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Systematic review

Reactivation of *Trypanosoma cruzi* infection in immunosuppressed patients: a systematic review and meta-analysis

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ABSTRACT

Background: The risk of *Trypanosoma cruzi* reactivation is poorly understood. Previous studies evaluating the risk of reactivation report imprecise findings, and recommendations for monitoring and management from clinical guidelines rely on consensus opinion.
Objectives: We conducted a systematic review and meta-analysis to estimate the cumulative *T. cruzi* reactivation incidence in immunosuppressed adults, summarize the available evidence on prognostic factors for reactivation, and examine its prognostic effect on mortality.
Data sources: MEDLINE, Embase, LILACS, Clinical Trials, and CENTRAL from inception to 4 July 2022.
Study eligibility criteria: Studies reporting the incidence of *T. cruzi* reactivation.
Participants: Immunosuppressed adults chronically infected by *T. cruzi*.
Methods: Two authors independently extracted data (including, but not limited to, incidence data, reactivation definition, follow-up, treatment, monitoring schedule, examined prognostic factors) and evaluated the risk of bias. We pooled cumulative incidence using a random-effects model.
Results: Twenty-two studies (806 participants) were included. The overall pooled incidence of *T. cruzi* reactivation was 27% (95% CI, 19–36), with the highest pooled proportion in the sub-group of transplant recipients (36%; 95% CI, 25–48). The highest risk period was in the first 6 months after transplant (32%; 95% CI, 17–58), decreasing drastically the number of new cases later. People living with HIV and patients with autoimmune diseases experienced significantly lower cumulative reactivation incidences (17%; 95% CI, 8–29 and 18%; 95% CI, 9–29, respectively). A single study explored the independent effect of benznidazole and found benefits for preventing reactivations. No studies evaluated the independent association between reactivation and mortality, while sensitivity analysis results using unadjusted estimates were inconclusive. The heterogeneity of diagnostic algorithms was substantial.
Conclusions: Reactivation occurs in three out of ten *T. cruzi*-seropositive immunosuppressed adults. These findings can assist clinicians and panel guidelines in tailoring monitoring schedules. There is a great need for an accurate definition of reactivation and targeted monitoring. **Alba Antequera, Clin Microbiol Infect 2024;30:980**

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Introduction

Infection with the protozoan *Trypanosoma cruzi* causes Chagas disease, a neglected tropical disease with a major impact on the health, social welfare, and economy of affected communities [1–4]. *T. cruzi* infection, with an estimated around 7 million infected people and 10 000 deaths per year [5], has become a global health challenge due to migratory flows, spreading to non-endemic areas such as the U.S. and European countries [6–9]. After an untreated acute infection, most patients evolve to a chronic stage, of which an estimated 20–30% develop cardiomyopathy over the years [10,11]. Reactivation of chronic *T. cruzi* infection, characterized by high levels of parasitaemia replication and lack of immunological control, may occur in patients with profound immunosuppression and lead to severe manifestations and fatal outcomes [12]. Clinical presentations of reactivation have been well-described in patients who become immunosuppressed due to HIV or transplantation, while clinical experience with other immunosuppressive conditions is scarce [13–15]. Among *T. cruzi*-HIV coinfected patients, reactivation behaves as other opportunistic infections [16], and it is considered an AIDS-defining disease [17,18]. Although several studies have reported a high rate of morbidity related to reactivation [19,20], low mortality may be expected with proper treatment [21]. There is a need to further study the epidemiology and predictors of reactivation due to most of the evidence relying on primary small case series and small cohorts [16,17,22].

No clinical trials have assessed the effectiveness and safety of trypanocidal drugs on immunosuppressed hosts. However, trypanocidal treatment is generally assumed to be more effective among immunosuppressed patients, both for potentially preventing disease flare-ups and for treating reactivation episodes [23,24]. Additionally, treatment is thought to lead to a sterile parasitological cure in very few chronically infected patients, as well as there are no reliable markers for cure, so treated patients (even those treated before the immunosuppression condition) should be considered to be at risk of reactivation and be followed-up [12].

An essential aspect of the clinical management of *T. cruzi* reactivation involves the early identification of cases. The scientific community has not established reference criteria for the diagnosis of reactivation due to techniques developing over time, accessibility to tests, and the controversial interpretation of parasitaemia if reactivation-related symptoms are lacking [25,26]. For example, the Brazilian Consensus statement and the Infectious Diseases Society of America (IDSA) guidelines define reactivation as parasite detection by microscopic or histological examination [18,22]. The IDSA guidelines also pinpoint that the earliest and most sensitive indicator of reactivation is the rise of parasite load over time using quantitative polymerase chain reaction (qPCR) [18]. However, there is no qPCR threshold to distinguish between reactivation and transient parasitaemia during the chronic phase [27]. The American Society of Transplantation Infectious Diseases Community of Practice (AST-IDCOP) guidelines recommend combining parasitological and molecular techniques [28]. Moreover, regarding the follow-up monitoring of immunosuppressed patients at risk of reactivation, there is no setup of a standardized periodicity either.

We conducted a systematic review to estimate the cumulative *T. cruzi* reactivation incidence in immunosuppressed adults, summarize the available evidence on prognostic factors associated with reactivation, and examine its prognostic effect on mortality.

Methods

We followed the PRISMA recommendations for reporting systematic reviews and meta-analysis [29] (Table S1 PRISMA checklist) and registered the protocol prospectively (PROSPERO CRD42022342963).

Eligibility criteria

We considered the following eligibility criteria: 1) Studies on immunosuppressed adults (age ≥16 years) chronically infected by *T. cruzi* (i.e. population at risk of reactivation). We defined immunosuppression as recipients of solid organ or haematopoietic stem cell transplant, patients on chronic corticosteroids, chemotherapy, immunosuppressive agents, HIV infection, or heritable immunodeficiency; 2) Studies (experimental or any observational design) investigating the *T. cruzi* reactivation. We accepted the study authors' definitions for reactivation; 3) Case series of adults at risk of reactivation were eligible only for the question on reactivation incidence. We attempted to minimize the potential overlapping of participants across studies by prioritizing the studies based on comparative cohorts and with larger samples. To further minimize selection bias, we excluded studies conducted only in reactivated patients when reactivation was the inclusion criterion. We also excluded reviews, editorials, and commentaries. We excluded conference abstracts when full texts were not available.

The coprimary outcomes were the cumulative *T. cruzi* reactivation incidence at the longest available follow-up and prespecified follow-up periods according to the recommended monitoring schedule of the AST-IDCOP (i.e. weekly for the first 2 months, every 2 weeks for the third month, monthly until at least the sixth-month after transplantation, and then larger intervals extended in stable patients). Secondary outcomes were clinical prognostic factors associated with reactivation and all-cause mortality.

Data sources and study selection

We searched MEDLINE (Ovid), Embase (Elsevier), and LILACS databases from inception to 4 July 2022 for published studies without date or language restrictions, using terms related to the population ((*T. cruzi* infection OR Chagas disease) AND immunosuppression) [30] and the outcome (reactivation) (see Supplementary Material). We also searched clinicaltrials.gov and CENTRAL and tracked the reference lists of included studies and the Pan American Health Organization (PAHO) guidelines to explore additional studies [23].

