Additional File 2. Details on Methods and Analyses

Increased mortality attributed to Chagas disease: a systematic review and meta-analysis

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Contents

1. Search strategy

Different algorithms were designed to search for the relevant literature; the final algorithms used for the search in the different datasets up to 30th September 2015, are presented in Table S1.

Table S1. Algorithms and databases used for the association between Chagas disease and mortality

Database	Algorithm	Number of titles	Specifications used
PubMed and MEDLINE	((((((mortality) OR death)) OR (((((progression) OR outcome) OR follow up) OR long term) OR prognos*)) OR (((((survival) OR cohort) OR clinical trial) OR hazard*) OR prospective))) AND ((((Chagas[Title/Abstract]) OR "Trypanosoma cruzi"[Title/Abstract]) OR chagas disease[MeSH Terms]) OR American trypanosomiasis)	2,242	Humans only filter
EMBASE	1. Chagas disease/ or Chagas.mp. 2. trypanosoma cruzi.mp. or Trypanosoma cruzi/ 3. american trypanosomiasis.mp. 4. 1 or 2 or 3 5. mortality/ or mortality.mp. 6. death.mp. or death/ 7. 5 or 6 8. progression.mp. 9. outcome.mp. 10. follow-up.mp. 11. long-term.mp. 12. prognos*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] 13. 8 or 9 or 10 or 11 or 12 14. survival.mp. 15. cohort.mp. 16. clinical trial.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] 17. hazard*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] 18. 14 or 15 or 16 or 17 19. 7 or 13 or 18 20. 4 and 19	2,837	Humans only filter

	21. prospective.mp. 22. 14 or 15 or 16 or 17 or 21 23. 7 or 13 or 22 24. 4 and 23		
Web of Science	((Chagas) OR (Trypanosoma cruzi) OR (American trypanosomiasis)) AND ((mortality) OR (death) OR (survival) OR (cohort) OR (clinical trial) OR (hazard) OR (prospective) OR (progression) OR (outcome) OR (follow up) OR (long term) OR (prognos*))	1,705	Humans only Search AND (humans OR patients OR cases)
LILACS	(tw:((chagas) OR (trypanosoma cruzi) OR (enfermedad de chagas) OR (doença de chagas) OR (American trypanosomiasis))) AND ((tw:(mortality OR mortalidad OR mortalidade OR death OR muerte OR morte)) OR (tw:(Progression OR Progresión OR Progressão OR Evolução da cardiopatia OR Outcome OR Desenlace* OR desfecho*)) OR (tw:(Survival OR Supervivencia OR Sobrevivência OR Follow-up OR Seguimiento OR Seguimento OR prospective OR prospective OR longitudinal OR Cohort* OR coort* OR prognos* OR pronóstico OR prognóstico OR (clinical trial) OR (ensayo clínico) OR (ensaio clínico) OR hazard)))	2,151	Humans only filter
TOTAL		**8,935**	

2. Quality assessment

The Newcastle–Ottawa Scale (NOS) for quality assessment of cohort studies was used (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp). Below, we describe the criteria used for this scale in the context of our cohort studies on Chagas disease.

Note: A study can be awarded a maximum of one star for each numbered item within the selection and outcome categories. A maximum of two stars can be given for comparability

Selection

1) Representativeness of the exposed cohort – **Chagas disease patients with clear definitions and tests to be classified in one of these clinical groups:**
- Asymptomatic/general population: asymptomatic or minimal ECG findings or from the general community (survey)
- Severe stage: proved by NYHA class III or IV
- Moderate Stage: proved by NYHA class III or IV
- All stages: studies with patients in several groups

a) truly representative of the in the community **(Star)**
b) somewhat representative of the average in the community **(Star)**
c) selected group of users, e.g. nurses, volunteers
d) no description of the derivation of the cohort

2) Selection of the non-exposed cohort – **Non-Chagas disease patients with clear definitions and tests to be classified in the same corresponding clinical groups:**

a) drawn from the same community as the exposed cohort **(Star)**
b) drawn from a different source
c) no description of the derivation of the non-exposed cohort

3) Ascertainment of exposure – **Chagas disease diagnosis**
a) secure record (e.g. surgical records) **(Star)**
b) structured interview **(Star)**
c) written self-report
d) no description

