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Authors: Yanina Sguassero, Karen N Roberts, Guillermina B Harvey, Daniel Comandé, Agustín Ciapponi, Cristina B Cuesta, Emmaría Danesi, Camila Aguiar, Ana L Andrade, Ana Mde Castro, Marta de Lana, Josep M Escribà, Diana L Fabbro, Cloé D Fernandes, Wendell SF Meira, María Flores-Chávez, Alejandro M Hasslocher-Moreno, Yves Jackson, Carlos D Lacunza, Girley F Machado-de-Assis, Marisel Maldonado, María M Monje-Rumi, Israel Molina, Catalina Muñoz-San Martín, Laura Murcia, Cleudson Nery de Castro, Celeste AN Silveira, Olga Sánchez Negrette, Manuel Segovia, Aldo Solari, Mário Steindel, Mirtha L Streiger, Ninfa Vera de Bilbao, Inés Zulantay, Sergio Sosa-Estani

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Course of serological tests in treated subjects with chronic Trypanosoma cruzi infection: a systematic review and meta-analysis of individual participant data.

Authors

Yanina Sguassero[1,35], Karen N Roberts[2], Guillermina B Harvey[2], Daniel Comandé[3], Agustín Ciapponi[3], Cristina B Cuesta[2], Emmaría Danesi[4], Camila Aguiar[5], Ana L Andrade[6], Ana M de Castro[7], Marta de Lana[8], Josep M Escribà[9], Diana L Fabbro[10], Cloé D Fernandes[11], Wendell SF Meira[12], María Flores-Chávez[13], Alejandro M Hasslocher-Moreno[14], Yves Jackson[15,16], Carlos D Lacunza[17], Girley F Machado-de-Assis[18], Marisel Maldonado[19], María M Monje-Rumi[20], Israel Molina[21,22], Catalina Muñoz-San Martín[23,24], Laura Murcia[25], Cleudson Nery de Castro[26], Celeste AN Silveira[27], Olga Sánchez Negrette[28,29], Manuel Segovia[30], Aldo Solari[31], Mário Steindel[32], Mirtha L Streiger[33], Ninfa Vera de Bilbao[19], Inés Zulantay[24], Sergio Sosa-Estani[3,34,35]

Affiliations

1. Centro Rosarino de Estudios Perinatales, Rosario, Argentina
2. Facultad de Ciencias Económicas y Estadística, Universidad Nacional de Rosario, Argentina
3. Instituto de Efectividad Clínica y Sanitaria, Buenos Aires, Argentina
4. Centro Nacional de Investigación y Diagnóstico en Endemoepidemias (CeNDIE-ANLIS), Buenos Aires, Argentina
5. Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Brasil
6. Departamento de Saúde Coletiva, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Goiás-Brasil
7. Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Goiás-Brasil
8. Departamento de Análise Clínica, Universidade Federal de Ouro Preto, Brasil
9. Medical Department, Doctors Without Borders, Barcelona, Spain
10. Centro de Investigaciones sobre Endemias Nacionales (CIEN), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina
11. Instituto de Pesquisa Biológica, Laboratório Central, Fundação Estadual de Produção e Pesquisa em Saúde, Porto Alegre, RS, Brasil
12. Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal do Triangulo Mineiro, Brasil
13. Unidad de Leishmaniasis, Servicio de Parasitología, Instituto de Salud Carlos III, Madrid, España
14. Instituto Nacional de Infectologia Evandro Chagas, Fiocruz, Brasil
15. Division of primary care medicine, Geneva University Hospitals, Geneva, Switzerland

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16. Institute of Global Health, University of Geneva, Geneva, Switzerland

17. Dirección de Primer Nivel de Atención, Área Operativa N° LV, Salta, Argentina

18. Departamento de Ciências Básicas da Vida, Universidade Federal de Juiz de Fora, Brasil

19. Departamento de Medicina Tropical, Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción, Paraguay

20. Laboratorio de Patología Experimental, Universidad Nacional de Salta, Salta, Argentina

21. Infectious Diseases Department, Hospital Universitario Vall d'Hebron, Barcelona, Spain

22. International Health Program of the Catalan Institute of Health, Barcelona, Spain

23. Laboratorio de Ecología, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile

24. Laboratorio de Parasitología Básico-Clínico Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile

25. Unidad Regional de Medicina Tropical, Servicio de Microbiología, Hospital Universitario Virgen de la Arrixaca, El Palmar Murcia, España

26. Faculdade de Medicina, Universidade Católica de Brasília, Brasil

27. Núcleo de Medicina Tropical, Faculdade de Medicina, Universidade de Brasília, Brasil

28. Cátedra de Inmunología, Facultad de Ciencias Agrarias y Veterinarias, Universidad Católica de Salta, Argentina

29. Cátedra de Química Biológica, Facultad de Ciencias Exactas, Universidad Nacional de Salta, Argentina

30. Departamento de Genética y Microbiología, Universidad de Murcia, Espinardo Murcia, España

31. Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile

32. Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Santa Catarina, Brasil

33. Centro de Investigaciones sobre Endemias Nacionales (CIEN), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina

34. Instituto Nacional de Parasitología, Fatala Chaben-ANLIS, Buenos Aires, Argentina

35. Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

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Corresponding Author

Yanina Sguassero M.D.

