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Monotherapy and combination chemotherapy for Chagas disease treatment: a systematic review of clinical efficacy and safety based on randomized controlled trials

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Review Article

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Abstract

From a systematic review framework, we analysed the clinical evidence on the effectiveness and safety of monotherapy and combination chemotherapy for Chagas disease (ChD) treatment. The research protocol was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses and patient, intervention, comparison and outcome strategy. Only randomized controlled trials (RCT) were retrieved from Embase, Medline, Scopus and Web of Science databases. Diagnostic tools, treatment protocols, seroconversion rates and adverse events were investigated. Fifteen RCT mainly concentrated in endemic countries were identified. ChD diagnosis was mainly based on haemagglutination, immunofluorescence, enzyme-linked immunosorbent assay and polymerase chain reaction. Benznidazole (BNZ), nifurtimox, fosravuconazole, posaconazole, allopurinol and thioctic acid were the identified drugs. The best negative seroconversion results (100, 96, 94 and 91.3%) were, respectively, based on BNZ (5 mg kg day^{-1}, 200 mg day^{-1}, 150 mg day^{-1} and 2.5 mg kg^{-1}) administration for 60 days. Negative seroconversion was not achieved with allopurinol (300 mg day^{-1} for 60 days). Adverse reactions ranged from 5 to 73% in patients receiving antiparasitic chemotherapy. Treatment discontinuation (1.5–57%) was mainly associated with gastrointestinal, cutaneous and neurological manifestations. Current RCT-based evidence indicates that BNZ is the most viable option for ChD treatment. However, new protocols need to be developed to mitigate side effects and increase patient adherence to antiparasitic chemotherapy. Therefore, shorter regimens, lower concentrations and treatments combining BNZ with posaconazole, fosravuconazole or ravuconazole may be viable to ensure comparable efficacy to BZN-based monotherapy, contributing to reduce dose- and time-dependent toxicity reactions.

Introduction

Chagas disease (ChD) or American trypanosomiasis is a life-threatening anthropozoonosis markedly related to poverty and caused by the protozoan parasite *Trypanosoma cruzi* (Bern, 2015; Torrico *et al.*, 2021). About 5.7–9.4 million people are infected with *T. cruzi* worldwide (Echeverría *et al.*, 2020*a*; Torrico *et al.*, 2021), especially in 21 Latin American endemic countries and 19 non-endemic countries in North America and Europe (Bivona *et al.*, 2020; Torrico *et al.*, 2021). Vector and oral transmission are the main forms of *T. cruzi* infection in endemic countries. However, donation of contaminated blood and organs from infected people, vertical transmission (e.g. mother to fetus) and laboratory accidents are the main causes of ChD spread in non-endemic areas (Nogueira *et al.*, 2018; Guhl and Ramirez, 2021).

ChD is clinically divided into acute and chronic phases (Bern, 2015). The acute phase courses with intense parasitaemia and marked cellular parasitism in multiple organs, especially the heart, skeletal muscles and cells of the phagocytic mononuclear system (Bivona *et al.*, 2020; Torrico *et al.*, 2021). However, acute infections are often asymptomatic and associated with low mortality rates (0.2–0.5%) (Pérez-Molina and Molina, 2018; Echeverría *et al.*, 2020*a*). Once parasitaemia is controlled, the disease progresses to the chronic phase, which may remain asymptomatic for decades or evolve into a symptomatic form associated with digestive, nervous and/or cardiovascular manifestations (Bern, 2015). Cardiac involvement characterizes chronic Chagas cardiomyopathy (CCC), which is the most severe form of ChD (Nogueira *et al.*, 2018; Bivona *et al.*, 2020).

Chronic cardiomyopathy is the leading cause of ChD-associated mortality (Nogueira *et al.*, 2018; Caldas *et al.*, 2019). In addition, CCC is the most common infectious cardiomyopathy

worldwide and the third most frequent cause of heart transplantation in endemic countries (Nogueira *et al.,* 2018). CCC exhibits a multifactorial and complex aetiology. It is often associated with parasite persistence, low-grade inflammation, autoimmunity, redox imbalance, thromboembolic events, myonecrosis, autonomic denervation and progressive myocardial fibrosis (Rassi *et al.,* 2017; Rodrigues *et al.,* 2017; Bonney *et al.,* 2019). Together, these events contribute to CCC progression, which often manifests electromechanical abnormalities such as conduction defects (e.g., bundle branch blocks), frequent and complex ventricular arrhythmias, and systolic ventricular dysfunction (Bonney *et al.,* 2019; Caldas *et al.,* 2019).

The specific ChD treatment is limited to the nitroheterocyclic compounds nifurtimox (NFx) and benznidazole (BNZ) (Muñoz *et al.,* 2011; Diniz *et al.,* 2013; Novaes *et al.,* 2016), which was developed almost 50 years ago (Muñoz *et al.,* 2011, Diniz *et al.,* 2013). Due to high toxicity and serious side effects (e.g. hypersensitivity reactions, anorexia, vomiting, polyneuritis and bone marrow depression), NFx is no longer available in most endemic countries (Urbina and Docampo, 2003; Caldas *et al.,* 2019). Thus, BNZ becomes the first-line drug for ChD treatment (Caldas *et al.,* 2019). Despite its limitations (e.g. systemic toxicity, prolonged treatment and limited efficacy in chronic infections), the risk–benefit of BNZ-based chemotherapy is still favourable (Muñoz *et al.,* 2011; Caldas *et al.*, 2019), especially in acute infections where high cure rates can be obtained (Martinez *et al.,* 2020; Caldas *et al.,* 2019). However, side effects often dictate treatment discontinuation, negatively influencing chemotherapy effectiveness and cure rates (Santos *et al.,* 2015; Torrico *et al.,* 2021).

Considering the limited effectiveness of the classic BNZ-based protocol in improving cardiac function, sustainably attenuating tissue parasitism, and achieving parasitological cure in chronic ChD (Molina-Morant *et al.,* 2020; Martín-Escolano *et al.,* 2020); developing more efficient therapeutic regimens is an urgent need (Molina *et al.,* 2014; Vallejo *et al.,* 2016; Torrico *et al.,* 2021). Thus, repositioning and combining drugs with antiparasitic effects and different mechanisms of action have emerged as potential alternatives to improve ChD treatment (Morillo *et al.,* 2017; Mendonça *et al.,* 2020; Torrico *et al.,* 2021). In addition to new NFx- and BNZ-based protocols, clinical trials currently registered on *ClinicalTrials.gov* for ChD treatment are based on drug repositioning, which admits that: (i) distinct diseases may share similar targets, (ii) drugs act on multiple targets and (iii) identifying new targets for a drug contributes to innovative therapeutic applications (Sardana *et al.,* 2011; Reddy and Zhang, 2013). Allied to this repositioning strategy, drug combinations have been investigated in search of synergistic or additive interactions to increase the effectiveness of anti-*T. cruzi* chemotherapy (Mazzeti *et al.,* 2019; Martinez *et al.,* 2020). As combinations can increase drug half-life, reduce dose and treatment time, minimize systemic toxicity and side effects; this strategy can also increase chemotherapy adherence and improve clinical outcomes in ChD patients (Muñoz *et al.,* 2011; Rassi *et al.,* 2017; Torrico *et al.,* 2021).

Currently, evidence on the types and effectiveness of different chemotherapy protocols applied to ChD treatment is based on diffuse initiatives. Thus, it becomes difficult to identify which drugs and combinations were investigated in clinical trials to delimit the most efficient protocols and potential risks associated with drug therapy. Therefore, we used a systematic review framework to map the evidence on specific ChD treatments included in randomized controlled trials (RCT). In addition to identifying the drugs administered (pharmacological class and dosimetry parameters), the effectiveness of the available chemotherapeutic protocols to control the parasite load, achieve negative seroconversion and therapeutic cure was investigated. Considering the pharmacological safety, adverse reactions and treatment discontinuation rates were also evaluated. Finally, the methodological quality of all studies reviewed and potential sources of bias associated with current evidence were objectively characterized, contributing to the refinement of further investigations.

Methods

Guiding question and protocol registration

Our research protocol was developed considering the patient, intervention, comparison and outcome (PICO) strategy (Huang *et al.,* 2006), which was used to define the following guiding question adopted in this systematic review: Could ChD patients undergoing different chemotherapy regimens exhibit better parasitological control, cardiovascular function, lower rates of adverse reactions and mortality compared to untreated control patients? The present review protocol has been registered within the PROSPERO (International Prospective Register of Systematic Reviews) database (registration number CRD42021276800).

