

# Impact of antiparasitic therapy on cardiovascular outcomes in chronic Chagas disease. A systematic review and meta-analysis



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## Summary

**Background** Endemic in more than 20 countries, Chagas disease affects 6.3 million people worldwide, leading to 28,000 new infections and 7700 deaths each year. Previous meta-analyses on antiparasitic treatment need updates to encompass recent studies and to assess key clinically meaningful endpoints. This study aims to evaluate the impact of antitrypanosomal therapy in preventing or reducing disease progression and mortality in chronic Chagas disease.

**Methods** We performed a systematic review and meta-analysis of studies reporting the cardiovascular outcomes of antitrypanosomal therapy in patients with chronic Chagas disease. We searched Ovid Embase, Ovid MEDLINE, Ovid Global Health, Scopus, Web of Science Core Collection, Cochrane Library, PubMed, Google Scholar, and Virtual Health Library databases from inception to May 18, 2024. We included aggregated data from randomized controlled studies and observational reports (full articles and abstracts) featuring antiparasitic interventions with benznidazole or nifurtimox compared to a control group. Primary outcomes were electrocardiogram (ECG) changes, disease progression, cardiovascular death, and overall mortality. A customized risk of bias scale assessed the methodological quality of studies, and a random-effects model estimated the pooled risk ratios. This investigation was registered in PROSPERO (CRD42023495755).

**Findings** Out of 4666 reports screened, 23 met the pre-specified inclusion criteria (8972 participants). Compared to no treatment or placebo, antiparasitic treatment led to a reduction in i) ECG changes (17 studies, 4994 participants: risk ratio (RR): 0.48, 95% CI 0.36–0.66,  $p < 0.001$ ;  $I^2 = 76.4\%$ ) with a number needed to treat (NNT) of 5; ii) disease progression (12 studies, 5722 participants: RR: 0.35, 95% CI 0.23–0.51,  $p < 0.001$ ;  $I^2 = 72.4\%$ ) NNT of 6; iii) cardiovascular death (7 studies, 5662 participants: RR: 0.44, 95% CI 0.21–0.95,  $p = 0.04$ ;  $I^2 = 50.5\%$ ) NNT of 22; and iv) overall mortality (10 studies, 7694 participants: RR: 0.54, 95% CI 0.34–0.87,  $p < 0.001$ ;  $I^2 = 60\%$ ) NNT of 23.

**Interpretation** We found compelling evidence that antiparasitic treatment significantly reduces the risk of ECG changes, disease progression, cardiovascular death, and overall mortality in chronic Chagas disease. Although the quality of evidence ranges from low to intermediate, with considerable heterogeneity across studies, the potential

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benefits are substantial. These findings support the broader use of trypanocidal therapy in the management of Chagas disease, though further research remains necessary.

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**Keywords:** Chagas disease; Chagas cardiomyopathy; *Trypanosoma cruzi*; Antitrypanosomal therapy; Benznidazole; Nifurtimox; Meta-analysis; Disease progression

### Research in context

#### Evidence before this study

Chagas disease, an infectious disease caused by the parasite *Trypanosoma cruzi*, poses a significant health burden, not only in Latin America, where transmission occurs but also in non-endemic countries due to the migration of infected individuals. It leads to severe complications such as chronic cardiomyopathy characterized by arrhythmias, heart failure, thromboembolic events, and sudden death. We examined the efficacy of antiparasitic treatment for chronic Chagas disease in a PubMed base search using search terms such as chronic Chagas disease, and antitrypanosomal therapy. We saw mixed results, often limited to surrogate endpoints like parasitemia negativization or seroreversion. We assessed several systematic reviews and meta-analyses, including two Cochrane reviews, that explored the efficacy and effectiveness of antitrypanosomal therapy. However, some studies focused only on serological or parasitological outcomes, while those that examined clinical endpoints found the evidence insufficient to reach definitive conclusions. For patients with chronic Chagas cardiomyopathy (CCC) or asymptomatic chronic Chagas disease, the available data does not indicate a clear benefit from etiological treatment. In one Cochrane meta-analysis, only two randomized controlled trials met strict inclusion criteria, resulting in insufficient data for firm conclusions. A second meta-analysis, which applied broader criteria, also failed to demonstrate treatment effectiveness due to inconsistent and low-quality data for key outcomes.

#### Added value of this study

More recently, new studies have been published, highlighting the need for an updated assessment of the evidence. This study bridges the gap by providing an extensive and updated evaluation of the clinical impact of antitrypanosomal therapy in chronic Chagas disease. We included full-text articles and abstracts published in English, Spanish, or Portuguese that reported primary data from prospective or retrospective cohorts, randomized or quasi-randomized trials, and cross-sectional studies from inception to May 18, 2024. Most studies (50%) exhibited a moderate risk of bias, with a substantial proportion (42%) showing a high risk of bias and a minority having a low risk of bias (8%). Compared to no

treatment or placebo, antiparasitic treatment led to a reduction in ECG changes (Risk ratio (RR): 0.48), disease progression (RR: 0.35), cardiovascular death (RR: 0.44), and overall mortality (0.54). Unlike previous analyses, our study incorporated a broader range of study designs and a more extended review period, providing a better understanding of the clinical efficacy of etiologic treatments. Overall, the evidence adds a positive effect of antitrypanosomal therapy in Chagas disease outcomes. The study findings suggest a more liberal approach to antitrypanosomal therapy should be implemented and validated in patients with Chagas disease.

#### Implications of all the available evidence

Despite several limitations of this meta-analysis, our findings suggest that antiparasitic therapy can significantly reduce the progression and mortality of chronic Chagas disease, challenging the previous belief that these treatments are ineffective in the chronic stage. The evidence suggests treatment benefits in patients with the indeterminate form and potentially even in the early stages of Chagas cardiomyopathy. However, the cutoffs when antiparasitic therapies fail to halt the progression of cardiac disease remain unknown. Nonetheless, our findings underscore the need for policy changes to enhance the availability and distribution of current antiparasitic drugs, ensuring they reach a broader patient population. Another point to consider is the urgency of identifying or developing definitive biological markers of cure. Due to the inherently complex nature of Chagas disease, finding a marker with 100% sensitivity is unlikely. Furthermore, even with such a marker, the limited availability of only two drugs with similar mechanisms of action diminishes the practical importance of declaring a patient cured. Efforts should focus on optimizing existing therapies and expanding their access. While researching new treatments with better efficacies and safety profiles is needed, we must recognize that developing and testing new drugs is lengthy and complex. This concept also applies to new immunotherapies. Balancing the enhancement of current treatments with the pursuit of improved therapies will potentially ensure both immediate and long-term advancements in managing Chagas disease.

## Introduction

Chagas disease (American trypanosomiasis) is an infectious disease caused by the blood and tissue parasite *Trypanosoma cruzi*, primarily transmitted through the faeces of Triatomines (kissing bugs), which contaminate mucous membranes or broken skin.<sup>1</sup> Transmission can also occur orally through food and beverage contamination, vertically (from infected mothers to their offspring), transfusion of blood products, solid organ transplantation, and laboratory accidents. Chagas disease is endemic in 21 countries across Latin America, as well as in parts of Texas and potentially other areas in the Southern U.S. According to recent estimates from the Global Burden of Disease Study 2021, Chagas disease affects approximately 6.3 million people worldwide, contributing to 239,000 disability-adjusted life years (DALYs), and causing 7700 deaths annually.<sup>2</sup> These data represent an update from previous estimates, which indicated an annual incidence of 28,000 cases, affecting about 6 million people globally and resulting in approximately 12,000 deaths per year.<sup>3</sup> The disease has become an important health problem in non-endemic countries due to the migration of infected individuals originating from endemic regions of Latin America.

Chagas disease has two sequential clinical phases: acute and chronic, with the former noted previously to be highly susceptible to parasitological cure. A more complicated picture emerges during the chronic phase, which has indeterminate (absence of clinical findings despite infection) and determinate (evidence of clinical expression) forms. The determinate form of chronic Chagas disease poses a significant burden to affected patients. As the illness progresses, it causes substantial morbidity through the development of angina, heart failure, arrhythmia, systemic or pulmonary embolism, megaesophagus, and megacolon.<sup>4</sup> Patients develop cardiomyopathy at an annual rate of 1.9% after being infected.<sup>5</sup> Once present, cardiomyopathy carries an annual mortality rate of 7.9%,<sup>6</sup> which is higher than that for acquired immunodeficiency syndrome (AIDS).<sup>7</sup> Despite advances in vector control and a reduction in disease transmission in some regions, clinical care for patients who are newly infected, as well as those who are diagnosed late, remains limited. Unfortunately, the majority of newly infected patients are asymptomatic and, therefore, go mostly undiagnosed. The window for treating acute Chagas disease successfully is missed, and these patients progress to the chronic phase.

Complicating this picture even further, when patients are diagnosed late, they often have advanced chronic Chagas cardiomyopathy (CCC), which necessitates specialized care that is both costly and time-consuming. Access to newer heart failure drugs, cardiac implantable electronic devices, catheter ablation, and cardiac transplantation is limited to only a few advanced centers. Given these challenges, early diagnosis and the timely use of effective antiparasitic drugs are both fundamental

strategies that could significantly improve patient outcomes.