Pediatrician and Researcher

Centro Rosarino de Estudios Perinatales (Argentinean Cochrane Centre)

Rosario, Argentina

ysguassero@crep.org.ar

Moreno 878 - S2000DKR - Rosario, Argentina

Tel.: +54 341 4483887

Highlights

- First meta-analysis of individual data in chronic *T. cruzi* infection after treatment
- Probability of seroreversion variable along the follow-up
- Interaction between age at treatment and country setting
- The course of parasitological/molecular tests after treatment needs to be assessed

ABSTRACT

Objective: To determine the course of serological tests in subjects with chronic *T. cruzi* infection treated with antitrypanosomal drugs.

Methods: We conducted a systematic review and meta-analysis using individual participant data. Survival analysis and Cox proportional hazards regression model with a random effect to adjust for covariates were applied. The protocol was registered at www.crd.york.ac.uk/PROSPERO (CRD42012002162).

Results: We included 27 studies (1296 subjects) conducted in eight countries. The risk of bias was low for all domains in 17 studies (63.0%). We assessed 913 subjects (149 seroreversion events, 83.7% censored data) for ELISA, 670 subjects (134 events, 80.0% censored) for IIF, and 548 subjects (99 events, 82.0% censored) for IHA. A higher probability of seroreversion was observed in subjects aged 1–19 years compared to adults at a shorter time span. The chance of seroreversion also varied according to the country where the infection might have been acquired. For instance, the pooled adjusted hazard ratio between children/adolescents and adults for IIF test was 1.54 (95% CI 0.64–3.71) and 9.37 (3.44–25.50) in some countries of South America and Brazil, respectively.

Conclusions: The disappearance of anti-*T. cruzi* antibodies was demonstrated along the follow-up. An interaction between age at treatment and country setting was shown.

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Keywords: Trypanosoma cruzi; Chronic Disease; Follow-Up Studies; Individual Participant Data; Meta-Analysis; Serologic Tests

Introduction

Rationale

Chagas disease is a potentially life-threatening endemic illness in the Latin America region.[1,2] It is caused by a protozoan parasite called *Trypanosoma cruzi (T. cruzi)* that has been classified into six genetic variants with approximate geographical distribution in domestic and wild transmission cycles.[3] It is mainly transmitted through vectors (namely triatomine bugs) in impoverished rural areas.[1,4] Blood transfusion and congenital transmission are other mechanisms of transmission. Alternative mechanisms are accidental, oral, and by organ transplantation.[5,6]

The duration and clinical presentation of the initial acute phase of the infection may be variable, depending on the patient's age, immunological status, presence of comorbidities and the transmission pathway. It usually lasts a few months; it may be symptomatic (prolonged febrile syndrome, asthenia, hepatosplenomegaly, and other characteristic but less frequent signs such as Romaña sign and "chagoma" of inoculation) or asymptomatic.[6]

Most subjects, who do not receive specific treatment during the acute phase, go on to develop chronic infection. If untreated, the chronic phase usually continues for the lifetime and 30% to 40% of patients will progress to chronic phase with a cardiac, digestive, neurological, or mixed form after 15 to 30 years of the initial infection. Progressive heart failure and sudden death are main causes of death in patients with chronic Chagas heart disease.[1,6]

Several systematic reviews about the effectiveness of treatment in chronically infected subjects were published.[7-10] The current recommendation of the World Health Organization is to offer antitrypanosomal drugs (benznidazole or nifurtimox) to subjects with chronic *T. cruzi* infection, particularly to those who are asymptomatic.[2] Based on the current techniques and their attributes, the general consensus is that treatment success is confirmed by the conversion from a positive to a negative serological state (seroreversion), while treatment failure is demonstrated through a positive parasitological or molecular test. The assessment of the response to treatment is uncertain in a large number of subjects because of the long span needed to demonstrate the disappearance of anti-*T. cruzi* antibodies.[11,12] In this scenario, we sought to answer the following question: when and to what extent does the administration of antitrypanosomal drugs entail the negativization of serological tests through the follow-up? We published a prognostic systematic review of follow-up studies including chronically infected and treated subjects as a first attempt to address our research question. These results showed a dynamic pattern of serological response; however, pre-planned secondary analyses were incomplete due to limited utility of aggregate data.[13] As a consequence, we conducted a meta-analysis of individual participant data (IPD) considering all laboratory tests performed after treatment.[14,15] For the purpose of this manuscript, we present the course of serological tests and describe the effect of explanatory factors on the time to seroreversion in treated subjects with chronic *T. cruzi* infection. Our hypothesis was based on previous research suggesting

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an earlier reversion of conventional serology to negative results in treated subjects undergoing an "early chronic infection" (children and adolescents) compared to "late chronic infection" (adults).

Methods

Study design

We undertook this meta-analysis according to a protocol of a systematic review registered at an international prospective register of systematic review protocols (http://www.crd.york.ac.uk/PROSPERO, Registration Number CRD42012002162) in March, 2013.[16] The protocol was approved by the Bioethical Committee of the Faculty of Medical Sciences of the National University of Rosario, Argentina (Record number: 42705/0345). All studies shared unidentified data; hence individual consent for the reuse of participants' data was not sought.