Search strategy and primary studies selection

Study selection was based on the updated Preferred Reporting Items for Systematic Reviews and Meta-Analyses strategy (PRISMA) (Page *et al.,* 2021). The retrieval of indexed studies on the aetiological treatment for ChD was operationalized from 2 complementary strategies: (i) electronic database search and (ii) indirect searches in citations and/or reference lists of all studies identified from electronic databases (Pereira *et al.,* 2017). The direct strategy was based on advanced searches in PubMed/Medline, Embase, Scopus and Web of Sciences databases. To identify relevant studies, search filters were initially built using the MeSH Terms, which are standardized descriptors obtained from the PubMed thesaurus (https://www.ncbi.nlm.nih.gov/mesh/) (Felizardo *et al.,* 2018). The search filters were developed considering 2 levels: (i) disease (ChD) and (ii) intervention (aetiological treatment). All descriptors were associated with specific algorithms [(MeSH Terms) and (TIAB)] to optimize the retrieval of relevant studies in PubMed/Medline (Altoé *et al.,* 2019), ensuring the identification of research records indexed and in the indexing process (Souza-Silva *et al.,* 2019). The 'human' and 'clinical trials' search limits provided by the search engine were applied to refine the quality of the PubMed/Medline search.

The same search filters built for the PubMed/Medline database were adapted for Embase, Scopus and Web of Science. Therefore, the respective syntax and algorithms required in the search engine of each database were used, such as: de,ab,ti, TITLE-ABS-KEY or TS = . To refine the quality of the Embase search, studies also indexed in Medline were automatically excluded from the Venn diagram tool (Sources tab) (Silva *et al.,* 2018), selecting studies exclusively indexed in Embase. In addition, the standardized limits 'human', 'major clinical study' and 'article' were used in our search strategy applied to Embase. The search limits 'human' and 'AND NOT INDEX (Medline)' were applied to the Scopus database. This last limit was used to exclude duplicate Medline studies from the results. For the Web of Science database, a filter was built to select clinical studies and specific animal species (humans). No chronological or language limits were used (Silva *et al.,* 2018). The complete search strategies used in each database can be accessed in the supplementary material (Table S1).

Studies screening, eligibility criteria and inter-rater agreement

Only clinical studies investigating the impact of ChD aetiological treatment were included in this systematic review. All relevant

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studies were selected according to the PRISMA flowchart (Page *et al.,* 2021). Two reviewers (S.S.N. and R.O.S.) retrieved and independently applied search strategies across all databases (Altoé *et al.,* 2019). Disagreements were resolved by arbitration in consultation with an expert researcher (R.D.N). Duplicate studies that were not directly excluded by the search algorithms were removed using Mendeley software (Mendeley Desktop Version 1.19.8). After this step, the titles and abstracts of all research records were screened and irrelevant records (not related to the investigated topic) were excluded. The remaining studies were retrieved in full text and well-defined eligibility criteria were analysed. The exclusion criteria applied in this review were: (i) grey literature (not peer-reviewed and formally published), (ii) studies unavailable in full text (title and/or abstract only), (iii) studies with non-pharmacological interventions, (iv) studies with multiple interventions where it was not possible to isolate the aetiologic treatment effect, (v) secondary studies (literature reviews, comments, letters to the editor and editorials), (vi) studies without control groups and (vii) studies unrelated to parasitological control. After selecting all relevant studies from the primary search, reference lists were screened to identify additional studies (Felizardo *et al.,* 2018; Nogueira *et al.,* 2018). These studies were retrieved in full text and the same eligibility criteria used in the direct search were analysed in this secondary strategy. The results obtained from the primary and secondary searches were compared and the inter-rater agreement (Kappa coefficient) was calculated (McHugh, 2012).

Data extraction

Qualitative and quantitative data were extracted from all relevant studies included in the systematic review. To this end, we use standardized spreadsheets (data extraction masks) (Marques *et al.,* 2018) structured from basic methodological requirements to characterize studies at different descriptive levels, such as: (i) publication characteristics: research design, authors, year of publication and country where the study was conducted; (ii) patient characteristics: age, sex, and disease stage; (iii) treatment characteristics: drugs and dosimetry (doses, administration frequency and rout, treatment duration, and patient follow-up); (iv) primary outcomes: parasite load (parasitological cure), seroconversion and mortality rates; (v) secondary outcomes: cardiovascular function (electrocardiographic and echocardiographic data), adverse reactions and treatment discontinuation rates.

Reporting quality as a risk of bias

Methodological quality and potential risk of bias in all studies reviewed were analysed using the Downs and Black (D&B) checklist, which is targeted at randomized and non-randomized trials of health care interventions (Downs and Black, 1998). The scale is based on 27 questions and is structured in 5 categories or domains, such as: (i) reporting quality, (ii) external validity, (iii) bias, (iv) confounding and (v) statistical power. This scale has high test–retest reliability ($r = 0.88$) and internal consistency (KR20 formula = 0.89). Due to previous recommendations and high ambiguity, question 27 (statistical power) was not applied (Nogueira *et al.,* 2018). The overall result obtained from the Downs and Black checklist was expressed graphically, and the average score was calculated (Nogueira *et al.,* 2018).

Results

Publication characteristics

In the primary search, 508 papers published between 1996 and 2021 were retrieved from PubMed, Embase, Scopus and Web of Science. After removing duplicates and evaluating eligibility criteria, 11 relevant papers were identified. Four additional papers were identified in the secondary search. Therefore, 15 original studies were included in the systematic review. Most studies ($n = 10$, 66.6%) were developed in Latin American countries. Three (20%) multicentre studies were also identified, followed by 2 studies developed in Spain (13.3%). The list of all papers included and the PRISMA flowchart with the complete strategy are shown in Table S2 and Fig. 1, respectively. The Kappa coefficient obtained from our search strategy ($\kappa = 0.842$) indicated substantial agreement between independent evaluators (Table S2).

Patient characteristics

From all the studies reviewed, 6 (40%) classified participants by sex, and female patients were investigated in 3 studies (26.6%). Men were exclusively recruited in 1 study (6%), and gender was not reported in 4 studies (26.6%). In general, the patients' ages ranged from 7 to 75 years, and 1 study (6%) investigated newborns. Body mass was underreported in most studies ($n = 12$, 80%). Considering disease stage, 11 studies (73.3%) included patients in the indeterminate chronic phase. One study (6.6%) investigated congenital disease (acute phase), and the disease phase was not reported in 3 studies (20%). ChD diagnosis was based on indirect haemagglutination assay (IAH), indirect immunofluorescence assay (IFA), enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA), or chemiluminescence immunoassay (CL-ELISA, A&T antigen) in 6 studies (40%). Polymerase chain reaction (PCR) was also used in 6 studies (40%). Xenodiagnostic alone or combined with IFA was applied in only 1 study (6%) each (Table 1).

Chemotherapy protocols, seroconversion and cure rates

As indicated in Table 2, BNZ was the drug mainly investigated in monotherapy in 8 studies (53.3%). Monotherapy based on NFx, allopurinol, posaconazole or the prodrug ravuconazole (E1224) was reported in 7 studies (46.6%). Drug combinations based on BNZ + thioctic acid, BNZ + posaconazole and BNZ + fosravuconazole were investigated in 3 studies (20%). Negative seroconversion rates reported in Latin American studies using BNZ were: 11.2–82.2% at 5 mg kg day^{-1}, 58–88.7% at 7.5 mg kg day^{-1}, 91.3% at 2.5 mg kg day^{-1} and 94% at 150 mg day^{-1}. Two other BNZ studies (developed in Canada and Spain), found 96% (200 mg day^{-1}) and 100% (5 mg kg day^{-1}) seroconversion rates, respectively. With respect to other treatments, seroconversion rates ranging from 10.9 to 28.9% were observed for E1224 (2000 and 4000 mg total^{-1}), and 10–20% for posaconazole (100 and 400 mg day^{-1}). Seroconversion was not achieved with allopurinol treatment. For drug combinations, a high seroconversion rate (96%) was obtained in patients treated with BNZ + posaconazole, while an 84% rate was achieved in patients receiving BNZ + fosravuconazole. This parameter was not investigated in studies with NFx and BNZ + thioctic acid. The parasitological cure was estimated from *T. cruzi* DNA detection in blood samples by PCR. This method was used in 6 studies (40%). Negative PCR ranged from 46.7 to 100% in BNZ-treated patients, 8.3–28.9% for E1224 treatment and 10–20% for posaconazole treatment. For drug combinations, negative PCR were obtained in 96% patients receiving BNZ + posaconazole, and 84% for patients treated with BNZ + fosravuconazole. Only 2 studies (13.3%) investigated potential changes in PCR results over time or over a year (Vallejo *et al.,* 2016; Morillo *et al.,* 2017). Negative PCR results were maintained in 46.7% to 100% of all patients investigated in a follow-up ranging from 1 to 5 years (Table 2).