Two authors independently screened the title and abstracts and, when appropriate, full texts against the eligibility criteria. Disagreements were resolved by discussion with a third author.

Data extraction and quality assessment

Two authors independently extracted data using a pre-piloted form. We used parts of the checklist for critical appraisal and data extraction for systematic reviews of prediction modelling studies for prognostic factors guidance for data collection [31]. We contacted study authors for additional information if required. We applied an outcome-level approach for assessing the study's risk of bias by using either a specific tool for prevalence studies [32] or the quality of prognosis studies tool [31,33] for prognostic factor studies, and we rated them as low, moderate, or high. We judged the certainty of evidence using the Grades of Recommendations, Assessment, Development, and Evaluation framework [34,35]. To judge the imprecision domain, we agreed on *a priori* an absolute risk difference of at least ±10% as the threshold that might influence clinical decision-making (i.e. when the confidence interval of absolute effect crossed the threshold of interest, we considered rating down for imprecision) [36].

Data synthesis and analysis

We pooled reactivation incidence data as proportions with 95% CIs using Dersimonian and Laird random-effects meta-analysis by stabilizing the variances using Freeman-Tukey double arcsin transformation [37,38]. We planned to combine adjusted prognostic effect estimate(s) using the Hartung-Knapp-Sidik-Jonkman method for random-effects. Instead, as no valid data were available, we tabulated the findings. Additionally, we meta-analysed unadjusted associations between reactivation and mortality. Given the low mortality frequency, we explored the prognostic effect of reactivation on mortality using Peto's method. We quantified statistical heterogeneity using I^2 and τ^2 statistics and 95% prediction intervals when data for at least three studies were available [39]. The pre-specified sub-group analyses were undertaken, except for the recruitment date, as reporting was ambiguous. We conducted sensitivity analyses restricted to studies at overall low risk of bias and a *post hoc* sensitivity analysis including only studies with at least one year of follow-up. In the peer-review process, we performed two additional *post hoc* sensitivity analyses, using the Paule and Mandel method [40] to explore whether the overall results were affected by heterogeneity and another removing the mixed population sub-group, which involved only outlying studies. Finally, we assessed the small-study effects using the funnel plot and the Egger test, as well as generating the doi plot [41] due to meta-analyses combined proportions [42]. Analyses were done with Stata (version 16) using the commands metaprop and meta [43].

Results

After removing duplicates from 798 records, we screened 571 unique registers and included 22 studies (23 references) [14,19,20,26,44–62] providing data on cumulative incidence, 14 of which were cohorts, six were case series, and two cross-sectional studies, published between 1996 and 2021 (Fig. 1). The study sample size ranged from 4 to 230, with a median of 18 participants (interquartile range, 10–43), and the follow-up spanned from 7 months to 11 years, with a median follow-up period of at least one year to <2 years for most studies when data were available. Studies were conducted in Argentina ($N = 8$), Brazil ($N = 6$), other endemic countries or multi-country collaborations ($N = 4$), and non-endemic countries (U.S. and Spain) ($N = 4$). The included studies involved 806 participants, of which 376 (29% women) were seropositive participants who underwent transplant, 342 (40% women) were people living with HIV (PLHIV), 64 (86% women) had autoimmune diseases under immunosuppressive therapy, and a further 24 (33% women) were mixed populations. The definition of reactivation varied substantially between studies. Details on reactivation monitoring schedule were reported in nine studies [19,20,26,47,48,51,53,57,58]. Ten studies (257 participants) informed participants received no pre-immunosuppression trypanocidal treatment, while six studies (100 participants) offered treatment, and a further six (450 participants) provided no information. Table 1 and Table S2 display the descriptive summary of included studies and the key characteristics of each study, respectively.

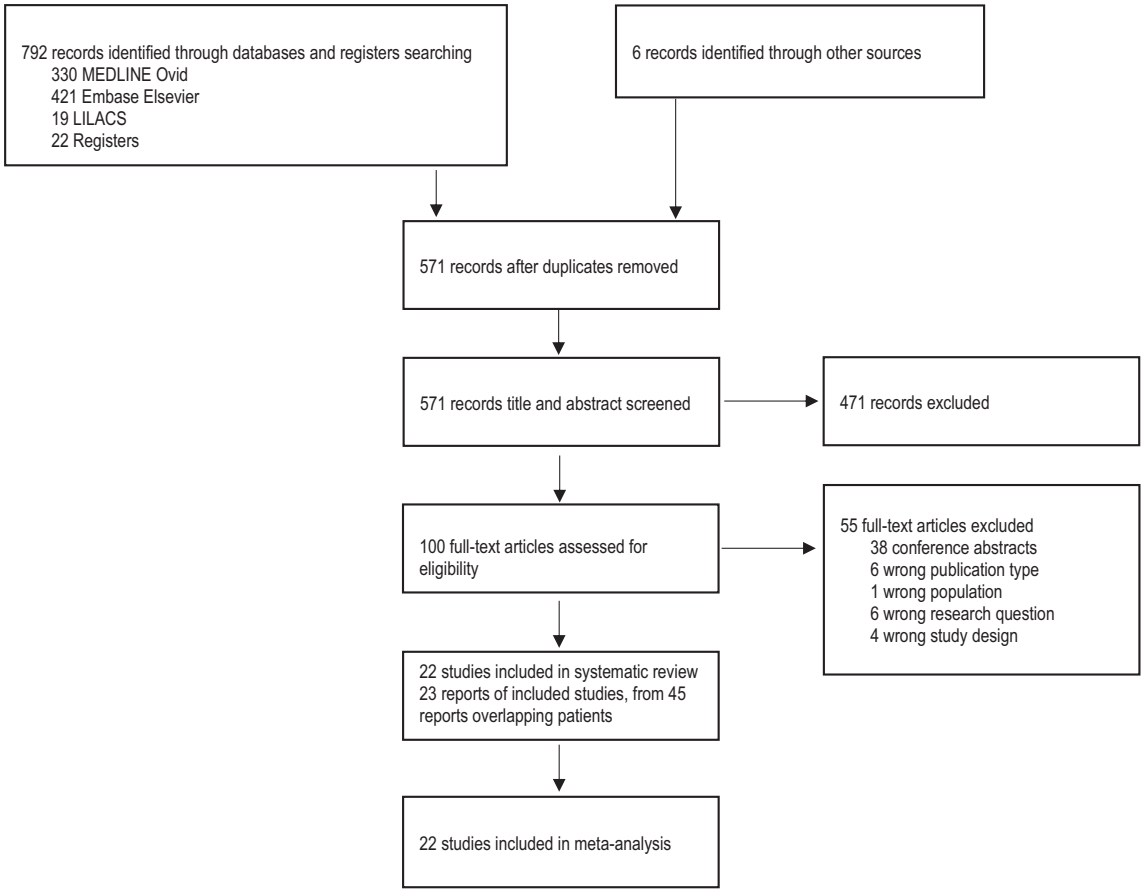

Fig. 1. Study selection.