The inclusion and exclusion criteria were detailed elsewhere.[13] Cohort studies and randomized controlled trials (RCTs) with follow-up data on serological, molecular, and parasitological outcomes measured in subjects with a definite diagnosis of chronic *T. cruzi* infection who received benznidazole and/or nifurtimox were included. Subjects in the acute phase, children aged 12 months or younger born to infected mothers, immunocompromised participants, and pregnant women were excluded. Two authors, one with training in systematic reviews (YS) and one expert in the field (SSE), identified potentially eligible studies. YS also screened the references of relevant studies. Disagreements were resolved by discussion. Electronic search strategies were previously described, with no restriction of language or year of publication.[13]

We updated the electronic searches in the Cochrane Database of Systematic Reviews (CDSR), Cochrane Central Register of Controlled Trials (CENTRAL), Database of Abstracts of Reviews of Effects (DARE), MEDLINE (Supplementary data, Annex A, Table S1), EMBASE, and LILACS on 1st July 2015. Three new eligible studies were identified.[17-19] In addition, we presented our protocol to experts in the field and we identified three studies by contacting the principal investigators.[20-23]

Two review authors (YS & AC) independently assessed the risk of bias (RoB) of included studies using the tools recommended by The Cochrane Collaboration.[24,25] We used a data extraction template with the following principal domains of bias: selection of participants into the study, measurement of the intervention, measurement of outcomes, and missing data. The overall RoB judgement for each serological outcome was rated as low, moderate, and serious. Studies with low RoB for all key domains or studies where it seemed unlikely for bias to alter the results were considered to have a low risk. Studies with a risk of bias in at least one domain were considered to have an overall RoB accordingly to the level of bias that decreased the certainty of the conclusions. Discrepancies were resolved through discussion with a third author (SSE).

Outcomes

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We considered the results of conventional serological tests such as enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence (IIF) assay, and indirect haemagglutination assay (IHA) as primary outcomes. The secondary outcomes were non-conventional "in-house" serological tests based on recombinant/synthetic or biochemically purified antigens specifically used to monitor changes after antitrypanosomal treatment. We dichotomised the results of serological tests as reactive or non-reactive (negative result) independently of each other. We decided to analyze techniques separately given the variability of their performance to show serological changes over time. The dependent variable was defined as the time elapsed from the end of treatment to a first negative result of each serological test as measured by the authors. No composite outcomes were defined at the protocol stage.

Data collection

A systematic approach to request, collect, and manage IPD was undertaken.[15] We invited all correspondent or primary authors to participate in our research by email. In the case of no response, we checked the contact details and explored the website of universities or other research organisations to find a new email address. At least three rounds of emails per investigator were launched by the same person (YS). In some cases, a member of the review team (SSE) sent a letter of invitation or made a phone call. The strategy developed had three basic steps. The first email intended to present our research providing a link to the systematic review protocol, to briefly explain the importance of the meta-analytical approach, and to invite investigators to participate. We sent a second email to those collaborators expressing their willingness to contribute with a link to a short online survey aimed at gathering baseline information (availability of primary study protocol, the format of the study dataset, the time needed to send the original data, etc.), and a section to update their contact details. Afterwards, we sent a third email including a detailed description of the piece of information needed for each outcome of interest, an agreement of data security and co-authorship to be signed, and recommendations for encrypted data sharing.
Concerning the variables of interest, collaborators provided individual-level data at baseline (sex, age at treatment, age at study entry, country of origin, the name of the antitrypanosomal drug, the dose and length of treatment) and during the follow-up (dates of measurements and results of all available laboratory results). The internal consistency of IPD from each study dataset was checked independently by two authors (YS & KR) taking into account the information published in the original reports. Minor queries were solved by reaching consensus; however, any specific IPD inconsistency was solved by seeking clarification from collaborators (YS). Any inaccuracies or errors were properly discussed and all amendments were registered before merging a study dataset into the master file (YS & KR). Missing data were also requested, whenever appropriate (YS). All collaborators answered an email about the risk of reinfection in their study population.

Statistical analysis

We followed the one-stage IPD meta-analysis as stated at the protocol stage. Two functions that are dependent on time for describing the distribution of event times (the survival and hazard functions) were estimated.[26,27] We used the Kaplan-Meier method to obtain univariate descriptive survival data on two or more groups of subjects.[26] The Cox proportional hazards regression model with a random effect was

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applied to adjust for prespecified covariates of clinical relevance. In our model, the time was assembled in years as provided in most datasets. We described the excess risk or frailty for distinct studies using the variance component of random effect and its corresponding Kendall´s Tau coefficient ($\hat{\tau}$).[28] Hazard ratios (HRs) and 95% of confidence interval (CI) were calculated.

Subgroup analyses were performed based on potential predictors of treatment response in the chronic phase of *T. cruzi* infection such as the age of subject at treatment (children/adolescents *vs* adults) and the country where the subject was born as a surrogate for the lineage of *T. cruzi* (Argentina, Bolivia, Chile, and Paraguay *vs* Brazil).[3]

Taking advantage of available IPD on polymerase chain reaction (PCR) or xenodiagnosis after treatment, we assign the study participants to the following three post-hoc categories: *potential responders* as subjects with three or more negative PCR or xenodiagnosis, *uncertain responders* as subjects with at least two negative PCR or xenodiagnosis, and *non-responders/potential non-responders* as subjects with just one PCR or xenodiagnosis (positive or negative).