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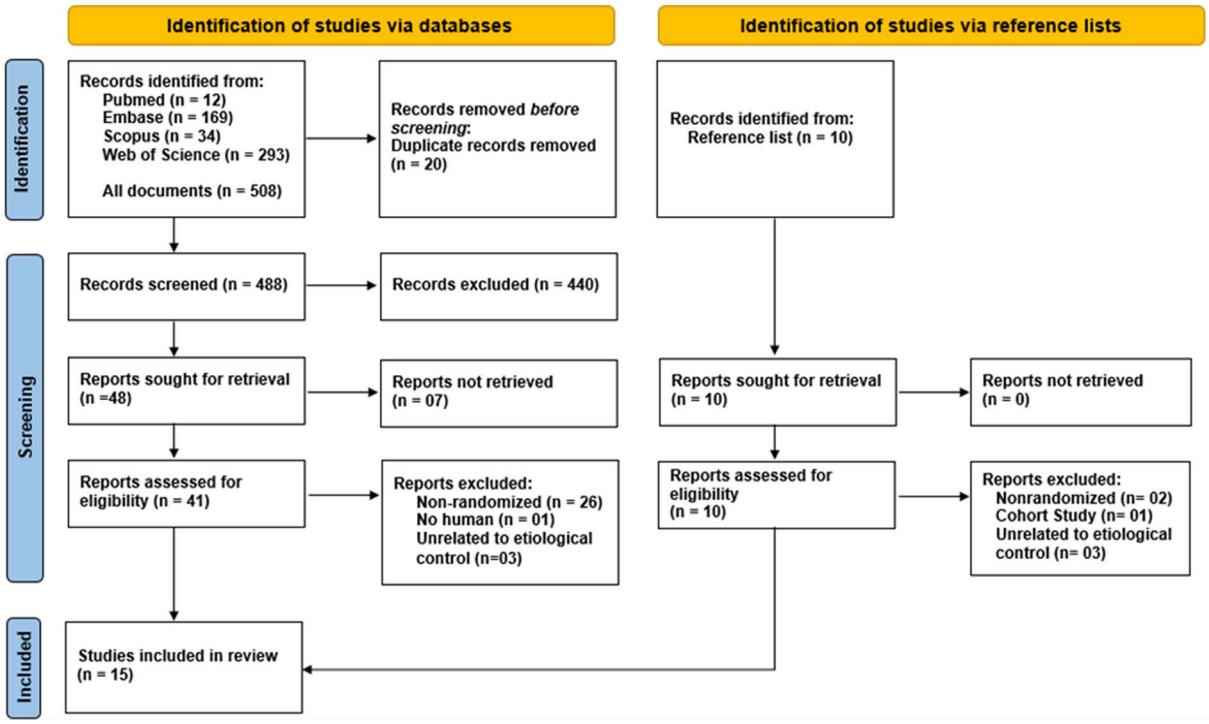

Fig. 1. Flow diagram of the systematic review literature search results. Based on the PRISMA statement 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses-http://www.prisma-statement.org/'.

Table 1. Patient characteristics, ChD stage and diagnostic method of *Trypanosoma cruzi* infection used in randomized clinical trials

Author and year of publication	Country	Sample size	Sex	Age /min–max (mean)	Clinical stage of disease	Methods used in ChD diagnostic
De Andrade *et al.* (1996)	Brazil	130	M/F	⩾07 and ⩽12	(–)	IHA, IFA, ELISA
Coura *et al.* (1997)	Brazil	77	(–)	(–)	Chronic	IFA, Xenodiagnosis
Sosa Estani *et al.* (1998)	Argentina	106	(–)	<13	Chronic indeterminate	IHA, IFA, EIA
Andrade *et al.* (2004)	Brazil	129	M/F	⩾14 and ⩽19	(–)	A&T CL-ELISA
Sosa-Estani *et al.* (2004)	Argentina	249	M/F	⩾15 and ⩽ 44	Chronic indeterminate	IHA, IFA, ELISA
Rassi *et al.* (2007)	Brazil	35	M/F	⩾18 and ⩽64	Chronic indeterminate	Xenodiagnosis
Marin-Neto *et al.* (2008)	Multicentre	3000	M/F	⩾18 and ⩽75	(–)	IHA, IFA, ELISA
Chippaux *et al.* (2010)	Bolivia	111	(–)	Newborn	Congenital	ELISA
Molina *et al.* (2014)	Bolivia	78	M/F	39 ± 9	Chronic	RT-PCR
Morillo *et al.* (2015)	Multicentre	2854	M/F	⩾18 and ⩽ 75	Chronic	RT-PCR
Vallejo *et al.* (2016)	Spain	14	M/F	⩾26 and ⩽ 57	Chronic indeterminate	RT-PCR
Morillo *et al.* (2017)	Canada	120	M	⩾18 and ⩽ 54	Chronic indeterminate	RT-PCR
Torrico *et al.* (2018)	Bolivia	231	M/F	⩾18 and ⩽ 50	Chronic indeterminate	RT-PCR
Molina-Morant *et al.* (2020)	Multicentre	240	(–)	⩾ 18	Chronic	IFA, IHA, ELISA
Torrico *et al.* (2021)	Bolivia	210	M/F	⩾18 and ⩽ 50	Chronic indeterminate	RT-PCR

–, data not reported; M, male; F, female; IHA, indirect haemagglutination assay; IFA, indirect immunofluorescence assay; EIA/ELISA, enzyme-linked immunosorbent assay; A&T CL-ELISA ELISA, chemiluminescent ELISA with A&T antigen; RT-PCR, real-time reverse transcription polymerase chain reaction.

Cardiovascular and laboratory outcomes, treatment discontinuation and adverse effects

As indicated in Table 3, pre-treatment and post-treatment cardiovascular parameters were reported in 9 studies (60%). Right bundle branch block, left anterior hemiblock, atrioventricular block, ectopic rhythm, atrial fibrillation, ventricular arrhythmia, ventricular tachycardia, stroke, transient ischaemic attack, systemic embolism, pulmonary embolism, pacemaker or implantable cardioverter-defibrillator, cardiac arrest and transplantation were the main cardiovascular abnormalities/outcomes reported. No study reported significant improvement or worsening of cardiac function in patients treated with the different drugs and therapeutic regimens investigated.

All treatments were associated with a low frequency of altered liver function estimated from alanine aminotransferase levels (2.2–38%) and reduced white blood cell counts (0.1–43%). Altered liver function, estimated from serum transaminase levels,

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Table 2. Characteristics of treatments used in the management of Chagas disease patients in randomized clinical trials.

Study	Patients / group (n)	Withdrawn[a]	Therapeutic schemes	Dose reported	Administration frequency and rout	Treatment (days)	Follow-up (years)	Seroconversion negative (n/%)
De Andrade *et al.* (1996)	64	06	BNZ	7.5 mg/kg/ day	b.d.i/oral	60	3	37/64 (58%)
	65	11	PLA	(–)	(–)			3/64 (5%)
Coura *et al.* (1997)	26	03	BNZ	5 mg/kg/day	b.d.i/oral	30	1	(?)
	27	08	NF	5 mg/kg/day				(?)
	24	02	PLA	(–)				(?)
Sosa Estani *et al.* (1998)	51	07	BNZ	5 mg/kg/day	(–)	60	2	5/44 (11.2%)
	50	06	PLA	(–)	(–)			2/44 (4.5%)
Andrade *et al.* (2004)	64	06	BNZ	7.5 mg/kg/day	b.d.i/oral	60	6	47/53 (88.7%)
	65	11	PLA	(–)	(–)			12/46 (26.1%)
Sosa-Estani *et al.* (2004)	62	35.5%	AT + BNZ	50 + 5 mg/kg/day	b.d.i/oral	1–37 + 5–37	0.14 (53 days)	(–)
	66	33.4%	AT + BNZ	100 + 5 mg/kg/day	b.d.i/oral	1–37 + 5–37		(–)
	59	28.8%	PLA + BNZ	(–) + 5 mg/kg/day	b.d.i/oral	1–37 + 5–37		(–)
	62	21%	PLA + AT + BNZ	(–) + 50 mg + 5 mg/kg/day	b.d.i/oral	1–4 + 1–37 + 5–37		(–)
Rassi *et al.* (2007)	23	06	ALLP	300 mg	3 daily	60	2	0/17 (0%)
	12	02	PLA	(–)	(–)			0/10 (0%)
Marin-Neto *et al.* (2008)	300	(–)	BNZ	5 mg/kg/day	(–)	40, 60 or 80	2	? (60%)
	300	(–)	PLA	(–)	(–)			? (30%)
Chippaux *et al.* (2010)	59	01	BNZ	2.5 mg/ kg	b.d.i/oral	60	1	53/58 (91.3%)
	52	02	BNZ	7.5 mg/ kg	o.d	30		45/50 (90%)
	68	(–)	Untreated	(–)	(–)	(–)		(–)
Molina *et al.* (2014)	26	09	BNZ	150 mg/day	b.d.i/oral	60	1	16/17 (94,1%)
	26	01	POS	400 mg/day				5/25 (20%)
	26	06	POS	100 mg/day				2/20 (10%)

POS, posaconazole; BNZ, benznidazole; PLA, placebo; NF, nifurtimox; ALLP, allopurinol; AT, thioctic acid; b.d.i, twice day; o.d, once a day; –, data not reported; ?, incomplete information.
[a]For discontinuity, adverse events and other reasons.