A central question in the antiparasitic treatment of chronic Chagas disease is whether patients in either the indeterminate or determinate forms of the illness would benefit from such therapy. The only specific antiparasitic treatment for this infection consists of two extensively investigated nitroheterocyclic trypanocidal drugs, benznidazole (BZN) and nifurtimox (NFT).<sup>8</sup> Previous studies have clearly shown that both compounds—certainly in the acute phase, but even in many cases in the chronic phase of the illness—frequently elicit a favorable parasitological and serological response.<sup>9,10</sup> However, seroreversion may take decades to be demonstrated, and the reversion of parasitological and molecular tests cannot be considered a surrogate for cure. An important question arises: are these favorable effects in terms of parasitological cure demonstrated by the disappearance of detectable parasites in the blood also translating into clinical improvement for patients? Or is it the case that during the chronic phase, host genetic factors and parasite characteristics become more important, rendering antiparasitic treatment unable to affect the outcome of CCC or other chronic sequelae? In this regard, the effect of antitrypanosomal therapy on disease outcomes has indeed been deemed inconclusive. Several systematic reviews and meta-analyses,<sup>9–11</sup> including two Cochrane reviews,<sup>12,13</sup> have attempted to assess the efficacy and effectiveness of antitrypanosomal therapy. Unfortunately, some reports evaluated only serological or parasitological outcomes,<sup>9,10</sup> while those that evaluated clinical endpoints<sup>11–13</sup> concluded that there was insufficient evidence to draw definitive conclusions. Specifically, for patients with CCC or asymptomatic chronic Chagas disease, the current available evidence suggests there is no clear benefit from etiological treatment. One Cochrane meta-analysis, which included only two randomized controlled trials (RCTs) due to rigid inclusion criteria, was unable to find enough data to provide useful conclusions<sup>12</sup>; the second meta-analysis, which applied broader inclusion criteria, also failed to demonstrate the utility of etiological treatment due to the low quality and inconsistency of the data for key outcomes.<sup>13</sup> Several studies have emerged since these reviews,<sup>14–16</sup> necessitating an updated evaluation of the evidence. We aim to conduct a comprehensive systematic review and meta-analysis to reassess the impact of trypanocidal therapy on Chagas disease progression and mortality, considering the latest available data and addressing the limitations of previous analyses.

## Methods

### Ethic statement

For this systematic review and meta-analysis, all data were anonymized and could not be traced back to the

original participants in the referenced clinical trials or cohort studies. As a result, obtaining informed consent or requesting an ethical permit specifically for this analysis was deemed unnecessary.

### Search strategy and selection criteria

This systematic review and meta-analysis was conducted in accordance with the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.<sup>17</sup> We systematically searched Ovid Embase, Ovid MEDLINE, Ovid Global Health, Scopus, Web of Science Core Collection, Cochrane Library, PubMed, Google Scholar, and Virtual Health Library databases to find relevant articles from inception to May 18, 2024, with no language or publication restrictions. We also screened the reference lists of included full texts and previous relevant systematic reviews and sought input from experts to identify additional articles. Databases were searched using a combination of controlled vocabulary and free text terms for Chagas disease and antitrypanosomal therapy. Details of the search strategy are presented in the [Appendix \(pp 3–6\)](#). We included full-text articles and abstracts published in English, Spanish, or Portuguese that reported primary data from prospective or retrospective cohorts, randomized or quasi-randomized trials, and cross-sectional studies. Case-control studies, case reports, and *in vitro* or animal studies were excluded to focus on human data and minimize publication bias.

The search of the literature was performed by a medical librarian and peer-reviewed by a second librarian using the Peer Review of Electronic Search Strategies (PRESS) guideline. All identified studies were uploaded into Endnote 20 (Clarivate Analytics, Pennsylvania, USA), and Covidence, and duplicate studies were removed. Three reviewers (AHM, ARJ, and FMR) independently screened the titles and abstracts of the studies for eligibility. If a study was judged as potentially relevant based on the title or abstract, the full text was obtained and assessed for inclusion. Disagreements were resolved by discussion among the reviewers or, if needed, by a fourth reviewer. Using Covidence, we generated a PRISMA document<sup>17</sup> detailing the initial number of reports, the number of duplicates removed, the number excluded during title/abstract screening, and the number excluded during full-text assessments, along with reasons for exclusion ([Appendix pp 7–13](#)). Reports that met the selection criteria were included in the final analysis.

Inclusion criteria for the studies were: (1) patients with chronic Chagas disease confirmed by serological tests (both children and adults) in either the indeterminate or cardiac forms; (2) studies comparing patients treated with either BZN or NFT to a control group (either untreated or treated with placebo), with no limit on the duration of follow-up; (3) studies reporting at least one of the following outcomes: ECG change, disease progression, cardiovascular death, or overall

mortality. The definitions of outcomes varied between studies, but these variations were accepted without the need for standardization, and (4) studies providing sufficient data to estimate a risk ratio.

We excluded reports that met any of the following criteria: use of historical controls, absence of a control group, administration of BZN or NFT in combination with other compounds, focus on acute or congenital infections, emphasis on preventing congenital Chagas disease through treatment of reproductive-age women, or those in the context of organ transplantation or immunocompromised patients. It is important to note that some studies did not distinguish between different groups and analyzed mixed populations together, such as children and adults, patients treated with BZN or NFT, and those with the indeterminate form alongside patients with the cardiac form. Finally, when multiple publications were based on the same cohort, we included data only from the most recent and comprehensive report.

### Data analysis

Two reviewers (AHM and ARJ) independently accrued data using a standardized Excel spreadsheet for initial data extraction, and disagreements were resolved through discussion. Once extracted, the data were transferred to Research Electronic Data Capture (REDCap) for further management and analysis. We extracted aggregated data as published, which included author, year and type of publication, country, type of study, population description (children and/or adults, disease stage or form, mean age, and percentage of men), number of participants (total and in each group), intervention (type of medication and dose), control group description, outcomes assessment (ECG change, disease progression, cardiovascular death, and overall mortality), and mean duration of follow-up. The disease stage was categorized into three groups: patients with cardiomyopathy, those exclusively in the indeterminate form, and those in the chronic phase (chronic Chagas disease). It's important to note that the studies classified as chronic Chagas disease included both patients with the indeterminate form and those with mild cardiomyopathy. However, the outcomes were analyzed collectively without distinguishing between patients with indeterminate or determinate forms. This classification approach allows for a comprehensive analysis of disease progression across different stages of Chagas disease while acknowledging the potential overlap between categories, particularly within the chronic phase group. For the pooled mean age and pooled mean follow-up, we used weighted values, which were calculated by multiplying each study's mean by its sample size (the "weight") and then dividing the sum of these values by the total sample size across all studies. This approach ensures that larger, more statistically robust studies have a greater impact on the overall mean, reducing the

potential bias introduced by smaller studies and providing more accurate results. Data related to side effects were not collected as they were either inconsistently reported or not reported at all across the various studies.

ECG alterations were measured as any new significant ECG change recorded during the study. However, in their analysis, some studies included all patients with ECG changes at the end of the follow-up rather than just the new cases. Disease progression was defined as any worsening disease parameter (e.g., worsening cardiomyopathy based on clinical symptoms, chest x-rays, ECG, or echocardiography). As mentioned earlier, there were some discrepancies in these definitions among the studies, but these differences do not significantly impact the analysis of the results.

All extraction forms, tools, and meta-analyses were pilot-tested on two studies and subsequently modified to ensure the extraction of relevant data and confirm the feasibility of our research question. We contacted the study authors to address any relevant ambiguities.

#### Risk of bias across studies

Two reviewers (AHM, ARJ) independently evaluated the included studies for risk of bias analysis, using a customized scale, with a third reviewer (FMR) resolving any disagreements. The risk of bias was classified as high, moderate, or low for each study (Appendix pp 14–15).

#### Statistical analysis

Risk ratios (RR) with 95% confidence intervals (CI) were used as the summary measure for all outcomes. These RRs were calculated based on the extracted frequencies and denominators, representing the ratio of outcomes in the antitrypanosomal drug group compared to the control group. Given the differences in study design among the included studies, we assumed a high potential for heterogeneity and used a random-effects model to calculate pooled effects size. Between-study heterogeneity was estimated using the  $I^2$  statistic, representing the proportion of variability not attributable to chance.  $I^2$  values equal to or greater than 50% indicate substantial heterogeneity.<sup>18</sup>

In a sensitivity analysis, the influence of individual studies on the summary statistics was examined by omitting one study at a time from the meta-analysis. We also conducted subgroup analyses for all outcomes based on the following potential sources of heterogeneity: publication type, disease stage, antiparasitic drug used, treatment duration of BZN, study design, population age, risk of bias of included studies, and country. For all subgroup analyses, we assessed whether subgroup effects (interaction) were present.

Meta-regression analyses of numerical variables were performed to examine factors associated with treatment efficacy and contributing to study heterogeneity. Contour-enhanced funnel plots were constructed to

assess publication bias, and the Egger test was used to evaluate for a small-study effect.<sup>19</sup> All tests were two-sided, and a p-value <0.05 was considered statistically significant. Statistical analyses were performed using Stata software, version 18.0 (StataCorp), and the protocol was prospectively registered in PROSPERO (CRD42023495755).