4) Demonstration that outcome of interest **(death)** was not present at start of study
a) yes **(Star)**
b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
a) study controls for **gender and/or age** **(Star)**
b) study controls for any additional factor **(Star)** (This criterion could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome **– mortality: clear description of how the outcome was investigated**
a) independent blind assessment **(Star)**
b) record linkage **(Star)**

c) self-report
d) no description

2) Cohort was follow up long enough for outcomes to occur – **at least 1 year**
a) yes (select an adequate follow up period for outcome of interest) **(Star)**
b) no

3) Adequacy of follow-up cohorts
a) complete follow up - all subjects accounted for **(Star)**
b) subjects lost to follow up unlikely to introduce bias - small number lost - > 10% follow up, or description provided of those lost) **(Star)**
c) follow-up rate < 10% and no description of those lost to follow up
d) no statement

Table S2. Newcastle–Ottawa Scale for quality assessment of the studies included in the meta-analysis

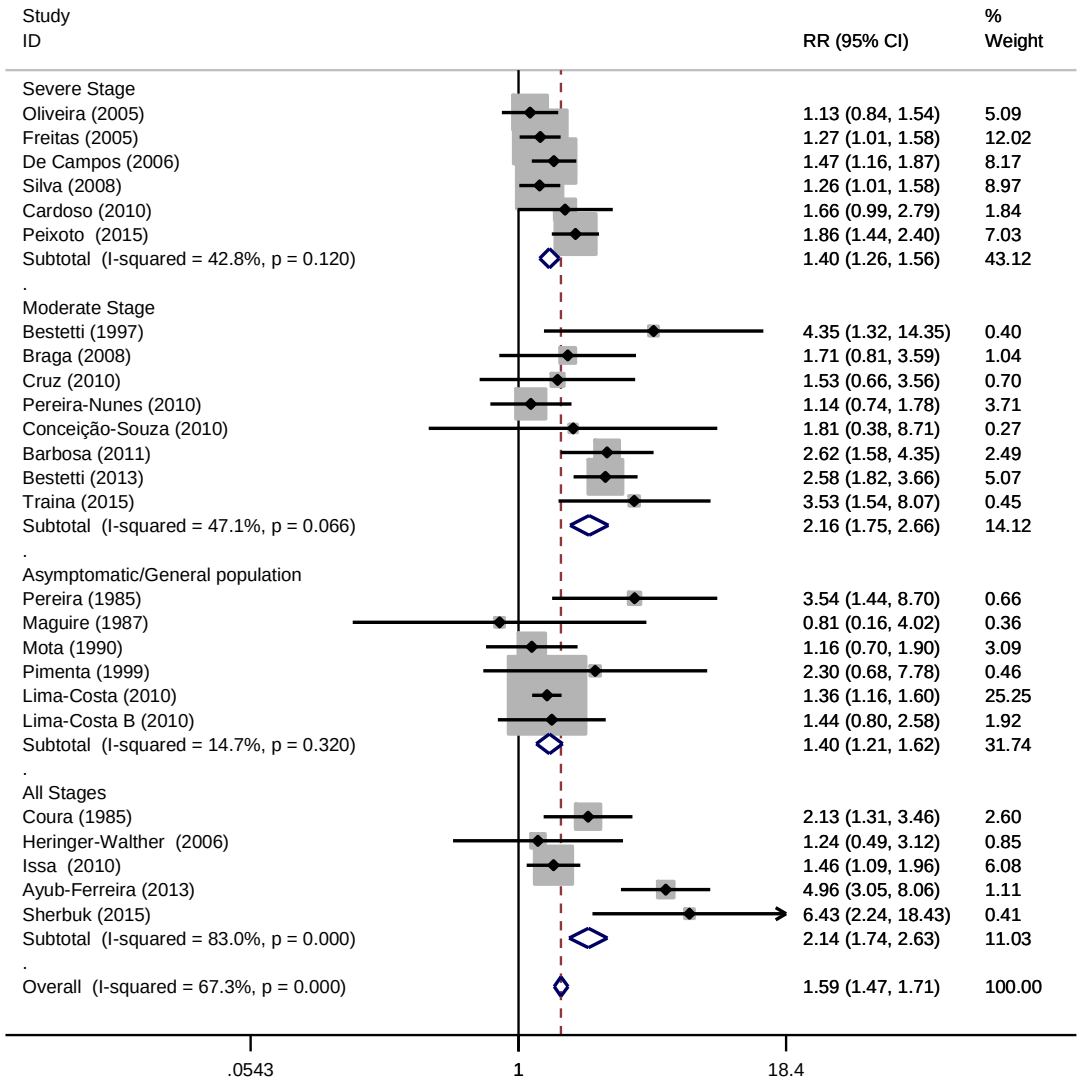
First author [Reference]	Year	Study type	Follow-up type	1st Star Represen-tativeness of the studied population	2nd Star Cohort from same community	3rd Star Ascertain-ment of exposure (diagnostic test)	4th Star Outcome is not present at the beginning	5th Star Compar-ability of cohorts Controlled for age and sex	6th Star Controlled for other factors	7th Star Ascertain-ment of outcome (death)	8th Star (At least 1 year of follow up)	9th Star l Lost to follow-up <10%	Total Stars	Overall Quality
Coura [1]	1985	Cohort	Prospective	1	1	1	1	1	0	1	1	0	7	High
Pereira [2]	1985	Cohort	Prospective	1	1	1	1	1	0	1	1	0	7	High
Maguire [3]	1987	Cohort	Prospective	1	1	1	1	1	0	1	1	1	8	High
Mota [4]	1990	Cohort/	Prospective	1	1	1	1	1	0	1	1	0	7	High
Bestetti [5]	1997	Cohort	Prospective	1	1	0	1	1	0	0	1	0	5	Moderate
Pimenta [6]	1999	Cohort	Prospective	1	1	1	1	0	0	1	1	0	6	Moderate
Freitas [7]	2005	Cohort	Retrospective	1	1	0	1	0	0	1	1	0	5	Moderate
De Oliveira [8]	2005	Cohort	Prospective	1	1	1	1	0	0	1	0	1	6	Moderate
De Campos Lopes [9]	2006	Cohort	Prospective	1	1	1	1	1	0	1	1	0	7	High
Heringer-Walther [10]	2006	Cohort	Prospective	1	1	0	1	1	1	0	1	0	6	Moderate
Braga [11]	2008	Cohort	Prospective	1	1	0	1	1	1	0	0	0	5	Moderate
Silva [12]	2008	Cohort	Prospective	1	1	0	1	0	0	0	1	1	5	Moderate
Lima-Costa A [13]	2010	Cohort	Prospective	1	1	1	1	1	0	1	1	0	7	High
Lima-Costa B [14]	2010	Cohort	Prospective	1	1	1	1	1	1	1	1	1	9	High
Pereira-Nunes [15]	2010	Cohort	Prospective	1	1	1	1	1	1	1	1	1	9	High
Issa [16]	2010	Cohort	Prospective	1	1	0	1	1	0	0	0	0	4	Low