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Table 2. (*Continued.*)

Study	Patients / group (*n*)	Withdrawn[a]	Therapeutic schemes	Dose reported	Administration frequency and rout	Treatment (days)	Follow-up (years)	Seroconversion negative (*n*/%)
Morillo *et al.* (2015)	1431	?/14	BNZ	300 mg day^{-1}	o.d	1431	⩾ 5	? (46.7%)
	1423	?/14	PLA	(–)	(–)	1423		? (33.1%)
Vallejo *et al.* (2016)	07	03	BNZ	5 mg kg day^{-1}	b.d.i/oral	60	1.5	7/7 (100%)
	07	01	Untreated	(–)	(–)	(–)		3/7 (42.8%)
Morillo *et al.* (2017)	32	00	POS	400 mg	b.d.i/oral	60	1	? (16%)
	30	01	PLA	10 mg				? (17%)
	28	09	BNZ + POS	200 mg + 400 mg				? (96%)
	30	10	BNZ + PLA	200 mg				? (96%)
Torrico *et al.* (2018)	45	03	E1224	4000 mg total^{-1}	400 mg/o.d for 1–3 days followed 400 mg/once wk for 7 wk/oral	65	1	13 (28.9%)
	46	(–)	E1224 + PLA	2000 mg total^{-1}	200 mg/o.d for 1–3 days + Placebo followed by 200 mg/E1224 and Placebo/once wk for 7 wk/oral			4 (8.3%)
	48	03	E1224 + PLA	2400 mg total^{-1}	400 mg/once wk for 3 wk followed by Placebo/4 wk or Placebo/8 wk/oral			5 (10.9%)
	45	03	BNZ	100 mg tablet^{-1}	5 mg/kg/day in 2 daily doses/oral			37 (82.2%)
	47	01	PLA	400 mg	4 tablets o.d for 1–3 days followed for 4 tablets once a wk for 7 wk/oral			4 (8.5%)

[a]For discontinuity, adverse events and other reasons. E1224 (ravuconazole prodrug).
BNZ, benznidazole; POS, posaconazole; PLA, placebo; o.d, once a day; b.d.i, twice day; wk, week; –, data not reported; ?, incomplete information.

Table 2. (*Continued.*)

Study	Patients/ group (*n*)	Withdrawn[a]	Therapeutic schemes	Dose reported	Administration frequency and rout	Treatment	Follow-up (years)	Seroconversion negative (*n*/%)
Molina-Morant *et al.* (2020)	80	(–)	BNZ	300 mg	(–)	60 days	1	(–)
	80	(–)	BNZ	150 mg	(–)			(–)
	80	(–)	BNZ	400 mg	(–)	15 days		(–)
Torrico *et al.* (2021)	30	11	BNZ	300 mg day^{-1}	b.d.i/oral	8 wk	1	18/23 (78%)
	30	09	BNZ	300 mg day^{-1}	b.d.i/oral	4 wk		24/27 (89%)
	30	10	BNZ	300 mg day^{-1}	b.d.i/oral	2 wk		22/28 (79%)
	30	06	BNZ	150 mg day^{-1}	b.d.i/oral	4 wk		24/29 (83%)
	30	06	BNZ + FOS	150 + 300 mg day^{-1}	b.d.i + o.d for 3 days followed by 300 mg once wk	4 wk		20/24 (83%)
	30	05	BNZ + FOS	300 + 300 mg day^{-1}	b.d.i + o.d for 3 days followed by 300 mg once wk	8 wk		21/25 (84%)
	30	01	PLA	(–)	(–)	(–)		1/30 (3%)

[a]For discontinuity, adverse events and other reasons.
BNZ, benznidazole; FOS, fosravuconazole; PLA, placebo; o.d, once a day, b.d.i., twice day; wk, week; –, data not reported; ?, incomplete information.

was identified in patients treated with BNZ (Molina *et al.*, 2014; Morillo *et al.*, 2015, 2017; Vallejo *et al.*, 2016; Torrico *et al.*, 2018, 2021), posaconazole (Molina *et al.*, 2014; Morillo *et al.*, 2017) and E1224 (Torrico *et al.*, 2018) alone, as well as BNZ combined with thioctic acid (Sosa-Estani *et al.*, 2004), posaconazole (Morillo *et al.*, 2017) or fosravuconazole (Torrico *et al.*, 2021). Leucopenia, neutropenia and/or lymphopenia were identified in patients treated with allopurinol, BNZ alone or combined with fosravuconazole (Rassi *et al.*, 2007; Morillo *et al.*, 2015; Torrico *et al.*, 2021) (Table 3). Treatment discontinuation ranged from 1.5 to 57% in patients receiving BNZ alone or combined with thioctic acid, posaconazole or fosravuconazole. Cutaneous, gastrointestinal and neurological reactions were the most common adverse effects identified in 5–73% of all patients investigated. The mean rate of serious adverse reactions was 22.78% in BNZ-treated patients, while this rate was 2.56% in patients receiving placebo (Table 4).

Sources of methodological bias

Based on the D&B checklist, compliance with the evaluated methodological criteria ranged from 46 to 100% (average result = 85.57%). Studies prior to 2014 were unable to meet all criteria. However, 4 studies (26.66%) after 2014 met all the methodological criteria analysed (Molina *et al.*, 2014; Morillo *et al.*, 2017; Torrico *et al.*, 2018, 2021). Considering the items evaluated in the D&B checklist, criteria 8 and 10 were the least observed by the authors, namely: were the statistical tests used to assess the main outcomes appropriate? and were the main outcome measures used accurate (valid and reliable)? These criteria were met by 73.3% of the studies reviewed. Items 3 (Are the outcomes to be measured clearly described in the introduction or in the methods section?), 4 (Are the interventions of interest clearly described?) and 23 (Were the study subjects randomized to the intervention groups?) were consistently attended in all studies. The individual and overall results of bias can be accessed in Fig. 2 and Table S3.

Discussion

In this review, we identified that the evidence provided by randomized controlled clinical trials targeting ChD treatment is based on BNZ (De Andrade *et al.*, 1996; Sosa Estani *et al.*, 1998; Andrade *et al.*, 2004; Marin-Neto *et al.*, 2008; Chippaux *et al.*, 2010; Morillo *et al.*, 2015; Vallejo *et al.*, 2016; Molina-Morant *et al.*, 2020), NFx (Coura *et al.*, 1997), allopurinol (Rassi *et al.*, 2007), posaconazole (Molina *et al.*, 2014; Morillo *et al.*, 2017), ravuconazole (Torrico *et al.*, 2018) and fosravuconazole (Torrico *et al.*, 2021); administered in monotherapy, as well as BNZ combined with thioctic acid (Sosa-Estani *et al.*, 2004), posaconazole (Morillo *et al.*, 2017) and fosravuconazole (Torrico *et al.*, 2021). In line with the perspective of efficacy for the treatment of neglected tropical diseases, most drugs used in monotherapy and in combination showed remarkable relevance in attenuating *T. cruzi* infection. Accordingly, therapeutic effects were primarily associated with better parasitological control, as evidenced by negative seroconversion rates obtained from different treatment protocols. Although studies do not report a definitive parasitological cure, a low frequency of functional cardiac deterioration has been identified. However, the therapeutic regimens administered were associated with important side effects, indicating variable systemic toxicity.