Table 1
Descriptive summary of included studies

Study variables		All included studies (*N* = 22)	Transplant (*N* = 15)	PLHIV (*N* = 3)	Autoimmune diseases (*N* = 2)	Mixed conditions (*N* = 2)
Year published[a]	<2010	6	6	0	0	0
	≥2010	16	9	3	2	2
Study design	Prospective cohort	5	4	0	1	0
	Retrospective cohort	9	7	1	0	1
	Case series	6	4	0	1	1
	Cross-sectional	2	0	2	0	0
Country	Endemic	17	13	2	2	0
	Non-endemic	4	2	0	0	2
	Both	1	0	1	0	0
Sample size, median (IQR)		18 (10–43)	15 (9–30)	80 (32–230)	32 (13–51)	12 (9–15)
Definition of reactivation	Microscopy (of blood or other body fluids)	1	0	1	0	0
	Histology	1	1	0	0	0
	PCR	4	1	0	1	2
	Microscopy or histology	2	1	1	0	0
	Microscopy or qPCR	2	1	0	1	0
	PCR or histology	1	1	0	0	0
	PCR or HC or histology	1	1	0	0	0
	PCR or HC or serology	1	1	0	0	0
	Direct/indirect parasitological methods or histology or serology	1	1	0	0	0
	Microscopy and clinical manifestations	2	2	0	0	0
	Microscopy/histology and clinical manifestations	2	1	1	0	0
	PCR and clinical manifestations	1	1	0	0	0
	Unclear	3	3	0	0	0
Follow-up times available median[b]	<1 y	1	1	0	0	0
	1–<2 y	6	5	0	0	1
	2–3 y	3	2	0	1	0
	4–5 y	1	1	0	0	0
	Not reported	9	7	1	1	0
	NA	2	0	2	0	0
Trypanocidal treatment before immunosuppression	No	10	7	1	2	0
	Yes–all participants	3	2	0	0	1
	Yes–some participants	3	2	0	0	1
	Not reported	6	4	2	0	0
Reactivation monitoring schedule	Reported	9	8	0	1	0
	Not reported	12	7	3	1	1
	Unclear	1	1	0	0	0

[a] Year of publication of the primary reference of included studies.
[b] Data reported directly by the study authors or calculated by the review authors when disaggregated data was provided. IQR, interquartile range; HC, haemoculture; NA, not applicable; PCR, polymerase chain reaction; qPCR, real-time quantitative PCR.

Table S3 depicts the risk of bias for each study providing *T. cruzi* reactivation incidence. The overall risk of bias was low for five studies [20,26,47,51,57], moderate for 11 studies [14,19,44,48,50,52,53,55,56,58,59], and high for six others [45,46,49,54,60–62]. The most frequent concerns referred to items assessing the measurement bias domain: eight studies [14,45,48,49,54,58,61,62] provided no acceptable reactivation definition, ten studies [14,44,45,49,52,54,56,60–62] were at high risk for data collection during the monitoring period due to a non-specified monitoring schedule, and half of the studies [19,45,48,50–55,59,60] were at high risk for appropriateness of the shortest follow-up period. Tables S4 and S5 present the risk of bias summary for studies reporting prognostic factors and mortality, respectively. The summary outcome estimates for each study are shown in Table S6. The certainty of the evidence was rated as low for cumulative incidence during the longest available follow-up, moderate for most cumulative incidences at a pre-specified period, and very low for all-cause mortality (Tables S7 and S8, respectively).

The overall pooled cumulative incidence of immunosuppressed adults infected by *T.cruzi* who experienced at least one reactivation (follow-up range, 7 months to 11 years) was 27% (95% CI, 19–36; $I^2 = 79\%$) (Fig. 2). The 95% prediction interval ranged from 1% to 64%. Sensitivity analysis results after restricting the meta-analysis to studies with an overall low risk of bias differed no from those of the primary analysis (Fig. S1). *Post hoc* sensitivity analysis excluding the study [58] with less than 1 year of follow-up found similar results (pooled proportion 27%, 95% CI, 19–36; $I^2 = 80\%$).

The highest reactivation incidence was in transplant recipients (pooled proportion 36%, 95% CI, 25–48; $I^2 = 76\%$), whereas PLHIV and patients with autoimmune diseases had significantly lower cumulative incidences (pooled proportion 17%, 95% CI, 8–29; and pooled proportion 18%, 95% CI, 9–29; respectively) ($p < 0.0001$). Among seropositive patients who undertook an organ transplant, heart recipients for end-stage Chagas' cardiomyopathy showed the greatest incidence (pooled proportion 41%, 95% CI, 26–56), yet the test for sub-group differences was not significant (p 0.269). There were country-related differences ($p < 0.0001$). Studies from the U.S. showed the highest reactivation incidence (pooled proportion 57%, 95% CI, 42–72), while those from Spain exhibit the lowest estimates (0%, 95% CI, 0–8). Geographical differences remained when the meta-analysis was restricted to the transplant recipient's subset (p 0.00), studies conducted in the U.S. had the highest reactivation proportion, and those performed in Colombia registered the lowest incidence (pooled proportion (18%, 95% CI, 9–30). Evidence of non-difference was found in sub-group analyses comparing studies by methods for reactivation diagnosis (p 0.70), treatment with benznidazole before immunosuppression (p 0.40)—neither when treatment status was stratified according to immunosuppression and country—, sex of participants (p 0.83), study design (p 0.39), and country income classification according to the World Bank (p 0.13) (Table S9).

