PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cohort Profile: Longitudinal Study of Patients with Chronic Chagas Cardiomyopathy in Brazil (SaMi-Trop Project)
AUTHORS	Cardoso, Clareci; Sabino, Ester; Oliveira, Claudia; Oliveira, Lea; Ferreira, Ariela; Neto, Edécio; Bierrenbach, Ana; Ferreira, João; Haikal, Desirée; Reingold, Art; Ribeiro, Antonio

VERSION 1 - REVIEW

REVIEWER	Rodolfo Viotti Hospital Eva Perón, Buenos Aires, Argentina
REVIEW RETURNED	08-Feb-2016

GENERAL COMMENTS	The manuscript "Cohort Profile: Longitudinal Study of Patients With chronic Chagas cardiomyopathy in Brazil (SaMi-Trop Project)" is a study planned to evaluate patients with Chagas disease and follow-up for 2 years. In the present manuscript the report is the basal results found in 20 rural communities of Brazil only, not data about the follow-up. The main study variables are epidemiological and socioeconomic data, ECG findings and measurement of BNP. The submitted manuscript is just one part of a larger manuscript, besides that not completed the planned follow-up. Relevant comments -While the study was designed for the diagnosis and prognosis of Chagas disease, a complete assessment of the type and severity of cardiac involvement was not performed. I understand whatever the difficulties of conducting a field study in rural areas, although a clinical stratification of the disease was not planned. -The Chronic Chagas cardiomyopathy presents different stages or degrees of severity, essentially ECG disorders with preserved LV systolic function, complex ventricular arrhythmias or LV systolic dysfunction with or without heart failure. Though the use of BNP is a marker of distension of myofibrils and heart failure, you can use a Chest-Rx to clinically classify patients or perform an Echocardiogram to obtain better and more complete information, which led to lost precision about the degree of dilation or systolic dysfunction of LV, and additionally the presence of aneurysms. -Follow-up to 2 years: why 2 years? Was the sample calculated? It will be enough 2 years? Specific comments -Page 3, line 10. What exactly "marginally effective therapeutic option" mean? -Page 3, line 13 "... with an incidence rate of 1.85% ..." from which these data and how reliable are they? Is it the same rate of

	progression in patients with LV increase and dysfunction that that with only conduction abnormalities? -Page 3, line 20. The ECG and chest radiography are possible and necessary in the first level of care. With these simple and none spends methods you can make the clinical classification of patients. -Page 3, line 27. Marginally treated or undertreated? It is not the same concept. - Page 4, line 10. Reported self-Chagas? Was reactive serology an inclusion criterion? -Page 4, line 23. Some ECG findings are not clearly related to disease in a sero-positive individual, as the likely overload or hypertrophy and abnormal T waves. -Page 5, line 12. Why was separated from the main study? Page 7, lines 13-14. The pro-BNP is a marker of advanced disease and heart failure, and it is unclear its predictive power in early stages and as an independent predictor of progression. -Page 7, lines 36-40. You should mention the results of the BENEFIT study. -Strengths and Limitations. The study population already have advanced disease and old age, target populations should be people without heart disease and younger. Another limitation mentioned above is the lack of clinical patient stratification. Finally, another important limitation is the lack of baseline echocardiograms. Those patients with abnormal ECG have preserved or reduced systolic function, and this has a strong prognostic value. -Page 10. References. The review of the literature is insufficient. Many indexed publications were omitted.

REVIEWER	Richard Schulz Departments of Pediatrics & Pharmacology Mazankowski Alberta Heart Institute University of Alberta Edmonton, Alberta Canada
REVIEW RETURNED	15-Feb-2016

