possible association, without a well-defined causal relationship[60]. In heart transplant scenarios, factors that were identified to be associated with reactivation included the number of rejection episodes, the presence of neoplasms, and the use of mycophenolate mofetil.

The relationship between different *T. cruzi* DTUs (TcI and TcVI) and the reactivation of Chagas disease in the presence of immunosuppression remains contentious in the literature[61].

Increased parasitaemia is the most crucial predictor of reactivation in people with immunosuppression, such as those with *T. cruzi*/HIV co-infection or immunosuppressed individuals. Its monitoring should be carried out highly vigilantly. The decrease in CD4+ T cell levels under 200 cells/mm^3 usually coincides with the rise in parasitaemia, suggesting the necessity to keep tabs on reactivation through constant monitoring. Furthermore, it is vital to monitor the escalation in viral load[62].

TREATMENT OF *T. CRUZI*/HIV CO-INFECTION AND CHAGAS DISEASE REACTIVATION

Patients with co-infection of *T. cruzi* and HIV should receive treatment targeting both diseases. This involves following specific indications for each situation and adhering to the guidelines outlined in the PCDT[4] and in the 2nd Brazilian Consensus on Chagas Disease[20] and applicable guidelines for HIV/AIDS infection, which are similar to those for non-infected cases.

When applying benznidazole to treat Chagas disease in cases of reactivated or un-reactivated *T. cruzi*/HIV co-infection, small case series or case reports show results that are consistent with those of cases without co-infection. In a prospective study of 53 cases of co-infection, eight patients exhibited persistent or high parasitaemia, as diagnosed through semiquantitative xenodiagnosis or rapid evolution of the clinical form of Chagas disease (133.5 months).

In cases of Chagas disease reactivation, with or without clinical symptoms, specific treatment for *T. cruzi* is an imperative. This applies even to immunocompetent individuals in the acute phase. When meningoencephalitis is present, studies show a lethality rate of up to 100% primarily affecting those who received delayed or no treatment. Early treatment has been found to be effective in reducing parasitaemia and providing stability to the clinical picture, as well as controlling tissue damage caused by the presence of parasites.

Upon completing more than 30 days of a specific treatment, patients experience a drop in lethality to approximately 20%[63]. Furthermore, a retrospective analysis of 241 cases revealed that all untreated patients who died as a result of reactivation succumbed within 36 hours of diagnosis, including 60 reactivation cases. Of the 18 patients who received treatment, 5.6% died within the first day after diagnosis, 11.1% within 7 days, 33.6% within 15 days, and 72.2% within 30 days after diagnosis. These findings indicate that complete treatment within 60 days is crucial for improving the prognosis of Chagas disease reactivation in individuals co-infected with *T. cruzi*/HIV. During reactivation, the patient should be admitted for initial treatment, including individuals with non-malignant conditions and those experiencing conditions beyond the CNS and cardiovascular system, due to the need for more rigorous clinical and laboratory observation[64].

In cases of co-infection without reactivation, immunocompetent patients who fail or lack ART treatment and present with CD4+ T cells < 200 cells/mm^3 may require specific treatment for Chagas disease in addition to usual therapy.

Studies examining co-infection treatment with benznidazole have produced conflicting data, potentially due to drug interactions or adverse events when ART is employed. In cases of co-infection treated with benznidazole, with or without reactivation, regardless of whether ART is employed, there have been no published adverse events that are more severe or distinct from those observed in individuals affected by Chagas disease.

• Medication and dosage

Prior to administering benznidazole treatment, the patient underwent a clinical assessment alongside several supplementary tests, consisting of a blood count, alanine aminotransferase (ALT), arginine aminotransferase (AST), alkaline phosphate (FA), urea, and creatinine screening. Once the treatment was complete, the same procedures were repeated to monitor the patient's progress, inclusive of an electrocardiogram, chest radiograph, and echocardiography.

For treatment of Chagas disease, benznidazole (100mg tablets) is prescribed at a dose of 5mg/kg/day for adults and 10mg/kg/day for children, every eight hours, for a minimum of 60 days and possibly extended up to 90 days[20]. The total daily dose should not surpass 300mg. Monitoring after treatment was the same for cases of Chagas disease without co-infection. If a patient is intolerant to benznidazole, the recommended treatment is to use

nifurtimox tablets. The dosage for adults is 10 mg/kg/day, orally, in three daily doses, for 60 days. For children, the dosage is 15 mg/kg/day, orally, in three daily doses, for 60 days. It should be noted that nifurtimox is not currently available on the pharmaceutical market. As a result, if the medication is required, the SVSA Chagas Disease Technical Group should be contacted to request information on how to obtain the drug from the Brazilian Ministry of Health. Azole antifungals such as posaconazole, itraconazole, fluconazole, and ketoconazole are acknowledged as inhibitors of *T. cruzi* parasitaemia and could be employed in specific circumstances, like reactivation of Chagas disease, when the use of benznidazole or nifurtimox is unfeasible due to contraindications, adverse reactions, therapeutic failure or the requirement for intravenous administration, especially in cases of oral incapacitation such as digestive disorders.

• Follow-up after antitrypanosomal treatment

The clinical and laboratory evaluation periods that follow the 2nd Brazilian Consensus on Chagas Disease and the PCDT guidelines should be pursued for cases without reactivation[4,20].

Given co-infection and reactivation, the length of the hospitalization period depends on the clinical evolution of the case. Complementary examinations, as per the guidelines directed by the clinical setting, should be maintained. The following protocol is advised for reactivation of Chagas disease:

1. Direct parasitological tests should be conducted weekly during treatment with benznidazole, until negative.
2. After obtaining negative results, direct parasitological tests should be carried out on a monthly basis until the third and sixth months, and every six months thereafter.
3. If there are signs of acute infection, particularly fever, direct parasitological examinations must be conducted promptly to eliminate the possibility of further Chagas disease reactivation.
4. Patients who are not on ART should receive it as soon as possible. However, treatment with benznidazole should be made for at least three weeks prior ART due to the risk of immune reconstitution syndrome. Starting ART treatment before completing antiparasitic treatment is acceptable, without waiting for its conclusion[13,65].

During treatment of *T. cruzi*, direct examinations of peripheral blood and biological fluid are necessary to identify the parasite, similar to the nervous system and cerebrospinal fluid examinations. It is important to adhere to this approach to ensure accurate diagnosis and monitoring during *T. cruzi* treatment.

For follow-up after treatment, direct study of peripheral blood is recommended, with biological liquids examined only when new symptoms emerge. If direct microscopy shows the presence of *T. cruzi* during follow-up after treatment, it is considered a new reactivation and requires additional treatment with benznidazole or another appropriate therapeutic option. Absence of positive results from direct tests does not necessarily imply a total recovery, and supervision and monitoring should be sustained. qPCR may be employed for this purpose under the same circumstances as direct parasitological inspections[66].

In the management of Chagas disease reactivation, serological tests are not as effective as the cure criteria observed during acute Chagas disease due to the potential for false-negative results resulting from immunosuppression.

PROPHYLAXIS FOR CHAGAS DISEASE REACTIVATION IN *T. CRUZI*/HIV CO-INFECTION

• Primary prophylaxis

Primary prophylaxis is not recommended for the reactivation of *T. cruzi*/HIV co-infection or other forms of immunosuppression.

• Secondary prophylaxis

Patients who have experienced reactivation of Chagas disease due to *T. cruzi*/HIV co-infection and have undergone specific treatment for *T. cruzi* should receive secondary prophylaxis when their CD4+ T lymphocyte levels are below 200 cells/mm^3. This recommendation is similar to that for other opportunistic infections among AIDS patients. Chagas disease can recurrently reactivate in individuals with co-infection. There is a lack of controlled trials investigating this indication. However, documented case reports with varying follow-up periods exist. Patients who did not receive secondary prophylaxis displayed positive progress after undergoing reactivation treatment. This could be attributed to the efficacy of ART, which facilitates immunological reconstitution.

• Medication and dosage

Secondary prophylaxis was administered with benznidazole at a dosage of 2.5 mg/kg/day, every 8 hours, three times a week.

EPIDEMIOLOGICAL SURVEILLANCE OF *T. CRUZI*/HIV CO-INFECTION

Since 1986, healthcare professionals, organizations, and both public and private health establishments have been mandated by law to report cases of AIDS. The Brazilian Ministry of Health recommends and oversees this responsibility, in compliance with Law 6259 of October 30, 1975 and Ordinance GM/MS nº 420 of March 2, 2022.

For the purposes of epidemiological surveillance and notification of *T. cruzi*/HIV co-infection, only cases of Chagas disease reactivation have been taken into account. Thus, it is necessary to obtain a conclusive diagnosis for *T. cruzi* infection through a parasitological method, which involves the direct examination of blood or other bodily fluids and must be associated with the following.

1. Meningoencephalitis can be identified by an image of a brain lesion with a mass effect as seen through computed tomography or nuclear magnetic resonance, with or without contrast injection.
2. Acute myocarditis can be confirmed by electrocardiography and echocardiography if arrhythmias or heart failure are present[67].

Acute phase Chagas disease is a compulsorily notifiable condition according to Ordinance No. and can be found at http://portalsinan.saude.gov.br/doenca-de-chagas-aguda. On May 18th, 2020, 1,061 updated the National Compulsory Notification List of diseases, conditions, and public health events to include chronic Chagas disease. Beginning in 2023, chronic cases will be notified through e-SUS Notifica (https://datasus.saude.gov.br/notifica/).

The primary sources of information for comprehending co-infection are:

1. The healthcare services found across all levels of the SUS network; primary care and reference centers play a crucial role in the referral and counter-referral processes.
2. Laboratories analyse organic fluids for the purpose of a direct investigation aimed at parasitological diagnosis.
3. Blood centers and other haemotherapy services investigate diagnostic suspicion based on either the screening of candidates for blood donation or the results of investigations conducted for hemovigilance purposes[68].- Seroepidemiological surveys and other research modalities.
4. Death certificates.

In addition to reporting cases, other surveillance strategies can be employed, such as the International Network for Care and Studies on *T. cruzi*/HIV Co-infection and other immunosuppressive conditions. The network allows for the monitoring of clinical events through the identification and blend of services dealing with instances of co-infection and reactivation of Chagas disease. This is crucial for addressing several outstanding queries highlighted in this manual. To achieve this objective, a more comprehensive examination of a larger series of cases is required, along with the establishment of protocols, standardization of procedures, and the development of better-controlled studies (contacts attached).

Figure 1 demonstrates recommended practices for managing cases of HIV/AIDS and those with an epidemiological history compatible with *T. cruzi* infection in regards to their conduct and antitrypanosomal treatment. **Figures 2 to 14** depict images captured during the reactivation of Chagas disease in *T. cruzi*/HIV co-infection.

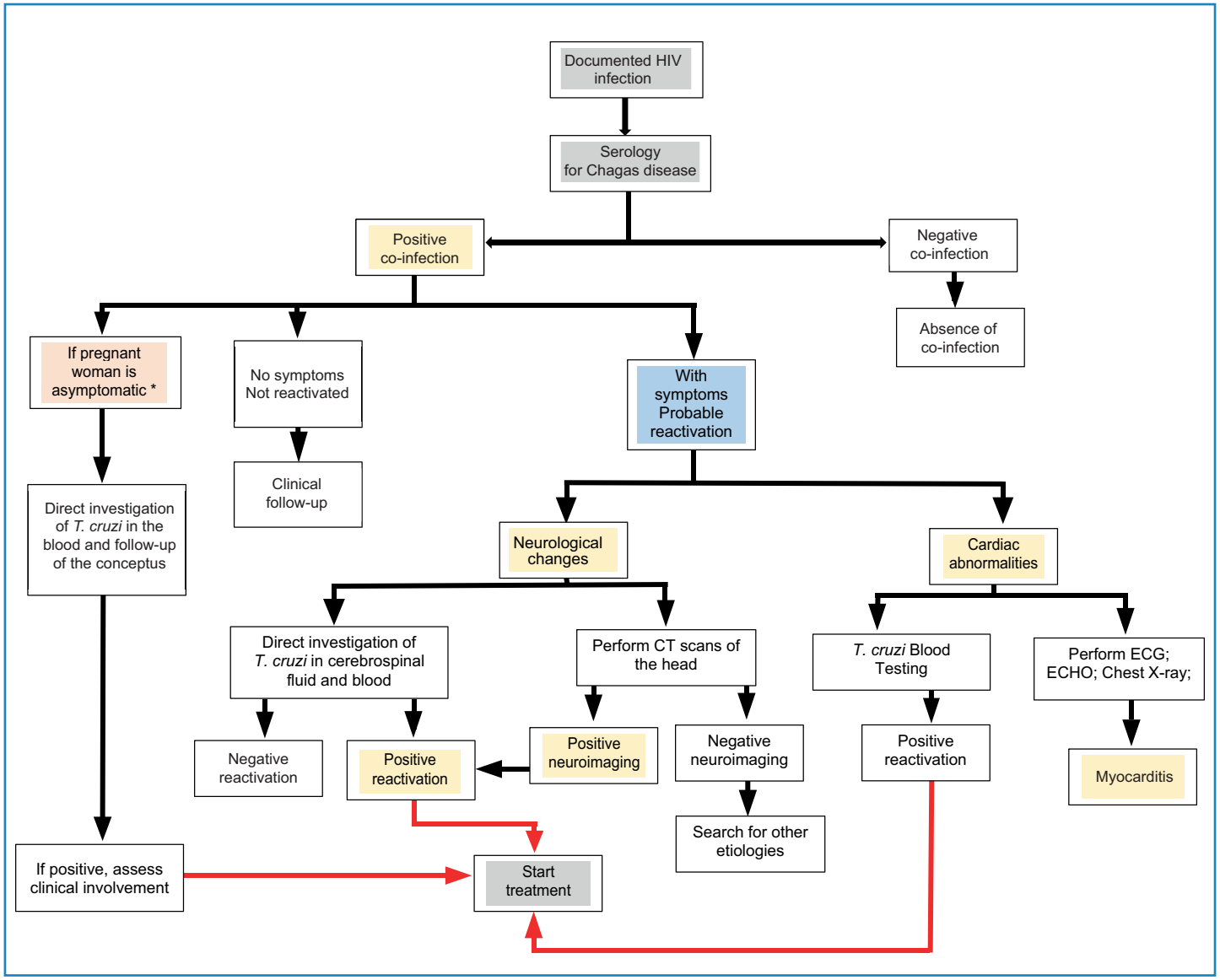

FIGURE 1: Flowchart: Systematizing Management of HIV-Infected Patients with Epidemiological History Compatible with *T. cruzi* Infection.
Caption: *High rate of congenital transmission with high morbidity and mortality in the fetus.