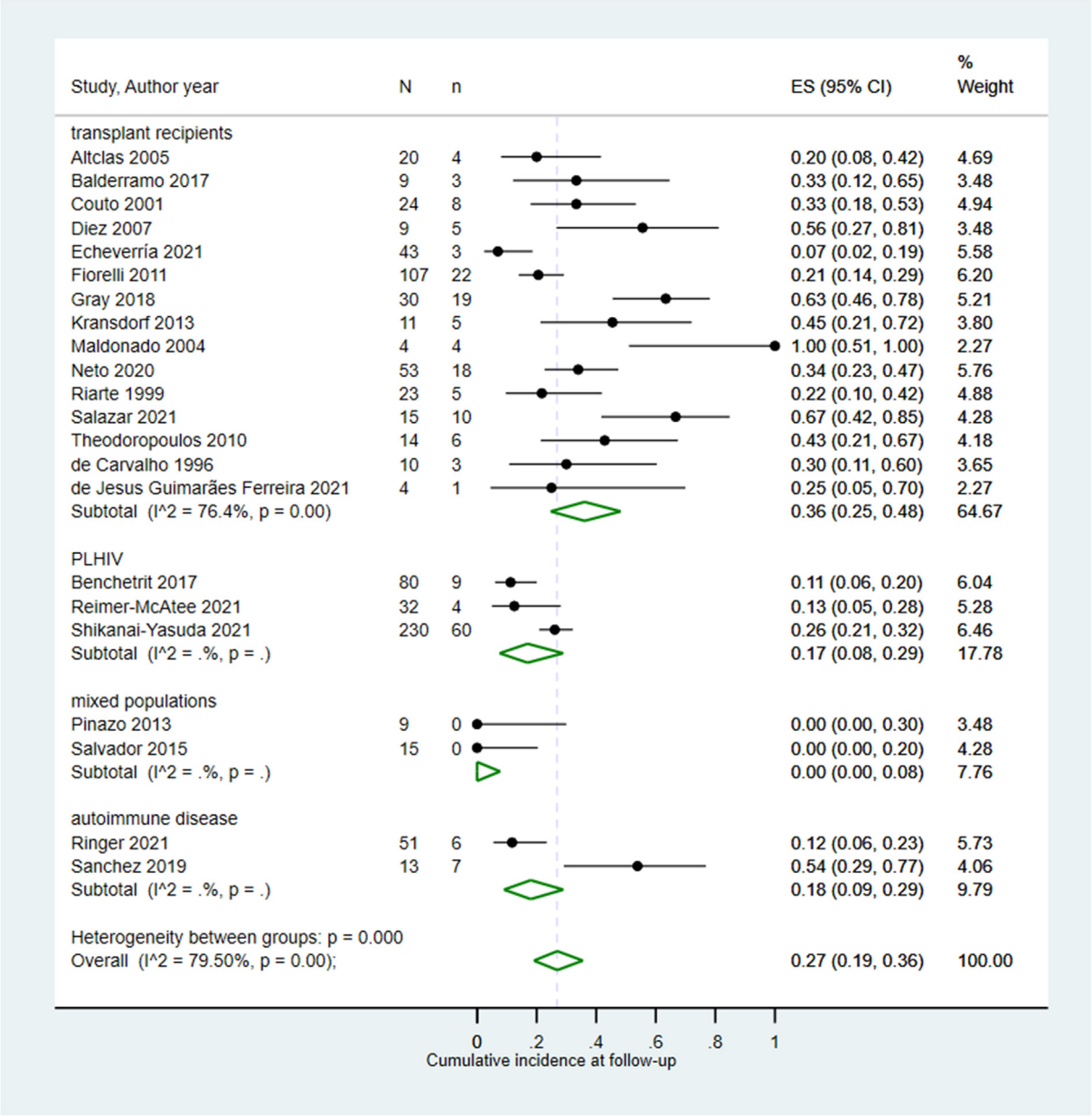

Fig. 2. Forest plots of cumulative *Trypanosoma cruzi* reactivation incidence at follow-up. ES, estimated proportion; I^2, I-squared statistics; PLHIV, people living with HIV.

Nine studies [19,20,48,51,52,57,58,61,63] involving transplant recipients provided data on reactivation cases over time. Reactivation incidence using pre-specified temporary cut-off points ranging from the first week to 11 years after transplantation showed pooled cumulative incidences of 0% to 13% (Table S7). Within the first 6 months after transplantation, the highest risk period for reactivation was from the first month up to the second (pooled proportion 5%, 95% CI, 1–11; Fig. S2). Sensitivity analysis results were similar (Fig. S3). Most patients who experienced a reactivation were at the first 6 months after transplantation (pooled proportion 32%, 95% CI, 17–58), whereas after 1 year, the risk was significantly reduced (pooled proportion, 4%; 95% CI, 0–10; p 0.001, Fig. 3). Sensitivity analysis results by pooling cumulative incidence at temporary cut-off points from studies with an overall low risk of bias remained unaltered (Fig. S4).

Among the six studies [26,46,49,55,56,59] that explored associations between potential prognostic factors and reactivation, only three [46,49,55] provided adjusted estimates. Two out of these three studies included patients with chagasic cardiomyopathy who underwent heart transplants. Neto et al. [49] reported that the pre-transplant therapy with benznidazole reduced the risk of reactivation (OR, 0.12; 95% CI, 0.02–0.76) while finding inconclusive results in the independent effect of sex (OR, 0.32; 95% CI, 0.07–1.48), corticosteroids (OR, 2.07; 95% CI, 0.27–15.86), tacrolimus (OR, 0.68; 95% CI, 0.03–14.41), cyclosporine (OR, 3.19; 95% CI 0.52–19.69), and mycophenolate (OR, 2.63; 95% CI, 0.41–16.90). Campos et al. [46] found the number of rejection episodes (HR, 1.31; 95% CI, 1.06–1.62) and neoplasms (HR, 5.07; 95% CI, 1.49–17.20) were independently associated with reactivation, whereas results on mycophenolate use-based differences in reactivation were inconclusive (HR, 3.14; 95% CI, 1.00–9.84). Shikanai-Yasuda et al. [55] found CD4+ count at *T. cruzi*/HIV co-infection was independently associated with reactivation (OR, 0.99; 95% CI, 0.994–0.999). However, this finding requires cautious interpretation, given the cross-sectional design.