Cardoso [17]	2010	Cohort	Prospective	1	1	0	1	1	0	0	1	1	6	Moderate
Conceição-Souza [18]	2010	Cohort	Prospective	1	1	0	1	1	1	0	1	0	6	Moderate
Cruz [19]	2010	Cohort	Prospective	1	1	1	1	1	0	0	1	0	6	Moderate
Barbosa [20]	2011	Cohort	Prospective	1	1	1	1	1	0	0	1	0	6	Moderate
Ayub-Ferreira [21]	2013	Cohort	Prospective	1	1	1	1	1	0	1	1	1	8	High
Bestetti [22]	2013	Cohort	Prospective	1	1	1	1	1	0	0	1	1	7	High
Peixoto [23]	2015	Cohort	Prospective	1	1	1	1	1	0	1	1	0	7	High
Traina [24]	2015	Cohort	Prospective	1	1	1	1	1	0	1	1	1	8	High
Sherbuk [25]	2015	Cohort	Retrospective	1	1	0	1	0	0	0	1	0	4	Low

Fixed-effects model

A fixed-effects model was run for comparison with the random-effects model presented in the main text (Fig. 2). The assumption for this model is that the effect size varies between studies due to sampling error (error in estimating the effect size). In this case, the weight of the study is mainly driven by the sample size.