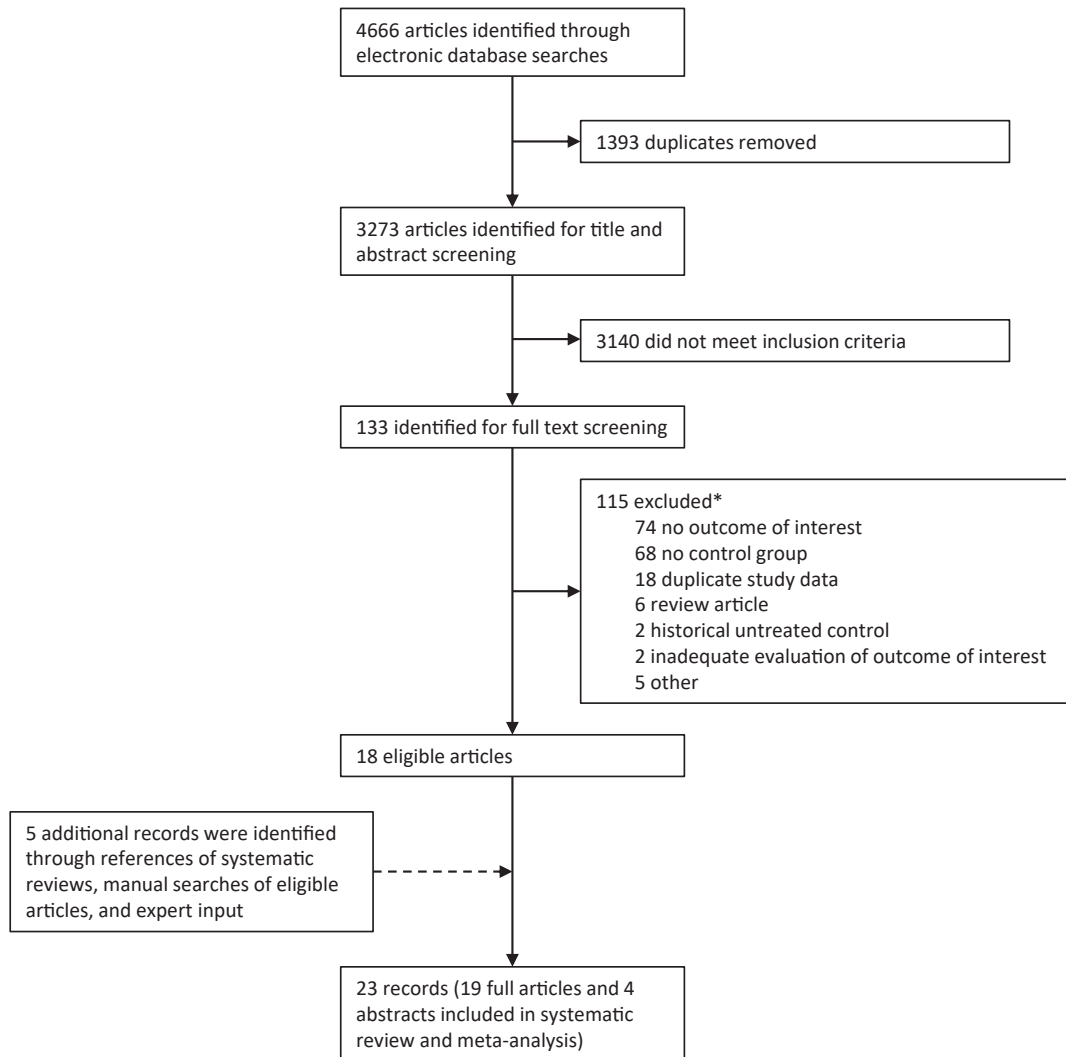
#### Role of the funding source

There was no funding source for this study. The corresponding authors had full access to all the data in the study and took final responsibility for the decision to submit the manuscript for publication.

## Results

### Literature search for Chagas disease studies with antitrypanosomal therapies

Database searches yielded 4666 citations. After automated duplicate removal, 3273 citations underwent title and abstract screening. Of these, 133 reports met the criteria for full-text review. Following the full-text review, 115 papers were excluded (Appendix pp 7–13), and 5 were manually added (4 abstracts<sup>20–23</sup> and 1 full-text article)<sup>24</sup> (Fig. 1). Ultimately, 23 studies<sup>14–16,20–39</sup> met the inclusion criteria (19 full-text articles<sup>14–16,24–39</sup> and 4 abstracts).<sup>20–23</sup> Notably, one study<sup>15</sup> employed two different designs: a cross-sectional design to evaluate ECG changes and a prospective design to evaluate mortality. Therefore, the percentages provided next refer to the 24 different study designs. Ten (42%) studies were retrospective, 8 (33%) were prospective, 5 (21%) were RCTs, and 1 (4%) was a cross-sectional study. One study<sup>32</sup> used an alternating sequence for randomization (quasi-randomization), and acknowledging the differences between RCT and quasi-randomized study, we included this study among the RCTs. The 23 included studies contained a total of 8972 participants (3859 treated and 5113 control). The number of participants in each study varied from a minimum of 33<sup>21</sup> and a maximum of 2854.<sup>36</sup> The weighted mean age of participants was 43.4 years, with individual study means ranging from 9 years<sup>28</sup> to 55 years<sup>36</sup> (Table 1). Our analysis included 43.6% male subjects. The weighted mean follow-up duration was 6.7 years, ranging from 1<sup>27</sup> to 25 years.<sup>39</sup> For the selected outcomes, we had 17 studies reporting on ECG change, 12 on disease progression, 7 on cardiovascular death, and 10 on overall mortality (Table 1). Disease progression was defined differently across studies. Five studies<sup>14,24,27,30,35</sup> defined it through clinical, electrocardiographic, or echocardiographic radiological changes. Four studies<sup>25,29,32,34</sup> used the Kuschnir classification, defining progression as a change to a more advanced group. Kuschnir groups are based on the presence of abnormal ECG, cardiomegaly, and symptoms of heart failure: Group 0–normal ECG and chest X-ray; Group I–abnormal ECG, normal chest



**Fig. 1:** PRISMA figure. Selection of reports for inclusion in meta-analysis. \*The total exceeds 115 because some studies met more than one exclusion criterion.

X-ray; Group II—abnormal ECG and cardiomegaly without heart failure; Group III—cardiomegaly with signs of heart failure. Two studies<sup>16,36</sup> used composite outcomes of cardiovascular events, and one study defined progression as the development of cardiomyopathy with left ventricular ejection fraction (LVEF) < 50%.<sup>23</sup> Most studies (50%) exhibited a moderate risk of bias, with a substantial proportion (42%) showing a high risk of bias and a minority having a low risk of bias (8%) (Appendix p 15).

**Key endpoint analysis**

In the random-effects model analysis, antitrypanosomal therapy significantly reduced the risk of several key outcomes in patients with chronic Chagas disease compared to placebo or no therapy (Table 2, Fig. 2). The

overall risk ratios (RR) were 0.48 (95% CI: 0.36–0.66,  $p < 0.001$ ) for ECG changes with a heterogeneity  $I^2$  of 76.4% and a number needed to treat (NNT) of 5, after a mean follow-up of 9.9 years (4994 participants from 17 studies, 4 RCTs and 13 observational studies); 0.35 (95% CI: 0.23–0.51,  $p < 0.001$ ) for disease progression with a heterogeneity  $I^2$  of 72.4% and an NNT of 6, after a mean follow up of 7.9 years (5722 participants from 12 studies, 3 RCTs and 9 observational studies); 0.44 (95% CI: 0.21–0.95,  $p = 0.04$ ) for cardiovascular mortality with a heterogeneity  $I^2$  of 50.5% and an NNT of 22, after a mean follow up of 7.2 years (5662 participants from 7 studies, 2 RCTs and 5 observational studies); and 0.54 (95% CI: 0.34–0.87,  $p < 0.001$ ) for overall mortality with a heterogeneity  $I^2$  of 60% and an NNT of 23, after a mean follow up of 6.2 years (7694 participants from 10