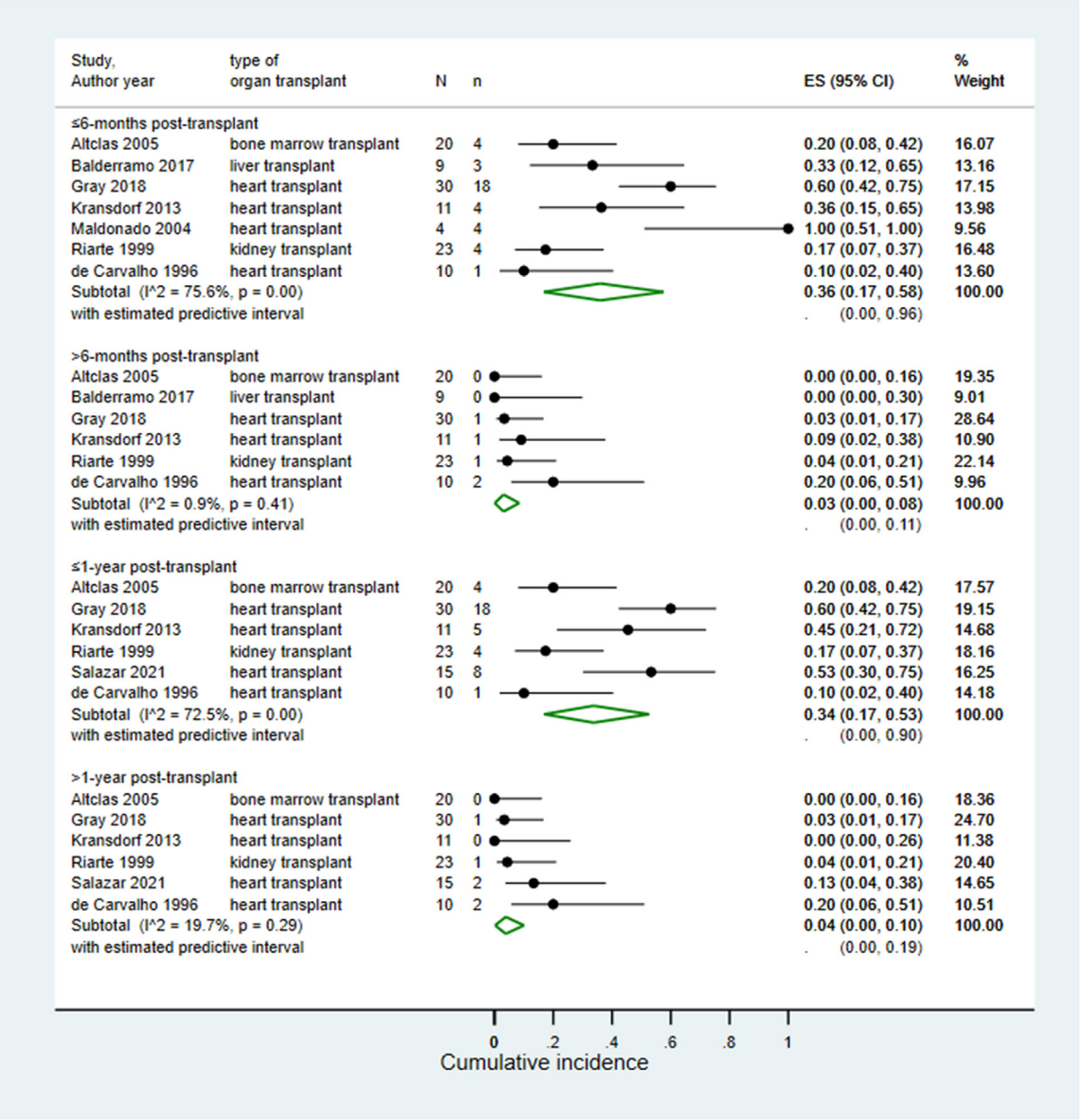

Fig. 3. Forest plots of cumulative *T. cruzi* reactivation incidence: the first 6 months to over 1-year post-transplant follow-up. ES, estimated proportion; I^2, I-squared statistics.

No study evaluated the independent prognostic role of *T. cruzi* reactivation on all-cause mortality. Sensitivity analysis results using unadjusted estimates were inconclusive (Peto's OR 0.60; 95% CI, 0.18–2.01, I^2 = 36%; Fig. S5). The 95% prediction interval ranged from 0.02 to 15.45.

We found no evidence of publication bias graphically and neither using Egger's test. We also assessed visual asymmetry using a *doi* plot (Fig. S6) [41]. The Luis Furuya-Kanamori index was 4.21, which indicates some degree of asymmetry and the possibility of small-study effects [41].

Discussion

This meta-analysis shows that three out of ten immunosuppressed adults chronically infected by *T. cruzi* experience reactivation, with seropositive transplant recipients presenting the greatest incidence. The first 6 months after transplantion is the highest risk period. Two cohorts of transplant recipients explored independent prognostic factors for reactivation and found that pre-transplant therapy with benznidazole reduced the risk of reactivation, whereas rejection episodes and neoplasm increased it [46,49]. One cross-sectional study on *T. cruzi*/HIV co-infection showed that CD4+ count was independently associated with reactivation [55]. No studies reported the independent prognostic effect of *T. cruzi* reactivation for all-cause mortality, whereas sensitivity analysis results using unadjusted estimates were inconclusive. Our pooled estimate of incidence for *T. cruzi* reactivation is consistent with the findings of the PAHO guidelines [23]. However, some methodological differences should be outlined. The reactivation question assessed by the PAHO guidelines involved 92 studies published up to 2017. Eleven out of the included studies overlapped between the PAHO guidelines and our systematic

review. We excluded the remaining 81 studies because of the following reasons: 62% were single case reports or case series that selected those patients with reactivation, 14% were narrative reviews and expert opinion articles, 24% addressed different research questions (e.g. the prevalence of co-infections) or populations (e.g. paediatric), and <1% other causes (e.g. conference abstract). Also, our findings confirm PAHO's findings regarding bone marrow and kidney recipients, whereas they differ for heart and liver recipients and PLHIV (pooled estimates by PAHO 2%, 31%, and 39%, respectively), probably due to the eligibility criteria above described. We detected a substantial statistical heterogeneity partially explained by type of immunosuppression and country. We found striking differences in reactivation between studies from the U.S [19,47]. (pooled proportion 59%) and Spain [14,53] (pooled proportion 0%), which may be explained by the participant profile. The studies conducted in the U.S. involved heart recipients, while those in Spain assessed mixed populations. Although benznidazole treatment before immunosuppression also differed between studies of both countries (non-treated in the U.S. and treated in Spain), our subgroup results suggested no previous treatment-based differences (Table S9). There was also a marked difference of reactivation incidence between studies from endemic countries (pooled proportion range, 13–31%) and the U.S [19,47]. The included studies from endemic countries reported different types of immunosuppressed patients. Differences in clinical guidelines for managing transplant recipients and changes over time may also have influenced. Six studies from endemic countries comprised transplant recipients before 2010, while the starting recruitment dates of studies from the U.S. were after 2006. We also explored heterogeneity by performing two additional sensitivity analyses. *Post hoc* sensitivity analysis applying the Paule and Mandel method found similar overall cumulative reactivation incidence (Fig. S7) [40]. Heterogeneity also might be due to two outlying studies conducted on mixed populations with zero events [14,53]. *Post hoc* sensitivity analysis by removing these studies yielded similar results (Fig. S8).

We noted high heterogeneity in reactivated *T. cruzi* infection definitions. The diversity may be partially explained not only by the development of new diagnostic tools over time but also by the lack of a standardized approach for reactivation diagnosis. We assert that the testing strategy should distinguish monitoring for presymptomatic detection from diagnosis in patients with symptoms compatible with reactivation. Thus, first, among transplant recipients, the AST-IDCOP [28] recommends molecular testing using PCR in peripheral blood or biopsy tissue for early reactivation diagnosis, and the IDSA guidelines [18] on opportunistic infections in PLHIV recommend monitoring performing qPCR assays on serial blood samples. Because a unique positive PCR result does not necessarily mean reactivation in individuals who are immunosuppressed [12,25,26], the sequential increase of parasite load by qPCR may provide a more accurate indicator [12], although the threshold has not been established. When qPCR is unavailable, monitoring can rely preferably on direct methods of parasitological diagnosis rather than xenodiagnosis or haemoculture [45,51]. Among patients with clinical manifestations, a definitive diagnosis of reactivation can be established by identification of the parasite in blood, other body fluid, or tissues [18,28].

Monitoring schedules also exhibit substantial heterogeneity. There is no standardized recommended schedule for immunosuppressed patients outside the transplant sub-group. Evidence of routine *T. cruzi* reactivation screening for PLHIV is lacking [64]. Previous evidence that had suggested a risk of reactivation in *T. cruzi*-HIV coinfected patients with a CD4+ count <200 cells/microL [22] was compiled by one of the included studies [55]. Thus, our review included two studies involving PLHIV [50,55] that found that CD4 count at reactivation was <100 cells/microL, but still, there are no data to support systematic monitoring. Among included studies on patients with autoimmune diseases (either exclusively or in mixed populations), two cohorts [26,53] performed monitoring every 2 to 3 months, or 6-monthly. None of the autoimmune sub-group studies sought independent prognostic factors for reactivation. Therefore, systematic data for supporting monitoring schedules in these patients is also lacking. Regarding transplant recipients, reported schedule monitoring was also heterogeneous and often different from the AST-IDCOP [28] recommendation, which states close monitoring during the first 6 months based on expert opinion. Our finding that most reactivation cases occurred during the first 6 months after transplantation contributes to informing evidence-based monitoring recommendations. Expert consensus [22,27,28] suggests offering trypanocidal treatment before transplantation to aim to reduce the parasitaemia. Thus, the PAHO guidelines [23] suggest treating immunosuppressed adults with chronic *T. cruzi* infection with indeterminate clinical form based on the potential greater benefit of preventing reactivations. Instead, some authors [44,51] challenge primary prophylactic treatment due to effectiveness-related concerns, especially when early detection and treatment are available. We found that robust evidence on the independent effect of treatment to prevent flare-ups is lacking. The single included study [49] that provided an adjusted estimate of benznidazole for preventing reactivations showed benefits, while our exploratory analyses found no previous treatment-based exploratory analyses. Therefore, further high-quality research assessing pre-emptive treatment is needed.