We explored the effect of the RoB through a sensitivity analysis. The survival analyses for this paper were done with SAS version 9.4. A p-value less than 0.05 was considered statistically significant. We were unable to use aggregate data from studies for which IPD were not obtained because time-to-event analysis requires individual-level data.

Results

We included 24 cohort studies and three RCTs (1296 subjects) reporting on serological outcomes (Figure 1; Table 1).[22, 29–54] For studies conducted before the 90s or for which the primary author has died, no attempt to obtain original raw data was made. We were not able to obtain IPD from 21 cohort studies (Figure 1); some of them are known to be lost (Supplementary data, Annex B). Three eligible studies in children could be reachable in the future, two studies are from Colombia (n=79) reporting up to 42.0% of seroreversion after 30 months of treatment and one is from Brazil (n=46) with almost 20.0% of seroreversion after 24 months of follow-up. Regarding the studies conducted in adults that may be still accessible, the published rate of seroreversion ranges from 5% after 5–10 years of treatment to 45.0% in just one cohort study reporting on 55 treated subjects living in a non-endemic area after more than 20-year-follow-up (Supplementary data, Annex A, Table S2).

The total available follow-up for serological outcomes was 6847 person-years (median 3.0 years per person, IQR 1.5–7.0). Benznidazole was used in 24 out of 27 included studies as the only treatment of choice. The prevalent duration of treatment was 31 to 60 days (959 subjects) (Supplementary data, Annex A, Table S3). Most of the included studies reported at least on two serological tests. Non-conventional serology and molecular or parasitological test results were provided in seven and 20 studies, respectively (Table 1). The RoB was low for all domains in 17 studies (63.0%), moderate in five (18.5%), and serious in another five (Supplementary data, Annex A, Figure S1).

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No issues were encountered when checking IPD integrity. The highest rate of missing IPD was around 15.0% for ELISA. Sample sizes beyond ten years of follow-up were small. Our approach for measuring the variation among studies revealed that $\hat{\tau}$ was 0.63 for ELISA, 0.58 for IIF, and 0.47 for IHA.

We assessed 913 subjects (149 seroreversion events, 83.7% censored data) for ELISA, 670 subjects (134 events, 80.0% censored data) for IIF, and 548 subjects (99 events, 82.0% censored data) for IHA. A higher probability of seroreversion was observed in treated subjects aged 1–19 years compared to adults at a shorter time span. In this respect, there was a 0.50 probability of seroreversion in the subgroup of treated children and adolescents living in Argentina, Bolivia, Chile, and Paraguay after 11 to 13 years of follow-up. When considering the same time after treatment for the subgroup of Brazilian children and adolescents, a similar probability of 0.55 was observed for ELISA test. In subjects treated as adults, this probability was around 0.90 after 11 years of treatment (Figure 2). There were no statistically significant differences in the risk of seroreversion between females and males for any of the serological tests (data not shown).

By using non-conventional serology, an earlier probability of seroreversion could also be inferred compared to conventional serology in children and adolescents (Supplementary data, Annex A, Table S4 and Figure S2). No statistically significant differences among survival curves stratified by category using available PCR or xenodiagnosis IPD were found (Supplementary data, Annex A, Table S5 and Figure S3).

The adjusted Cox model demonstrated the interaction between age at treatment and country setting. The pooled adjusted HR between subjects aged 1–19 years at treatment in comparison to adults varied according to the country were the infection might have been acquired. In the region of Brazil, a higher chance of seroreversion was found in children and adolescents compared to adults [HR 6.60 (IC 95% 2.03–21.42) for ELISA, HR 9.37 (3.44–25.50) for IIF, and HR 5.55 (1.46–21.11) for IHA]. No statically significant differences were found for this comparison in Argentina, Bolivia, Chile, and Paraguay (Table 3). The sensitivity analysis was not informative (Supplementary data, Annex A, Table S6).

Discussion

To the best of our knowledge, this is the first meta-analysis using IPD in subjects with chronic *T. cruzi* infection who received antitrypanosomal drugs. Our results supplied with a better picture of the kinetics of anti-*T. cruzi* antibodies in different country settings and revealed that the serological course after treatment is variable along the follow-up as previously reported.[11,12] In this regard, we highlight that the single-arm character of the study poses limitations to the inferences coming from this meta-analysis. This approach relates directly to the aim of our research and the preliminary published results showing scatter plots of negative serological tests in non-treated subjects with chronic *T. cruzi* infection based on aggregate data.[13] The techniques used to measure the serological course presented differences that could be explained by the affinity during the formation of the antigen-antibody complex, with an ELISA test having a

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better performance to detect seroreversion. This phenomenon was described before in two RCTs in the medium-term.[52,53] Non-conventional serology appears to formerly detect the disappearance of antibodies after treatment in children and adolescents.