Author, publication type	Country	Study type	Population age and disease stage	Total of participants (treated/control)	Intervention vs control	Outcomes assessment	Mean follow up, years	BZN/NFT dose (mg/kg/day) and duration of therapy (days)	Age at treatment (mean)	Men (%)	Risk of Bias
Macedo et al. (1987), <sup>20</sup> abstract	Brazil	Retrospective	Adults with chronic indeterminate form	171 (103/68)	BZN/NFT vs placebo	ECG change	7	BZN (7) (30–60) NFT (7–8) (60–90)	NI	NI	High
Ianni et al. (1993), <sup>21</sup> abstract	Brazil	Retrospective	Adults with chronic indeterminate form	33 (15/18)	BZN vs placebo	ECG change	8.1	BZN (5) (60)	NI	NI	High
Miranda et al. (1994), <sup>24</sup> full article	Brazil	Prospective	Children and adults with chronic Chagas disease	120 (76/44)	BZN vs untreated	Disease progression	13 <sup>a</sup>	BZN (5) <sup>b</sup> (60)	34	41.7	High
Viotti et al. (1994), <sup>25</sup> full article	Argentina	Retrospective	Children and adults with chronic Chagas disease	201 (131/70)	BZN vs untreated	ECG change, disease progression, CV death and overall mortality	8	BZN (5) (30)	46.6 <sup>c</sup>	41.3	Moderate
De Andrade et al. (1996), <sup>26</sup> full article	Brazil	RCT	Children with chronic Chagas disease	129 (64/65)	BZN vs placebo	ECG change	3	BZN (7,5) (60)	9.5 <sup>d</sup>	58.9	Moderate
Coura et al. (1997), <sup>27</sup> full article	Brazil	RCT	Adults with chronic indeterminate form <sup>e</sup>	77 (53/24)	BZN/NFT vs placebo	ECG change and disease progression	1	BZN (5) (30) NFT (5) (30)	NI	NI	Moderate
Cataliotti et al. (1998), <sup>22</sup> abstract	Venezuela	Prospective	Adults with chronic Chagas disease	539 (74/465)	BZN vs untreated	CV death and overall mortality	5	BZN (5) (60)	41.0 <sup>n</sup>	38.6	High
Sosa-Estani et al. (1998), <sup>28</sup> full article	Argentina	RCT	Children with chronic Chagas disease	106 (55/51)	BZN vs placebo	ECG change	4	BZN (5) (60)	9 <sup>d</sup>	NI	Moderate
Fabbro de Suasnábar et al. (2000), <sup>29</sup> full article	Argentina	Prospective <sup>e</sup>	Children and adults with chronic Chagas disease	198 (68/130)	BZN/NFT vs untreated	Disease progression, CV death and overall mortality	14	BZN (5) <sup>f</sup> (30) NFT (5–8) (60)	36.5 <sup>d</sup>	42.9	High
Gallerano et al. (2000), <sup>30</sup> full article	Argentina	Prospective <sup>g</sup>	Children and adults with chronic Chagas disease	894 (226/668)	BZN/NFT <sup>h</sup> vs untreated	ECG change, disease progression, CV death and overall mortality	6.6 <sup>i</sup>	BZN (4–8) (45–60) NFT (10) (45–60)	33.4	49.1	Moderate
Lauria-Pires et al. (2000), <sup>31</sup> full article	Brazil	Retrospective	Adults with chronic Chagas disease	91 (45/46)	BZN/NFT vs untreated	ECG change and overall mortality	10	BZN (10) (20–60) NFT (10) (20–60)	45.5 <sup>d</sup>	NI	High
Viotti et al. (2006), <sup>32</sup> full article	Argentina	RCT <sup>l</sup>	Adults with chronic Chagas disease	566 (283/283)	BZN vs untreated	ECG change, disease progression, CV death and overall mortality	9.8	BZN (5) (30)	39.4	46.1	Low
Fabbro et al. (2007), <sup>33</sup> full article	Argentina	Prospective <sup>g</sup>	Adults with chronic indeterminate form	111 (54/57)	BZN/NFT vs untreated	ECG change	21	BZN (5) <sup>f</sup> (30) NFT (8–10) (60)	31.5 <sup>d</sup>	31.5	Moderate
Bertocchi et al. (2013), <sup>34</sup> full article	Argentina	Prospective	Adults with chronic Chagas disease	107 (82/25)	BZN vs untreated	ECG change and disease progression	8.8 <sup>i</sup>	BZN (5) (30)	35 <sup>d</sup>	36.4	Moderate
Machado-de-Assis et al. (2013), <sup>35</sup> full article	Brazil	Retrospective <sup>e</sup>	Children and adults with chronic Chagas disease	58 (29/29)	BZN vs untreated	Disease progression	13	BZN (5) (60)	NI	34.5	Moderate
Morillo et al. (2015), <sup>36</sup> full article	Multiple	RCT	Adults with chronic Chagas cardiomyopathy	2854 (1431/1423)	BZN vs placebo	Disease progression, CV death and overall mortality	5.4	BZN (5) (40–80)	55	49.3	Low
Colantonio et al. (2016), <sup>37</sup> full article	Argentina	Retrospective	Children with chronic indeterminate form	86 (48/38)	BZN vs placebo	ECG change	10.3	BZN (5) (60)	10	48.8	Moderate

(Table 1 continues on next page)

Author, publication type	Country	Study type	Population age and disease stage	Total of participants (treated/control)	Intervention vs control	Outcomes assessment	Mean follow up, years	BZN/NFT dose (mg/kg/day) and duration of therapy (days)	Age at treatment (mean)	Men (%)	Risk of Bias
(Continued from previous page)											
Fragata-Filho et al. (2016), <sup>14</sup> full article	Brazil	Retrospective	Adults with chronic indeterminate form	310 (263/47)	BZN vs untreated	ECG change, disease progression, CV death and overall mortality	19.6	BZN (5) (60)	38.4	34.5	Moderate
Cardoso et al. (2018), <sup>15</sup> full article	Brazil	Cross-sectional/prospective <sup>o</sup>	Adults with chronic Chagas cardiomyopathy	1813 (493/1320)	BZN vs untreated	ECG change and overall mortality	2 <sup>k</sup>	BZN (NI) (NI)	NI	32.2	High/Moderate <sup>l</sup>
Soverow et al. (2019), <sup>38</sup> full article	USA	Prospective	Adults with chronic Chagas disease	89 (59/30)	BZN/NFT vs untreated	ECG change	3.3 <sup>j</sup>	BZN (5) (60) NFT (8–10) (90)	46.7 <sup>n</sup>	NI	High
Haslocher-Moreno et al. (2021), <sup>16</sup> full article	Brazil	Retrospective	Adults with chronic indeterminate form	228 (114/114)	BZN vs untreated	ECG change, disease progression and overall mortality	15.1	BZN (5) <sup>m</sup> (30–60)	31.3	70.2	Moderate
Suasnábar et al. (2021), <sup>39</sup> full article	Argentina	Retrospective	Children with chronic Chagas disease	82 (41/41)	BZN/NFT vs untreated	ECG change	25	BZN (5) <sup>l</sup> (30) NFT (12–15) (45–60)	12.1 <sup>n</sup>	35.4	High
Jiang et al. (2024), <sup>23</sup> abstract	USA	Retrospective	Adults with Chagas disease <sup>e</sup>	109 (52/57)	BZN/NFT vs untreated	Disease progression	NI	BZN (NI) (NI) NFT (NI) (NI)	57.8	37.6	High

BZN, Benznidazole. CV, cardiovascular. ECG, 12-lead electrocardiogram. NI, not informed. NFT, Nifurtimox. RCT, randomized controlled trial. <sup>a</sup>For studies reporting a range of follow-up periods as this one (10–16 years), the mean follow-up duration was calculated as the average of the minimum and maximum values within the range (13 years). <sup>b</sup>The Benznidazole dose was corrected from 15 mg/kg/day, as stated in the paper, to 5 mg/kg/day, based on communication with the author. <sup>c</sup>For studies not reporting the mean age for the overall population as this one, the weighted mean age was calculated using the mean ages and sample sizes of the treatment and control groups. For example, the authors reported mean ages of 46.0 years (BZN, 131 patients) and 47.7 years (control, 70 patients), resulting in a weighted mean age of 46.6 years [(46 × 131) + (47.7 × 70)], divided by 201. <sup>d</sup>Average of the minimum and maximum values. <sup>e</sup>Deduced from us based on the details provided in the paper, although not specified by the authors. <sup>f</sup>Half dose during the first week. <sup>g</sup>Actually, the authors affirmed that the study was partially retrospective and partially prospective. <sup>h</sup>Patients treated with Allopurinol (n = 309) were excluded. <sup>i</sup>Weighted mean follow-up. <sup>j</sup>Although the authors used an alternating sequence for randomization (quasi-randomization), we included this study among the RCT, acknowledging the differences between RCT and quasi-randomized trials. <sup>k</sup>For the prospective cohort. <sup>l</sup>High for the cross-sectional design and moderate for the prospective cohort. <sup>m</sup>Or 200 mg/day as a fixed dose. <sup>n</sup>Weighted mean age. <sup>o</sup>This study had two epidemiological designs, one cross-sectional and the other prospective.

**Table 1: Characteristics of 23 studies included in the meta-analysis.**

studies, 2 RCTs and 8 observational studies). The intervention may benefit 19.3% of additional patients for ECG changes, 15.6% for disease progression, 4.6% for cardiovascular mortality, and 4.4% for overall mortality (absolute risk reductions). Omitting each study individually did not result in significant changes to the risk ratios in any outcomes, indicating robustness in the findings (Appendix p 16).