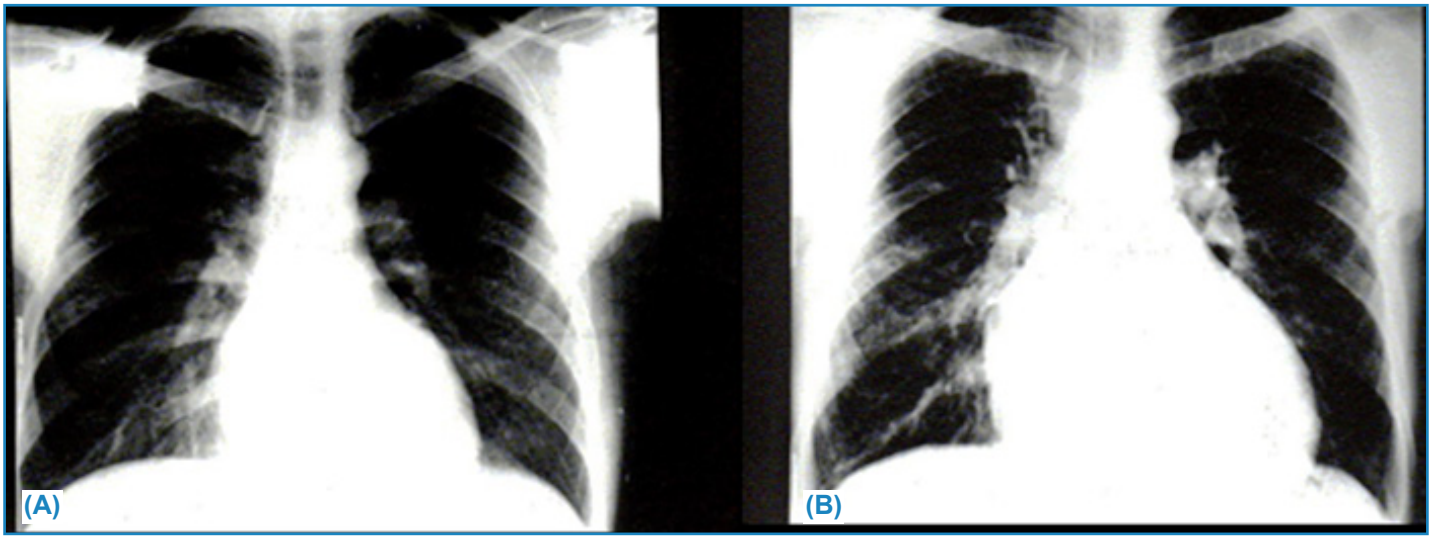

FIGURE 2: Images of chest radiological examination of a male patient, 36 years old, seropositive for HIV. The examination was carried out in Oct/1994 when the patient started experiencing dyspnea **(A)**. In Feb/2005 the patient presented with congestive heart failure, functional class IV. The electrocardiogram showed a complete right bundle branch block, anterior superior divisional block, and frequent polymorphic ventricular arrhythmia, and echocardiogram with an ejection fraction of 31% **(B)**.

Caption 2: The images were generously supplied by Dr. Ana Marli Sartori from the Clinical Hospital of the University of São Paulo.

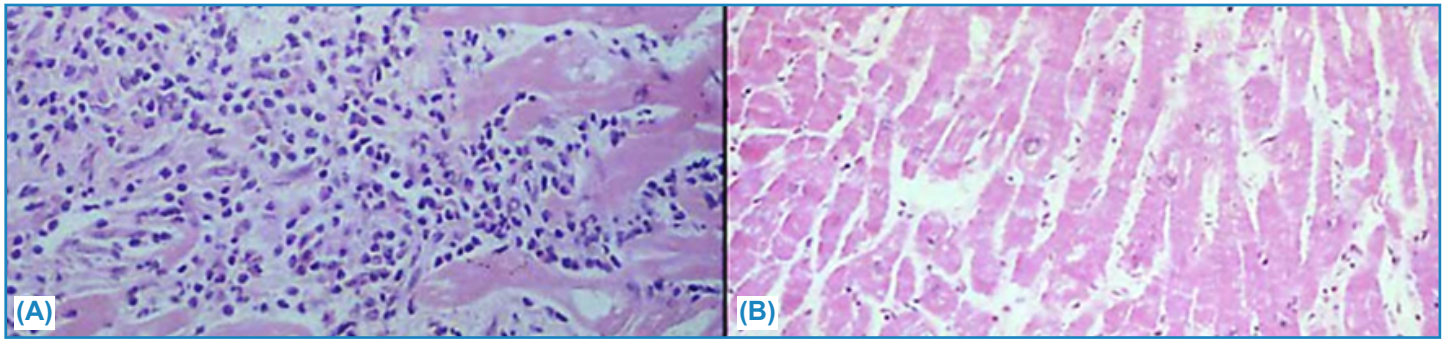

FIGURE 3: Images from the anatomopathological examination depict fragments obtained through an endomyocardial biopsy of the same patient. The examination conducted in February 1995 revealed profound lymphocytic myocarditis with myocyte degeneration and necrosis **(see Fig. 3(A)**. By April 1995, the direct peripheral blood test yielded a positive result for the first time, leading to the prescription of benznidazole treatment. Subsequent biopsy in April 1996 displayed moderate myocyte hypertrophy, devoid of inflammatory infiltrate, myocyte injury, or interstitial fibrosis **(see Fig. 3(B)**.

Caption 3: The images were generously supplied by Dr. Ana Marli Sartori from the Clinical Hospital of the University of São Paulo. (Source: Am J Trop Med Hyg. 1998 Nov;59(5):784-6. doi: 10.4269/ajtmh.1998.59.784).

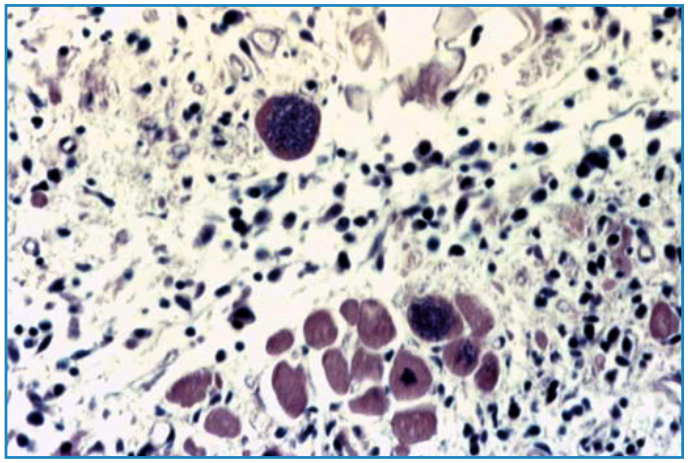

FIGURE 4: Pathological examination of a patient with acute Chagas myocarditis resulting from the reactivation of Chagas disease associated with AIDS reveals parasitized myocardiocytes (located at the top left and bottom right). Additionally, there is a discreet and diffuse mononuclear exudate in the interstitium, accompanied by edema. The examination further indicates necrosis of myocells on the right and hypertrophy of the remaining ones below. Hematoxylin-Eosin staining was employed for this analysis.

Caption 4: The images were generously supplied by Dr. Ademir Rocha from the Clinical Pathology Laboratory at the Clinical Hospital of the Federal University of Uberlândia.

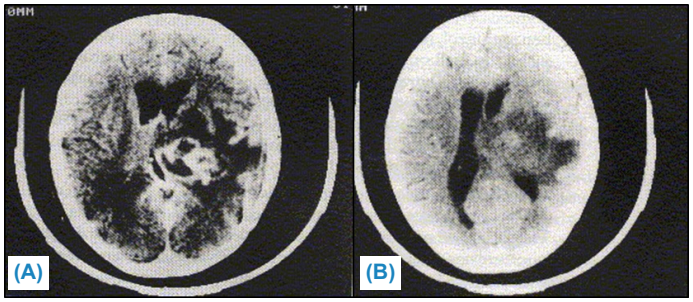

FIGURE 5: Tomography images of the skull the female patient revealed *T. cruzi* meningoencephalitis associated with AIDS, presenting a CD4 count of 88 cells/mm^3. Notably, **Fig. (A)** displays an expansive lesion compressing the lateral ventricles, accompanied by intense perilesional edema, with contrast capture. In **Fig. (B)**, the same condition is depicted without the use of contrast.

Caption 5: The images were generously supplied by Dr. Eduardo Alexandrino Servolo de Medeiros, Federal University of São Paulo. (Source: J Acquir Immune Defic Syndr Hum Retrovirol. 1999 Apr 1;20(4):342-9. doi: 10.1097/00042560-199904010-00004).

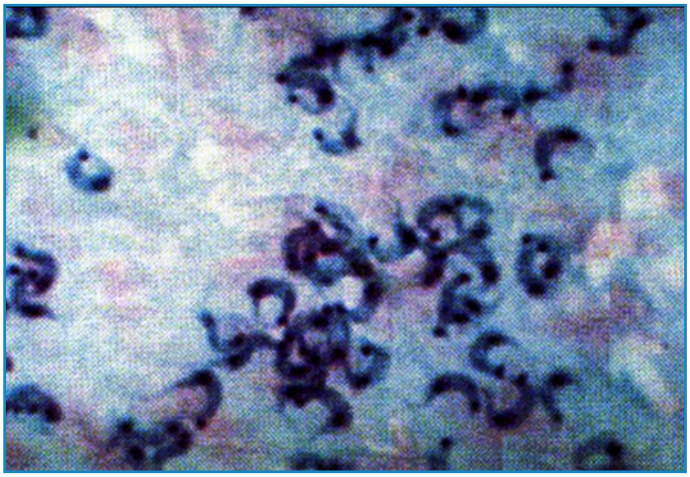

FIGURE 6: The image of cerebrospinal fluid from a patient with meningoencephalitis had been showcasing numerous trypomastigote forms of *T. cruzi*. The staining technique used is Giemsa.

Caption 6: The images were generously supplied by Dr. Eduardo Alexandrino Servolo de Medeiros, Federal University of São Paulo. (Source: J Acquir Immune Defic Syndr Hum Retrovirol. 1999 Apr 1;20(4):342-9. doi: 10.1097/00042560-199904010-00004).

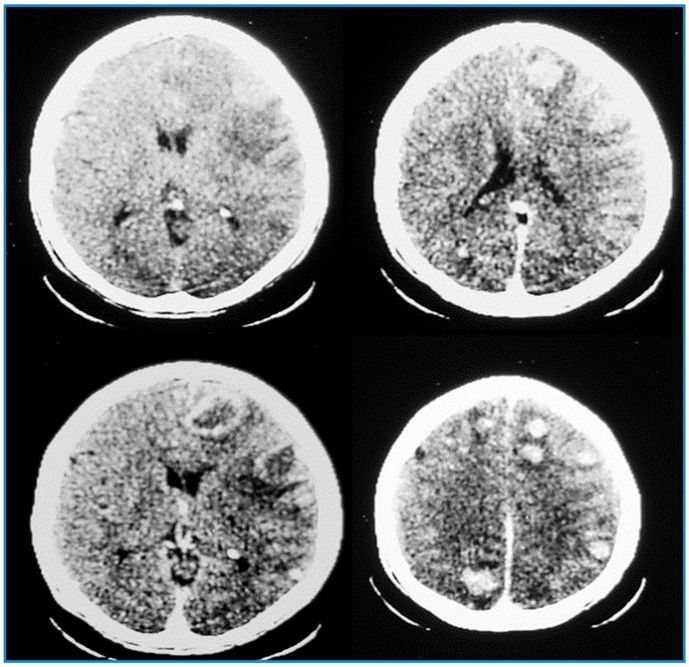

FIGURE 7: Tomography images of a patient with reactivated Chagas disease associated with AIDS.

Caption 7: The images were generously supplied by Dr. Marcelo Simão Ferreira, Federal University of Uberlândia.

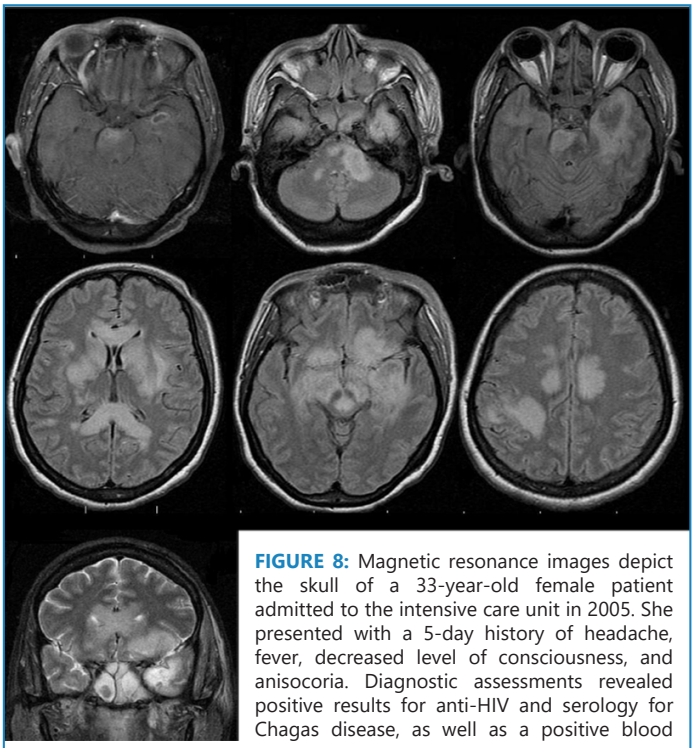

FIGURE 8: Magnetic resonance images depict the skull of a 33-year-old female patient admitted to the intensive care unit in 2005. She presented with a 5-day history of headache, fever, decreased level of consciousness, and anisocoria. Diagnostic assessments revealed positive results for anti-HIV and serology for Chagas disease, as well as a positive blood culture for *T. cruzi*. Cerebrospinal fluid analysis showed 10 cells per mm^3, with 92% of lymphocytes, proteins at 78, and glucose at 40 mg/dl, along with the presence of numerous forms of *T. cruzi*. Immunological parameters indicated CD4 at 26 mm^3 and HIV Viral Load at 1,160,000 copies.

Caption 8: The images were generously provided by the Intensive Care Unit of the Infectious and Parasitic Diseases Clinic at the Clinical Hospital, Faculty of Medicine, University of São Paulo.