Interestingly, the RCT identified were mainly concentrated in 3 Latin American countries (i.e., Brazil, Argentina and Bolivia) (De Andrade *et al.*, 1996; Coura *et al.*, 1997; Sosa Estani *et al.*, 1998; Andrade *et al.*, 2004; Sosa-Estani *et al.*, 2004; Rassi *et al.*, 2007; Chippaux *et al.*, 2010; Molina *et al.*, 2014; Torrico *et al.*, 2018,

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Table 3. Adverse events, cardiac function, immune response and laboratory findings in ChD patients

Study	Groups treatment	Pre-treatment cardiac condition	Post-treatment cardiac condition	Laboratory findings
De Andrade *et al.* (1996)	BNZ 7.5 mg/kg/60d	Right bundle branch block ($n = 2$) Left anterior hemiblock ($n = 1$) Atrioventricular block ($n = 2$) Ectopic rhythm ($n = 1$)	Right bundle branch block BNZ: $n = 1$ (1.7%) PLA: $n = 4$ (6.9%)	The frequency of anaemia (haemoglobin $\leqslant 110\ g\ L^{-1}$) was similar in the 2 groups no patient developed leucopenia (white-cell count $<0.3 \times 10^9$/L) or neutropenia (neutrophil count $<0.75 \times 10^9$/L)
	PLA	Right bundle branch block ($n = 7$)		
Coura *et al.* (1997)	BNZ 5 mg/kg/30d	(–)	ECG of patients did not suffer changes after treatment	(–)
	NTX 5 mg/kg/30d	(–)		(–)
	PLA	(–)		(–)
Sosa Estani *et al.* (1998)	BNZ 5 mg/kg/60d	ECG: Left anterior hemiblock or right bundle branch block BNZ: $n = ?$ (5%) PLA: $n = ?$ (4.8%)	Changes in ECG after 48 months follow-up: ventricular ectopic beats. BNZ: $n = 1$ (2.5%) PLA: $n = 1$ (2.4%)	(–)
	PLA			(–)

Author and year of publication	Groups treatment	Pre-treatment cardiac condition	Post-treatment cardiac condition	Laboratory findings
Andrade *et al.* (2004)	BNZ 7.5 mg/kg/b.d.i	(–)	3 years follow-up ECG: complete right bundle branch block ($n = 1$) 6 years follow-up No incident case of ECG abnormality was found	(–)
	PLA	(–)	3 years follow-up ECG: complete right bundle branch block ($n = 4$) 6 years follow-up No incident case of ECG abnormality was found	(–)
Sosa-estani *et al.* (2004)	TA 50 mg day^{-1} 1–37d + BNZ 5 mg kg^{-1}/5–37d	(–)	(–)	↑ALT ($n = 1$)
	TA 100 mg/1–37d + BNZ 5 mg kg^{-1}/5–37d	(–)	(–)	↑ALT ($n = 1$)
	PLA 1–37d + BNZ 5 mg kg^{-1}/5–37d	(–)	(–)	(–)
	PLA 1–4d + TA 50 mg/1–37d + BNZ 5 mg kg^{-1}/5–37d	(–)	(–)	(–)
	Untreated	(–)	(–)	(–)
Rassi *et al.* (2007)	ALLP 300 mg, 3 × d/60d	(–)	(–)	Leucopenia ($n = 2$)
	PLA	(–)	(–)	(–)

Study	Groups treatment	Pre-treatment cardiac condition	Post-treatment cardiac condition	Laboratory findings
Molina *et al.* (2014)	BNZ 150 mg/b.d.i	(–)	No significant effects on QT interval	↑ ALT ($n = 5$) ↑ AST ($n = 3$) ↑ ALP ($n = 13$)
	POS 400 mg/b.d.i	(–)		↑ ALT ($n = 6$) ↑ AST ($n = 4$) ↑ ALP ($n = 11$)
	POS 100 mg/b.d.i	(–)		↑ ALT ($n = 10$) ↑ AST ($n = 8$) ↑ ALP ($n = 19$)
Morillo *et al.* (2015)	BNZ 5 mg/kg/60d	Atrial fibrillation ($n = 107$) Ventricular arrhythmia ($n = 221$) Resuscitated cardiac arrest ($n = 19$)	Deaths ($n = 246$) Resuscitated cardiac arrest ($n = 10$) Ventricular tachycardia ($n = 33$)	↑ ALT ($n = 75$) Neutrophil < 1900/mm^3 ($n = 2$)

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Table 3. (*Continued.*)

Study	Groups treatment	Pre-treatment cardiac condition	Post-treatment cardiac condition	Laboratory findings
		Heart failure ($n = 142$) Pacemaker ($n = 205$) Implantable cardioverter-defibrillator ($n = 39$) Stroke or transient ischaemic attack ($n = 61$) Systemic or pulmonary embolism ($n = 7$)	Heart failure ($n = 109$) Pacemaker or implantable cardioverter-defibrillator ($n = 109$) Stroke or transient ischaemic attack, systemic embolism, or pulmonary embolism ($n = 54$) Cardiac transplantation ($n = 3$)	
	PLA	Atrial fibrillation ($n = 90$) Ventricular arrhythmia ($n = 189$) Resuscitated cardiac arrest ($n = 16$) Heart failure ($n = 128$) Pacemaker ($n = 198$) Implantable cardioverter–defibrillator ($n = 31$) Stroke or transient ischaemic attack ($n = 62$) Systemic or pulmonary embolism ($n = 11$)	Deaths ($n = 257$) Resuscitated cardiac arrest ($n = 17$) Ventricular tachycardia ($n = 41$) Heart failure ($n = 122$) Pacemaker or implantable cardioverter-defibrillator ($n = 125$) Stroke or transient ischaemic attack, systemic embolism, or pulmonary embolism ($n = 61$) Cardiac transplantation ($n = 9$)	↑ ALT ($n = 28$)

Author and year of publication	Groups treatment	Pre-treatment cardiac condition	Post-treatment cardiac condition	Laboratory findings
Vallejo *et al.* (2016)	BNZ 5 mg/kg/60 d	Normal echocardiography	None developed cardiomyopathy	↑ Naïve CD4 T cell, KLGR 1 receptor expression on CD4 memory cells, Th17, Th1; INF-ɣ and TNF-α-producing CD4 T cells, Th1/Th2, INFγ/IL4 and TNFα/IL4 ratio ↑ Liver test values ($n = 01$)
	Untreated	(–)	(–)	(–)
Morillo *et al.* (2017)	POS 400 mg/b.i.d	Normal QT interval	No significant effects on QT interval	↑ ALT ($n = 1$)
	PLA 10 mg/b.i.d.			↑ ALT ($n = 2$)
	BNZ 200 mg/b.i.d. + POS 400 mg/b.i.d.			↑ ALT ($n = 4$)
	BNZ 200 mg + PLA b.i.d			↑ ALT ($n = 2$)
Torrico *et al.* (2018)	E1224 4000 mg/8w			↑ ALT and/or AST ($n = 5$)
	E1224 2000 mg/8w			(–)
	E1224 2400 mg/4w + 4 w placebo	Normal QT interval	No significant increases in QT interval	(–)
	BNZ 5 mg/kg/60d			↑ ALT ($n = 1$)
	PLA 8w			(–)

Study	Groups treatment	Pre-treatment cardiac condition	Post-treatment cardiac condition	Laboratory findings
Torrico *et al.* (2021)	BNZ 300 mg/d/8w	Normal electrocardiogram	There were no meaningful safety signals from standard haematology, biochemistry and ECG assessments.	Neutropenia ($n = 8$) Leucopenia ($n = 5$) Lymphopenia ($n = 3$) ↑ ALT ($n = 4$) ↑ AST ($n = 1$)
	BNZ 300 mg/d/4w			Neutropenia ($n = 2$) Leucopenia ($n = 2$) lymphopenia ($n = 3$) ↑ ALT ($n = 1$) ↑ AST ($n = 1$)
	BNZ 300 mg/d/2w			Lymphopenia ($n = 1$) ↑ ALT ($n = 2$)
	BNZ 150 mg/d/4w			Neutropenia ($n = 2$) Lymphopenia ($n = 1$) ↑ ALT ($n = 4$) ↑ AST ($n = 1$)
	BNZ 150 mg mg/d/4w + FOS			Neutropenia ($n = 4$) Leucopenia ($n = 1$) Lymphopenia ($n = 1$) ↑ ALT ($n = 4$) ↑ AST ($n = 5$)

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Table 3. (*Continued.*)

Study	Groups treatment	Pre-treatment cardiac condition	Post-treatment cardiac condition	Laboratory findings
	BNZ 300 mg/w/8 w + FOS			Neutropenia (*n* = 2) Leucopenia (*n* = 2) ↑ ALT (*n* = 3) ↑AST (*n* = 7)
	PLA			Normal: ALT and AST

BNZ, benznidazole; PLA, placebo; POS, posaconazole; TA, thioctic acid; ALLP, allopurinol; –, data not reported; ?, incomplete information; ALT, alanine aminotransferase; ECG, electrocardiogram; d, day; NTX, nifurtimox; AST, aspartate aminotransferase; ALP, alkaline phosphatase; E1224, ravuconazole prodrug; w, week; FOS, fosravuconazole; b.d.i, twice day.

Table 4. Adherence, discontinuation and adverse events associated with the treatment administered in ChD patients.