**Subgroup analysis**

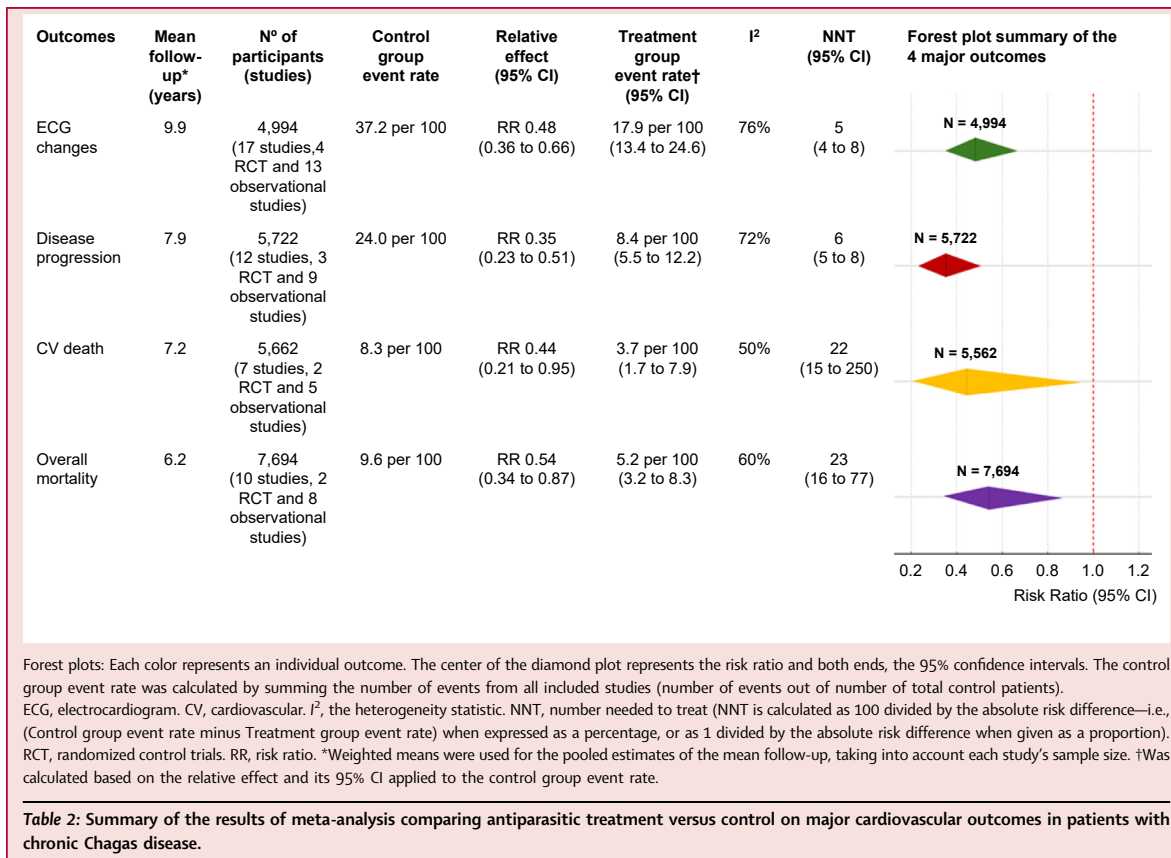
The subgroup analyses for ECG changes, disease progression, cardiovascular death, and overall mortality consistently show that antiparasitic treatment is beneficial compared to control (no treatment or placebo) (Appendix pp 17–19). For ECG changes, significant differences in the magnitude of the treatment effect were observed for disease stage (p < 0.001), study type (p < 0.001), and country (p = 0.02). This indicates that while the treatment effect remains beneficial across all subgroups, its strength varies among them. Other factors such as publication type, selection of antiparasitic drug, treatment duration of BZN, population age, and risk of bias of the studies (low, moderate, or high) showed no significant interaction effects. For disease

progression and cardiovascular death, significant quantitative differences were found based on disease stage and country, again indicating variability in effect size but a consistent benefit derived from treatment. The analysis of overall mortality revealed significant differences by country, but the treatment consistently reduced mortality across all subgroups. No qualitative interactions were found for any outcome, meaning the treatment effect was consistently favorable.

**Additional analysis**

The meta-regression analyses for the four outcomes provide insights into the factors influencing treatment efficacy (Appendix pp 20–24). For ECG changes, age showed a significant negative association (p = 0.04), suggesting that older patients benefited more from the treatment. However, it is crucial to note that most observations are based on a mean age of up to 50 years, aligning with current guidelines focused on this age group. Only two studies included patients with a mean age over 50 years.<sup>23,36</sup> These results should not be interpreted as indicating greater benefit for patients older than 50 nor suggesting universal adult benefit over children. Within the studied age range (childhood to





middle adulthood), a trend towards increased benefit with age was observed, possibly driven by differences between pediatric and young adult populations rather than among adults of various ages. Considering changes in life expectancy, particularly for migrants in Western countries with better healthcare access, older patients may exhibit distinct treatment responses. This highlights the need for further research to clarify treatment effects across more specific age groups, especially focusing on potential differences between children, young adults, and older adults. The dose of BZN showed a significant positive association, with 5 mg/kg proving more effective ( $p < 0.001$ ) than higher dose regimens. Notably, only one study in our analysis utilized a 10 mg/kg dose, which limits the generalizability of this finding. Moreover, several confounding factors may have influenced these results. No significant effects were observed for the percentage of men ( $p = 0.38$ ), mean follow-up duration ( $p = 0.95$ ), or NNT dose ( $p = 0.98$ ) regarding ECG changes. In terms of disease progression, cardiovascular death, and overall mortality, none of the factors analyzed showed significant associations.

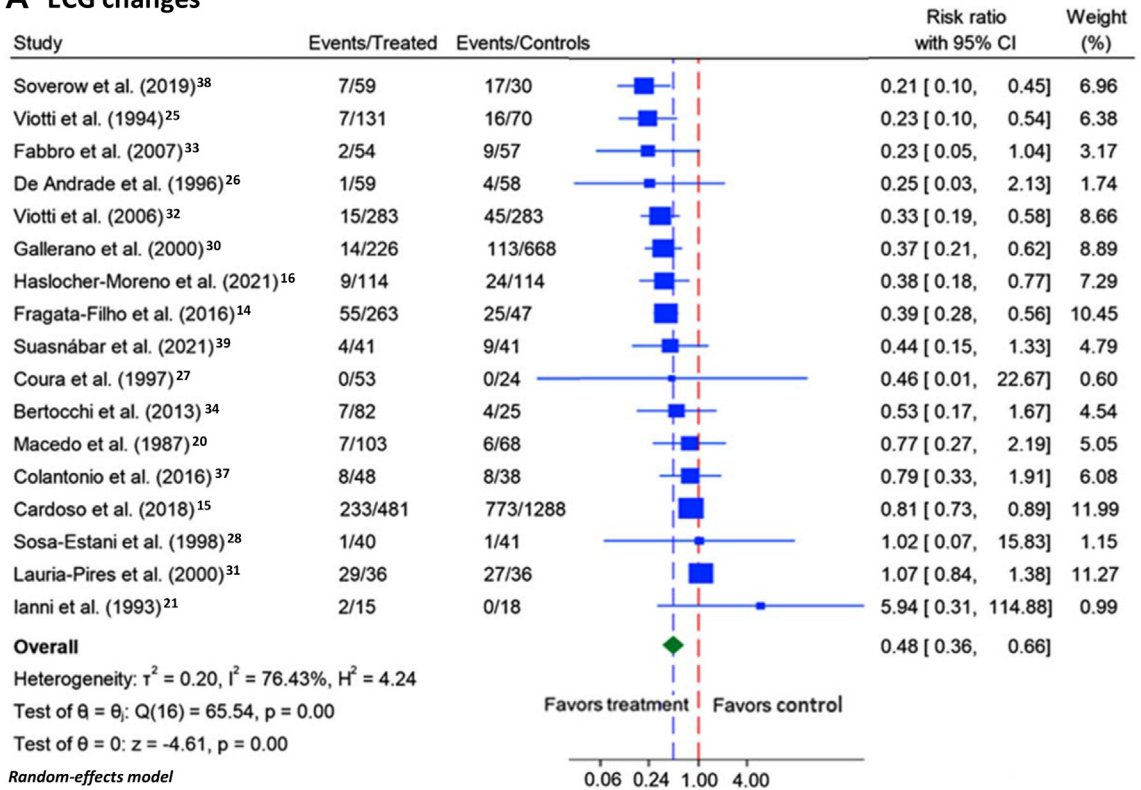
For analysis of publication bias, the funnel plots for the four outcomes are depicted in the [Appendix \(pp 25–26\)](#). For ECG changes, the symmetrical plot and Egger test

( $p = 0.83$ ) indicate no significant bias. Disease progression shows slight asymmetry with a borderline Egger test result ( $p = 0.06$ ). Cardiovascular death appears symmetrical, and the Egger test ( $p = 0.15$ ) confirms no significant bias. Overall mortality is mostly symmetrical, with the Egger test ( $p = 0.67$ ) indicating no significant bias. Overall, publication bias is not a major concern for these outcomes, although the findings for disease progression suggest that negative studies may have been slightly less likely to be published.