Some limitations of this review arise from poor conducting and reporting in the included studies. First, some studies provided unclear or inconsistent reactivation definitions. Another issue is the completeness of reporting on monitoring schedules, immunosuppressive protocols after transplantations, length follow-up, and additional variables to characterise the profile of reactivated patients (e.g. CD4+ count at reactivation in PLHIV, cumulative dose of immunosuppressive drugs at reactivation in patients with autoimmune diseases). However, the lack of agreed reactivation definitions and monitoring schedules represents a relevant review finding, which highlights the need for consensus. We extracted patient-level data at case series when available, carried out analyses of our interest using a supplemental dataset, and requested additional clarifications to study authors. Secondary outcomes on prognostic factors for reactivation and prognostic effect of reactivation for all-cause mortality were addressed narratively and by pooling unadjusted estimates, respectively. Lastly, we included 22 studies with more than doubled registers overlapping patients that frequently omitted to reference previous publications on the same cohort. We handled this challenge by carefully checking affiliations, scanning characteristic data at-patient level in included case series, contacting the study authors, and reviewing appendixes when available.

This systematic review and meta-analysis offer information for further research. To our knowledge, this is the first systematic review addressing the overall cumulative incidence of reactivating *T. cruzi* among immunosuppressed patients, along with the cumulative incidence across temporal thresholds. Our findings can assist in tailoring monitoring schedules according to the type of immunosuppressive condition, including the risk associated with each type of organ transplantation. This review also contributes to identifying data gaps. Some patients presented several reactivations over time, even when they completed treatment at the first event [45]. So far, little evidence has addressed prognostic factors for reactivation, and when it was done, study designs were only suitable for providing hypothesis-generating evidence of potential associations [33]. Further research to confirm the independent associations is needed, specifically related to drugs used in the immunosuppressive protocols after transplantation.

In conclusion, this systematic review provides comprehensive evidence on the risk of immunosuppressed adults for reactivating *T. cruzi*. Our findings can contribute to generate evidence-based recommendations for the timing of *T. cruzi* reactivation monitoring and management. There is a great need for an accurate definition of reactivation and targeted monitoring.

Author contributions

Conceptualisation: A.A.; Methodology: A.A., J.L.A., A.M., N.A-D., J.M. Literature searches: N.A-D. Data acquisition: A.A., A.D.M-V. Data analysis: A.A., A.M. Data interpretation: A.A., A.D.M-V, J.L.A., A.M., J.M. Writing-Original draft: A.A. Writing-Review and Editing: all authors.

Transparency declaration

The authors declare that they have no conflicts of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data

All data relevant to the study are included in the article or uploaded as online supplemental information. The study protocol is available online at PROSPERO CRD42022342963. Included studies are publicly available, main data supporting the conclusions of this systematic review are included in the article and supplemental material.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2024.04.013.

References

[1] Olivera MJ, Buitrago G. Economic costs of Chagas disease in Colombia in 2017: a social perspective. Int J Infect Dis 2020;91:196–201. https://doi.org/10.1016/j.ijid.2019.11.022.
[2] Forsyth C, Meymandi S, Moss I, Cone J, Cohen R, Batista C. Proposed multidimensional framework for understanding Chagas disease healthcare barriers in the United States. PLOS Negl Trop Dis 2019;13:e0007447. https://doi.org/10.1371/journal.pntd.0007447.
[3] Dell'Arciprete A, Braunstein J, Touris C, Dinardi G, Llovet I, Sosa-Estani S. Cultural barriers to effective communication between Indigenous communities and health care providers in Northern Argentina: an anthropological contribution to Chagas disease prevention and control. Int J Equity Health 2014;13:6. https://doi.org/10.1186/1475-9276-13-6.
[4] Chadalawada S, Sillau S, Archuleta S, Mundo W, Bandali M, Parra-Henao G, 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. https://doi.org/10.1001/jamanetworkopen.2020.15072.
[5] WHO. Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021-2030. Geneva: World Health Organization; 2020. https://www.who.int/publications/i/item/9789240010352. [Accessed 18 March 2023].
[6] Irish A, Whitman JD, Clark EH, Marcus R, Bern C. Updated estimates and mapping for prevalence of Chagas disease among adults, United States. Emerg Infect Dis 2022;28:1313–20. https://doi.org/10.3201/eid2807.212221.
[7] Manne-Goehler J, Umeh CA, Montgomery SP, Wirtz VJ. Estimating the burden of Chagas disease in the United States. PLOS Negl Trop Dis 2016;10:e0005033. https://doi.org/10.1371/journal.pntd.0005033.
[8] Navarro M, Reguero L, Subirà C, Blázquez-Pérez A, Requena-Méndez A. Estimating Chagas disease prevalence and number of underdiagnosed, and undertreated individuals in Spain. Travel Med Infect Dis 2022;47:102284. https://doi.org/10.1016/j.tmaid.2022.102284.
[9] Colombo V, Giacomelli A, Casazza G, Galimberti L, Bonazzetti C, Sabaini F, et al. *Trypanosoma cruzi* infection in Latin American pregnant women living outside endemic countries and frequency of congenital transmission: a systematic review and meta-analysis. J Travel Med 2021;28:taaa170. https://doi.org/10.1093/jtm/taaa170.
[10] Pérez-Molina JA, Molina I. Chagas disease. Lancet 2018;391:82–94. https://doi.org/10.1016/S0140-6736(17)31612-4.
[11] Ribeiro AL, Nunes MP, Teixeira MM, Rocha MOC. Diagnosis and management of Chagas disease and cardiomyopathy. Nat Rev Cardiol 2012;9:576–89. https://doi.org/10.1038/nrcardio.2012.109.
[12] Bern C. Chagas disease in the immunosuppressed host. Curr Opin Infect Dis 2012;25:450–7. https://doi.org/10.1097/QCO.0b013e328354f179.
[13] Bern C. Chagas' disease. N Engl J Med 2015;373:456–66. https://doi.org/10.1056/NEJMra1410150.
[14] Pinazo M-J, Espinosa G, Cortes-Lletget C, Posada Ede J, Aldasoro E, Oliveira I, et al. Immunosuppression and Chagas disease: a management challenge. PLOS Negl Trop Dis 2013;7:e1965. https://doi.org/10.1371/journal.pntd.0001965.
[15] Lattes R, Lasala MB. Chagas disease in the immunosuppressed patient. Clin Microbiol Infect 2014;20:300–9. https://doi.org/10.1111/1469-0691.12585.
[16] Clark EH, Marquez C, Whitman JD, Bern C. Screening for Chagas disease should be included in entry-to-care testing for at-risk people with human immunodeficiency virus (HIV) living in the United States. Clin Infect Dis 2022;75:901–6. https://doi.org/10.1093/cid/ciac154.
[17] Definición de la OMS de caso de infección por el VIH a efectos de vigilancia y revisión de la estadificación clínica y de la clasificación inmunológica de la enfermedad relacionada con el VIH en adultos y niños. Washington, DC: Organización Panamericana de la Salud; 2009. https://www.paho.org/es/documentos/definicion-oms-caso-infeccion-por-vih-efectos-vigilancia-revision-estadificacion-clinica. [Accessed 8 August 2023].
[18] Panel on Opportunistic Infections in adults and adolescents with HIV. Guidelines for the prevention and treatment of opportunistic infections in adults and adolescents with HIV: recommendations from the Centers for Disease Control and Prevention. the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. [Accessed 19 March 2023].
[19] Gray EB, La Hoz RM, Green JS, Vikram HR, Benedict T, Rivera H, et al. Reactivation of Chagas disease among heart transplant recipients in the United States, 2012-2016. Transpl Infect Dis 2018;20:e12996. https://doi.org/10.1111/tid.12996.
[20] Diez M, Favaloro L, Bertolotti A, Burgos JM, Vigliano C, Lastra MP, et al. Usefulness of PCR strategies for early diagnosis of Chagas' disease reactivation and treatment follow-up in heart transplantation. Am J Transplant 2007;7:1633–40. https://doi.org/10.1111/j.1600-6143.2007.01820.x.
[21] Perez CJ, Lymbery AJ, Thompson RCA. Reactivation of Chagas disease: implications for global health. Trends Parasitol 2015;31:595–603. https://doi.org/10.1016/j.pt.2015.06.006.
[22] Dias JCP, Ramos AN, Gontijo ED, Luquetti A, Shikanai-Yasuda MA, Coura JR, et al. 2nd Brazilian consensus on Chagas disease, 2015. Rev Soc Bras Med Trop 2016;49:3–60. https://doi.org/10.1590/0037-8682-0505-2016.
[23] Guidelines for the diagnosis and treatment of Chagas disease. Washington, DC: Pan-American Health Organization; 2019. https://iris.paho.org/bitstream/handle/10665.2/49653/9789275120439_eng.pdf. [Accessed 31 October 2022].
[24] Chagas disease: migrant health guide. Advice and guidance on the health needs of migrant patients for healthcare practitioners. London: Office of health improvement and disparities; 2014. https://www.gov.uk/guidance/chagas-disease-migrant-health-guide. [Accessed 1 May 2023].
[25] Marcon GEB, De Jesus Guimarães Ferreira J, De Almeida EA, Delicio AM, Pereira MB, Wanderley JDS, et al. Parasite load evaluation by qPCR and blood culture in Chagas disease and HIV co-infected patients under antiretroviral therapy. PLoS Negl Trop Dis 2022;16:e0010317. https://doi.org/10.1371/journal.pntd.0010317.
[26] Ringer A, Ruffino JP, Leiva R, Cuadranti N, Argento MC, Martínez MF, et al. Chagas disease reactivation in rheumatologic patients: association with immunosuppressive therapy and humoral response. Clin Rheumatol 2021;40:2955–63. https://doi.org/10.1007/s10067-021-05581-2.
[27] Pierrotti LC, Carvalho NB, Amorin JP, Pascual J, Kotton CN, López-Vélez R. Chagas disease recommendations for solid-organ transplant recipients and