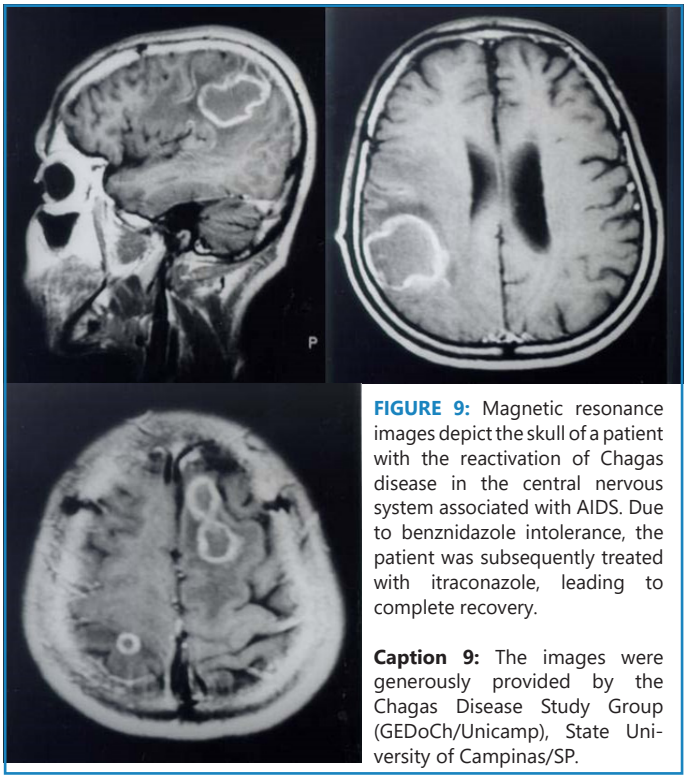

FIGURE 9: Magnetic resonance images depict the skull of a patient with the reactivation of Chagas disease in the central nervous system associated with AIDS. Due to benznidazole intolerance, the patient was subsequently treated with itraconazole, leading to complete recovery.

Caption 9: The images were generously provided by the Chagas Disease Study Group (GEDoCh/Unicamp), State University of Campinas/SP.

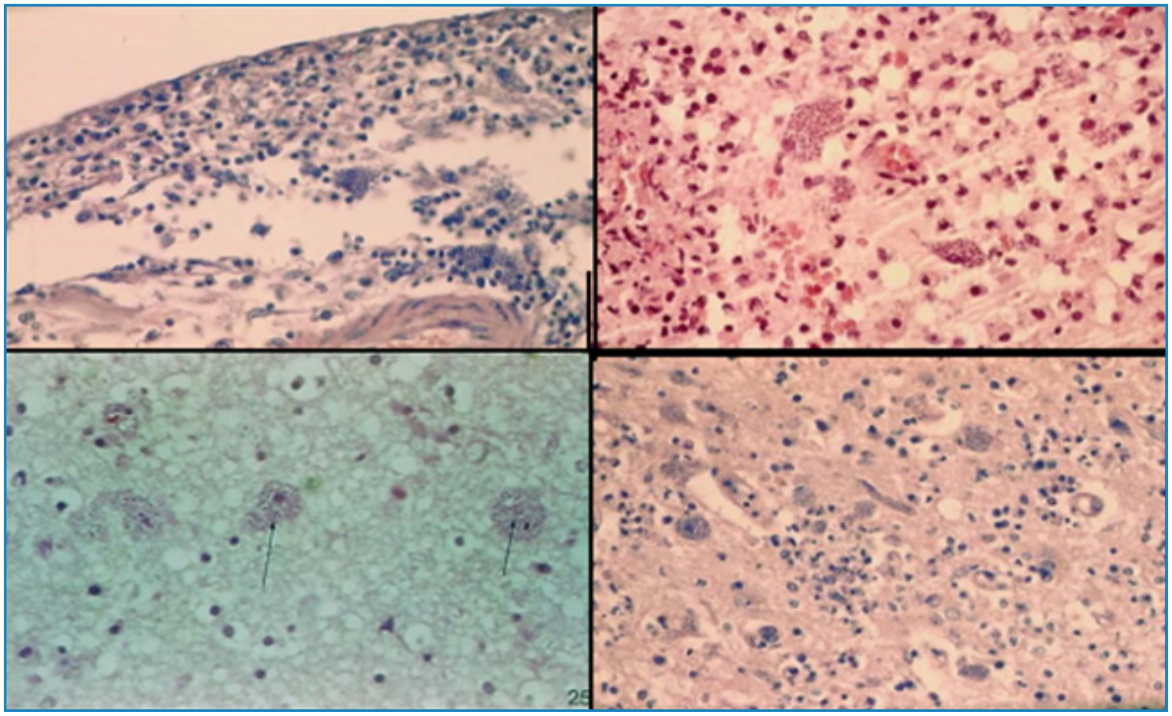

FIGURE 10: Pathological examination images of patients with meningoencephalitis due to reactivated Chagas disease associated with AIDS.

Caption 10: The images were generously supplied by Dr. Marcelo Simão Ferreira, Federal University of Uberlândia.

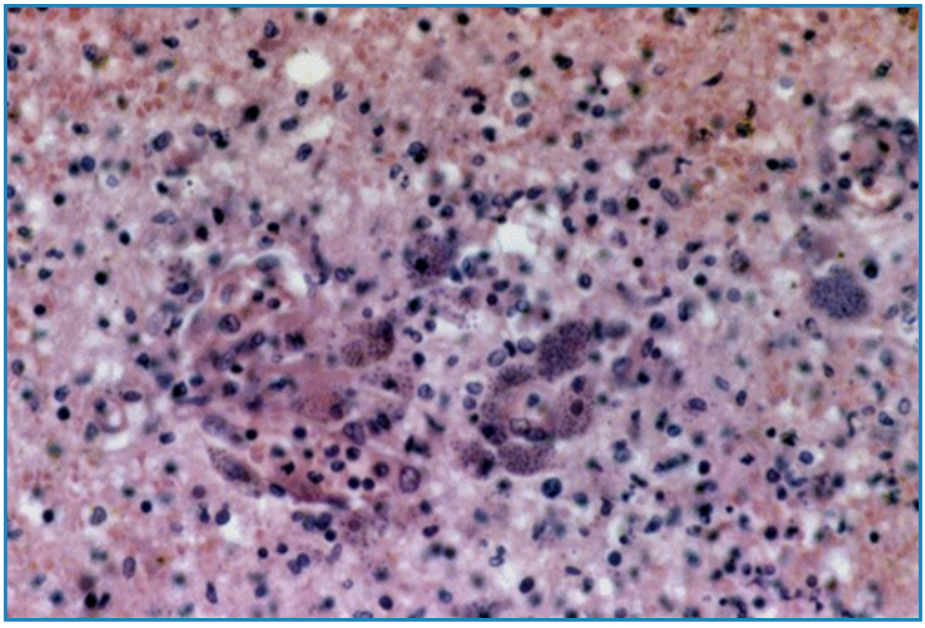

FIGURE 11: Anatomopathological examination image of a patient with meningoencephalitis due to reactivated Chagas disease associated with AIDS. The image reveals multiple parasitized cells (macrophages and microglial cells) in the center and right, along with a diffuse mononuclear and neutrophilic exudate. Additionally, areas of recent hemorrhage are evident. Hematoxylin-Eosin staining was employed for this analysis.

Caption 11: The images were generously supplied by Dr. Ademir Rocha from the Clinical Pathology Laboratory at the Clinical Hospital of the Federal University of Uberlândia.

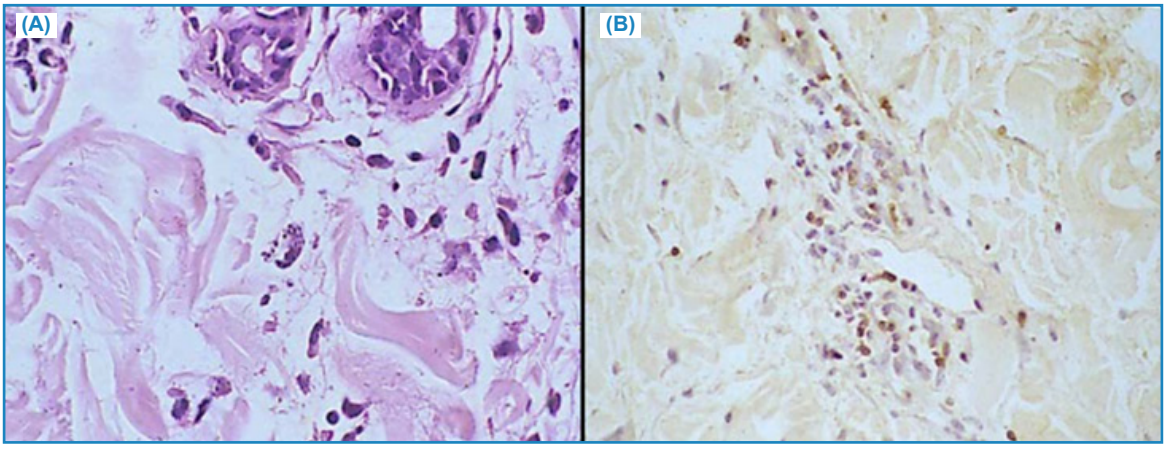

FIGURE 12: Biopsy images of a 37-year-old female patient diagnosed with *T. cruzi*/HIV coinfection and pulmonary pneumocystosis in Nov/1997. Following the initiation of antiretroviral treatment in December 1997, the patient developed painful, erythematous nodules on the right lower limb. Histological examination revealed a superficial dermis with dilated vessels, follicles, and sweat glands involved in a moderate lymphohistiocytic inflammatory infiltrate. The dermis displayed collagen fiber dissociation with the deposition of amorphous material and rare inflammatory cells. Notably, rare macrophages exhibited structures suggestive of amastigote forms in the cytoplasm (**see Fig. 12(A**). Immunohistochemistry confirmed positivity for *T. cruzi* (**see Fig. 12(B)**.

Caption 12: The images were generously supplied by Dr. Ana Marli Sartori from the Clinical Hospital of the University of São Paulo.

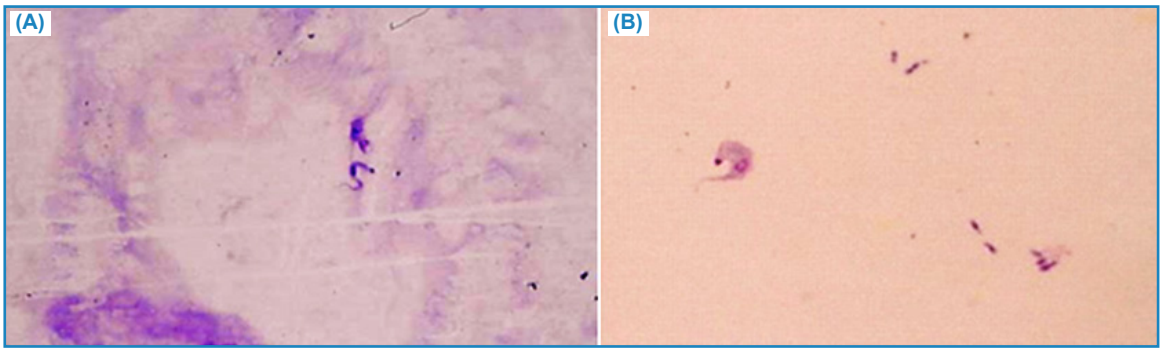

FIGURE 13: Image depicting cerebrospinal fluid **(A)** and pericardial fluid **(B)** containing trypomastigote forms of *T. cruzi*.

Caption 13: The images were generously supplied by Dr. Marcelo Simão Ferreira, Federal University of Uberlândia.

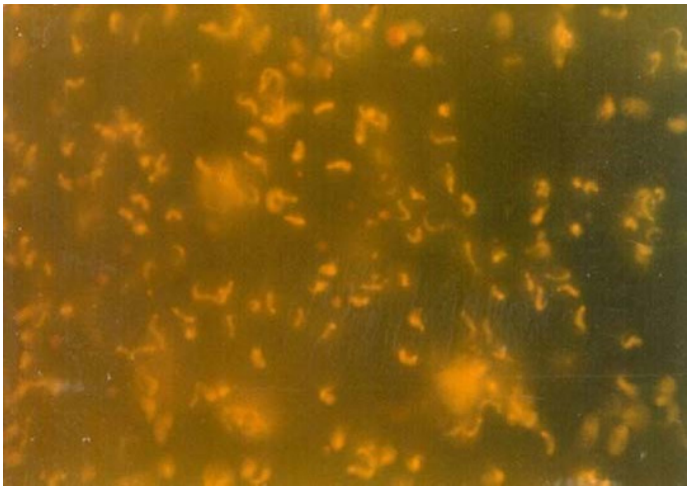

FIGURE 14: Quantitative Buffy Coat (QBC) image of a patient with Chagas disease.

Caption 14: The images were generously supplied by Prof. Vicente Amato Neto and Dr. Pedro Luiz Silva Pinto from the Medical Research Laboratory in Parasitology at the Clinical Hospital, Faculty of Medicine, University of São Paulo.

OTHER IMMUNOSUPPRESSION CONDITIONS AND CHAGAS DISEASE

The first technical manual, Recommendations for Diagnosis, Treatment and Monitoring of *T. cruzi*/HIV Co-infection, was published in 2006. This period followed the recognition of reactivation of Chagas disease as an AIDS-defining disease in people living with HIV.

The ongoing progression of epidemiological transformations within communities impacted by these two illnesses emphasised the requirement for immunosuppression to be intensified in its technological norms. In 2016, the 2nd Brazilian Consensus on Chagas Disease was published[20], which included a chapter on the management of other immunosuppressive conditions. At the 2017 XXXII Annual Meeting of Applied Research in Chagas Disease, hosted by the International Network for Care and Studies on *T. cruzi*/HIV Co-infection and other immunosuppressive conditions, the responsibility of updating the 2006 technical manual with added sections regarding immunosuppression was assigned to the network.

The rise in the number of people experiencing immunosuppression as a result of transplant rejection, neoplasms, and autoimmune diseases in patients with Chagas disease has played a part in this. The progress and availability of symptomatic treatment for chronic chagasic heart disease implies that those who would have died at a young age can now live with refractory heart failure syndrome or other related disorders. Therefore, individuals with this condition may be considered suitable recipients for heart, kidney, and liver transplants, but are also vulnerable to other immunosuppressive disorders[69].

TRANSPLANTATION AND CHAGAS DISEASE

Recent epidemiological trends, the mobility of people chronically infected with Chagas disease to large urban centers, increased life expectancy and better access to health care are factors that have led to a greater association with conditions of low immunity. Concerning transplants, the increased number of infected individuals residing in non-endemic regions has heightened the likelihood of potential donors or recipients becoming infected[70].

The indication for organ transplantation in individuals with Chagas disease raises concerns regarding both recipients and donors. Certain unique circumstances arise when transplanting organs in recipients with chronic Chagas disease, specifically:

1. The disease is prevalent in chronic chagasic heart disease, with a seropositivity prevalence of 13-35% amongst individuals awaiting heart transplants in Brazil[71].
2. Terminal organ failure is not associated with Chagas disease. The most prevalent types of failure are kidney, liver, lung and kidney-pancreas, with a seropositivity prevalence of 0.46%, 2.84%, 1.05% and 2.25% respectively, or haematological diseases[72,73].
3. Digestive diseases may also occur. Severe megaviscera diagnoses can disqualify patients from transplantation due to increased morbidity and challenges in absorbing orally-administered immunosuppressive therapy for megaoesophagus cases. The individual risk-benefit and treatment plans prior to digestive involvement must be carefully assessed[74].