Study	Adherence	Discontinuation	Adverse events	Serious adverse events	Most common adverse events
De Andrade *et al.* (1996)	Completed the treatment and follow-up *n* = 112 (87%) BNZ: *n* = 58 PLA: *n* = 54	BNZ: *n* = 1 (1.5%)	Nausea, anorexia, headache, stomach ache, arthralgia (<5% patients). Cutaneous rash and pruritus BZN (*n* = 8), placebo (*n* = 2)	BNZ: Cutaneous rash and pruritus (*n* = 1)	(–)
Coura *et al.* (1997)	Completed the treatment *n* = 64 (83.1%)	BNZ: *n* = 3 (11.5%) NF: *n* = 8 (29.6%)	(–)	BNZ: *n* = 3 (11.5%) NF: *n* = 8 (29.6%)	NF: gastrointestinal disorders, paraesthesia and neurological BNZ: gastrointestinal, rash, neurological
Sosa Estani *et al.* (1998)	Completed the treatment *n* = 101 (95.2%)	(–)	BNZ: Intestinal colic, cutaneous rash, headache, anorexia, vomiting, nausea, diarrhoea, dizziness, paraesthesia, hands shivering (*n* = <20%)	BNZ: *n* = 6 (10%)	Intestinal colic and rush were more frequent in BNZ than PLA
Sosa-Estani *et al.* (2004)	Completed the treatment *n* = 175 (70.3%)	BNZ: *n* = 74 (17.7%)	Patients affected by adverse events was similar between the 4 groups: 54.8 to 58%	Cutaneous rash, itching and fever (*n* = ?)	Rash 28%, pruritus 13.6%, headache 8%, epigastric pain 6.2%, fever 6.2%, asthenia 4.3%, nausea 4%, myalgia 4.3%, vomiting 3.2%, fatigue 4.3%, others 21.5%
Rassi *et al.* (2007)	Completed the treatment ALLP: *n* = 17 PLA: *n* = 10	ALLP: *n* = 6 (26%)	ALLP: 11/23, fever (*n* = 5), cutaneous allergy (*n* = 5), pyrosis (*n* = 3), pruritus (*n* = 3), leucopenia (*n* = 2), lymph node enlargement (*n* = 1), vomiting (1), stomachache (*n* = 1), red eye (*n* = 1) PLA: 1/12, vertigo (*n* = 1)	(–)	(–)
Molina *et al.* (2014)	Completed the treatment and follow-up BNZ: *n* = 17 High-dose POS: *n* = 25 Low-dose POS: *n* = 20	BNZ: *n* = 5 (19%).	BNZ: Cutaneous rash (*n* = 16), Gastrointestinal (*n* = 7), Dysgeusia (*n* = 2), High-dose POS: Cutaneous (*n* = 5), gastrointestinal (*n* = 4), mucosal dryness (*n* = 3) Low-dose POS: Cutaneous reaction (*n* = 4), gastrointestinal (*n* = 3), mucosal dryness (*n* = 2)	BNZ: Dermatitis (*n* = 5), anaphylaxis with angioedema (*n* = 1)	Headache, asthenia, sleepiness, arthralgia, dizziness

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Table 4. (*Continued.*)

Study	Adherence	Discontinuation	Adverse events	Serious adverse events	Most common adverse events
Morillo *et al.* (2015)	Completed the treatment and follow-up BZN: 1429/1431 (99.9%) PLA: 1422/1423 (99.9%)	BZN: n = 288 (20.1%) PLA: *n* = 66 (4.6%)	BNZ: Cutaneous rash (*n* = 9.59%), gastrointestinal (*n* = 7.84%), neurological (*n* = 3.64%) PLA: Cutaneous rash (*n* = 1.27%), gastrointestinal (*n* = 2.88%), Neurological (*n* = 1.34%)	BNZ: Cutaneous rash (1.16%), gastrointestinal (1.82%) neurological (0.98%) PLA: Cutaneous rash (*n* = 0.14%), gastrointestinal (*n* = 0.63%), neurological (*n* = 0.42%)	(–)
Vallejo *et al.* (2016)	Completed the treatment and follow-up BNZ: *n* = 3 (42%) PLA: *n* = 6 (85%)	BZN: *n* = 4 (57%) PLA: *n* = 1 (15%)	BNZ: Rash (*n* = 3), change in liver function (*n* = 1), rash and fever (1)	BNZ: Rash (*n* = 2), fever (*n* = 1)	(–)
Morillo *et al.* (2017)	Completed the treatment and follow-up POS: *n* = 32 (100%) PLA: *n* = 29 (97%) POS + BNZ: *n* = 19 (70%) BNZ + PLA: *n* = 20 (60%)	32% patients discontinue therapy due to side effects POS + BNZ: *n* = 9 (30%) BNZ + PLA: *n* = 10 (33%) PLA: *n* = 1 (3%)	POS: Cutaneous (*n* = 2), gastrointestinal (*n* = 12), neurological (*n* = 4) PLA: Cutaneous (*n* = 3), gastrointestinal (*n* = 5), neurological (*n* = 3) POS + BNZ: Cutaneous (*n* = 12), gastrointestinal (*n* = 10), neurological (*n* = 9) BNZ + PLA: Cutaneous (*n* = 18), gastrointestinal (*n* = 8), neurological (*n* = 10)	POS + BNZ: Hepatitis (*n* = 1), rash (n = 1) BNZ + PLA: Peripheral neuropathy (*n* = 1), Abortion (*n* = 1), cutaneous rash (*n* = 1) PLA: Head injury (*n* = 1)	Headache (14%), nausea (10%), rash (10%)
Torrico *et al.* (2018)	Completed the treatment and follow-up *n* = 217 (?)	HD E1224 = 5 (11.1%) BNZ = 4 (8.9%)	81% patients affected by adverse events. BZN = 64.4%. LD E1224 = 31.3% SD E1224 = 52.2%. HD E1224 = 44.4%	Short-dose E1224: 1 (2%) High-dose E1224: 3 (7%) BNZ: 2 (4%)	(–)
Torrico *et al.* (2021)	Completed treatment and follow-up. *n* = 202 (96%)	BNZ 300 mg/day/8w: *n* = 6 (20%) BNZ 300 mg/day/4w: *n* = 1 (3%) BNZ 300 mg/day/2w: *n* = 0 (0%) BNZ 150 mg/day/4w: *n* = 1 (3%) BNZ 150 mg/day/4w + FOS: *n* = 3 (10%) BNZ 300 mg/day/8w + FOS: *n* = 4 (13%) PLA: *n* = 0 (0%)	70% patients affected by adverse events. BNZ 300 mg/day/8w: *n* = 23 (73%) BNZ 300 mg/day/4w: *n* = 19 (63%) BNZ 300 mg/day/2w: *n* = 21 (70%) BNZ 150 mg/day/4w: *n* = 19 (63%) BNZ 150 mg/day/4w + FOS: *n* = 22 (73%) BNZ 300 mg/day/8w + FOS: 19 (63%) PLA: *n* = 14 (47%)	BNZ 300 mg/day/8w + FOS: neutropenia *n* = (1), elevated AST (*n* = 2) and GGT (*n* = 1), breast cancer (*n* = 1) BNZ 300 mg/day/8w: neutropenia (*n* = 2), leucopenia (*n* = 1) BNZ 150 mg/day/4w + FOS: Elevated AST (2) and GGT (1) BNZ 300 mg/day/8w: leucopenia (*n* = 1), leucopenia and neutropenia (*n* = 1) BNZ 300 mg/day/8w + FOS: neutropenia and leucopenia (*n* = 1), breast cancer (*n* = 1) BNZ 300 mg/day/4w: pyrexia and rash (*n* = 1) BNZ 150 mg/day/4w + FOS: acute cholecystitis and biliary polyp (*n* = 1)	BNZ 300 mg/day/8w: Headache, neutropenia and leucopenia were reported more frequently than other BZN regimens

BNZ, benznidazole; PLA, placebo; POS, posaconazole; ALLP, allopurinol; AT, thioctic acid; NF, nifurtimox; E1224, ravuconazole prodrug; LD, low-dose; SD, short-dose; HD, high-dose; –, data not reported; FOS, fosravuconazole; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ?, incomplete information.

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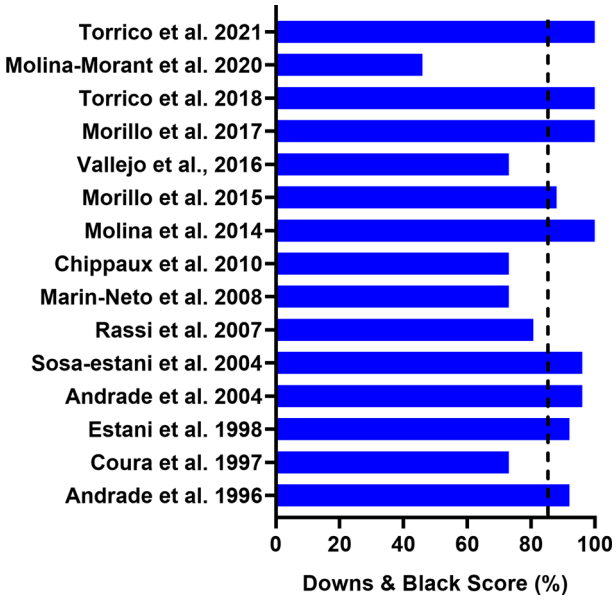

Fig. 2. Percentage of items met in the methodological bias analysis (reporting quality) for all RCTs included in the systematic review. Bias analysis was based on the Downs & Black checklist for randomized and non-randomized studies. The dotted line indicates the average percentage of methodological criteria met (85.51%). The complete bias analysis stratified by domains and items assessed can be found in Table S3.