donors. Transplantation 2018;102:S1–7. https://doi.org/10.1097/TP.0000000000002019.
[28] La Hoz RM, Morris MI. Infectious diseases community of Practice of the American society of transplantation. Tissue and blood protozoa including toxoplasmosis, Chagas disease, leishmaniasis, babesia, acanthamoeba, balamuthia, and naegleria in solid organ transplant recipients- guidelines from the American society of transplantation infectious diseases community of Practice. Clin Transplant 2019;33:e13546. https://doi.org/10.1111/ctr.13546.
[29] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. PLoS Med 2021;18:e1003583. https://doi.org/10.1371/journal.pmed.1003583.
[30] Galmiche S, Luong Nguyen LB, Tartour E, de Lamballerie X, Wittkop L, Loubet P, et al. Immunological and clinical efficacy of COVID-19 vaccines in immunocompromised populations: a systematic review. Clin Microbiol Infect 2022;28:163–77. https://doi.org/10.1016/j.cmi.2021.09.036.
[31] Riley RD, Moons KGM, Snell KIE, Ensor J, Hooft L, Altman DG, et al. A guide to systematic review and meta-analysis of prognostic factor studies. BMJ 2019;364:k4597. https://doi.org/10.1136/bmj.k4597.
[32] Hoy D, Brooks P, Woolf A, Blyth F, March L, Bain C, et al. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. J Clin Epidemiol 2012;65:934–9. https://doi.org/10.1016/j.jclinepi.2011.11.014.
[33] Hayden JA, Côté P, Steenstra IA, Bombardier C, QUIPS-LBP Working Group. Identifying phases of investigation helps planning, appraising, and applying the results of explanatory prognosis studies. J Clin Epidemiol 2008;61:552–60. https://doi.org/10.1016/j.jclinepi.2007.08.005.
[34] Huguet A, Hayden JA, Stinson J, McGrath PJ, Chambers CT, Tougas ME, et al. Judging the quality of evidence in reviews of prognostic factor research: adapting the GRADE framework. Syst Rev 2013;2:71. https://doi.org/10.1186/2046-4053-2-71.
[35] Foroutan F, Guyatt G, Zuk V, Vandvik PO, Alba AC, Mustafa R, et al. GRADE Guidelines 28. GRADE Guidelines 28: use of GRADE for the assessment of evidence about prognostic factors: rating certainty in identification of groups of patients with different absolute risks. J Clin Epidemiol 2020;121:62–70. https://doi.org/10.1016/j.jclinepi.2019.12.023.
[36] Zeng L, Brignardello-Petersen R, Hultcrantz M, Mustafa RA, Murad MH, Iorio A, et al. GRADE Guidance 34: update on rating imprecision using a minimally contextualized approach. J Clin Epidemiol 2022;150:216–24. https://doi.org/10.1016/j.jclinepi.2022.07.014.
[37] Doi SA, Xu C. The Freeman–Tukey double arcsine transformation for the meta-analysis of proportions: recent criticisms were seriously misleading. J Evid Based Med 2021;14:259–61. https://doi.org/10.1111/jebm.12445.
[38] Barker TH, Migliavaca CB, Stein C, Colpani V, Falavigna M, Aromataris E, et al. Conducting proportional meta-analysis in different types of systematic reviews: a guide for synthesisers of evidence. BMC Med Res Methodol 2021;21:189. https://doi.org/10.1186/s12874-021-01381-z.
[39] Borenstein M. Common mistakes in meta-analysis and how to avoid them. Englewood: First; 2019.
[40] Veroniki AA, Jackson D, Viechtbauer W, Bender R, Bowden J, Knapp G, et al. Methods to estimate the between-study variance and its uncertainty in meta-analysis. Res Synth Methods 2016;7:55–79. https://doi.org/10.1002/jrsm.1164.
[41] Furuya-Kanamori L, Barendregt JJ, Doi SAR. A new improved graphical and quantitative method for detecting bias in meta-analysis. Int J Evid-Based Healthc 2018;16:195–203. https://doi.org/10.1097/XEB.0000000000000141.
[42] Hunter JP, Saratzis A, Sutton AJ, Boucher RH, Sayers RD, Bown MJ. In meta-analyses of proportion studies, funnel plots were found to be an inaccurate method of assessing publication bias. J Clin Epidemiol 2014;67:897–903. https://doi.org/10.1016/j.jclinepi.2014.03.003.
[43] Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. Arch Public Health 2014;72:1–1039. https://doi.org/10.1186/2049-3258-72-39.
[44] Echeverría LE, Figueredo A, Rodriguez MJ, Salazar L, Pizarro C, Morillo CA, et al. Survival after heart transplantation for Chagas cardiomyopathy using a conventional protocol: a 10-year experience in a single center. Transpl Infect Dis 2021;23:e13549. https://doi.org/10.1111/tid.13549.
[45] Fiorelli AI, Santos RHB, Oliveira Jr JL, Lourenço-Filho DD, Dias RR, Oliveira AS, et al. Heart transplantation in 107 cases of Chagas' disease. Transplant Proc 2011;43:220–4. https://doi.org/10.1016/j.transproceed.2010.12.046.
[46] Campos SV, Strabelli TMV, Amato Neto V, Silva CP, Bacal F, Bocchi EA, et al. Risk factors for Chagas' disease reactivation after heart transplantation. J Heart Lung Transplant 2008;27:597–602. https://doi.org/10.1016/j.healun.2008.02.017.