Figure S1. Forest plot of the fixed-effects model for the association between Chagas disease and mortality



3. Heterogeneity analysis

Heterogeneity among studies was measured using Cochran's Q test and I^2 statistic [26]. Cochran's Q is calculated by adding the squared deviations of each study's estimate from the overall meta-analytic estimate, weighting each study's contribution. For the results of the random-effects model presented here and in the main text, those weights account not only for the sample size of the particular study, but also for the variance between studies (tau-squared) within the particular group (i.e. the clinical categories or random effect). The p-values for this test are obtained by comparing the Q statistic with a chi-square distribution with *k*-1 degrees of freedom (where *k* is the number of studies). The I^2 statistic describes the percentage of variation across studies that is due to study heterogeneity rather than chance (a measure of the degree of inconsistency in the studies' results), $I^2 = 100\% \times (Q–df)/Q$, where Q is the Cochran's chi-squared statistic and df its degrees of freedom [26].

The most common guide to interpreting I^2 is as follows:

- 0% to 40%: heterogeneity may not be important
- 30% to 60%: may represent moderate heterogeneity
- 50% to 90%: may represent substantial heterogeneity
- 75% to 100%: represents considerable heterogeneity

In our analysis, heterogeneity was mostly present in specific clinical groups (Table S3).

Table S3. Results of heterogeneity tests in both fixed- and random-effects models

Clinical classification	Cochran's Q test	df	p-value	I^2 statistic	Fixed-effects model		Random-effects model		
					Significant test of RR=1		Tau-squared	Significant test of RR=1	
					z	p-value		z	p-value
Severe	8.74	5	0.120	42.8%	6.14	<0.001	0.014	4.51	<0.001
Moderate	13.24	7	0.066	47.1%	7.24	<0.001	0.09	3.12	0.002
Asymptomatic/ general population	5.86	5	0.320	14.7%	4.56	<0.001	0.014	3.12	0.002
All stages	23.59	4	0.000	83.0%	7.24	<0.001		3.04	0.002
Overall	73.37	24	0.000	67.3%	12.08	<0.001	0.079	6.98	<0.001

4. Meta-regression

Meta-regression formally compares the differences in event rates across the selected study-level covariates and estimates the among-study variance, allowing the effects of multiple factors to be investigated simultaneously. In meta-regression, the outcome variable is the effect estimate, so the regression coefficient obtained will describe how the outcome variable (RR in this case) changes with a unit increase in the explanatory variable (the potential effect modifier) [27].

For this study a meta-regression was used to test the impact of potential effect modifiers, namely, clinical characteristics, follow-up time, starting year of the study, and proportion of men, on effect size of the RR (Table S4).

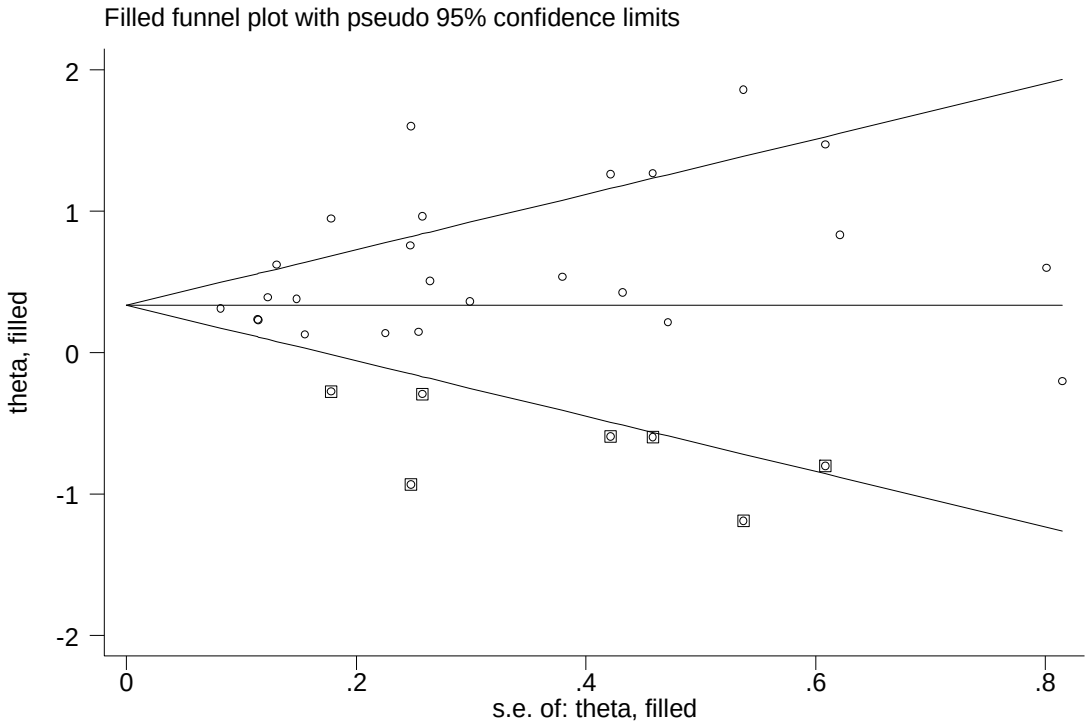
Table S4. Meta-regression on the RR effect of Chagas on mortality (measured as logRR).