## Discussion

We found that antitrypanosomal therapy positively affected relevant cardiovascular outcomes in chronic Chagas disease. The intervention resulted in an NNT of 5, 6, 22, and 23 to prevent one case of ECG change, disease progression, cardiovascular death, and overall mortality, respectively. Despite the considerable risk of bias in most included studies, this comprehensive review synthesized all relevant research on antiparasitic treatment in chronic Chagas disease. The analysis yielded interpretable data within acceptable scientific parameters for the field, providing valuable insights despite limitations. Consistent trends across studies,

**A ECG changes**



**B Disease progression**

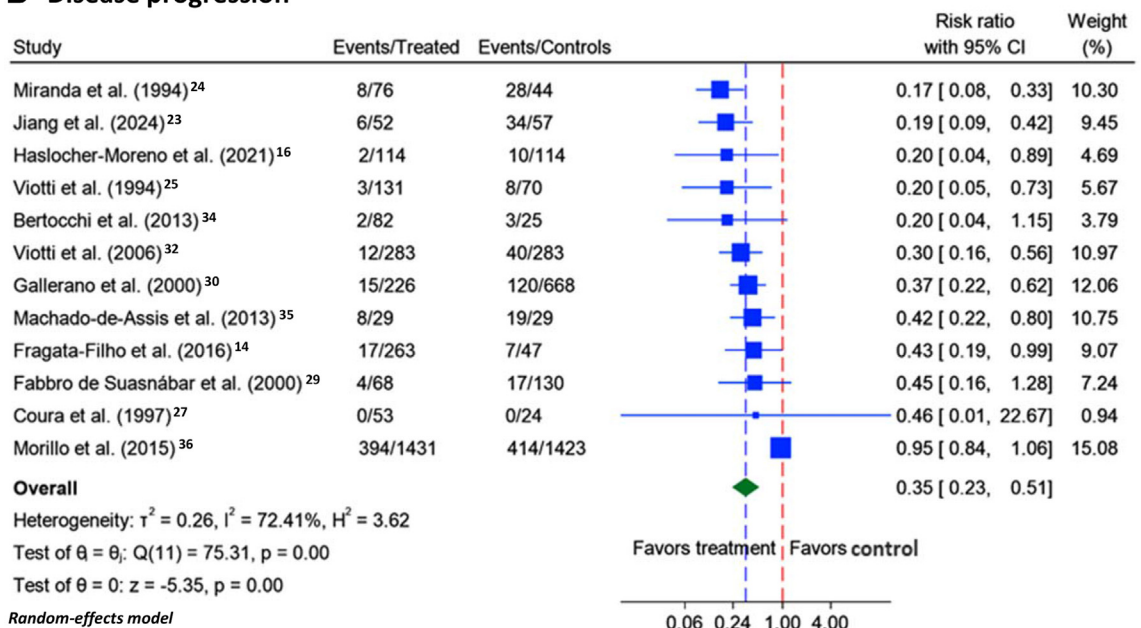
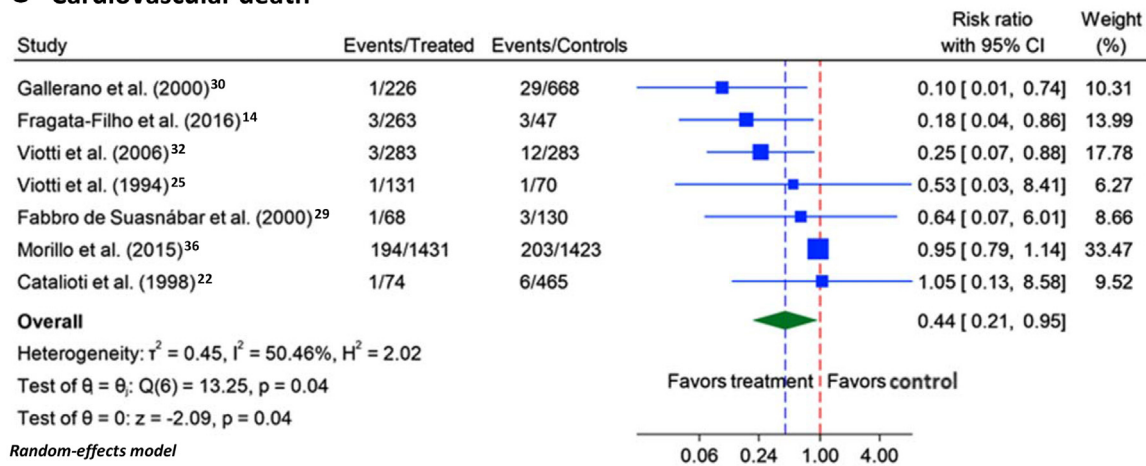


Fig. 2: Forest plots for pooled risk ratios for outcomes: (A) electrocardiogram changes, (B) disease progression, (C) Cardiovascular mortality, and (D) Overall mortality.

### C Cardiovascular death



### D Overall mortality

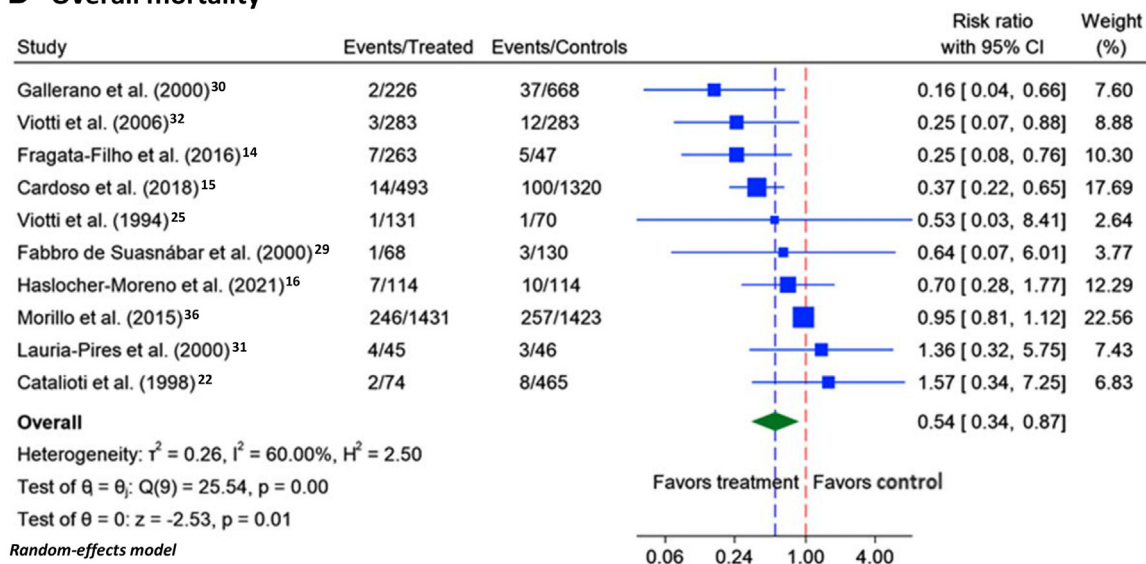


Fig. 2: Continued.

showing reduced risks of various adverse outcomes, strengthen the findings' validity. While acknowledging the need for more robust research, this review offers important guidance for clinical decision-making and future investigations in Chagas disease management.

Our results differ from a recent meta-analysis investigating the use of BZN to treat chronic Chagas disease, which concluded that more data is needed due to a lack of significant impact on cardiovascular outcomes.<sup>11</sup> However, that meta-analysis included only five studies (one randomized and four prospective observational) and considered studies up to 2021, excluding research on NFT, another treatment option. In contrast, our more comprehensive analysis included 23 reports,

encompassing a broader range of study designs and extending the review period to 2024. By incorporating studies on BZN and NFT, we aimed to provide a more extensive evaluation of their efficacy in treating chronic Chagas disease. This broader approach highlights potential benefits associated with these treatments that were not evident in the previous, more limited meta-analysis and aligns with the most recent recommendations from the Brazilian Society of Cardiology guidelines.<sup>4</sup>

Direct parasite-mediated cardiac injury, long-standing myocardial fibrosis due to tissue parasite persistence, adverse immune reactions, and reinfections are pivotal in the pathogenesis of CCC.<sup>40-42</sup> Morbidity

and mortality arise primarily from CCC complications, including arrhythmias, biventricular myocardial dysfunction, and thromboembolic events. A recent review highlighted the conspicuous arrhythmogenic feature of CCC, starting with basic electric remodeling at the cellular level and culminating with widespread fibrosis, alongside additional remodeling processes driven by immunological disturbances, coronary microvascular derangements, dysautonomia, and contractile impairment.<sup>43</sup>

Our study endpoints, including ECG alterations and clinical disease progression, are instrumental in detecting ongoing cardiac injury and informing the risk of cardiomyopathy development.<sup>44</sup> In addition, subgroup analysis indicated that earlier stages of the disease (patients with the indeterminate form) might benefit more from trypaocidal therapy, aligning with the potential benefit of reducing parasite tissue burden before further damage occurs.

Recommendations for antiparasitic treatment in chronic Chagas disease are usually based on surrogate outcomes such as short-term parasitemia negativization or long-term seroreversion, which do not clearly relate to cardiac pathogenesis and disease progression. Our findings suggest that antiparasitic treatment may impact disease progression and, to a lesser degree, mortality, even in those with mild cardiomyopathy. Therefore, it is critical to assess each patient individually for therapy, regardless of the stage of the disease, as some patients with early and minor cardiac alterations may still benefit from treatment.