[47] Kransdorf EP, Czer LSC, Luthringer DJ, Patel JK, Montgomery SP, Velleca A, et al. Heart transplantation for Chagas cardiomyopathy in the United States. Am J Transplant 2013;13:3262–8. https://doi.org/10.1111/ajt.12507.
[48] Maldonado C, Albano S, Vettorazzi L, Salomone O, Zlocowski JC, Abiega C, et al. Using polymerase chain reaction in early diagnosis of re-activated *Trypanosoma cruzi* infection after heart transplantation. J Heart Lung Transplant 2004;23:1345–8. https://doi.org/10.1016/j.healun.2003.09.027.
[49] Neto JMR, Finger MA, dos Santos CC. Benznidazole as prophylaxis for Chagas disease infection reactivation in heart transplant patients: a case series in Brazil. Trop Med Infect Dis 2020;5:512. https://doi.org/10.3390/tropicalmed5030132.
[50] Reimer-McAtee MJ, Mejia C, Clark T, Terle J, Pajuelo MJ, Cabeza J, et al. HIV and Chagas disease: an evaluation of the use of real-time quantitative polymerase chain reaction to measure levels of *Trypanosoma cruzi* parasitemia in HIV patients in Cochabamba, Bolivia. Am J Trop Med Hyg 2021;105:643–50. https://doi.org/10.4269/ajtmh.20-1141.
[51] Riarte A, Luna C, Sabatiello R, Sinagra A, Schiavelli R, De Rissio A, et al. Chagas' disease in patients with kidney transplants: 7 years of experience, 1989-1996. Clin Infect Dis 1999;29:561–7. https://doi.org/10.1086/598634.
[52] Salazar L, Medina R, Ramirez JA. Reactivación de enfermedad de Chagas en pacientes trasplantados por cardiopatía chagásica. Fundación Cardioinfantil 2020. https://repository.urosario.edu.co/items/018f7c2d-c8a2-4b3d-9726-65c55e1dc488. [Accessed 9 August 2023].
[53] Salvador F, Sánchez-Montalvá A, Valerio L, Serre N, Roure S, Treviño B, et al. Immunosuppression and Chagas disease; experience from a non-endemic country. Clin Microbiol Infect 2015;21:854–60. https://doi.org/10.1016/j.cmi.2015.05.033.
[54] Sánchez AG, Baenas DF, Bonisconti F, Salinas MJH, Alvarellos A, Saurit V, et al. Reactivation of Chagas disease in patients with rheumatic autoimmune diseases diagnosed by molecular quantification techniques. J Clin Rheumatol 2021;27:S533–6. https://doi.org/10.1097/RHU.0000000000001108.
[55] Shikanai-Yasuda MA, Mediano MFF, Novaes CTG, Sousa AS, Sartori AMC, Santana RC, et al. Clinical profile and mortality in patients with *T. cruzi*/HIV co-infection from the multicenter data base of the 'network for healthcare and study of *Trypanosoma cruzi*/HIV co-infection and other immunosuppression conditions'. PLoS Negl Trop Dis 2021;15:e0009809. https://doi.org/10.1371/journal.pntd.0009809.
[56] Theodoropoulos TAD, Silva AG, Bestetti RB. Eosinophil blood count and anemia are associated with *Trypanosoma cruzi* infection reactivation in Chagas' heart transplant recipients. Int J Cardiol 2010;145:55–6. https://doi.org/10.1016/j.ijcard.2009.04.011.
[57] Altclas J, Sinagra A, Dictar M, Luna C, Verón MT, De Rissio AM, et al. Chagas disease in bone marrow transplantation: an approach to preemptive therapy. Bone Marrow Transplant 2005;36:123–9. https://doi.org/10.1038/sj.bmt.1705006.
[58] Balderramo D, Bonisconti F, Alcaraz A, Giordano E, Sánchez A, Barrabino M, et al. Chagas disease and liver transplantation: experience in Argentina using real-time quantitative PCR for early detection and treatment. Transpl Infect Dis 2017;19. https://doi.org/10.1111/tid.12782.
[59] Benchetrit AG, Fernández M, Bava AJ, Corti M, Porteiro N, Martínez Peralta L. Clinical and epidemiological features of chronic *Trypanosoma cruzi* infection in patients with HIV/AIDS in Buenos Aires, Argentina. Int J Infect Dis 2018;67:118–21. https://doi.org/10.1016/j.ijid.2017.11.027.
[60] Couto WJ, Branco JNR, Almeida D, Carvalho AC, Vick R, Teles CA, et al. Transplante cardíaco e infecção. Rev Bras Cir Cardiovasc 2001;16:141–51. https://doi.org/10.1590/S0102-76382001000200008.
[61] De Carvalho VB, Sousa EFL, Vila JHA, da Silva JP, Caiado MR, Araujo SR, et al. Heart transplantation in Chagas' disease: 10 years after the initial experience. Circulation 1996;94:1815–7. https://doi.org/10.1161/01.cir.94.8.1815.
[62] Jesus Guimarães Ferreira J, Antonio de Almeida E, Barbosa Marcon GE, Gonçalves Lima R, Barroso Pereira M, Ramos Gadelha F, et al. Evaluation of *Trypanosoma cruzi* parasitic load by real-time PCR and blood culture in long-term kidney transplant recipients. J Infect Dev Ctries 2021;15:1774–81. https://doi.org/10.3855/jidc.13973.
[63] Kransdorf E, Kittleson M, Patel J, Rafiei M, Osborne A, Chang D, et al. High rate of reactivation of Chagas disease after heart transplantation in the United States. J Heart Lung Transplant 2013;32:S130. https://doi.org/10.1016/j.healun.2013.01.289.
[64] Pérez-Molina JA. Management of *Trypanosoma cruzi* coinfection in HIV-positive individuals outside endemic areas. Curr Opin Infect Dis 2014;27:9–15.