DONORS

In areas where Chagas disease is endemic, serology should be carried out for the diagnosis of this illness in all cases. The risk of transmitting the infection to recipients, the potential occurrence of acute disease or the progression to the chronic phase in immunocompromised patients are of concern. Furthermore, it is imperative to maintain a standard of diagnostic procedure.

In cases where the disease was not previously diagnosed or the transplant was performed in an emergency situation, the transmission rate of Chagas disease was between 13% and 22% for kidney or liver transplantation, rising to as high as 75%. However, for heart transplants, the transmission rate is 100%.

Due to the high transmission rates of Chagas disease, transplant centers in certain countries worldwide, including Brazil, Spain, and the United States, do not accept organs or tissues from donors with this condition[75].

SOLID ORGAN TRANSPLANTS

In solid organ transplantation, a typical immunosuppressive regimen combines varied agents that act on different sites within the immune response cascade. The regimen typically involves both induction and maintenance phase and draws upon immunosuppressants from multiple groups of action:

Drugs that operate at different levels of the cascade, including corticosteroids (prednisone, prednisolone and methylprednisolone).

Drugs that inhibit the action of interleukin-2 (IL-2) include calcineurin inhibitors such as cyclosporine and tacrolimus.

Drugs that interfere with the synthesis of nucleic acids include purine synthesis inhibitors like azathioprine and mycophenolate mofetil.

Drugs that inhibit the proliferation signal of smooth muscle cell growth and hematopoietic lineages are mammalian target of rapamycin inhibitors, such as sirolimus and everolimus).

Maintenance therapy typically involves a combination of three drug classes, including corticosteroids, which are usually tapered six months post-transplantation. Induction therapy may be necessary to modulate effector T cell responses to antigen presentation in certain cases. The aim was to decrease the occurrence of acute rejection, which is exclusively employed in conditions of elevated rejection risk during the immediate pre-transplant period, and where interleukin-2 receptor blockers and T-lymphocyte-depleting agents can be administered[76].

In terms of solid organs, the kidney and liver are at the top globally, with Brazil ranking as the second country with the most transplants of these organs[77]. As reported in the Brazilian Transplant Association's publication (Year XXVIII, No. 4). From 2014 to 2021, there were 4,750 kidney transplants performed in Brazil, with 581 of these from living donors. Liver transplantation followed with 2,033 cases, and heart transplantation with 332 cases in 2016.

Patients with advanced heart failure and Chagas disease are at an independent risk of death, making heart transplantation a significant and highly recommended treatment option in these cases. Chagas disease is the third most common reason for heart

transplantation in Brazil, accounting for 35% of all cases. The infectious disease is linked to the need for transplantation, and therefore, the prevalence of Chagas disease is higher among heart recipients compared to other solid organ candidates. Consequently, heart recipients have the highest risk of Chagas disease reactivation in solid organ transplants[78].

• Kidney and liver transplants

From a donor with Chagas disease to a recipient without the disease (D+/R-), certain pretransplant procedures should be followed.

The recipient's informed consent form must be collected.

In the event of a living donor, they must receive benznidazole treatment for Chagas disease prior to transplantation to reduce parasitaemia and the possibility of parasite transmission. In cases that necessitate transplantation before concluding the donor's treatment, or if that is not feasible, transplantation can be carried out with continuous monitoring of the recipient, as outlined below.

The post-transplant care plan for the recipient (D+/R-) regardless of treatment, untreated living donor, or deceased donor includes:

1. Perform clinical-parasitological monitoring with the following frequency:

- Conduct weekly direct parasitological tests of peripheral blood or qualitative PCR/qPCR for 60 days. If either test is positive, specific treatment for Chagas disease is necessary.
- Serological examinations should be carried out 30 and 60 days after transplantation, and subsequently at 3, 6, 9, and 12 months. Repeat serology once every six months, starting from twelve months after the procedure. Due to its low sensitivity in cases with immunosuppression, serology should not be solely relied upon for post-therapeutic and/or transplantation follow-up. It should only be considered valuable if the test result is positive. Alternatively, PCR/qPCR is deemed the best option. If either PCR/qPCR or serology test results are positive, specific treatment for Chagas disease must be administered immediately.
- Direct parasitological tests or PCR/qPCR should be carried out for any suspected infectious condition. If either test comes back positive, specific treatment for Chagas disease is required.

2. If monitoring the recipient is not possible, prophylactic treatment with benznidazole should be administered for 60 days post-transplant. The recipient should also undergo follow-ups at 3, 6, and 12 months or have at least 3 post-therapeutic controls using PCR/qPCR, as serology is not a sensitive method in cases of immunosuppressed patients and only valuable if the test is positive[79].
3. In the event of heightened immunosuppression, for instance, during rejection episode treatment, it is necessary to resume monitoring in accordance with the aforementioned sequence.
4. If the donor does not exhibit Chagas disease, but the recipient does (D-/R+), reactivation is a concern, with procedures being specified as such in *T. cruzi*/HIV co-infection[80].

• Heart transplants

In heart transplantations, it is not possible for a donor to have Chagas disease; only a recipient can have the disease (D-/R+). Therefore, the focus should be on the reactivation of Chagas disease, and the particular procedures outlined in this manual for reactivation in *T. cruzi*/HIV co-infection should be followed.

However, some special considerations regarding heart transplantations in patients with Chagas disease must be highlighted:

1. Reactivation may occur with other forms of immunosuppression, but myocarditis typically presents with arrhythmias, advanced atrioventricular blocks, graft failure, or cardiogenic shock.
2. A differential diagnosis of rejection is crucial, as graft dysfunction may manifest similarly. Definitive detection of high-intensity amastigote forms among lymphocytic inflammatory infiltrates, oedema, and fibrosis is possible through histopathological examination. An incorrect diagnosis can have fatal consequences. Intensifying immunosuppression to treat rejection, for example, can exacerbate the infection process. In post-heart transplant follow-up, it is typical to use endomyocardial biopsy for monitoring rejection. This technique enables a histopathological differential diagnosis and detection of any parasite reactivation, which is not found in other transplant procedures[81].
3. Quantitative polymerase chain reaction (qPCR) analysis of whole blood specimens can identify an elevation in parasitaemia some days or even weeks before positive results are obtained from direct parasitological or reactive clinical tests. Therefore, a particular preemptive therapy involving benznidazole is necessary to be administered[82].
4. In cases of timely detection and treatment of reactivation, the result is promising, with an estimated mortality rate of about 0.9%. The prognosis for patients with Chagas disease who receive heart transplants is superior to that observed in heart transplants indicated for other diseases. Fatalities were caused by rejection (10%) or infections unrelated to Chagas disease (10%), comparable with the mortality rates among transplant recipients suffering from different aetiologies.
5. In terms of immunosuppressants, induction therapy with thymoglobulin should be steered clear of. If required, basiliximab or daclizumab should be preferred whenever possible. Furthermore, mycophenolate mofetil may be a risk factor for reactivation, alongside incidents of rejection or concurrent neoplasms, and could be replaced by azathioprine. However, this approach appears to be warranted solely in instances with a high risk of reactivation, as azathioprine exhibits lower immunosuppressive effectiveness, decreased anti-proliferative impact on T lymphocytes, and rejection is the primary cause of mortality in these cases[83].

• Hematopoietic transplants

In hematopoietic transplants, it is important to acknowledge that patients are immunosuppressed prior to the transplant, during the pre-infusion conditioning of the marrow. The level of immunosuppression varies depending on the underlying illness, type of chemotherapy, immunosuppressive drugs used, and the

pre-infusion marrow conditioning routine. Thus, it is crucial to be mindful of the possibility of heightened parasitaemia risk in recipients with Chagas disease, even prior to the transplantation[84].

Conditioning regimens focus on eradicating any remaining disease and reducing immune activity, thereby enabling effective integration of transplanted bone marrow cells. Immunosuppression is particularly intense during the initial phase and in the first few months following transplantation. After infusion, aplasia persisted in the bone marrow for around two to three weeks. In addition to the effects of the spinal function deficit and the conditioning regimen's side effects, a heightened risk of infection exists during this period, including Chagas disease reactivation or acute infection in immunocompromised patients[85].

There are two options for hematopoietic transplantation: (1) autologous, which employs the recipient's personal cells, during which pre-transplant elimination represents the period of most significant immunosuppression, and Chagas disease reactivation is a concerning factor; and (2) allogeneic, from related or unrelated donors, which necessitates intense immunosuppression in the post-transplant period due to an increased risk of graft versus host disease (GVHD). Functional recovery of the bone marrow may take a year or more in these instances. The issue at hand pertains to acute Chagas disease transmitted via marrow infected with *T. cruzi* (D+/R-) as well as reactivation in recipients with Chagas disease (D-/R+). If both donor and recipient are affected by Chagas disease (D+/R+), reactivation poses a concern. Attention should be paid to the treatment of GVHD as it is a risk factor for this disease[86].

Donors with Chagas disease (D+/R-) must have their restrictions taken into consideration in hematopoietic transplantation, as with other forms of transplant, and these may be accepted in special situations. People affected by Chagas disease (D-/R+) face no limitations for transplantation or autologous transplantation.

HEMATOPOIETIC STEM CELL TRANSPLANTATION - STRATEGIES AND MONITORING OF CHAGAS DISEASE

• Pre-transplant

In D+/R- transplantation, the donor must receive the same treatment as indicated in solid organ transplant procedures. Nevertheless, in urgent situations, the lack of such treatment should not hinder donation[87].

Pre-transplant treatment of infected (D-/R+) or autologous recipients should only be given in cases of detectable parasitaemia due to benznidazole toxicity.

Parasitological monitoring should take place during the chemotherapy phase before transplantation to determine if preemptive therapy is necessary.

• Post-transplant

For D+/R- transplantations, it is recommended:

1. Direct parasitological tests or qualitative PCR of peripheral blood be conducted on a weekly basis for 60 days, fortnightly in the third month, and monthly during the immunosuppression period for allogeneic transplants. If any of the aforementioned examinations yield a positive result, it is essential to administer specialised treatment for Chagas disease.
2. Qualitative PCR testing must be conducted 30- and 60-days post-transplantation, followed by examinations at the 3, 6, 9, and 12-month marks. After 12 months, the PCR test should be repeated every six months, as it is the most effective follow-up method for Chagas disease treatment. Serology is not sensitive enough for post-therapeutic follow-up, and is only useful if it returns a positive result. PCR is considered to be the best alternative. Specific treatment for Chagas disease must be provided when any of the methods return a positive result.
3. If there is any suspicion of an infectious condition, direct parasitological tests or qualitative PCR/qPCR should be conducted. If either of the tests is positive, then specific treatment for Chagas disease needs to be administered.

• Suggested approach for transplantation with D-/R+ or autologous

The reactivation of Chagas disease poses a potential risk. The recommended procedure is similar to that for reactivation of *T. cruzi*/HIV co-infection, and preemptive treatment may be given if parasitaemia is elevated.

• Specificities of hematopoietic transplantation and Chagas disease

The risk of Chagas disease reactivation varies depending on the type of hematopoietic transplantation. Reactivation can occur in up to 40% of cases for the allogeneic type as the intensity of chemotherapy or radiotherapy is greater, mainly owing to the increased risk of GVHD. The prevalence for autologous transplantation is 16.6%. As with any other type of transplant, rejection is managed by intensifying immunosuppression, which in turn heightens the risk of Chagas disease reactivation[88].

It is important to consider the adverse effects of benznidazole during the severe pancytopenia stage, with particular attention to myelotoxicity. Nonetheless, in cases of Chagas disease reactivation, antiparasitic treatment is a formal requirement. Following the transplantation procedure, there is a gradual rise in granulocytes during the initial month. This is subsequently succeeded by an augmentation in red blood cells and platelets. It is imperative to take into account the risk-benefit assessment of benznidazole treatment, closely evaluating its employment on an individual basis and restricting it to essential cases[89].

There is a lack of prospective randomized studies supporting the use of pre-transplant antiparasitic treatment to prevent transmission or reactivation of Chagas disease. Most scientific reports recommend post-transplant clinical-parasitological monitoring instead.

Antiparasitic treatment should only be administered in cases of confirmed reactivation or acute infection.

CHAGAS DISEASE REACTIVATION IN TRANSPLANTATION

Reactivation of Chagas disease during organ transplantation is defined as being caused by other forms of immunosuppression or immunodeficiency[90]. Due to the global spread of the disease, reactivation represents a growing concern for public health in countries with chronic Chagas disease. This warrants particular

attention as an emerging and significant problem. There are two possible scenarios to be considered: Firstly, reactivation in recipients who are chronically infected with *T. cruzi* (D-/R+), and secondly, transmission of the disease from donors who have the disease (D+/R-)[91]. In both scenarios, there is a severe illness with high levels of parasitaemia that necessitates immediate antiparasitic intervention. **Table 1** highlights varying rates of disease reactivation and transmission based on the transplant type.

The risk of Chagas disease reactivation or acute infection mainly manifests during the initial post-transplant period when immunosuppression is heightened, particularly within the first six months or after treating rejection episodes in solid organ transplants[92].

TABLE 1: Frequency of Chagas disease reactivation and transmission in various transplant types.

Transplant	Reactivation (D-/R+)	Transmission (D+/R-)
Kidney	8-35%	13-21,7%
Liver	0-18,75%	18,7-22%
Heart	26,4-40%	75-100%
Hematopoietic Cells-Allogeneic	27,3-40%	
Hematopoietic cells- Autologous	8,3%	

CLINICAL DIAGNOSIS - CHAGAS DISEASE REACTIVATION IN TRANSPLANTATION

The clinical manifestations of Chagas disease reactivation or transmission after transplantation are similar to the acute phase symptoms which include fever, anorexia, myalgia, lymph node enlargement, hepatosplenomegaly, myocarditis, and meningoencephalitis. The existence of panniculitis or subcutaneous nodules (chagomas) substantiates this finding, although these are less common in the acute phase of other forms of *T. cruzi* transmission. In addition to nodules, skin lesions may present as reddish and measles-like rashes with sporadic itching. The appearance of bubbles or vesicles is frequent in cases of high parasitic load. Ultimately, asymptomatic status was achieved by the patient, with the occurrence of patent parasitaemia[93].

FACTORS ASSOCIATED WITH CHAGAS DISEASE REACTIVATION IN TRANSPLANTATION

Corticosteroids are linked to reactivation during transplantation. In heart transplant cases, reactivation has been associated with the number of rejection episodes, the presence of neoplasms, and the use of mycophenolate mofetil[94].

Prospective studies have shown that a high parasitaemia is a predictive factor for reactivation in the role of protozoan. Furthermore, there is discussion in the literature regarding the role of *T. cruzi* strains (TcI to TcVI) in producing clinical manifestations of reactivation, although these findings remain controversial[95].