logRR	Coefficient	Standard Error	*t*	p>|*t*|	95% Confidence Interval	
Clinical classification	0.32	0.18	1.76	0.10	–0.07	0.70
Star year of the study	0.01	0.01	0.74	0.47	–0.02	0.04
Proportion of men	–2.11	1.33	1.59	0.14	–4.96	0.74
Location	–0.22	0.27	0.83	0.42	–0.80	0.35
Constant	–19.47	28.89	0.67	0.51	–81.44	42.49

5. Publication bias

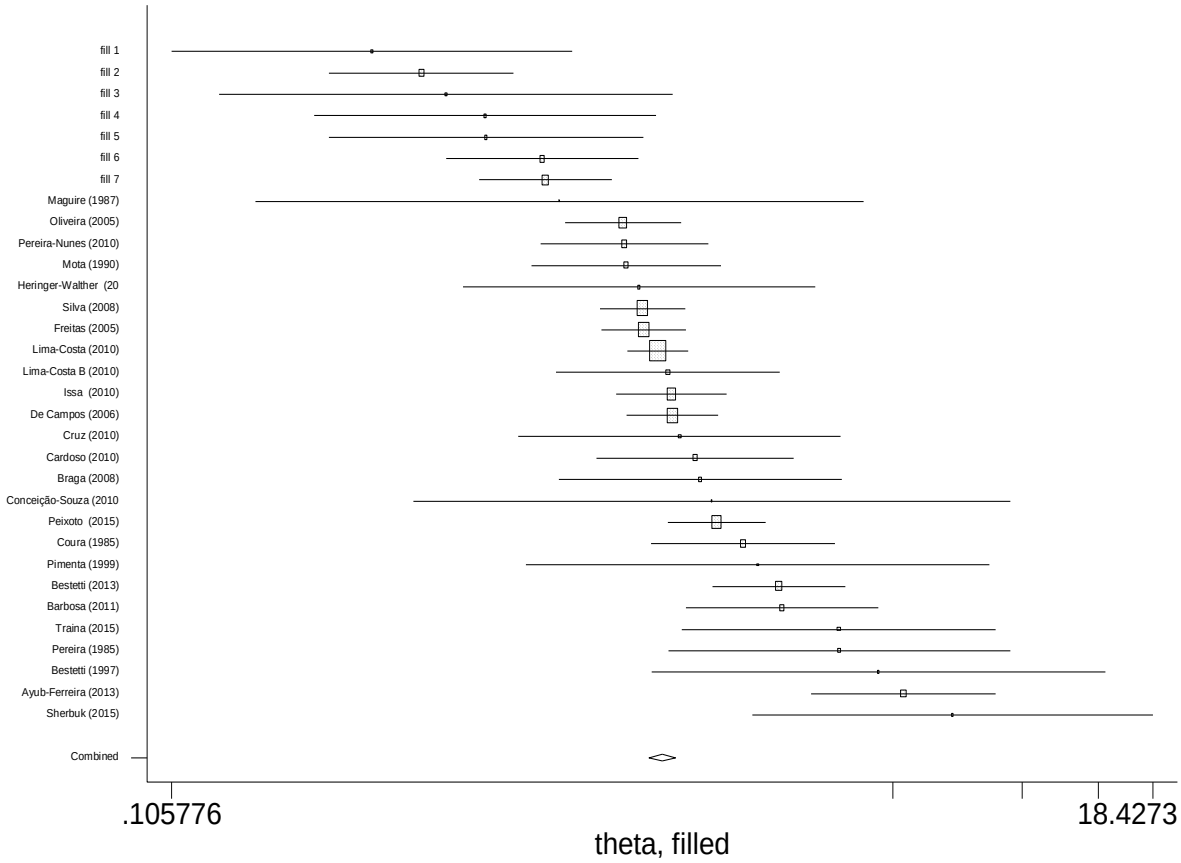
To explore the potential impact of publication bias on our estimates of excess mortality, we used a trim-and-fill technique to re-estimate the effect incorporating hypothetically missing studies [28]. The results are presented in Fig. S3 and Fig. S4.