This review has several limitations. There was significant heterogeneity between studies, as demonstrated by a high  $I^2$  statistic. Differences in study designs, heterogeneous populations, varying follow-up durations, and the inclusion of studies with moderate to high risk of bias could distort our findings and misrepresent the effect of antiparasitic therapy. Additionally, the variation in outcome definitions and measurement methods among the studies presents a limitation. Another key issue is that while some studies used only BZN, others included both BZN and NFT (analyzed together), and the control groups varied between placebo and no treatment, which are not equivalent comparisons. Furthermore, several studies combined children and adults, raising concerns about whether the conclusions can be generalized across these different populations.

Another limitation is the use of RR as the effect size metric in retrospective cohort studies. While RR is typically used in prospective designs, we applied it in retrospective cohort studies as well, given the available data on the number of events and total patients in each group. However, this approach may introduce some bias, as odds ratios or rate ratios are often recommended for certain observational designs. That said, since we included only one cross-sectional study and excluded case-control studies, the use of RR becomes less problematic and, in fact, more suitable for integrating these

different study designs. Critics may also highlight the methodological weaknesses in mixing randomized and observational studies due to inherent biases and confounders. Nonetheless, non-randomized studies provide the most comprehensive and relevant data currently available. The advantages of incorporating both types of studies in a meta-analysis may outweigh the disadvantages, suggesting that observational studies should not be excluded a priori.<sup>45</sup>

It is important to note that we performed subgroup analyses based not only on study type (RCTs, prospective, retrospective, and cross-sectional) but also on other factors, such as treatment type (BZN vs BZN or NFT), age groups, and publication type, among others. The benefit of antiparasitic therapy remained consistent across these various subgroups. Conducting large-scale, high-quality RCTs in Chagas disease is exceptionally challenging due to the intrinsic characteristics of the disease and logistical and economic constraints. Including abstracts alongside full papers could be seen as another limitation, but as long as the information in the abstracts is sufficient for analysis, their inclusion minimizes potential publication bias and ensures all available studies are considered.

In Chagas disease research, several widely claimed yet inadequately supported narratives dominate the literature. These include the urgent call for novel therapeutic agents, based on the assumption that existing drugs, BZN and NFT, are primarily effective only in the acute phase and pediatric populations, are associated with significant toxicity, and require prolonged treatment durations.<sup>46</sup> Another persistent belief emphasizes the need for a definitive gold-standard test or biomarker for early cure determination, driven by the prolonged persistence of seropositivity post-treatment and the limited reliability of parasitological and molecular assays in chronic cases.<sup>47,48</sup>

However, emerging data challenge these long-standing assertions, suggesting the need to recalibrate research and clinical priorities. Recent evidence supports the tolerability of BZN in chronic Chagas disease. Data from the largest RCT<sup>36</sup> showed that BZN when administered with close monitoring, required permanent discontinuation in only about 10% of cases due to adverse events, a rate comparable to that observed with the newer drug sacubitril/valsartan for treating heart failure from other etiologies.<sup>49</sup> Furthermore, a 30 to 60-day treatment regimen, albeit longer than many other infectious disease treatments, is feasible and justifiable given the chronic nature of Chagas disease and its severe consequences. Some incipient findings suggest that shorter protocol treatments with BZN were well tolerated and effective in adult patients with chronic Chagas disease.<sup>50,51</sup> Nevertheless, it is crucial to recognize that these studies focused solely on parasitological responses and did not evaluate long-term clinical outcomes.

The relentless pursuit of a definitive biomarker of cure warrants reconsideration. In chronic Chagas disease, the serological response to treatment can take decades to resolve, complicating efforts to establish a rapid, reliable marker of successful therapy. Parasitological assays, while valuable when positive, are limited by the low parasitemia in chronic infection, reducing their utility in confirming cure. Therefore, focusing on a single biomarker may divert resources from more impactful, immediate, but potentially beneficial interventions. Given that BZN and NFT have demonstrated efficacy and a manageable safety profile, the immediate focus should be expanding access to these treatments. It's crucial to develop standardized follow-up protocols to address the current gaps in treatment monitoring. This would ensure better patient outcomes and strengthen the conclusions drawn from ongoing and future research. Expanding drug availability through policy reforms, improving distribution systems, and moving these medications beyond governmental facilities to wider commercial availability will help reach a broader patient population.

The emphasis on developing new pharmacotherapies, though important, should not overshadow the immediate need to optimize current treatments. Developing new drugs is a lengthy and uncertain process. In contrast, maximizing the use of existing effective treatments offers a tangible opportunity to mitigate the burden of Chagas disease. This requires efforts to enhance drug availability, optimize treatment protocols, and implement robust public health strategies.

In conclusion, while searching for novel treatments and effective biomarkers of cure and disease progression with trypanocidal therapy remains important, there is an urgent need to adopt a pragmatic approach focused on effectively utilizing existing treatments. Their proven BZN and NFT efficacy—as demonstrated by this systematic review and meta-analysis—and their manageable safety profiles should call for more widely accessible patient treatments. Prioritizing this approach will address immediate therapeutic needs and lay a stronger foundation for future advancements in Chagas disease management. Realigning research and clinical strategies towards solutions that provide an immediate and significant impact on the patient population is imperative.

#### Contributors

ARJ: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing-original draft, writing-review & editing, visualization, supervision, project administration, AG: conceptualization, methodology, software, validation, resources, visualization, writing-review & editing, supervision, AS: investigation, data curation, writing-review & editing, RS: investigation, data curation, writing-review & editing, SS: investigation, data curation, writing-review & editing, NIAH: supervision, writing-review & editing, FMR: investigation, data curation, writing-review & editing, MFC: investigation, data curation, writing-review & editing, HMK: investigation, data curation, writing-review & editing, JS: investigation, data curation, writing-review & editing, SK: writing-review &

editing, LAM: writing-review & editing, EAR: writing-review & editing, MC: writing-review & editing, PH: writing-review & editing, MEB: writing-review & editing, SP: writing-review & editing, CFP: supervision, writing-review & editing, JAMN: supervision, writing-review & editing, AFHM: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing-original draft, writing-review & editing, visualization, supervision, project administration.

#### Data sharing statement

The corresponding authors have full access to data in the study and had final responsibility for the decision to submit the manuscript for publication. The datasets generated and analyzed in the current study are available from the corresponding authors at reasonable request.

#### Declaration of interests

Maria Elena Bottazzi and Peter Hotez are part of a team of scientists advancing research toward the development of a therapeutic Chagas disease vaccine and are listed among the inventors on a Chagas disease vaccine patent submitted by Baylor College of Medicine. Dr. Andres F. Henao-Martinez received educational funds from F2G: Novel Therapeutics for Rare Fungal Infections; and support to attend 3ra Jornada Internacional de Enfermedades Infecciosas y Tropicales as a lecturer in Lima, Peru.

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#### Appendix. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.eclim.2024.102972>.

#### References

- Rassi A Jr, Rassi A, Marin-Neto JA. Chagas disease. *Lancet*. 2010;375:1388–1402.
- Institute for Health Metrics and Evaluation. 2021 chagas disease: level 3 disease factsheet. <https://www.healthdata.org/research-analysis/diseases-injuries-risks/factsheets/2021-chagas-disease-level-3-disease>. Accessed August 31, 2024.
- Pereira Pereira AC. Guidelines for the diagnosis and treatment of Chagas disease. *Lancet*. 2019;393:1486–1487.
- Marin-Neto JA, Rassi A Jr, Oliveira GMM, et al. SBC Guideline on the diagnosis and treatment of patients with cardiomyopathy of Chagas disease – 2023. *Arq Bras Cardiol*. 2023;120:e20230269.
- Chadalawada S, Sillau S, Archuleta S, et al. Risk of chronic cardiomyopathy among patients with the acute phase or indeterminate form of Chagas disease: a systematic review and meta-analysis. *JAMA Netw Open*. 2020;3:e2015072.
- Chadalawada S, Rassi A Jr, Samara O, et al. Mortality risk in chronic Chagas cardiomyopathy: a systematic review and meta-analysis. *ESC Heart Fail*. 2021;8:5466–5481.
- Maron BJ. Clinical course and management of hypertrophic cardiomyopathy. *N Engl J Med*. 2018;379:1977.
- Bern C. Antitrypanosomal therapy for chronic Chagas' disease. *N Engl J Med*. 2011;364:2527–2534.
- Sguassero Y, Roberts KN, Harvey GB, et al. Course of serological tests in treated subjects with chronic *Trypanosoma cruzi* infection: a systematic review and meta-analysis of individual participant data. *Int J Infect Dis*. 2018;73:93–101.
- Sguassero Y, Cuesta CB, Roberts KN, et al. Course of Chronic *Trypanosoma cruzi* Infection after treatment based on parasitological and serological tests: a systematic review of follow-up studies. *PLoS One*. 2015;10:e0139363.
- Crespillo-Andujar C, Comeche B, Hamer DH, et al. Use of benznidazole to treat chronic Chagas disease: an updated systematic review with a meta-analysis. *PLoS Negl Trop Dis*. 2022;16:e0010386.