REGULATIONS FOR ORGAN OR TISSUE DONATIONS IN BRAZIL

The Brazilian Ministry of Health's Ordinance 2600, issued on October 21, 2009, governs the complete operation of Brazil's National Transplant System (SNT), which includes its architecture, overall transplant facility guidelines, and the management of the Single Technical Registry (CTU). The ordinance mandates transplantation teams or dialysis units to furnish essential information about patients and data for the evaluation of potential organ donors (Brazilian Ministry of Health's Ordinance, no. 2,600, dated October 21st, 2009); approves the Technical Regulation of the National Transplant System - Official Gazette of the Federative Republic of Brazil, Brasília-DF, 21 Oct 2009; Section 1:77).

It is mandatory for organ recipients to undergo serology using indirect immunofluorescence for Chagas disease before enrolling in CTU, regardless of the type of organ to be transplanted. According to WHO guidelines, two distinct serological methods are required to confirm a Chagas disease diagnosis. This is crucial in preventing transmission in instances where the initial serological test produces inconclusive results and no retesting is conducted.

All potential donors of deceased organs, tissues, cells or body parts must also undergo Chagas disease diagnosis evaluation, which is not necessary for corneal donors who solely undergo serological testing for HIV, hepatitis B and hepatitis C. The initial assessment of a possible brain-dead donor necessitates indirect immunofluorescence testing for Chagas disease along with the essential serologies. However, it would be ideal for all transplant centers to employ two highly sensitive and standardised serological tests to prevent disease transmission. This is particularly important as using only one serological test to exclude donors with Chagas disease may lead to disease transmission[96].

It should be noted that reactive serology for Chagas disease is not an absolute donor exclusion factor, as opposed to reactive HIV and HTLV infections, active tuberculosis, and refractory sepsis. The agency will evaluate the acceptance of individuals with positive serology for *T. cruzi* based on the criteria outlined in the Ministry of Health document on a case-by-case basis:

1. For kidney, liver, lung, or pancreatic transplants from a deceased donor with positive serology for Chagas disease, the transplant team bears responsibility for deciding whether to accept the organ.
2. In the case of heart and intestine transplantation, an organ from a donor with positive serology for Chagas disease is not accepted.
3. Positive serology for Chagas disease disqualifies donors from donating musculoskeletal tissue, cardiovascular tissue, or skin for banks.
4. There are no specific provisions for Chagas disease serology in hematopoietic stem cell transplantation. This directive is aimed at the National Cancer Institute (INCA), which manages the National Registry of Voluntary Bone Marrow Donors (REDOME) and the National Registry of Bone Marrow Recipients (REREME). Once a suitable donor is identified, they are directed to one of the blood center for additional serological testing. During this testing process, all necessary tests for blood product donation, including a test for *T. cruzi*, are conducted. However, positive serology results for Chagas disease do not preclude donation. Registration with REDOME is only prohibited for those with HIV/AIDS or viral hepatitis.

While the potential to carry out transplants using living kidney, liver, and lung donors has been outlined, there is currently no dedicated section governing compulsory tests for such donors. Nevertheless, they must adhere to serology guidelines established for deceased donors[97].

The guidelines for the operation of banks that store musculoskeletal, cardiovascular, or skin tissue specify the requirement of using diagnostic kits validated by ANVISA for serological testing for *T. cruzi*. This serological testing should be conducted for donors who have died due to brain death (with maintained blood circulation) or cadavers (cardiac arrest), employing diagnostic kits duly registered with ANVISA.

CANCER, AUTOIMMUNE DISEASES AND CHAGAS DISEASE

The risk of reactivation of chronic Chagas disease should be taken into account in instances of depressed cellular immunity, either as a result of the presence of neoplasms (solid or haematological), or use of immunosuppressive therapy for treating autoimmune diseases such as rheumatoid arthritis and disseminated lupus erythematosus, vasculitis, polymyalgia, spondylarthritis, and autoimmune hepatitis, or control rejection during organ transplants using high doses of corticosteroids, cyclosporine, methotrexate, cyclophosphamide, mycophenolate mofetil, cytotoxic and immunobiological medications, or other forms of immunodeficiency[98].

Controlling parasitaemia through indirect parasitological methods revealed that the administration of corticosteroids led to an escalation in parasitaemia for patients with pemphigus foliaceus, vasculitis, and other indications. Furthermore, Chagas disease reactivation was observed in connection with chemotherapy applied in the treatment of haematological malignancies like Hodgkin and non-Hodgkin lymphomas, leukaemia, and multiple myeloma, among other forms.

However, the introduction of new immunobiological drugs for treating autoimmune diseases, which act in various phases of the cell cycle of anti-*T. cruzi*, creates fresh risks for the reactivation of parasitosis[99].

The lack of systematic and prospective monitoring of trypanosomiasis reactivation in situations like *T. cruzi*/HIV co-infection management and transplant recipients makes it vital to monitor parasitaemia in these patients. This surveillance aims to diagnose reactivation and can be accomplished through direct parasitological methods or qPCR [100,101].

In light of cost benefits, absence of contraindications, and parasitaemia confirmed by direct examinations, specific treatment for *T. cruzi* should be prescribed in accordance with the recommendations of the 2nd Brazilian Consensus on Chagas Disease[20]. Regardless of the treatment, regular clinical and parasitological monitoring is essential.

SUPPLEMENTARY MATERIAL

APPENDIX 1: State/municipal reference services for assistance/research in cases of co-infection or reactivation of Chagas disease.

APPENDIX 2: Clinical-epidemiological record of cases of *T. cruzi*/HIV co-infection.

APPENDIX 3: Contacts of the "Network for healthcare and study of *Trypanosoma cruzi*/HIV co-infection and other immunosuppression conditions".

APPENDIX 4: Central Public Health Laboratories for Chagas disease referral.

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REFERENCES

1. Dias JC, Ramos AN Jr, Gontijo ED, Luquetti A, Shikanai-Yasuda MA, Coura JR, et al. 2nd Brazilian Consensus on Chagas Disease, 2015. Rev Soc Bras Med Trop. 2016;49:(Suppl 1):3-60. Available from: https://doi.org/10.1590/0037-8682-0505-2016.
2. Ramos Jr AN et al. Recomendações para diagnóstico, tratamento e acompanhamento da co-infecção *Trypanosoma cruzi*: vírus da imunodeficiência humana [Recommendations for diagnosis, treatment and follow-up of the *Trypanosoma cruzi*: human immunodeficiency virus co-infection]. Rev Soc Bras Med Trop. 2006;39(4):392-415. Portuguese. Available from: https://doi.org/10.1590/S0037-86822006000400017
3. Shikanai-Yasuda MA, Mediano MFF, Novaes CTG, Sousa AS, Sartori AMC, Santana RC, et al. Correction: Correction: Clinical profile and mortality in patients with *T. cruzi*/HIV co-infection from the multicenter data base of the "Network for healthcare and study of *Trypanosoma cruzi*/HIV co-infection and other immunosuppression conditions". PLoS Negl Trop Dis. 2023;17(1):e0011036. Available from: https://doi.org/10.1371/journal.pntd.0011036. Erratum for: PLoS Negl Trop Dis. 2021 Sep 30;15(9):e0009809.
4. Ministério da Saúde (MS). Comissão Nacional de Incorporação de Tecnologias no SUS (CONITEC). Protocolo Clínico e Diretrizes Terapêuticas Doença de Chagas [Internet]. Brasília: MS; 2018. 135 p. Available at: http://antigo-conitec.saude.gov.br/images/PCDT_Doenca_de_Chagas.pdf
5. Stanaway JD, Roth G. The burden of Chagas disease: estimates and challenges. Glob Heart. 2015;10(3):139-44. Available from: https://doi.org/10.1016/j.gheart.2015.06.001
6. Shikanai Yasuda MA. Emerging and reemerging forms of *Trypanosoma cruzi* transmission. Mem Inst Oswaldo Cruz. 2022;117:e210033. Available from: https://doi.org/10.1590/0074-02760210033
7. Chagas C. Nova tripanozomiaze humana: estudos sobre a morfolojia e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen., n. sp., ajente etiolojico de nova entidade morbida do homem. Mem Inst Oswaldo Cruz [Internet]. 1909;1(2):159–218. Available from: https://doi.org/10.1590/s0074-02761909000200008
8. World Health Organization (WHO). Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Wkly Epidemiol Rec. 2015;90(6):33-43. English, French.
9. Apt W, Zulantay I. Estado actual en el tratamiento de la enfermedad de Chagas [Update on the treatment of Chagas' disease]. Rev Med Chil. 2011;139(2):247-57. Spanish. Epub 2011 Jul 11. Available from: http://doi.org/10.4067/S0034-98872011000200016

10. Sartori AM, Ibrahim KY, Nunes Westphalen EV, Braz LM, Oliveira OC Jr, Gakiya E, et al. Manifestations of Chagas disease (American trypanosomiasis) in patients with HIV/AIDS. Ann Trop Med Parasitol. 2007;101(1):31-50. Available from: https://doi.org/10.1179/136485907X154629

11. Acquatella H. Echocardiography in Chagas heart disease. Circulation. 2007;115(9):1124-31. Available from: https://doi.org/10.1161/CIRCULATIONAHA.106.627323

12. Dias JC. The indeterminate form of human chronic Chagas' disease A clinical epidemiological review. Rev Soc Bras Med Trop. 1989;22(3):147-56. Available from: https://doi.org/10.1590/S0037-86821989000300007

13. Almeida EA. Epidemiologia e clínica da coinfecção *Trypanosoma cruzi* e vírus da imunodeficiência adquirida. 1st ed. Campinas: Unicamp; 2015. 328 p.

14. Lattes R, Linares L, Radisic M. Emerging parasitic infections in transplantation. Curr Infect Dis Rep. 2012;14(6):642-9. Available from: https://doi.org/10.1007/s11908-012-0295-z

15. Cavalcanti MAF, Neta FI, Benevides DHJ, Andrade CM, Nascimento EGC. Caracterizando o perfil de mortalidade da doença de Chagas no Brasil. In: ANAIS MEDTROP [Internet]. 2016. Available from: https://www.sbmt.org.br/medtrop2016/wp-content/uploads/2016/10/10193-Caracterizando-o-perfil-de-mortalidade-da-doença-de-Chagas-no-Brasil.pdf

16. Pérez-Molina JA, Molina I. Chagas disease. Lancet. 2018;391(10115):82-94. Available from: https://doi.org/10.1016/S0140-6736(17)31612-4

17. Pinto Dias JC. Human chagas disease and migration in the context of globalization: some particular aspects. J Trop Med. 2013;2013:789758. Available from: https://doi.org/10.1155/2013/789758

18. Ferreira I de LM, Silva TPT e. Eliminação da transmissão da doença de Chagas pelo *Triatoma infestans* no Brasil: um fato histórico. Rev Soc Bras Med Trop [Internet]. 2006;39(5):507–9. Available from: https://doi.org/10.1590/S0037-86822006000500018

19. World Health Organization (WHO). Chagas disease (American trypanosomiasis) – fact sheet (revised in August 2012). Wkly Epidemiol Rec. 2012;87(51/52):519-22. English, French.

20. Organización Panamericana de la Salud. Guía para el diagnóstico y el tratamiento de la enfermedad de Chagas. [Internet]. 2019. Available at: http://iris.paho.org/xmlui/handle/10665.2/49653

21. Freilij H, Altcheh J, Muchinik G. Perinatal human immunodeficiency virus infection and congenital Chagas' disease. Pediatr Infect Dis J. 1995;14(2):161-2.

22. Rassi A Jr, Rassi A, Marcondes de Rezende J. American trypanosomiasis (Chagas disease). Infect Dis Clin North Am. 2012;26(2):275-91. Available from: https://doi.org/10.1016/j.idc.2012.03.002

23. Martins-Melo FR, Ramos AN Jr, Alencar CH, Heukelbach J. Mortality Related to Chagas Disease and HIV/AIDS Coinfection in Brazil. J Trop Med. 2012;2012:534649. Available from: https://doi.org/10.1155/2012/534649

24. D'Albuquerque LA, Gonzalez AM, Filho HL, Copstein JL, Larrea FI, Mansero JM, et al. Liver transplantation from deceased donors serologically positive for Chagas disease. Am J Transplant. 2007;7(3):680-4. Available from: https://doi.org/10.1111/j.1600-6143.2007.01663.x

25. Organización Panamericana de la Salud. Enfermedad de Chagas e inmunosupresión. Decálogo para la prevención, el diagnóstico y el tratamiento. [Internet]. 2021. Available from: https://iris.paho.org/handle/10665.2/54561

26. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. 2nd ed. Geneva: World Health Organization; 2016. Available from: https://www.who.int/publications/i/item/9789241549684

27. Almeida EA, Ramos Júnior AN, Correia D, Shikanai-Yasuda MA. Co-infection *Trypanosoma cruzi*/HIV: systematic review (1980-2010). Rev Soc Bras Med Trop. 2011;44(6):762-70. Available from: https://doi.org/10.1590/S0037-86822011000600021

28. Jannin J. Présentation de l'atelier de consensus sur la maladie de Chagas en zone non endémique (26 Juin 2009, Paris, France) [Presentation of the consensus workshop about the Chagas disease in non-endemic areas (26 June 2009, Paris, France)]. Bull Soc Pathol Exot. 2009;102(5):275. French.

29. Almeida EA, Lima JN, Lages-Silva E, Guariento ME, Aoki FH, Torres-Morales AE, et al. Chagas' disease and HIV co-infection in patients without effective antiretroviral therapy: prevalence, clinical presentation and natural history. Trans R Soc Trop Med Hyg. 2010;104(7):447-52. Available from: https://doi.org/10.1016/j.trstmh.2010.02.004

30. Cordova E, Boschi A, Ambrosioni J, Cudos C, Corti M. Reactivation of Chagas disease with central nervous system involvement in HIV-infected patients in Argentina, 1992-2007. Int J Infect Dis. 2008;12(6):587-92. Available from: https://doi.org/10.1016/j.ijid.2007.12.007

31. Lopez-Albizu C, Bravo MP, Pico M, Fernandez ML. Case report of Chagas disease reactivation: new diagnosis tool by direct microscopic observation of biopsy specimen and its preservation fluid. Rev Soc Bras Med Trop. 2020;54:e20200326. Available from: https://doi.org/10.1590/0037-8682-0326-2020

32. Sartori AM, Neto JE, Nunes EV, Braz LM, Caiaffa-Filho HH, Oliveira Oda C Jr, et al. *Trypanosoma cruzi* parasitemia in chronic Chagas disease: comparison between human immunodeficiency virus (HIV)-positive and HIV-negative patients. J Infect Dis. 2002;186(6):872-5. Available from: https://doi.org/10.1086/342510

33. Benvenuti LA, Roggério A, Sambiase NV, Fiorelli A, Higuchi Mde L. Polymerase chain reaction in endomyocardial biopsies for monitoring reactivation of Chagas' disease in heart transplantation: a case report and review of the literature. Cardiovasc Pathol. 2005;14(5):265-8. Available from: https://doi.org/10.1016/j.carpath.2005.06.001

34. Benvenuti LA, Roggério A, Coelho G, Fiorelli AI. Usefulness of qualitative polymerase chain reaction for *Trypanosoma cruzi* DNA in endomyocardial biopsy specimens of chagasic heart transplant patients. J Heart Lung Transplant. 2011;30(7):799-804. Available from: https://doi.org/10.1016/j.healun.2011.02.012

35. Burgos JM, Diez M, Vigliano C, Bisio M, Risso M, Duffy T, et al. Molecular identification of *Trypanosoma cruzi* discrete typing units in end-stage chronic Chagas heart disease and reactivation after heart transplantation. Clin Infect Dis. 2010;51(5):485-95. Available from: https://doi.org/10.1086/655680

36. Cuna WR, Choque AG, Passera R, Rodriguez C. Pro-inflammatory cytokine production in chagasic mothers and their uninfected newborns. J Parasitol. 2009;95(4):891-4. Available from: https://doi.org/10.1645/GE-1927.1

37. Carlier Y, Sosa-Estani S, Luquetti AO, Buekens P. Congenital Chagas disease: an update. Mem Inst Oswaldo Cruz. 2015;110(3):363-8. Available from: https://doi.org/10.1590/0074-02760140405

38. Alonso-Vega C, Hermann E, Truyens C, Rodriguez P, Torrico MC, Torrico F, et al. Estatus inmunológico de las madres infectadas por *T. cruzi* [Immunological status of mothers infected with *Trypanosoma cruzi*]. Rev Soc Bras Med Trop. 2005;38 Suppl 2:101-4. Spanish.