Whereas a positive parasitological or molecular test confirms the failure of treatment, a negative result cannot rule out the absence of infection because of the lack of sensitivity in the chronic phase of Chagas disease. The pattern of serology showed according to the number and results of available PCR or xenodiagnosis merits a more comprehensive assessment. Our preliminary observations suggest a better course in treated subjects with persistent parasite clearance measured by three or more negative PCR after treatment. Among other plausible explanations, seroreversion in subjects classified as *non-responders/potential non-responders* might be explained by the transient nature of the PCR results and by the conservative approach adopted to define the categories on a post-hoc basis.

We also explored possible interactions that allow estimating, as a function of time, the probability of occurrence of seroreversion. The age at treatment and the country setting are two variables that merit further attention when assessing treatment success during the chronic phase.

This study has the following strengths. We had a pragmatic approach in adopting current recommendations for IPD meta-analysis (Supplementary data, Annex C). The rate of response to our invitation to participate was high and most of the studies for which IPD was not provided include adults. We restricted the risk of bias assessment to those domains that are relevant to our meta-analysis. Included studies benefited from a well-defined intervention status and objective outcome measures, hence the measurement of interventions and the measurement of outcomes were judged as low risk of bias in all studies. We conducted a pilot test for the selection of the statistical methodology to ensure a rigorous IPD analysis.

This study has some limitations. To be relevant, a meta-analysis should include all the available studies. The inherent conflicts for the provision of IPD were in line with the barriers of data sharing described in the literature, including concerns about protecting subject confidentiality and anonymity, lack of time, and not available or unusable datasets.[55] We were not able to explore potential sources of heterogeneity as planned given the lack of comparison groups to estimate the hazard ratios of each study. Some datasets included children while others included only adults and all studies were conducted in one single country. Our meta-analysis has not enough IPD from countries where TcI *T. cruzi* lineage may be prevalent.[3]

We updated electronic searches on 28th August 2017 to seek for new studies published since the last search and we identified three potentially eligible studies reporting on serological outcomes (two studies including adults living in Argentina and Spain, and one study including children living in Guatemala) that will be considered in future updates.

Our survival analyses may shed light on current recommendations for clinical management of chronic *T. cruzi* infection. The notion of factors such as the age of the patient attending the clinical appointment and the age at treatment (time elapsed between treatment and clinical assessment), the country of origin (or the country where the infection was picked up), and the complementary assessment of conventional serology and PCR results is critical to monitoring after treatment.

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The use of participant-level data was intended to overcome pragmatic constraints of a new follow-up study such as the long-time elapsed between exposure to antitrypanosomal drugs and seroreversion, the need for appropriate sample sizes for children and adults, and the care of adequate follow-up rates. We hope to provide an encouraging example of the importance of anonymizing and sharing IPD for secondary analysis. This strategy may decrease the burden on research resources through the meaningful reuse of existing data.
The next steps will be to determine the course of parasitological and molecular tests after treatment during chronic *T. cruzi* infection and to conduct new analysis using available IPD according to the standard definition of cure (seroreversion defined as having negative results to at least two different serology techniques) as the primary result. The period would likely be one where health care providers expect to make decisions based on official guidelines, for example, up to three years. Our ultimate goal is to provide a monitoring tool to help with treatment assessment of chronically infected patients.

Ethical Approval: The protocol was approved by the Bioethical Committee of the Faculty of Medical Sciences of the National University of Rosario, Argentina (Record number: 42705/0345). All studies shared unidentified data; hence individual consent for the reuse of participants' data was not sought.

Conflict of Interest: All the authors declare no competing interests.

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Figure 1

PRISMA IPD Flow Diagram

¶ One multicountry study including four cohorts of children. ***** Number obtained from published reports.

♣ A subject could have more than one reason for exclusion. IPD=Individual Participant Data. RCT=randomized controlled trial.

Figure 2

Kaplan-Meier plots of progression of conventional serology in treated subjects with chronic *T. cruzi* infection stratified by age at treatment and country setting with 95% IC.

Plots show the proportion of treated subjects progressing towards seroreversion according to ELISA (1), IIF (2), and HAI (3) tests during the follow-up, stratified by age at treatment (1–19 years *vs* > 19 years) and country setting (Group A: Argentina, Bolivia, Chile and Paraguay *vs* Group B: Brazil). IC=interval confidence. ELISA=enzyme linked immunosorbent assay. IIF= indirect immunofluorescence. IHA= indirect haemagglutination assay.