Figure S2. Funnel plot of trim-and-fill technique for re-estimating the effect size



On the left axis the log(RR) (theta) from each study is plotted against its corresponding standard error (s.e.). Circles represent the studies included in the meta-analysis and the squares represent the hypothetically missing studies. Lines represent pseudo 95% confidence limits for the re-estimate using the 'filled' studies.

Figure S3. Forest plot for the trim-and-fill technique



The effect estimate (RR, theta) from the studies included in the meta-analysis (with authors and years) and the hypothetically missing studies (fill 1, 2, 3, 4, 5, 6, 7) are plotted. The missing studies would have a bias towards lower excess mortality.

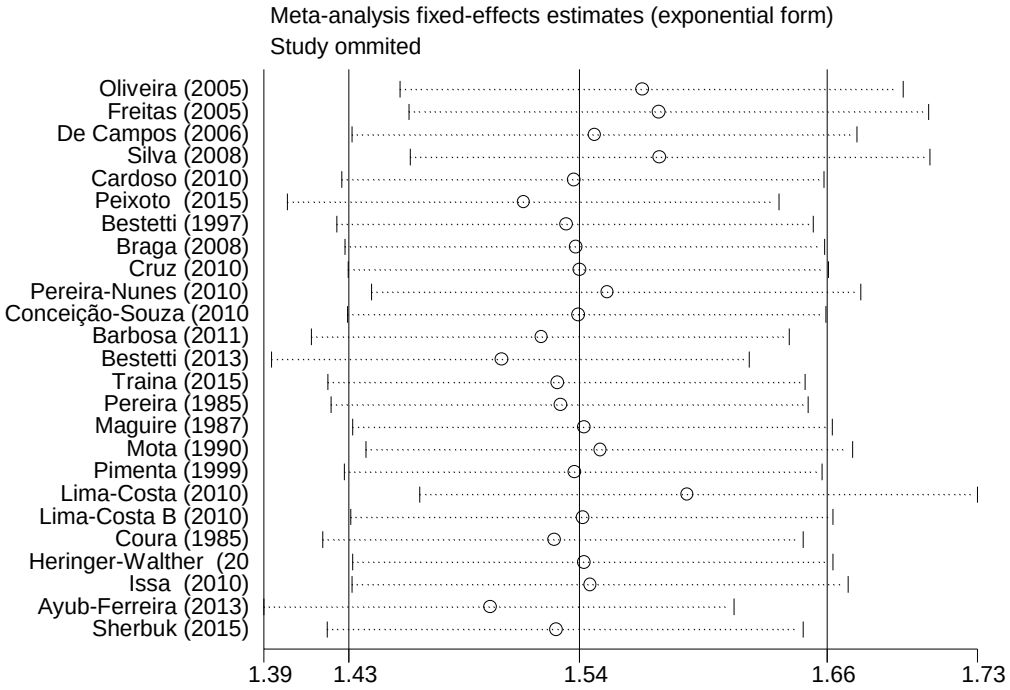
The magnitude of the RR re-estimated after applying the Trim and Fill technique (as a random-effects model) is 1.42 (95%CI 1.19–1.70).

This result indicates that the RR estimate and its uncertainty bounds obtained from the meta-analysis are not strongly affected by missing studies.