39. Scapellato PG, Bottaro EG, Rodríguez-Brieschke MT. Mother-child transmission of Chagas disease: could coinfection with human immunodeficiency virus increase the risk? Rev Soc Bras Med Trop. 2009;42(2):107-9. Available from: https://doi.org/10.1590/S0037-86822009000200002

40. Bisio M, Altcheh J, Lattner J, Moscatelli G, Fink V, Burgos JM, et al. Benznidazole treatment of chagasic encephalitis in pregnant woman with AIDS. Emerg Infect Dis. 2013;19(9):1490-2. Available from: https://doi.org/10.3201/eid1909.130667

41. Castro Jr CG de, Gregianin LJ, Brunetto AL. Transplante de medula óssea e transplante de sangue de cordão umbilical em pediatria. J Pediatr (Rio J) [Internet]. 2001;77(5):345–60. Available from: https://doi.org/10.1590/S0021-75572001000500004

42. Nisida IV, Amato Neto V, Braz LM, Duarte MI, Umezawa ES. A survey of congenital Chagas' disease, carried out at three health institutions in São Paulo City, Brazil. Rev Inst Med Trop Sao Paulo. 1999;41(5):305-11. Available from: https://doi.org/10.1590/S0036-46651999000500007

43. Vekemans J, Truyens C, Torrico F, Solano M, Torrico MC, Rodriguez P, et al. Maternal *Trypanosoma cruzi* infection upregulates capacity of uninfected neonate cells To produce pro- and anti-inflammatory cytokines. Infect Immun. 2000;68(9):5430-4. Available from: https://doi.org/10.1128/IAI.68.9.5430-5434.2000

44. Agosti MR, Ercoli P, Dolcini G, Andreani G, Martínez Peralta L, González Ayala S. Two cases of mother-to-child transmission of HIV and *Trypanosoma cruzi* in Argentina. Braz J Infect Dis. 2012;16(4):398-9. Available from: https://doi.org/10.1016/j.bjid.2012.06.008

45. Fernández ML, Marson ME, Mastrantonio GE, Corti MA, Fleitas U, Lloveras SC, et al. Benznidazole in Cerebrospinal Fluid: a Case Series of Chagas Disease Meningoencephalitis in HIV-Positive Patients. Antimicrob Agents Chemother. 2021;65(3):e01922-20. Available from: https://doi.org/10.1128/AAC.01922-20

46. Portela-Lindoso AA, Shikanai-Yasuda MA. Doença de Chagas crônica: do xenodiagnóstico e hemocultura à reação em cadeia da polimerase [Chronic Chagas' disease: from xenodiagnosis and hemoculture to polymerase chain reaction]. Rev Saude Publica. 2003;37(1):107-15. Portuguese. Available from: https://doi.org/10.1590/s0034-89102003000100016

47. Ferreira MS, Borges AS. Some aspects of protozoan infections in immunocompromised patients- a review. Mem Inst Oswaldo Cruz. 2002;97(4):443-57. Available from: https://doi.org/10.1590/S0074-02762002000400001

48. Duffy T, Bisio M, Altcheh J, Burgos JM, Diez M, Levin MJ, et al. Accurate real-time PCR strategy for monitoring bloodstream parasitic loads in chagas disease patients. PLoS Negl Trop Dis. 2009;3(4):e419. Available from: https://doi.org/10.1371/journal.pntd.0000419

49. Cura CI, Lattes R, Nagel C, Gimenez MJ, Blanes M, Calabuig E, et al. Early molecular diagnosis of acute Chagas disease after transplantation with organs from *Trypanosoma cruzi*-infected donors. Am J Transplant. 2013;13(12):3253-61. Available from: https://doi.org/10.1111/ajt.12487

50. Moreira OC, Ramírez JD, Velázquez E, Melo MF, Lima-Ferreira C, Guhl F, et al. Towards the establishment of a consensus real-time qPCR to monitor *Trypanosoma cruzi* parasitemia in patients with chronic Chagas disease cardiomyopathy: a substudy from the BENEFIT trial. Acta Trop. 2013;125(1):23-31. Available from: https://doi.org/10.1016/j.actatropica.2012.08.020

51. Martinez-Perez A, Norman FF, Monge-Maillo B, Perez-Molina JA, Lopez-Velez R. An approach to the management of *Trypanosoma cruzi* infection (Chagas' disease) in immunocompromised patients. Expert Rev Anti Infect Ther. 2014;12(3):357-73. Available from: https://doi.org/10.1586/14787210.2014.880652

52. Burgos JM, Begher S, Silva HM, Bisio M, Duffy T, Levin MJ, et al. Molecular identification of *Trypanosoma cruzi* I tropism for central nervous system in Chagas reactivation due to AIDS. Am J Trop Med Hyg. 2008;78(2):294-7.

53. Pacheco RS, Ferreira MS, Machado MI, Brito CM, Pires MQ, Da-Cruz AM, et al. Chagas' disease and HIV co-infection: genotypic characterization of the *Trypanosoma cruzi* strain. Mem Inst Oswaldo Cruz. 1998;93(2):165-9. Available from: http://doi.org/10.1590/S0074-02761998000200005

54. Burgos JM, Begher SB, Freitas JM, Bisio M, Duffy T, Altcheh J, et al. Molecular diagnosis and typing of *Trypanosoma cruzi* populations and lineages in cerebral Chagas disease in a patient with AIDS. Am J Trop Med Hyg. 2005;73(6):1016-8.

55. Schijman AG, Bisio M, Orellana L, Sued M, Duffy T, Mejia Jaramillo AM, et al. International study to evaluate PCR methods for detection of *Trypanosoma cruzi* DNA in blood samples from Chagas disease patients. PLoS Negl Trop Dis. 2011;5(1):e931. Available from: https://doi.org/10.1371/journal.pntd.0000931

56. Duffy T, Cura CI, Ramirez JC, Abate T, Cayo NM, Parrado R, et al. Analytical performance of a multiplex Real-Time PCR assay using TaqMan probes for quantification of *Trypanosoma cruzi* satellite DNA in blood samples. PLoS Negl Trop Dis. 2013;7(1):e2000. Available from: https://doi.org/10.1371/journal.pntd.0002000

57. Andrade JP, Marin Neto JA, Paola AA, Vilas-Boas F, Oliveira GM, Bacal F, et al. I Latin American Guidelines for the diagnosis and treatment of Chagas' heart disease: executive summary. Arq Bras Cardiol. 2011;96(6):434-42. English, Portuguese, Spanish. Available from: https://doi.org/10.1590/S0066-782X2011000600002

58. Marin-Neto JA, Rassi A Jr, Oliveira GMM, Correia LCL, Ramos Júnior AN, Luquetti AO, et al. SBC Guideline on the Diagnosis and Treatment of Patients with Cardiomyopathy of Chagas Disease - 2023. Arq Bras Cardiol. 2023;120(6):e20230269. English, Portuguese. Available from: https://doi.org/10.36660/abc.20230269

59. Vacas AS, Gomez-Santana LV, Torre AC, Galimberti RL. Reactivation of Chagas-Mazza disease during treatment with infliximab. An Bras Dermatol. 2017;92(6):899-900. Available from: https://doi.org/10.1590/abd1806-4841.20177346

60. Rassi A, Amato Neto V, de Siqueira AF, Doles J, Leite MS, Silva OQ, et al. Influência de corticóide, na doença de Chagas crônica, administrado em virtude de afecções associadas [The influence of corticoids, in Chronic Chagas disease, administered in virtue of associated disorders]. Rev Soc Bras Med Trop. 1997;30(2):93-9. Portuguese. Available from: https://doi.org/10.1590/S0037-86821997000200002

61. Lages-Silva E, Ramirez LE, Silva-Vergara ML, Chiari E. Chagasic meningoencephalitis in a patient with acquired immunodeficiency syndrome: diagnosis, follow-up, and genetic characterization of *Trypanosoma cruzi*. Clin Infect Dis. 2002;34(1):118-23. Available from: https://doi.org/10.1086/324355

62. Pinazo MJ, Espinosa G, Cortes-Lletget C, Posada Ede J, Aldasoro E, Oliveira I, et al. Immunosuppression and Chagas disease: a management challenge. PLoS Negl Trop Dis. 2013;7(1):e1965. Available from: https://doi.org/10.1371/journal.pntd.0001965

63. Molina I, Gómez i Prat J, Salvador F, Treviño B, Sulleiro E, Serre N, et al. Randomized trial of posaconazole and benznidazole for chronic Chagas' disease. N Engl J Med. 2014;370(20):1899-908. Available from: https://doi.org/10.1056/NEJMoa1313122

64. Bacal F, Silva CP, Pires PV, Mangini S, Fiorelli AI, Stolf NG, et al. Transplantation for Chagas' disease: an overview of immunosuppression and reactivation in the last two decades. Clin Transplant. 2010;24(2):E29-34. Available from: https://doi.org/10.1111/j.1399-0012.2009.01202.x

65. Shikanai-Yasuda M A, Novaes C G, Freitas VLT, Carvalho NB. Experiência brasileira sobre a coinfecção *T. cruzi*/HIV. In: Almeida EA editor. Epidemiologia e clínica da coinfecção *Trypanosoma cruzi* e vírus da imunodeficiência adquirida. 1st ed. Campinas: Unicamp; 2015. p. 298–308.

66. Schijman AG, Vigliano C, Burgos J, Favaloro R, Perrone S, Laguens R, et al. Early diagnosis of recurrence of *Trypanosoma cruzi*

infection by polymerase chain reaction after heart transplantation of a chronic Chagas' heart disease patient. J Heart Lung Transplant. 2000;19(11):1114-7. Available from: https://doi.org/10.1016/S1053-2498(00)00168-6

67. Ministério da Saúde (MS). Secretaria de Vigilância em Saúde. Departamento de Doenças de Condições Crônicas e Infecções Sexualmente Transmissíveis. Relatório de monitoramento clínico do HIV 2021 [Internet]. Brasília: MS; 2022. p. 153. Available from: https://bvsms.saude.gov.br/bvs/publicacoes/relatorio_monitoramento_clinico_hiv_2021.pdf

68. Silva VLC, Albuquerque LEJ. Prevalência de infecção pelo *T. cruzi* em doadores de sangue nos hemocentros coordenadores do Brasil em 2007. Epidemiol. Serv. Saúde [Internet]. 2013;22(1):103-10. Available from: https://doi.org/10.5123/S1679-49742013000100011

69. Kransdorf EP, Zakowski PC, Kobashigawa JA. Chagas disease in solid organ and heart transplantation. Curr Opin Infect Dis. 2014;27(5):418-24. Available from: https://doi.org/10.1097/QCO.0000000000000088

70. Salvador F, Sánchez-Montalvá A, Valerio L, Serre N, Roure S, Treviño B, et al. Immunosuppression and Chagas disease; experience from a non-endemic country. Clin Microbiol Infect. 2015;21(9):854-60. Available from: https://doi.org/10.1016/j.cmi.2015.05.033

71. Stauffert D, Silveira MF, Mesenburg MA, Manta AB, Dutra AD, Bicca GL, et al. Prevalence of *Trypanosoma cruzi*/HIV coinfection in southern Brazil. Braz J Infect Dis. 2017;21(2):180-4. Available from: https://doi.org/10.1016/j.bjid.2016.10.006

72. McCormack L, Quiñónez E, Goldaracena N, Anders M, Rodríguez V, Orozco Ganem F, et al. Liver transplantation using Chagas-infected donors in uninfected recipients: a single-center experience without prophylactic therapy. Am J Transplant. 2012;12(10):2832-7. Available from: https://doi.org/10.1111/j.1600-6143.2012.04160.x

73. Sousa AA, Lobo MC, Barbosa RA, Bello V. Chagas seropositive donors in kidney transplantation. Transplant Proc. 2004;36(4):868-9. Available from: https://doi.org/10.1016/j.transproceed.2004.03.113

74. Lattes R, Lasala MB. Chagas disease in the immunosuppressed patient. Clin Microbiol Infect. 2014;20(4):300-9. Available from: https://doi.org/10.1111/1469-0691.12585

75. Bern C, Kjos S, Yabsley MJ, Montgomery SP. *Trypanosoma cruzi* and Chagas' Disease in the United States. Clin Microbiol Rev. 2011;24(4):655-81. Available from: https://doi.org/10.1128/CMR.00005-11

76. Hermann E, Truyens C, Alonso-Vega C, Rodriguez P, Berthe A, Torrico F, et al. Congenital transmission of *Trypanosoma cruzi* is associated with maternal enhanced parasitemia and decreased production of interferon- gamma in response to parasite antigens. J Infect Dis. 2004;189(7):1274-81. Available from: https://doi.org/10.1086/382511

77. Riarte A, Luna C, Sabatiello R, Sinagra A, Schiavelli R, De Rissio A, et al. Chagas' disease in patients with kidney transplants: 7 years of experience 1989-1996. Clin Infect Dis. 1999;29(3):561-7. Available from: https://doi.org/10.1086/598634

78. Chagas' Disease Argentine Collaborative Transplant Consortium; Casadei D. Chagas' disease and solid organ transplantation. Transplant Proc. 2010;42(9):3354-9. Available from: https://doi.org/10.1016/j.transproceed.2010.09.019

79. Diez M, Favaloro L, Bertolotti A, Burgos JM, Vigliano C, Lastra MP, et al. Usefulness of PCR strategies for early diagnosis of Chagas' disease reactivation and treatment follow-up in heart transplantation. Am J Transplant. 2007;7(6):1633-40. Available from: https://doi.org/10.1111/j.1600-6143.2007.01820.x