- 12 Vallejo M, Reyes PPA, Martinez Garcia M, Gonzalez Garay AG. Trypanocidal drugs for late-stage, symptomatic Chagas disease (Trypanosoma cruzi infection). *Cochrane Database Syst Rev*. 2020;12:CD004102.
- 13 Villar JC, Perez JG, Cortes OL, et al. Trypanocidal drugs for chronic asymptomatic Trypanosoma cruzi infection. *Cochrane Database Syst Rev*. 2014;5:CD003463.
- 14 Fragata-Filho AA, França FF, Fragata Cda S, Lourenço AM, Faccini CC, Costa CA. Evaluation of parasiticide treatment with benznidazol in the electrocardiographic, clinical, and serological evolution of Chagas disease. *PLoS Negl Trop Dis*. 2016;10:e0004508.
- 15 Cardoso CS, Ribeiro ALP, Oliveira CDL, et al. Beneficial effects of benznidazole in Chagas disease: NIH SaMi-Trop cohort study. *PLoS Negl Trop Dis*. 2018;12:e0006814.
- 16 Hasslocher-Moreno AM, Saraiva RM, Sangenis LHC, et al. Benznidazole decreases the risk of chronic Chagas disease progression and cardiovascular events: a long-term follow up study. *eClinicalMedicine*. 2020;31:100694.
- 17 Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71.
- 18 Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327:557–560.
- 19 Sterne JA, Sutton AJ, Ioannidis JP, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ*. 2011;343:d4002.
- 20 Macedo V, Silveira CA. Perspectivas da terapêutica específica na doença de Chagas. Experiências na forma indeterminada. *Rev Soc Bras Med Trop*. 1987;20(supl II):M-24 (abstr).
- 21 Ianni BM, Arteaga E, Mady C, Barreto ACP, da Luz PL, Pileggi F. Uso do benznidazol em chagásicos na forma indeterminada: resultados a longo prazo. *Arq Bras Cardiol*. 1993;61(supl II):130 (abstr).
- 22 Cataliotti F, Acquatella H. Comparación de la mortalidad durante seguimiento por 5 años en sujetos con enfermedad de Chagas crónica con y sin tratamiento de benznidazol. *Rev Pat Trop*. 1998;27(supl.):29–31 (abstr).
- 23 Jiang M, Gulhar R, Malik A, et al. Association between antiparasitic treatment and development of cardiomyopathy in patients with Chagas disease. *J Am Coll Cardiol*. 2024;83(Suppl A):415 (abstr).
- 24 Miranda L, Miranda L, Campos G, Marins N, Lobo V. História natural da forma crônica da doença de Chagas x tratamento específico. *Rev Centro-Oeste Cardiol*. 1994;1:25–29.
- 25 Viotti R, Vigliano C, Armenti H, Segura E. Treatment of chronic Chagas' disease with benznidazole: clinical and serologic evolution of patients with long-term follow-up. *Am Heart J*. 1994;127:151–162.
- 26 de Andrade AL, Zicker F, de Oliveira RM, et al. Randomised trial of efficacy of benznidazole in treatment of early Trypanosoma cruzi infection. *Lancet*. 1996;348:1407–1413.
- 27 Coura JR, de Abreu LL, Willcox HP, Petana W. Estudo comparativo controlado com emprego de benznidazole, nifurtimox e placebo, na forma crônica da doença de Chagas em uma área de campo com transmissão interrompida. I. Avaliação preliminar. *Rev Soc Bras Med Trop*. 1997;30:139–144.
- 28 Sosa Estani S, Segura EL, Ruiz AM, Velazquez E, Porcel BM, Yampotis C. Efficacy of chemotherapy with benznidazole in children in the indeterminate phase of Chagas' disease. *Am J Trop Med Hyg*. 1998;59:526–529.
- 29 Fabbro De Suasnabar D, Arias E, Streiger M, et al. Evolutionary behavior towards cardiomyopathy of treated (nifurtimox or benznidazole) and untreated chronic chagasic patients. *Rev Inst Med Trop Sao Paulo*. 2000;42:99–109.
- 30 Gallerano RR, Sosa RR. Estudio de intervención en la evolución natural de la enfermedad de Chagas. Evaluación del tratamiento antiparasitario específico. Estudio retrospectivo-prospectivo de terapéutica antiparasitaria. *Rev Fac Cienc Méd (Córdoba)*. 2000;57:135–162.
- 31 Lauria-Pires L, Braga MS, Vexenat AC, et al. Progressive chronic Chagas heart disease ten years after treatment with anti-Trypanosoma cruzi nitroderivatives. *Am J Trop Med Hyg*. 2000;63:111–118.
- 32 Viotti R, Vigliano C, Lococo B, et al. Long-term cardiac outcomes of treating chronic Chagas disease with benznidazole versus no treatment: a non-randomized trial. *Ann Intern Med*. 2006;144:724–734.
- 33 Fabbro DL, Streiger ML, Arias ED, Bizai ML, del Barco M, Amicone NA. Trypanocide treatment among adults with chronic Chagas disease living in Santa Fe city (Argentina), over a mean follow-up of 21 years: parasitological, serological and clinical evolution. *Rev Soc Bras Med Trop*. 2007;40:1–10.
- 34 Bertocchi GL, Vigliano CA, Lococo BG, Petti MA, Viotti RJ. Clinical characteristics and outcome of 107 adult patients with chronic Chagas disease and parasitological cure criteria. *Trans R Soc Trop Med Hyg*. 2013;107:372–376.
- 35 Machado-de-Assis GF, Diniz GA, Montoya RA, et al. A serological, parasitological and clinical evaluation of untreated Chagas disease patients and those treated with benznidazole before and thirteen years after intervention. *Mem Inst Oswaldo Cruz*. 2013;108:873–880.
- 36 Morillo CA, Marin-Neto JA, Avezum A, et al. BENEFIT Investigators. Randomized trial of benznidazole for chronic Chagas' cardiomyopathy. *N Engl J Med*. 2015;373:1295–1306.
- 37 Colantonio LD, Prado N, Segura EL, Sosa-Estani S. Electrocardiographic abnormalities and treatment with benznidazole among children with chronic infection by Trypanosoma cruzi: a retrospective cohort study. *PLoS Negl Trop Dis*. 2016;10:e0004651.
- 38 Soverow J, Hernandez S, Sanchez D, et al. Progression of baseline electrocardiogram abnormalities in Chagas patients undergoing antitrypanosomal treatment. *Open Forum Infect Dis*. 2019;6:ofz012.
- 39 Suasnabar S, Olivera LV, Arias E, et al. Trypanocidal therapy among children infected by Trypanosoma cruzi. Serological and electrocardiographic changes over a mean twenty-five-years follow-up period. *Acta Trop*. 2021;222:106050.
- 40 Olivo Freites C, Sy H, Gharamti A, et al. Chronic Chagas disease—the potential role of reinfections in cardiomyopathy pathogenesis. *Curr Heart Fail Rep*. 2022;19:279–289.
- 41 Marin-Neto JA, Cunha-Neto E, Maciel BC, Simões MV. Pathogenesis of chronic Chagas heart disease. *Circulation*. 2007;115:1109–1123.
- 42 Henaó-Martínez AF, Schwartz DA, Yang IV. Chagasic cardiomyopathy, from acute to chronic: is this mediated by host susceptibility factors? *Trans R Soc Trop Med Hyg*. 2012;106:521–527.
- 43 Roman-Campos D, Marin-Neto JA, Santos-Miranda A, Kong N, D'Avila A, Rassi A Jr. Arrhythmogenic manifestations of Chagas disease: perspectives from the bench to bedside. *Circ Res*. 2024;134:1379–1397.
- 44 Brito BOF, Ribeiro ALP. Electrocardiogram in Chagas disease. *Rev Soc Bras Med Trop*. 2018;51:570–577.
- 45 Shrier I, Boivin JF, Steele RJ, et al. Should meta-analyses of interventions include observational studies in addition to randomized controlled trials? A critical examination of underlying principles. *Am J Epidemiol*. 2007;166:1203–1209.
- 46 Ramos LG, de Souza KR, Júnior PAS, et al. Tackling the challenges of human Chagas disease: a comprehensive review of treatment strategies in the chronic phase and emerging therapeutic approaches. *Acta Trop*. 2024;256:107264.
- 47 Pinazo MJ, Thomas MC, Bustamante J, Almeida IC, Lopez MC, Gascon J. Biomarkers of therapeutic responses in chronic Chagas disease: state of the art and future perspectives. *Mem Inst Oswaldo Cruz*. 2015;110:422–432.
- 48 Alonso-Padilla J, Abril M, Alarcón de Noya B, et al. Target product profile for a test for the early assessment of treatment efficacy in Chagas disease patients: an expert consensus. *PLoS Negl Trop Dis*. 2020;14:e0008035.
- 49 McMurray JJ, Packer M, Desai AS, et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med*. 2014;371:993–1004.
- 50 Torrico F, Gascón J, Barreira F, et al. New regimens of benznidazole monotherapy and in combination with fosravuconazole for treatment of Chagas disease (BENDITA): a phase 2, double-blind, randomised trial. *Lancet Infect Dis*. 2021;21:1129–1140.
- 51 Bosch-Nicolau P, Fernández ML, Sulleiro E, et al. Efficacy of three benznidazole dosing strategies for adults living with chronic Chagas disease (MULTIBENZ): an international, randomised, double-blind, phase 2b trial. *Lancet Infect Dis*. 2024;24:386–394.