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Figure 1

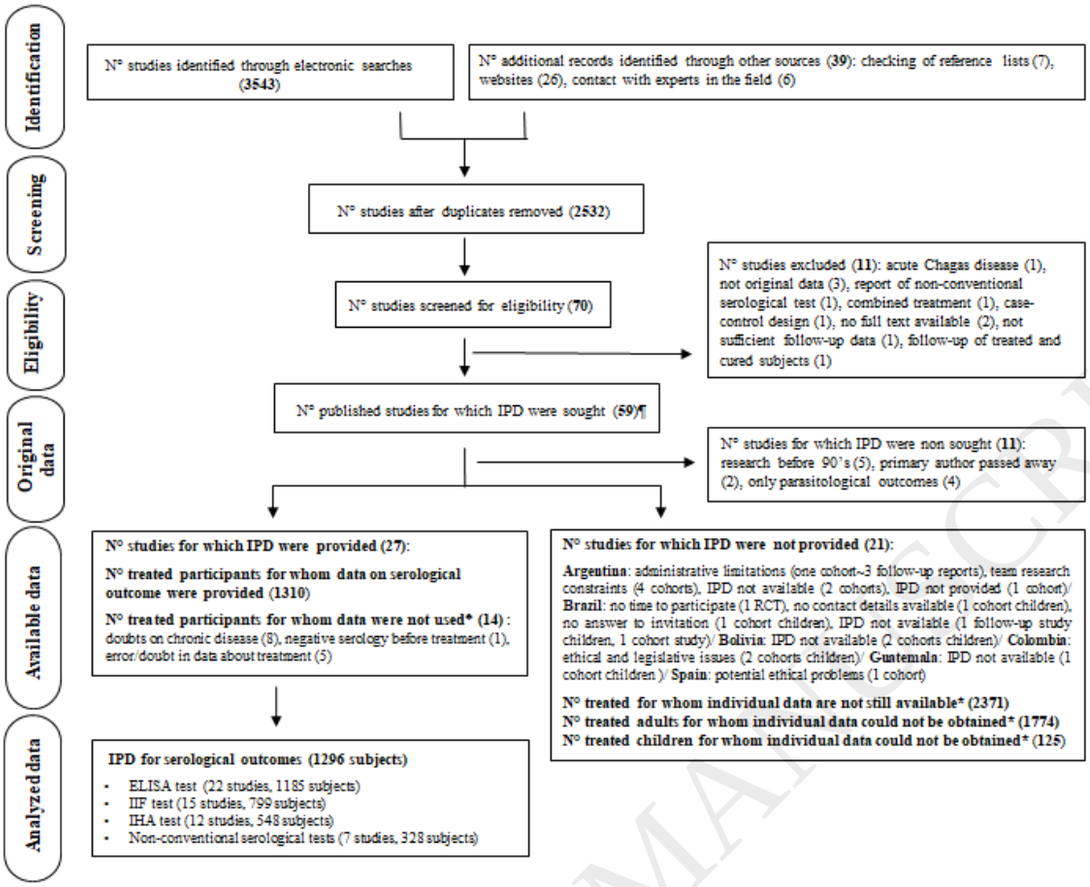

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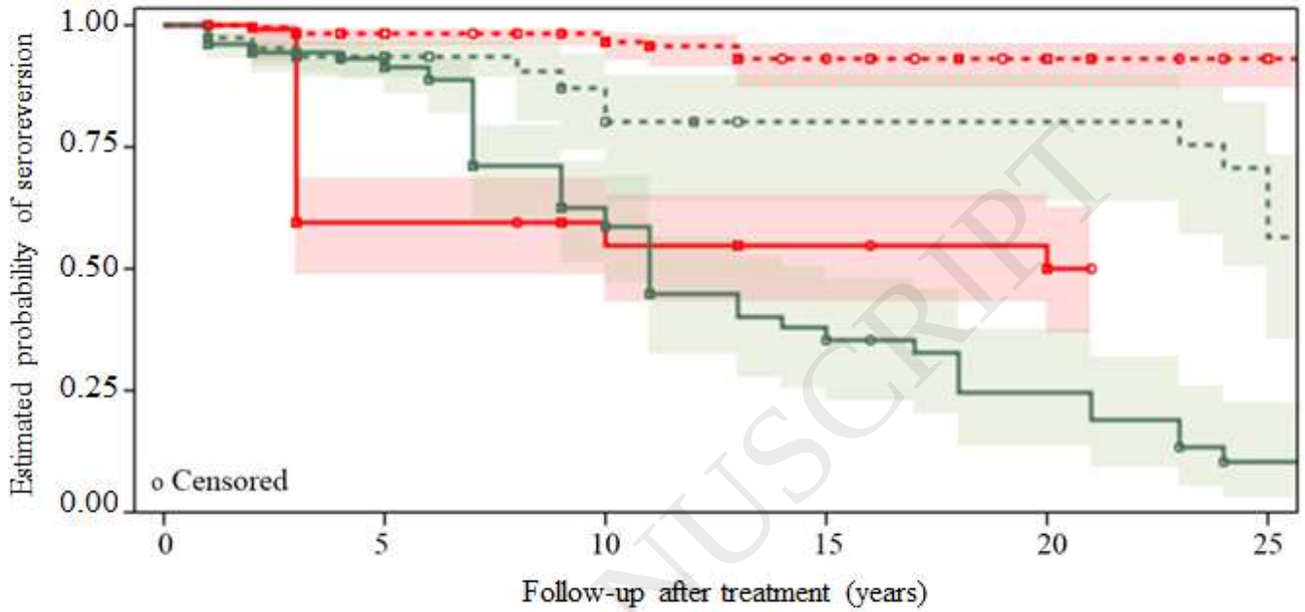
Figure 2

1



Number at risk

1–19 years, group B	96	33	26	13	12	0
1–19 years, group A	243	97	34	16	9	2
>19 years, group B	297	137	127	55	46	22
>19 years, group A	277	38	25	17	17	15