80. de Freitas VL, da Silva SC, Sartori AM, Bezerra RC, Westphalen EV, Molina TD, et al. Real-time PCR in HIV/*Trypanosoma cruzi* coinfection with and without Chagas disease reactivation: association with HIV viral load and CD4 level. PLoS Negl Trop Dis. 2011;5(8):e1277. Available from: https://doi.org/10.1371/journal.pntd.0001277

81. Lescure FX, Le Loup G, Freilij H, Develoux M, Paris L, Brutus L, et al. Chagas disease: changes in knowledge and management. Lancet Infect Dis. 2010;10(8):556-70. Available from: https://doi.org/10.1016/S1473-3099(10)70098-0

82. Britto CC. Usefulness of PCR-based assays to assess drug efficacy in Chagas disease chemotherapy: value and limitations. Mem Inst Oswaldo Cruz. 2009;104(Suppl 1):122-35. Available from: https://doi.org/10.1590/S0074-02762009000900018

83. Campos SV, Strabelli TM, Amato Neto V, Silva CP, Bacal F, Bocchi EA, et al. Risk factors for Chagas' disease reactivation after heart transplantation. J Heart Lung Transplant. 2008;27(6):597-602. Available from: https://doi.org/10.1016/j.healun.2008.02.017

84. Dictar M, Sinagra A, Verón MT, Luna C, Dengra C, De Rissio A, et al. Recipients and donors of bone marrow transplants suffering from Chagas' disease: management and preemptive therapy of parasitemia. Bone Marrow Transplant. 1998;21(4):391-3. Available from: https://doi.org/10.1038/sj.bmt.1701107

85. Altclas J, Sinagra A, Dictar M, Luna C, Verón MT, De Rissio AM, et al. Chagas disease in bone marrow transplantation: an approach to preemptive therapy. Bone Marrow Transplant. 2005;36(2):123-9. Available from: https://doi.org/10.1038/sj.bmt.1705006

86. Sánchez-Montalvá A, Salvador F, Ruiz-Camps I, Barba P, Valcárcel D, Sulleiro E, et al. Imported Disease Screening Prior to Chemotherapy and Bone Marrow Transplantation for Oncohematological Malignancies. Am J Trop Med Hyg. 2016;95(6):1463-8. Available from: https://doi.org/10.4269/ajtmh.16-0458

87. Huprikar S, Bosserman E, Patel G, Moore A, Pinney S, Anyanwu A, et al. Donor-derived *Trypanosoma cruzi* infection in solid organ recipients in the United States, 2001-2011. Am J Transplant. 2013;13(9):2418-25. Available from: https://doi.org/10.1111/ajt.12340

88. Pinazo MJ, Miranda B, Rodríguez-Villar C, Altclas J, Brunet Serra M, García-Otero EC, et al. Recommendations for management of Chagas disease in organ and hematopoietic tissue transplantation programs in nonendemic areas. Transplant Rev (Orlando). 2011;25(3):91-101. Available from: https://doi.org/10.1016/j.trre.2010.12.002

89. Coura JR, de Abreu LL, Willcox HP, Petana W. Estudo comparativo controloado com emprego de benznidazole, nifurtimox e placebo, na forma crônica da doença de Chagas; em uma área de campo com transmissão interrompida. I. Avalição preliminar [Comparative controlled study on the use of benznidazole, nifurtimox and placebo, in the chronic form of Chagas' disease, in a field area with interrupted transmission. I. Preliminary evaluation]. Rev Soc Bras Med Trop. 1997;30(2):139-44. Portuguese. Available from: https://doi.org/10.1590/S0037-86821997000200009

90. Dias JCP and Coura JR. Clínica e terapêutica da doença de Chagas: uma abordagem prática para o clínico geral [Internet]. 1 st. Ed. Rio de Janeiro: Editora Fiocruz; 1997. 486 p.

91. Rassi A, Amato Neto V, de Siqueira AF, Ferriolli Filho F, Amato VS, Rassi Júnior A. Efeito protetor do benznidazol contra a reativação parasitária em pacientes cronicamente infectados pelo *Trypanosoma cruzi* e tratados com corticóide em virtude de afecções associadas [Protective effect of benznidazole against parasite reactivation in patients chronically infected with *Trypanosoma cruzi* and treated with corticoids for associated diseases]. Rev Soc Bras Med Trop. 1999;32(5):475-82. Portuguese. Available from: https://doi.org/10.1590/S0037-86821999000500002

92. Bern C. Chagas disease in the immunosuppressed host. Curr Opin Infect Dis. 2012;25(4):450-7. Available from: https://doi.org/10.1097/QCO.0b013e328354f179

93. Lazo J, Meneses AC, Rocha A, Ferreira MS, Marquez JO, Chapadeiro E, et al. Chagasic meningoencephalitis in the immunodeficient. Arq Neuropsiquiatr. 1998;56(1):93-7. Available from: https://doi.org/10.1590/S0004-282X1998000100015

94. Benatti RD, Oliveira GH, Bacal F. Heart Transplantation for Chagas Cardiomyopathy. J Heart Lung Transplant. 2017;36(6):597-603. Available from: https://doi.org/10.1016/j.healun.2017.02.006

95. Reimer-McAtee MJ, Mejia C, Clark T, Terle J, Pajuelo MJ, Cabeza J, et al. HIV and Chagas Disease: An Evaluation of the Use of Real-Time Quantitative Polymerase Chain Reaction to Measure Levels of Trypanosoma cruzi Parasitemia in HIV Patients in Cochabamba, Bolivia. Am J Trop Med Hyg. 2021;105(3):643-50. Available from: https://doi.org/10.4269/ajtmh.20-1141

96. Gray EB, La Hoz RM, Green JS, Vikram HR, Benedict T, Rivera H, et al. Reactivation of Chagas disease among heart transplant recipients in the United States, 2012-2016. Transpl Infect Dis. 2018;20(6):e12996. Available from: https://doi.org/10.1111/tid.12996

97. Salvador F, Len O, Molina I, Sulleiro E, Sauleda S, Bilbao I, et al. Safety of liver transplantation with Chagas disease-seropositive donors for seronegative recipients. Liver Transpl. 2011;17(11):1304-8. Available from: https://doi.org/10.1002/lt.22346

98. dos Santos-Neto LL, Polcheira MF, Castro C, Lima RA, Simaan CK, Corrêa-Lima FA. Alta parasitemia pelo *Trypanosoma cruzi* em paciente com lupus eritematoso sistêmico [*Trypanosoma cruzi* high parasitemia in patient with systemic lupus erythematosus]. Rev Soc Bras Med Trop. 2003;36(5):613-5. Portuguese. Epub 2003 Oct 21.

99. Sánchez AG, Baenas DF, Bonisconti F, Salinas MJH, Alvarellos A, Saurit V, et al. Reactivation of Chagas Disease in Patients With Rheumatic Autoimmune Diseases Diagnosed by Molecular Quantification Techniques. J Clin Rheumatol. 2021;27(8S):S533-S536. Available from: https://doi.org/10.1097/RHU.0000000000001108

100. Balouz V, Agüero F, Buscaglia CA. Chagas Disease Diagnostic Applications: Present Knowledge and Future Steps. Adv Parasitol. 2017;97:1-45. Available from: https://doi.org/10.1016/bs.apar.2016.10.001

101. United Nations Programme on HIV/AIDS (UNAIDS). 90-90-90: uma meta ambiciosa de tratamento para contribuir para o fim da epidemia de Aids. 2015. [Internet]. Available from: https://unaids.org.br/wp-content/uploads/2015/11/2015_11_20_UNAIDS_TRATAMENTO_META_PT_v4_GB.pdf

APPENDIX 1: STATE/MUNICIPAL REFERENCE SERVICES FOR ASSISTANCE/RESEARCH IN CASES OF CO-INFECTION OR REACTIVATION OF CHAGAS DISEASE

SOUTH REGION

STATE – Rio Grande do Sul

1. Name of Service: Infectious and Parasitic Diseases Outpatient Clinic of UFCSPA/Santa Casa de Misericórdia de Porto Alegre/RS

Name of Person Responsible: Marília Maria dos Santos Severo

Address: Rua Professor Annes Dias, nº 295, Centro Histórico, Porto Alegre/RS/Brazil/ ZIP Code: 90020-090.

Telephone: +55 51 3214-8035

E-mail: mariliass@ufcspa.edu.br

Website: https://instagram.com/infecto.ufcspa/

SOUTHEAST REGION

STATE - São Paulo

1. Name of Service: Chagas Disease Study Group-GEDoCh/HC/Unicamp

Name of Person Responsible: Eros Antonio de Almeida

Address: Hospital de Clínicas da Unicamp/Rua Alexander Fleming, nº 40/Campus Universitário Zeferino Vaz/Distrito de Barão Geraldo/ Campinas/SP/Brazil/ ZIP Code: 13083-887.

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2. Name of Service: Chagas disease Outpatients clinic. Clínica de Moléstias Infecciosas, Hospital das Clínicas da Faculdade de Medicina, Universidade de São Paulo.

Name of the Person Responsible: Noêmia Barbosa de Carvalho and Maria Aparecida Shikanai Yassuda.

Address: Avenida Dr. Enéas Carvalho de Aguiar, nº 255. Insitituo Central

Paulo/SP/Brazil/ZIP Code: 05403-000

Telephone: +55 11 2662-6397

E-mail: nobc@uol.com.br

Website: https://www.usp.br/imt/portal/

3. Name of Service: Neglected Diseases Outpatient Clinic/Institute of Infectious Diseases Emílio Ribas

Name of Person Responsible: Guilherme Assis dos Anjos

Address: Avenida Dr. Arnaldo, nº 165. Bairro Pacaembu, São Paulo/SP/Brazil/ZIP Code: 01246-900

Telephone: +55 11 3896-1317

E-mail: guilherme.anjos@emilioribas.sp.gov.br

Website: https://www.emilioribas.org

STATE: Minas Gerais

Name of Service: Reference Outpatient Clinic for Chagas Disease/Federal University of Triângulo Mineiro (UFTM)

Name of Person Responsible: Dalmo Correia / Rodrigo Molina

Address: Hospital de Clínicas da UFTM, Av. Getúlio Guaritá, S/N, Abadia, Uberaba-MG, Brazil, ZIP Code: 38025-440

Telephone: +55 34 3318-5254

E-mail: dalmocorreiadip@gmail.com

STATE: Rio de Janeiro

Name of Service: National Institute of Infectious Diseases, Evandro Chagas/INI/Fundação Oswaldo Cruz (Fiocruz)

Name of Person Responsible: Mauro Felippe Felix Mediano

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Telephone: +55 21 3865-9648

E-mail: mffmediano@gmail.com/mauro.mediano@ini.fiocruz.br

Website: https://www.ini.fiocruz.br

NORTHEAST REGION:

STATE: Pernambuco

Name of Service: Chagas Outpatient Clinic/IC-PROCAPE-UPE/House of patients with Chagas disease

Name of Person Responsible: Wilson Oliveira Júnior

Address: Rua Álvares de Azevedo, nº 220. Bairro Santo Amaro/Recife/PE/Brazil/ZIP Code : 50100-040

Telephone: +55 81 3181-7211

Email: casadechagas@yahoo.com.br

Website: https://chagas.fiocruz.br/recife/

STATE: Ceará

1. Name of service: Department of Community Health and Postgraduate Program in Public Health/Faculty of Medicine/Federal University of Ceará.

Name of Person Responsible: Alberto Novaes Ramos Jr.

Address: Rua Professor Costa Mendes, 1608 – Bloco didático, 5th Floor – Rodolfo Teófilo/Fortaleza/CE/Brazil/ZIP Code: 60430-140.

Telephone: +55 85 3366-8044/8050

Email: novaes@ufc.br/saudecom@ufc.br

Website: https://www.medicina.ufc.br

2. Name of Service: Cardiology Service of the Walter Cantídio University Hospital, Federal University of Ceará.

Name of Person Responsible: Eduardo Arrais Rocha

Address: Rua Pastor Samuel Munguba, 1290 - Rodolfo Teófilo/Fortaleza/CE/Brazil/ZIP Code: 60430-372.

Telephone: +55 85 3366-8167 / (85) 99121-5386.

Email: eduardoa@cardiol.br

Website: https://www.gov.br/ebserh/pt-br/hospitais-universitarios/regiao-nordeste/ch-ufc.

MIDWEST REGION

STATE: Goiás

Name of Service: Hospital de Clínicas of the Federal University of Goiás

Name of Person Responsible: Moara Alves Borges Santa Bárbara.

Address; Rua 235 QD. 68, Lote Área, s/nº. Setor Leste Universitário,

Goiânia/GO/Brasil/ZIP Code: 74605-050

Telephone: +55 62 98628-6606

Email: moarassb@ufg.br

NORTH REGION

STATE: Pará

Name of Service: Instituto Evandro Chagas/Fiocruz/PA

Name of Person Responsible: Ana Yecê das Neves Pinto

Address: Avenida Almirante Barroso, nº 492, São Brás, Belém/PA/Brazil/ZIP Code: 66093-020;

Telephone: +55 91 3214-2170

Email: secctiab@iec.pa.gov.br

Website: https://www.gov.br/iec/pt-br

STATE: Tocantins

Name of Service: Palmas General Hospital/State Health Department

Name of Person Responsible: Flávio Augusto de Pádua Milagres

Address: 201 Sul, Avenida NS1, Conjunto 02, Lot 02, Palmas/TO/Brasil/ZIP Code: 77015-202;

Telephone: +55 63 3218-7801

Email: flaviomilagres@uft.edu.br

Website: https://www.to.gov.br/saude

APPENDIX 2: CLINICAL-EPIDEMIOLOGICAL RECORD OF CASES OF *T. CRUZI*/HIV CO-INFECTION

1 Health Unit: _____

2 Doctor responsible: _____ **3** Notification Date: ___/___/___

4 HIV Infection Diagnosis: ___/___/___ **5** Diagnosis of Chagas disease: ___/___/___

6 Diagnosis Reactivation: ___/___/___

7 Situation at the time of notification: |__| 1= Death; 2= As it follows; 3= Abandonment; 9=Ign

8 If dead, date: ____/____/____

9 Basic causes of death according to medical interpretations: |__| 1= Reactivation Chagas disease; 2= Chronic Chagas disease; 3= AIDS opportunist; 4= Others; 9=Ign

IDENTIFICATION (data at the time of diagnosis and definition of co-infection)

10 Patient Name: _____

11 Date of birth: ____/_____/____ **12** Age: |__|__| |__| days |__| months |__| years **13** Sex: |__| **1**= Men; **2**=Fem; **9**=Ign