6. Sensitivity analysis

To identify potential outliers and assess their potential impact on the results of the meta-analysis, we re-evaluated the (fixed-effects) model after removing each study one at a time. The original RR estimate was compared to that obtained after omitting each study (Figure S4).

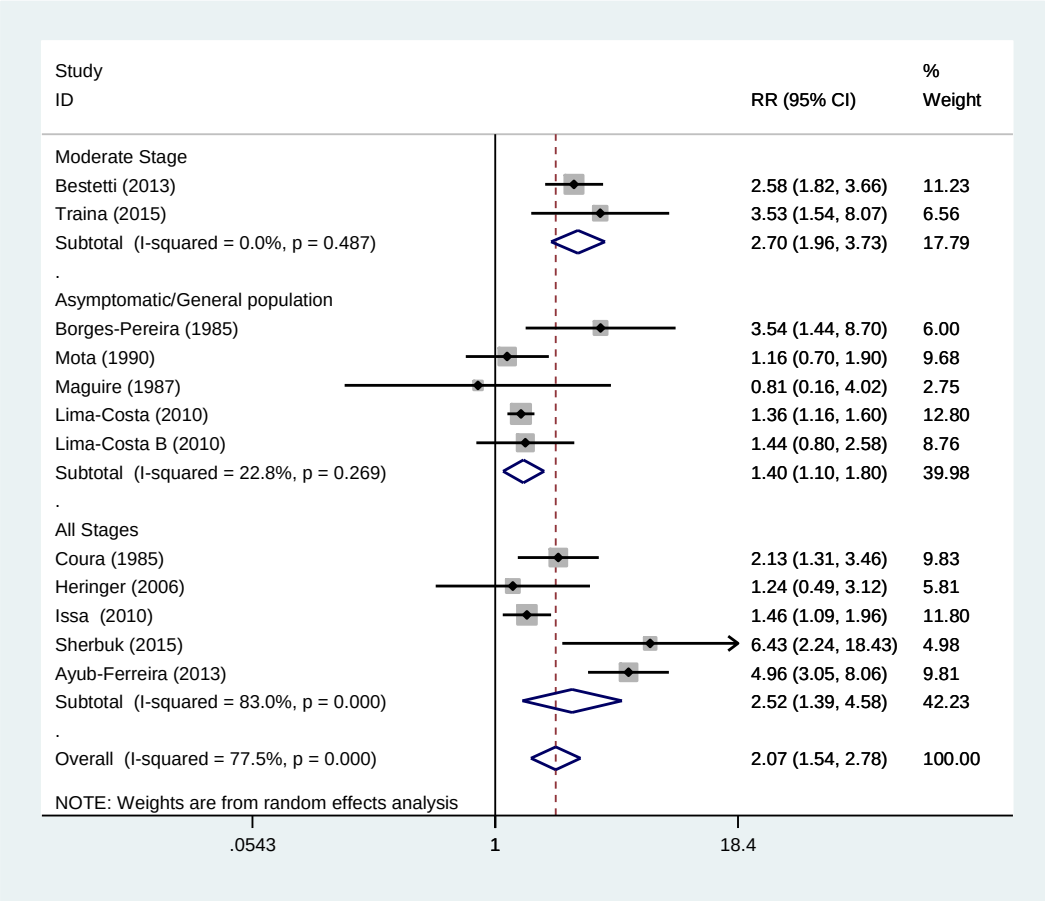
Figure S4. Sensitivity analysis for the fixed-effects model based on omitting one study at a time



The re-estimated effect size (RR, open circles) and its 95% confidence interval (dotted horizontal lines) are plotted on the horizontal axis after removing each study in turn (left axis). Values and 95%CI were hardly affected by removing any single study, indicating no evidence of outliers in the studies selected for meta-analysis.

Figure S5 presents the results of restricting the analysis to only those ranked as ‘high quality’ studies (see Table S2). This sub-analysis was conducted to obtain robust estimates of excess mortality among clinical categories, and to assess the impact on our results of study quality. The results of the meta-analysis conducted using only high quality papers were very similar to those of the meta-analysis presented in the main text, indicating that study quality did not strongly influence the results.

Fig. S5. Forest plot of the fixed-effects model for the association between Chagas disease and mortality including only "high quality" studies



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