GENERAL COMMENTS	The manuscript by Cardoso et al describes a longitudinal cohort study of nearly 2000 patients living across 21 municipalities in northern part of the state of Minas Gerais, Brazil living with Chagas' cardiomyopathy, also known more commonly as Chagas' disease. This is one of the most common causes of heart failure in Central and South America and is a neglected tropical disease primarily affects impoverished people which has no known cure. The patients were found through a national telehealth program whereby electrocardiograms (ECGs) of patients self-reporting as having Chagas' disease were screened for abnormalities consistent with the 20-25% subgroup of persons infected with Trypanosoma cruzi (T. cruzi) that progress to heart failure. Approximately 6 million persons are infected with T. cruzi and several more million are at risk in endemic regions throughout Central and South America. In those susceptible patients infected with T. cruzi, over a extraordinary time course of 20-40 years they develop an auto-immune like cardiac inflammation which leads to cardiac dysrhythmias, heart

failure and death. Finding biomarkers that could help to identify those who may progress to cardiac complications and a cure to prevent or treat Chagas disease are sorely needed. The results of the first ever trial in Chagas disease patients unfortunately showed that antiparasitic therapy with benznidazole in those with Chagas cardiomyopathy showed no benefit in preventing the progression to heart failure (ref. 14 in manuscript).

This important and useful study is a prospective cohort designed to evaluate whether a clinical prediction rule based on ECG and brain natriuretic peptide (BNP) levels in the blood could be useful in clinical practice in the prediction of those who may progress to severe heart outcomes. This paper outlines the study and the baseline socio-demographic, clinical information and co-morbidities of the cohort of 1959 patients and invites other researchers to participate in the study which will have a open access for two years following the end of the data collection in August 2018.

Comments

Major
1. Although it may be common to refer to Chagas' cardiomyopathy as Chagas' disease, this is not clearly stated or defined. Does a patient self-reporting to have Chagas' disease actually have Chagas cardiomyopathy? Does T. cruzi seropositivity, plus self-reporting, equate to a diagnosis of Chagas cardiomyopathy? A clearly explanation of the study rationale in the abstract, introduction and study objectives would help, as it appears that the study was designed to provide a clinical prediction rule.

2. There is no explanation of the statistics used in the methods. It seems that more statistical comparisons of these data could be made. Why is it that the majority of the subjects (68%) are female? Does this correspond to known gender predictions of this disease and/or does this have perhaps reasons in the socio-economic conditions of Brazil, including migration of the poor for jobs and difficulties in follow-up within Brazil? Some analysis and discussion of this may be required.

3. Although there is a lack of predictive biomarkers for Chagas cardiomyopathy, some more background and references may be useful for comparison. In the first paragraph of the Introduction which 'complex prognostic scores' are available? Briefly discuss and cite. A recent biomarker study showing the potential utility of plasma matrix metalloproteinase-2 and -9 activities in a Colombian cohort as diagnostic markers in the progression to Chagas disease should be cited (Batista-Lopez et al. Am Heart J 2013 165:558).

Minor
3. Please provide the current university department and business address of the corresponding author.

4. I believe that the better term to describe the regions selected is 'municipality' or 'municipal district' and not 'city'. Please replace throughout the manuscript and in Fig 1 legend. In Fig. 1, for those less familiar with the geography of Brazil it would be useful to state in the figure legend that the insert is the entire country (mark the biggest population 5 cities on the map and label the dark area as 'State of Minais Gerais', with Belo Horizonte as its capital. In the larger map of Minas Gerais again it would be useful for reference to

	give at least the 5 largest cities overall. Please provide a table of the names of the 21 municipalities that were selected. It was also not obvious as to why exactly these 21 places were selected (what were the criteria)? 5. Please explain what kind of blood sample was taken (serum or plasma) and if the latter, what was the anticoagulant used. What were the conditions of centrifugation (time, g, temperature?). Were these samples aliquoted and kept at -20 C during shipment within Brazil? 6. Many abbreviations are either unnecessary or undefined. Please reduce to a minimum for clarity. Those to be deleted include ChD and CCC. What are SIM and EIA on pg. 5? SAMI-TROP needs to be explained. 7. Note in Table 2 that this is monthly income data. 8. Having cited the NEJM BENEFIT trial study, I think it would be useful to state more clearly in the discussion for the reader that this study was multi-national in scope (including Brazil) and showed that antiparasitic treatment in those with established Chagas cardiomyopathy has no treatment benefit. 9. On pg. 8 it should rather read as 'As stated by Maguire'. The first initial of this author is missing in the cited reference list.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name
Rodolfo Viotti

Institution and Country
Hospital Eva Perón, Buenos Aires, Argentina

Please state any competing interests or state ‘None declared’:
None declared

Please leave your comments for the authors below

The manuscript "Cohort Profile: Longitudinal Study of Patients With chronic Chagas cardiomyopathy in Brazil (SaMi-Trop Project)" is a study planned to evaluate patients with Chagas disease and follow-up for 2 years.
In the present manuscript the report is the basal results found in 20 rural communities of Brazil only, not data about the follow-up.
The main study variables are epidemiological and socioeconomic data, ECG findings and measurement of BNP.
The submitted manuscript is just one part of a larger manuscript, besides that not completed the planned follow-up.