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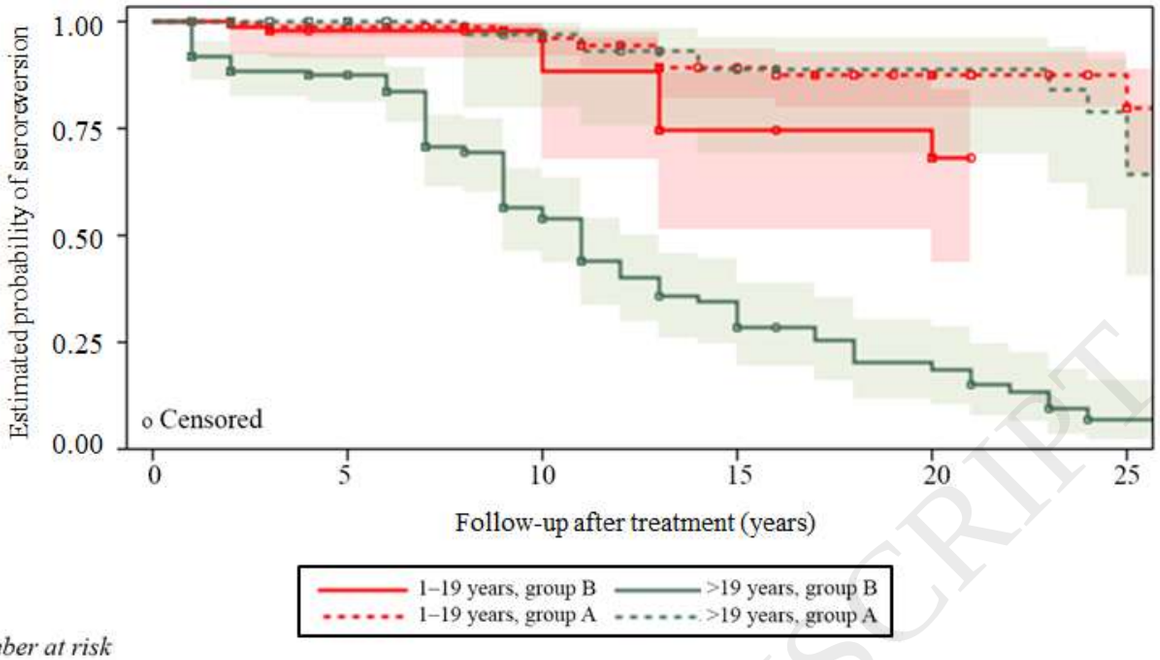

Number at risk

1–19 years, group B	91	28	21	13	12	0
1–19 years, group A	156	94	48	24	12	2
>19 years, group B	305	161	128	59	46	22
>19 years, group A	118	52	29	20	18	16

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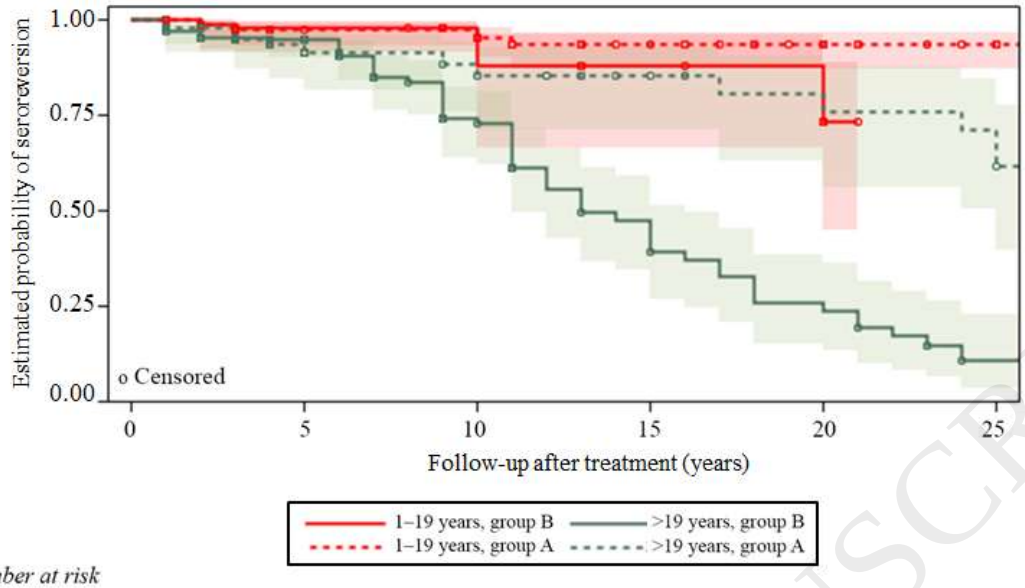
3



Number at risk

1–19 years, group B	88	27	20	13	12	0
1–19 years, group A	200	115	47	23	12	2
>19 years, group B	169	103	102	53	44	20

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Table 1

Studies for which individual participant data for serological outcomes was provided by country, duration of follow-up, area of endemicity, treatment and type of test (27 studies, 1296 treated subjects with chronic *T. cruzi* infection).