2021). In these endemic countries, environmental and socioeconomic factors create favourable conditions for *T. cruzi* infection (Mejía-Jaramillo *et al.*, 2014; Santos *et al.*, 2020). However, poor early diagnosis favours the transmissibility of this parasite in non-endemic areas, which is mainly linked to migratory movements of infected people (Guarner, 2019; Echeverría *et al.*, 2020*a*). In the studies reviewed, ChD was rigorously confirmed by one or more diagnostic tools, including xenodiagnosis, indirect haemagglutination, indirect immunofluorescence, ELISA and PCR. Although xenodiagnosis has traditionally been proposed to confirm ChD, this method has shown a marked decline in the last 3 decades (Zingales, 2018). On the other hand, indirect haemagglutination, indirect immunofluorescence and ELISA are the most used tests for ChD diagnosis (Andrade *et al.*, 2011; Nogueira *et al.*, 2018; Zingales, 2018). These methods exhibit high analytical sensitivity (96% – 99%) (Castro *et al.*, 2002; Nogueira *et al.*, 2018), a characteristic consistent with most randomized studies published up to 2014. In the last 2 decades, PCR-based methods were frequently incorporated into clinical studies, increasing the sensitivity and specificity of parasitological diagnosis (Nogueira *et al.*, 2018; Caldas *et al.*, 2019; Molina-Morant *et al.*, 2020). This method was predominant from 2014 onwards, being widely applied for ChD diagnosis, as well as for evaluating chemotherapy effectiveness. PCR is a remarkable tool for early detection of therapeutic failure (Caldas *et al.*, 2019), which has been clearly demonstrated in patients with chronic ChD treated with BNZ during recent (Silveira *et al.*, 2000; Solari *et al.*, 2001) and late (Morillo *et al.*, 2017) infections. Although PCR is the gold standard for *T. cruzi* detection, its applicability is quite restricted to clinical studies and specialized laboratories due to the higher cost, need for specialized devices and professionals with greater technical qualification (Caldas *et al.*, 2019). Thus, expanding access to this technology can improve the monitoring of populations exposed to greater risk of *T. cruzi* infection and the health care of newly infected patients.

Although BNZ is the first-line treatment for acute (Caldas *et al.*, 2019), recent (Silveira *et al.*, 2000; Solari *et al.*, 2001) and late (Morillo *et al.*, 2017) chronic infections, its effectiveness in achieving parasitological cure is still controversial, especially in chronic cases. Apparently, divergent parasitological results are influenced by parasite strain (e.g., tropism, virulence and pathogenicity), patient characteristics (e.g., age, immunological status and comorbidities) and infection phase (De Andrade *et al.*, 1992; Filardi and Brener, 1987). In addition, the toxicity profile and numerous side effects of different antiparasitic drugs have a marked impact on treatment adherence (Morillo, *et al.*, 2015, 2017). Accordingly, longer drug regimens that require high doses represent a greater clinical challenge, as dose-dependent and time-dependent toxicity favours treatment discontinuation and therapeutic failure (e.g., poor parasite control) (Vallejo *et al.*, 2016; Caldas *et al.*, 2019; Molina-Morant *et al.*, 2020).

Currently, there is an objective recommendation to administer BNZ between 5 and 8 mg kg day^{-1}, twice a day for 60 days (Andrade *et al.*, 2011). However, marked variations in BNZ dose and treatment period are recurrent in clinical studies (De Andrade *et al.*, 1996, Sossa-Estani *et al.*, 1998; Andrade *et al.*, 2004; Marin-Neto *et al.*, 2008; Chippaux *et al.*, 2010, Morillo *et al.*, 2015; Vallejo *et al.*, 2016; Mollina-Morant *et al.*, 2020). Accordingly, we identified that therapeutic schemes based on 2–7.5 mg kg day^{-1} for 60 or 30 days were respectively reported for newborns (Chippaux *et al.*, 2010); while conventional dosimetry (5 mg kg day^{-1} for 60 days) was investigated in children aged 13 years and older (Andrade *et al.*, 2004), and patients aged 26–57 years (Vallejo *et al.*, 2016). The same conventional dose was assigned for patients aged 18–75 years; however, different administration periods (40, 60 or 80 days) were evaluated (Marin-Neto *et al.*, 2008). In addition, significant adaptations were recently incorporated into randomized studies with BNZ, which used 150 and 300 mg kg day^{-1} by 60 days or 400 mg kg day^{-1} by 15 days in patients aged 50–80 years (Molina-Morant *et al.*, 2020). Objectively, these variations express the urgent need to define more efficient protocols for the reference drug, whose dose ranges and administration periods were mainly established in clinical practice and not in unequivocal evidence of therapeutic efficacy.

Regardless of the therapeutic regimen adopted, parasitological clearance, negative seroconversion, as well as low systemic toxicity are desirable clinical outcomes of drug treatment, including BNZ (Caldas *et al.*, 2019). Thus, we found that 5 mg BNZ kg^{-1} day^{-1} per60 days administered to patients with chronic ChC aged 26–57 years was associated with 100% negative seroconversion compared to 45% in untreated patients after 1 year follow-up (Vallejo *et al.*, 2016). Even using similar therapeutic regimens (2–7.5 mg kg day^{-1} for 30–80 days), BNZ-induced divergent negative seroconversion rates, ranging from 11.2 to 91.3% (De Andrade *et al.*, 1996; Sosa Estani *et al.*, 1998; Andrade *et al.*, 2004; Chippaux *et al.*, 2010). Thus, the best parasitological results were obtained by Vallejo *et al.* (2016), followed by Morillo *et al.* (2017), Molina *et al.* (2014) and Chippaux *et al.* (2010), who reported respectively 100, 96 and 94.1% cure rates for young adults and 91.3% cure for newborns, both after 1 year follow-up. Despite this response, clinical trials support BNZ efficacy for congenital ChD, corroborating a consistent trend of negative serology maintenance over time, an outcome that can be more easily reversed in adults (Blanco *et al.*, 2000).

Although NFx is the second choice for ChD treatment, RCT with this drug are scarce. Thus, NFx was evaluated in only one trial identified in this review (Coura *et al.*, 1997). Unfortunately, this study reinforced the controversial effectiveness of this drug for chronic ChD. Accordingly, Coura *et al.* (1997) did not identify negative seroconversion in patients receiving 5 mg kg day^{-1} NFx for 30 days or BNZ after 1 year follow-up. Thus, the lack of evidence of parasitological cure attributed to NFx in addition to its recognized toxicity profile (e.g., polyneuritis and bone marrow depression) makes the clinical use of this drug inadvisable when BNZ is available and well tolerated (Urbina and Docampo,

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2003; Muñoz *et al.,* 2011; Nogueira *et al.,* 2018). Despite clinical evidence generated in recent decades supporting BNZ-based treatment for indeterminate chronic ChD (Molina *et al.,* 2014; Morillo *et al.,* 2017; Torrico *et al.,* 2018), more efficient and safer drugs are still needed. Thus, several studies reviewed were based on repositioning strategies involving drugs with trypanocidal potential, such as allopurinol (Rassi *et al.,* 2007), posaconazole (Molina *et al.,* 2014; Morillo *et al.,* 2017), ravuconazole (Torrico *et al.,* 2018) and fosravuconazole (Torrico *et al.,* 2021).

Studies with allopurinol have confirmed its potent trypanostatic effect *in vitro* on 5 *T. cruzi* strains (Marr *et al.,* 1978; Avila and Avila, 1981). Interestingly, Berens *et al.* (1982) confirmed that allopurinol is metabolized by bloodstream trypomastigotes and intracellular amastigotes, which can be eradicated *in vitro* by this drug. Contrary to expectations, allopurinol administration (300 mg kg day^{-1} for 60 days) was not associated with negative seroconversion in patients with indeterminate chronic ChD (Rassi *et al.,* 2007). In contrast, posaconazole and ravuconazole (e.g., antifungal drugs used in humans) have proven their trypanocidal activity *in vitro* and *in vivo* (Urbina *et al.,* 1998; Molina *et al.,* 2014). Accordingly, posaconazole induced marked parasitological cure compared to BNZ-based monotherapy in acute experimental ChD (Olivieri *et al.,* 2010; Calvet *et al.,* 2020). Thus, all *T. cruzi*-infected mice (100%) receiving posaconazole and 50% receiving BNZ had negative blood cultures for this parasite (Calvet *et al.,* 2020). Surprisingly, this effect was even better in chronic *T. cruzi* infection, with parasitological cure rates reaching 60 and 0% when these animals were respectively treated with posaconazole and BNZ (Urbina *et al.,* 1998; Molina-Morant *et al.,* 2020). Conversely, BNZ (150 mg^{-1}kg per 60 days) showed better results compared to posaconazole (100 or 400 mg^{-1}kg per 60 days), returning a higher negative seroconversion rate (94.1%) for BNZ compared to posaconazole (10–20%) (Molina *et al.,* 2014). Torrico *et al.* (2018) identified a similar response in patients with chronic ChD receiving ravuconazole. Accordingly, this drug (4000 or 2000 mg for 65 days) determined lower negative seroconversion rates (28.9% or 8.3%) compared to BNZ (82.2%, 5 mg^{-1}kg per 60 days).