14 Mother's Name: _____

15 Place of Birth: (city/state; rural/urban): _____-_____

16 Municipality of Residence: _____

18 State: _____

19 Country (if resident outside Brazil): _____

20 Race/Color: |__| **1**= White; **2**= Black; **3**= Yellow; **4**= Brown; **5**= Indigenous; **6**= Other; **9**=Ign

INITIAL CLINICAL AND LABORATORY DATA (consider the first 6 months after diagnosis of co-infection)

21 Megaesophagus: |__| **1**= yes; **2**= no; **9**=ign **22** Megacolon: |__| **1**= yes; **2**= no; **9**=ign

23 Cardiomyopathy: |__| **1**= yes; **2**= no **9**=ign **24** Indeterminate form: |__| **1**= yes; **2**= no.

25 Serological tests for Chagas disease*: |__| **1**= positive; **2**= negative; **3**= Inconclusive; **4**= It was not done; **5**= Ign

26 Date: __/__/__

27 Parasitological diagnosis: |__| **1**= direct search (+) *T. cruzi* blood**; 2** = histopathological; **3** = direct search for *T. cruzi* in fluids associated with histopathology; **4 =** others (cite) _____**; 9** = ign

28 Xenodiagnosis |__| **1**=positive; **2**=negative; **3**= unrealized; **9**=Ign

29 Date of first positive result __/__/__

30 Blood culture |__| **1**=positive; **2**=negative; **3**= unrealized; **9**=Ign

31 Date of first positive result __/__/__

32 PCR |__| **1**=positive; **2**=negative; **3**=unrealized; **9**=Ign

33 Date of first positive result __/__/__

34 Others: |_____| **35** First test date __/__/__

36 Lymphocyte count: CD4 = _____ cels/µl **37** Collection Date ____/____/____

38 HIV Viral Load _____ copies/µl **39** Collection Date ____/____/____

40 Did co-infected patients reactivate?|__| 1=yes; 2=no; 9=Ign. **(If there was reactivation, skip to 42)**

41 If there was no reactivation, did you receive a specific treatment? |__| 1=yes; 2=no; 3= Others; 9=ign. **(If there was no reactivation, end at 41)**

APPENDIX 2: CLINICAL-EPIDEMIOLOGICAL RECORD OF CASES OF *T. CRUZI*/HIV CO-INFECTION

CLINICAL AND LABORATORY DATA ON REACTIVATION
Fill in the following information for patients who REACTIVATED

42 Type of Reactivation: |__| **1**= meningoencephalitis; **2**= myocarditis; **3**= other (cite); _____
9= ign

43 Reactivation Diagnostics: |__| **1**= Direct Search (+) *T. cruzi* blood**; 2** = Histopathological; **3** = direct investigation of *T. cruzi* in fluids associated with histopathology; **4 =** Other (cite)_____**; 9** = ign

44 Lymphocyte count: CD4 = _____ cels/µl **45** Date of Collection ____/____/____

46 HIV Viral Load _____ Copies/µl **47** Date of Collection ____/____/____

48 Use of benznidazole? I__I 1-yes; 2-no

Benznidazole dose:_____ Date: ____/____/____

49 Use of imidazoles? I__I 1-yes; 2-no. Specify which:_____

Dose of imidazolic:_____ Date: ____/____/____

INSTRUCIONAL

Definition of co-infection case: Patients who have confirmed concomitant infection with the protozoan *Trypanosoma cruzi* and the Human Immunodeficiency Virus.
Case definition of Chagas disease reactivation: In an immunocompromised patient, presence of trypomatism-drops of *T. cruzi* directly observed by microscopic examination of blood, pericardial fluid, cerebrospinal fluid or other body fluids (e.g. ascites fluid), or occurrence of histopathological changes compatible with acute inflammatory process and presence of amastigote nests.
Serological tests: Consider ELISA and/or Immunofluorescence and/or Hemagglutination and/or Complement Fixation:
* Instructional: + = 2 or 3 positive in 2 or 3. Negative 2 from 2 or 3 de 3 Negative. Inconclusive: no + no Negative. NFF It wasn't done.

Estadiamento do comprometimento miocárdico na cardiopatia chagásica crônica.

Estádios	Eletrocardiograma	Ecocardiograma	Insuficiência cardíaca
A	Alterado	Normal	Ausente
B1	Alterado	Alterado FEVE ≥ 45%	Ausente
B2	Alterado	Alterado FEVE < 45%	Ausente
C	Alterado	Alterado	Compensável
D	Alterado	Alterado	Refratária

Staging of myocardial involvement in chronic Chagas cardiomyopathy

Stage	Electrocardiogram	Echocardiogram	Cardiac insufficiency
A	Altered	Normal	Absent
B1	Altered	Altered, LVEF ≥ 45%	Absent
B2	Altered	Altered, LVEF < 45%	Absent
C	Altered	Altered	Compensable
D	Altered	Altered	Refractory

APPENDIX 2: CLINICAL-EPIDEMIOLOGICAL RECORD OF CASES OF *T. CRUZI*/HIV CO-INFECTION

Classificação de comprometimento esofágico (Rezende, 1960)

Grupos	Alterações
I	Esôfago de calibre aparentemente normal (exame radiológico) Trânsito lento. Pequena retenção na radiografia tomada um minuto após a ingestão
II	Esôfago com pequeno a moderado aumento do calibre. Apreciável retenção de contraste. Presença freqüente de ondas terciárias, associadas ou não a hipertonia do esôfago
III	Esôfago com grande aumento de diâmetro. Atividade motora reduzida. Hipotonia do esôfago inferior. Grande retenção de contraste
IV	Dolicomegaesôfago. Esôfago com grande capacidade de retenção, atônico, alongado, dobrando-se sobre a cúpula diafragmática

Classification of esophageal involvement (Rezende, 1960)

Groups	Changes
I	Esophagus of apparently normal caliber (radiological examination) Slow transit. Small retention on x-ray taken one minute after ingestion.
II	Esophagus with small to moderate increase in caliber. Appreciable contrast retention. Frequent presence of tertiary waves, associated or not with esophageal hypertonia
III	Esophagus with large increase in diameter. Reduced motor activity. Hypotonia of the lower esophagus. Great contrast retention
IV	Dolichomegaesophagus. Esophagus with great retention capacity, atonic, elongated, folding over the diaphragmatic dome

Observations: _____

APPENDIX 3: CONTACTS OF THE "NETWORK FOR HEALTHCARE AND STUDY OF *TRYPANOSOMA CRUZI*/HIV CO-INFECTION AND OTHER IMMUNOSUPPRESSION CONDITIONS".

1. GT-Chagas: Chagas Disease Technical Group/SVS/Brazilian Ministry of Health Portal.Gov.br: https://www.gov.br/saude. Email: chagas@saúde.gov.br.

2. Department of HIV/AIDS, Tuberculosis, Viral Hepatitis, and Sexually Transmitted Infections//SVSA/Ministry of Health of Brazil – Portal. Gov.br: https://www.gov.br/saude.

3. Universidade Estadual de Campinas/SP/Brazil/Study Group on Chagas Disease-GEDoCh. Email: dcmedfcm@unicamp.br. Fone: 55 11 2662-6397.

4. University of São Paulo-USP/São Paulo/SP/Brazil/Chagas disease Outpatients clinic. Clínica de Moléstias Infecciosas, Hospital das Clínicas da Faculdade de Medicina, Universidade de São Paulo. https://www.usp.br/imt/portal/. Fone: + 55 11 3061 7011.

5. Evandro Chagas, National Institute of Infectious Diseases/INI/Oswaldo Cruz Foundation/Ministry of Health. https://www.ini.fiocruz.br. Fone: + 55 21 3865 9595.

APPENDIX 4: CENTRAL PUBLIC HEALTH LABORATORIES FOR CHAGAS DISEASE REFERRAL

NORTHERN REGION				
STATE	**INSTITUTION**	**ADDRESS**	**TELEPHONE**	**E-mail**
Acre	Central Public Health Laboratory Dr. Djalma da Cunha Batista	Av. Getúlio Vargas, Travessa do HEMOACRE, s/n. **CEP:** 69.900-614. Rio Branco/AC	+55 68 3228 2720	lacen.saude@ac.gov.br
Amazonas	Central Public Health Laboratory	Rua Emílio Moreira, 510, Praça 14, Centro. **CEP:** 69.020-040. Manaus/AM	+55 92 31828750	lacen@fvs.am.gov.br
Amapá	Central Public Health Laboratory Prof. Reinaldo Damasceno	Rua Tancredo Neves nº 1118, Bairro São Lázaro.**CEP**: 68.908-530. Macapá/AP	+55 96 32511233	diretoria@lacen.ap.gov.br
Pará	Central Public Health Laboratory	Av. Augusto Montenegro, Km 10, Bairro Icoaraci. **CEP**: 66.823.010. Belém/PA	+55 91 32024903	lacen@sespa.pa.gov.br
Roraima	Central Public Health Laboratory	Av. Brigadeiro Eduardo Gomes, s/n, Novo Planalto.**CEP:** 69.305-650. Boa Vista/RR	+55 95 36232455	lacen_rr@yahoo.com.br
Rondônia	Central Public Health Laboratory	Rua Anita Garibaldi, nº 4130, Bairro Costa e Silva. **CEP**: 78903-770. Porto Velho/RO	+55 69 32165302	lacen_ro@hotmail.com
Tocantins	Central Public Health Laboratory	601 SUL Av. LO 15 conj. 02 Lote 01, Planalto Diretor Sul. **CEP:** 77054-970. Palmas/TO	+55 63 32183238	lacen.palmas@gmail.com
NORTHEAST REGION				
STATE	**INSTITUTION**	**ADDRESS**	**TELEPHONE**	**Email**
Maranhão	Central Public Health Laboratory Oswaldo Cruz Institute	Rua Afonso Pena, 198, Centro.**CEP**: 65010-030. São Luís/MA	+55 98 32323410	lacenmara@yahoo.com.br
Pernambuco	Central Public Health Laboratory Dr. Milton Bezerra Sobral/ FUSAN	Rua Fernandes Vieira, s/nº, Bairro Boa Vista. **CEP**: 50050-220. Recife/PE	+55 81 31816416	lacen@lacen.pe.gov.br
Alagoas	Central Public Health Laboratory Dr. Aristeu Lopes	Av. Marechal Castelo Branco, 1773, Bairro Jatiúca.**CEP**: 57036-340. Maceió/AL	+55 82 33152702	coordenacao@lacen.com.br
Ceará	Central Public Health Laboratory	Av. Barão de Studart, 2405 – Aldeota. **CEP**: 60120-002. Fortaleza/CE	+55 85 31011472	lacen@lacen.ce.gov.br
Paraíba	Central Public Health Laboratory	Av. Cruz das Armas, s/n, Bairro Cruz das Armas. **CEP**: 58085-000. João Pessoa/PB	+55 83 32185926	marta.lacenpb@gmail.com

APPENDIX 4: CENTRAL PUBLIC HEALTH LABORATORIES FOR CHAGAS DISEASE REFERRAL

Piauí	Central Public Health Laboratory Dr. Costa Alvarenga	Rua 19 de Novembro, 1945, Bairro Primavera.**CEP:** 64002-570. Teresina/PI	+55 86 32163657	diretoria.lacen@lacen.pi.gov.br
Rio Grande do Norte	Central Public Health Laboratory	Rua Cônego Monte, s/n – Quintas.**CEP**: 59037-170. Natal/ RN	+55 84 32326195	lacenrn@yahoo.com.b
Sergipe	Central Public Health Laboratory Parreiras Horta Health Foundation	Rua Campo do Brito, 55, Bairro São José.**CEP**: 49020-380. Aracaju/SE	+55 79 32346020	lacen.fsph@fsph.se.gov.br
Bahia	Central Public Health Laboratory Prof. Gonçalo Moniz	Rua Waldemar Falcão, 123 – Horto Florestal. **CEP**: 40295-001. Salvador/BA	+55 71 33561414	lacen.diretoria@saude.ba.gov.br
SOUTHEAST REGION				
STATE	**INSTITUTION**	**ADDRESS**	**TELEPHONE**	**Email**
Rio de Janeiro	Central Public Health Laboratory Noel Nutels	Rua do Resende, 118 - Bairro de Fátima. **CEP**: 20231-092. Rio de Janeiro/RJ	+55 21 23328597	dgnnutels@saude.rj.gov.br
Espírito Santo	Central Public Health Laboratory	Avenida Marechal Mascarenhas de Moraes, 2025 – Bento Ferreira.**CEP**: 29052-121. Vitória/ES	+55 27 33258275	lacen@saude.es.gov.br;
Minas Gerais	Octávio Magalhães Institute / Ezequiel Dias Foundation	Rua Conde Pereira Carneiro, 80 – Gameleira. **CEP**: 30510-010. Belo Horizonte/ MG	+55 31 33144655	iomlacen@funed.mg.gov.br
São Paulo	Institute Adolfo Lutz - IAL	Av. Dr. Arnaldo, 355 - Cerqueira Cesar.**CEP**: 01246-902. São Paulo/SP	+55 11 30682800	expedientedg@ial.sp.gov.br
SOUTHERN REGION				
STATE	**INSTITUTION**	**ADDRESS**	**TELEPHONE**	**Email**
Paraná	Central Public Health Laboratory	Rua Sebastiana Santana Fraga, 1.001, Guatupê, São José dos Pinhais. **CEP:** 83060-500. Curitiba/PR	+55 41 32993200	diretorialacen@sesa.pr.gov.br
Santa Catarina	Central Public Health Laboratory	Av. Rio Branco, 152 - Fundos – Centro. **CEP**: 88015-201. Florianópolis/SC	+55 48 32517800	lacen@saude.sc.gov.br
Rio Grande do Sul	Central Public Health Laboratory	Av. Ipiranga 5.400, Bairro Jardim Botânico. **CEP**: 90610-000. Porto Alegre/RS	+55 51 32884000	lacen@saude.rs.gov.br

APPENDIX 4: CENTRAL PUBLIC HEALTH LABORATORIES FOR CHAGAS DISEASE REFERRAL

MIDWEST REGION				
STATE	**INSTITUTION**	**ADDRESS**	**TELEPHONE**	**Email**
Distrito Federal	Central Public Health Laboratory	SGAN Q. 601 - Lotes O e P.**CEP**: 70830-010. Brasília/DF	+55 61 33255288	lacen.df@gmail.com
Goiás	Central Public Health Laboratory Dr. Giovanni Cysneiros	Alameda do Contorno, 3556 - Jardim da Luz. CEP: 74853-120. Goiânia/GO	+55 62 32013888	lacen.dirgeral@saude.go.gov.br
Mato Grosso	Central Public Health Laboratory	Rua Thogo da Silva Pereira, nº 63, Centro.**CEP**: 78020-500. Cuiabá/MT	+55 65 36236404	dgmtlab@ses.mt.gov.br
Mato Grosso do Sul	Central Public Health Laboratory	Av. Senador Felinto Muller, 1666, Bairro Ipiranga.**CEP**: 79074-460. Campo Grande/MS	+55 67 33451300	lacendiretoria@saude.ms.gov.br