Source	Study ID*	Maximum duration follow-up (months)	Area of endemicity	Risk of reinfection	Age at treatment (years)	Antitrypanosomal drug	Number of treated subjects with IPD for time-to-event analysis						Total number of subjects with serology♣
							Parasitological tests		Conventional serological tests			Non-conventional serological tests	
							XD	PCR	ELISA	IIF	IHA		
Argentina	Lacunza CD et al. 2015 [22]	69	Yes	No	16 to 34	Benznidazole	-	52	38	-	39	-	39
	Fabbro DL et al. 2014 [30]	401	No	No	6 to 45	Benznidazole & Nifurtimox	-	-	47	52	52	-	52
	Monje Rumi MM et al. 2013 [34]	60	Yes	No	3 to 16	Benznidazole	-	45	45	-	45	-	45
	Streiger ML et al. 2004 [48]	288	No	No	1 to 14	Benznidazole & Nifurtimox	21	-	26	48	48	-	48
	Sosa-Estani S et al. 1998 [52]	48	Yes	No	6 to 14	Benznidazole	49	46	53	53	52	53	53
	Sosa-Estani S et al. 2009 [42]	144							16	16	16	16	
	Sánchez Negrette O et al. 2008 [43]	66	Yes	No	19 to 41	Benznidazole	-	-	18	-	18	18	18
Total number of subjects-Argentina													**255**
Brazil	Machado-de-Assis GF et al. 2013 [32]	156	Yes	No	6 to 37	Benznidazole	-	27	26	-	-	22	26
	de Lana M et al. 2009 [39]												
	Aguiar C et al. 2012 [35]	348	Yes	No	8 to 56	Benznidazole	-	29	29	29	-	-	29
	Machado-de-Assis GF et al. 2012 [36]	432	Yes	No	2 to 60	Benznidazole	-	94	94	94	94	94	94
	Hassslocher-Moreno AM 2010 [37]	204	No	No	16 to 56	Benznidazole	62	-	-	62	-	-	62
	Fernandes CD et al. 2009 [41]	36	Yes	No	17 to 48	Benznidazole	-	80	80	80	-	-	80
	de Castro AM et al. 2006 [44]	44	Yes	NK	23 to 76	Benznidazole	-	37	37	37	37	-	37
	Meira WSF et al. 2004 [47]	50	Yes	No	10 to 61	Benznidazole	-	31	31	-	31	-	31
	Silveira CAN et al. 2000 [51]	204	Yes	NK	7 to 12	Benznidazole & Nifurtimox	38	28	37	37	37	-	37
	de Andrade AL et al. 1996 [53]	36	Yes	No	8 to 11	Benznidazole	-	-	58	58	58	58	58
Total number of subjects-Brazil													**454**
Bolivia	Flores-Chavez M et al. 2006 [45]	12	Yes	No	5 to 10	Benznidazole	22	33	33	22	-	33	33
Total number of subjects-Bolivia													**33**
Chile	Muñoz C et al. 2013 [33]	57	Yes	No	22 to 48	Nifurtimox	21	21	-	21	-	-	21
	Solari A et al. 2001 [50]	36	Yes	No	1 to 10	Nifurtimox	37	37	37	-	37	-	37
Total number of subjects-Chile													**58**
Honduras	Escribà JM et al. 2009 [40]	51	Yes	No	1 to 18	Benznidazole	-	-	227	-	-	-	227
Total number of subjects-Honduras													**227**
Paraguay	Vera de Bilbao N et al. 2006 [46]	120	Yes	No	9 to 11	Benznidazole	-	-	5	5	-	-	5
	Vera de Bilbao N et al. 2004 [49]	24	Yes	No	7 to 14	Benznidazole	20	20	20	20	-	-	20
	Maldonado M et al. 1995 [54]												
Total number of subjects-Paraguay													**25**
Spain	Molina I et al. 2014 [29]	12	No	No	27 to 60	Benznidazole	-	25	26	-	-	-	26
	Murcia L et al. 2010 [38]	30	No	No	2 to 74	Benznidazole	-	138	181	181	-	-	181
Total number of subjects-Spain													**207**
Switzerland	Jackson Y et al. 2013 [31]	36	No	No	25 to 59	Nifurtimox	-	37	37	-	-	-	37
Total number of subjects-Switzerland													**37**
Grand total													**1296**

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*Study identification includes surname of primary author and year of publication [number of reference]. ♣The estimation of total number of subjects was based on the test with the best number of individual participant data. ¶Individual level data extracted from tables of published reports.

ELISA=enzyme-linked immunosorbent assay. IIF=indirect immunofluorescence assay. IHA=indirect haemagglutination assay. IPD= individual participant data. NK=not known. PCR=protein chain reaction. XD=xenodiagnosis

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Table 3

Hazard ratios (95% IC) corresponding to the adjusted Cox interaction model for conventional serology in treated children or adolescents (1–19 years) *vs* adults (> 19 years) with chronic *T. cruzi* infection.

Serological test	HR (95% IC)		p-value*
	Brazil	**Argentina, Bolivia, Chile and Paraguay**	
ELISA	6.60 (2.03–2.42)	1.71 (0.77–3.81)	0.062
IIF	9.37 (3.44–25.50)	1.54 (0.64–3.71)	0.007
IHA	5.55 (1.46–21.11)	1.09 (0.44–2.70)	0.047

*The p-value corresponds to the effect of the interaction obtained from adjusted Cox proportional hazards model.

ELISA=enzyme-linked immunosorbent assay. IC=interval confidence. IIF=indirect immunofluorescence assay. IHA=indirect haemagglutination assay. HR=hazard ratio.

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