Currently, available evidence indicates that negative seroconversion rates obtained with these azoles are lower than BNZ-based monotherapy (Moraes *et al.,* 2014; Chatelain, 2015). However, combining these drugs has been suggested as a means to improve treatment efficacy, with the prospect of simultaneously interfering with multiple molecular pathways associated with *T. cruzi* parasitism (Bustamante *et al.,* 2014; Morillo *et al.,* 2017). Accordingly, Torrico *et al.* (2021) identified that therapeutic regimens combining BNZ (e.g., 150 or 300 mg kg^{-1} for 2, 4 or 8 weeks, respectively) and fosravuconazole (300 mg kg^{-1} for 4 or 8 weeks) achieved better effects than BNZ-based monotherapy. Interestingly, this study supported the proposition that BNZ dose could be reduced without losing its effectiveness (Bustamante *et al.,* 2014; Álvarez *et al.,* 2020). In this sense, Torrico *et al.* (2021) demonstrated similar negative seroconversion rates and cardiac function in patients exposed to conventional and low BNZ doses when combined with fosravuconazole in a 4-week protocol. Conversely, BNZ + posaconazole (200 + 400 mg kg^{-1} twice daily for 60 days) did not change seroconversion rates or improved cardiac function compared to BNZ-based monotherapy after 1-year follow-up (Morillo *et al.,* 2017). A similar effect was reported combining BNZ and thioctic acid (50 and 100 + 5 mg kg^{-1} twice daily for 1–37 days), which showed no superior benefit over reference chemotherapy after 53 days follow-up (Sosa-Estani *et al.,* 2004). Thus, the efficacy of pharmacological combinations is not unequivocal, even considering drugs with potent antiparasitic effects in preclinical models (Diniz *et al.,* 2013; Echeverría *et al.,* 2020*b*).

Despite successful examples, evidence of therapeutic failure indicates that ChD treatment is still challenging, especially considering that the long-term prognosis may not be favourable even with negative seroconversion in up to 1-year follow-up (Caldas *et al.,* 2019). Thus, parasite clearance may not improve or prevent cardiac deterioration in patients with chronic ChD (Marin-Neto *et al.*, 2008; Bern *et al.,* 2007). Although this finding is discouraging, it also indicates that negative seroconversion and parasitological cure may not be ideal indicators for estimating the full spectrum of benefits related to antiparasitic chemotherapy (Gonçalves and Novaes, 2018). From this perspective, De Andrade *et al.* (1996) identified a positive association between negative seroconversion rates and cardiac function in ChD patients receiving BNZ, whose electrical abnormalities (e.g., right bundle branch block) were attenuated even in the absence of parasitological cure. Although Sosa Estani *et al.* (1998) found low negative seroconversion rates (11.2%) in BNZ-treated children, no electrical conduction disturbances were identified after 48-months follow-up. Likewise, radiological or electrocardiographic changes were not detected in BNZ-treated adults (5 mg kg day^{-1} twice a day), who maintained positive serology 12 months after treatment (Coura *et al.,* 1997). These findings indicate the need to reframe the perception of therapeutic failure and success, since preventing progression to the symptomatic chronic phase and CCC evolution may be equally or more relevant outcomes than parasitological cure (Gonçalves and Novaes, 2018).

Considering the evidence based on randomized clinical trials, BNZ clearly showed greater overall efficacy compared to NFx, allopurinol, posaconazole, ravuconazole and fosravuconazole. However, the toxicity profile of BNZ is still a serious limitation to be overcome, especially considering the negative impact on treatment adherence (Molina-Morant *et al.,* 2020; Martín-Escolano *et al.,* 2020). In line with clinical experience, laboratory findings such as neutropenia, leucopenia, lymphopenia (De Andrade *et al.,* 1996; Morillo *et al.,* 2015; Torrico *et al.,* 2018, 2021), increased transaminases circulating levels, and upregulation in pro-inflammatory effectors (Vallejo *et al.,* 2016) were reported in the studies reviewed. Accordingly, BNZ/placebo ratios for adverse events ranging from 1.64 (Morillo *et al.,* 2015) to 3.85 (Torrico *et al.,* 2021) were identified in these studies. These findings were consistent with gastrointestinal, neurological and cutaneous adverse events, which can be reported in up to 90% of patients receiving trypanocidal chemotherapy (Francisco *et al.,* 2020). In general, treatment discontinuation is most often attributed to serious adverse effects associated with therapeutic regimens with higher doses administered for longer periods (Norman and López-Vélez, 2019; Morillo *et al.,* 2017). However, the incidence of adverse effects does not always follow this quantitative logic, exhibiting marked variability even in similar therapeutic regimens (Torrico *et al.,* 2018, 2021). Accordingly, adverse effects ranging from less than 20% (Sosa Estani *et al.,* 1998) to 64.4% (Torrico *et al.,* 2018) in patients with chronic ChD receiving the same treatment (e.g., 5 mg kg day^{-1} BNZ for ≅ 60 days), reinforce the proposition that patient characteristics influence the organic tolerability to antiparasitic drugs (Salvador *et al.,* 2015; Echeverría *et al.,* 2020*b*). Apparently, sensitivity to BNZ may have an important genetic component, as a higher treatment discontinuation rate related to cutaneous adverse reactions was detected in patients carrying the HLA-B*3505 allele (Salvador *et al.,* 2015).

From a critical interpretation of the evidence, we identified that 85.51% ± 15.49% of all the basic methodological criteria investigated in the bias analysis instrument were met, with adherence scores ranging from 46% (Molina-Morant *et al.,* 2020) to 100% (Molina *et al.,* 2014; Morillo *et al.,* 2017; Torrico *et al.,* 2018, 2021). Interestingly, these findings indicate that most of

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the studies reviewed presented high methodological rigour. Contrary to expectations, the quality index did not show a clearly time-dependent behaviour (influence of the publication year). Thus, the variability detected may be linked to the systematic replication of confounding factors (sources of bias) despite the advances applied to the design, operationalization and monitoring of randomized clinical trials, as well as greater availability of sensitive and specific analytical tools applicable to parasitology research. In cases of partial methodological adherence, the least met criteria were related to incomplete reporting of random variability estimates, specific delimitation of statistical probability, definition of appropriate statistical tests and precise use (validity and reliability) of the main outcome measures. Admittedly, these methodological limitations undermine the reproducibility, internal and external validity of the studies reviewed, limiting evidence reliability (Downs and Black, 1998; Torrico *et al.*, 2018, 2021). However, it is important to consider that these quality scores do not indicate flaws in the experimental protocols, as they exclusively point out limitations in the research reports. Thus, by mapping potential bias sources in all investigated studies, this review provides objective support to delimit further clinical trials with greater methodological rigour.

Taken together, the RCT provide robust evidence that BNZ is the most viable therapeutic option for the aetiological treatment of acute and chronic ChD. However, adjustments in therapeutic protocols based on this drug are still underway in search of optimized responses to increase adherence to antiparasitic chemotherapy and therapeutic success rates (Molina-Morant *et al.*, 2020; Morillo *et al.*, 2017; Torrico *et al.*, 2018, 2021). Therefore, shorter regimens or lower BNZ doses appear to be viable options to ensure similar efficacy compared to the reference protocol, reducing the incidence of adverse effects potentially linked to dose- and time-dependent toxicity reactions. In addition, combining BNZ with other antiparasitic drugs such as posaconazole, fosravuconazole or ravuconazole may be relevant to attenuate the frequency of adverse effects, despite not having a significant impact on negative seroconversion and parasitological cure compared to BNZ-based monotherapy. In order to improve the pharmacological management of ChD patients, longer clinical follow-up is required to evaluate aetiological treatment efficacy, allowing objectively characterizing the relevance of negative seroconversion and parasitological cure as primary endpoints of therapeutic success or failure. Thus, an ambitious proposal is to design robust methodological protocols incorporating more sensitive and specific diagnostic methods (e.g., PCR), allowing the reassessment of patients included in these randomized trials to clarify the relationship between parasitological cure and ChD progression.

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