Relevant comments
-While the study was designed for the diagnosis and prognosis of Chagas disease, a complete assessment of the type and severity of cardiac involvement was not performed.

I understand whatever the difficulties of conducting a field study in rural areas, although a clinical stratification of the disease was not planned.

R: Thank you for your comment. The main goal of the study is exactly to develop a simple, prognostic model for Chagas cardiopathy patients without performing all complimentary exams required in current predictive models. Indeed, 24h Holter monitoring, exercise stress testing and echocardiogram, methods used to stratify the type and severity of cardiac involvement, are not easily available in endemic areas, as in the North of Minas Gerais, and the underlying hypothesis of the present study is that clinical evaluation, ECG findings and BNP levels would be sufficient to recognize high risk patients.

-The Chronic Chagas cardiomyopathy presents different stages or degrees of severity, essentially ECG disorders with preserved LV systolic function, complex ventricular arrhythmias or LV systolic dysfunction with or without heart failure.
Though the use of BNP is a marker of distension of myofibrils and heart failure, you can use a Chest-Rx to clinically classify patients or perform an Echocardiogram to obtain better and more complete information, which led to lost precision about the degree of dilation or systolic dysfunction of LV, and additionally the presence of aneurysms.

R: Thank you for your comment. As stated in the previous answer, we recognize the importance of evaluating LV systolic function by an echocardiogram and to recognize the presence of complex ventricular arrhythmias using 24h Holter monitoring and exercise stress testing. However, the aim of the study was exactly to recognize those patients with worse prognosis without performing these exams, not readily available in rural areas.
Concerning Chest X-rays, we should stress that X-Ray equipments are not easily available in remote rural areas, at least in those we performed this study. Moreover, the evaluation of the cardiac silhouette by chest X-ray is not an accurate marker of left ventricular dysfunction, as we showed in a previous study (Perez et al, 2003). Indeed, we showed that to perform BNP and ECG is more efficiently in recognizing left ventricular dysfunction than to perform chest X-ray and ECG (Ribeiro et al. 2006).

1: Perez AA, Ribeiro AL, Barros MV, de Sousa MR, Bittencourt RJ, Machado FS, Rocha MO. Value of the radiological study of the thorax for diagnosing left ventricular dysfunction in Chagas' disease. Arq Bras Cardiol. 2003 Feb;80(2):208-13, 202-7. Epub 2003 Feb 25. PubMed PMID: 12640514.

2: Ribeiro AL, Teixeira MM, Reis AM, Talvani A, Perez AA, Barros MV, Rocha MO. Brain natriuretic peptide based strategy to detect left ventricular dysfunction in Chagas disease: a comparison with the conventional approach. Int J Cardiol. 2006 Apr 28;109(1):34-40. Epub 2005 Jul 14. PubMed PMID: 16023747.

-Follow-up to 2 years: why 2 years? Was the sample calculated? It will be enough 2 years?

R: Yes, two years of follow-up will be enough, considering death as the main outcome. Preliminary results of follow up show us that mortality is 5% a year, the same rate expected in initial project was presented, as stated in the next item. The sample size calculation was added to the manuscript.

“Sample size”
Considering the minimal number of events per variable acceptable in a proportional hazards regression analysis of 10 events per variable (Peduzzi et al, 1995), and maximal number of studied variables of ten, the number of events would be 100 in the whole study. Since the prediction model has to be developed and validated and the whole sample will be divided in two, the number of events

should be 200. For a 2-year follow-up period and annual mortality rate of 5% in CCC (10% in 2 years), the calculated sample size is 2,000 subjects."

Specific comments
-Page 3, line 10. What exactly "marginally effective therapeutic option" mean?

R: We agree that the meaning of the sentence is not clear. Since this sentence is not necessary in the introduction of the text, we decided to withdraw it.

-Page 3, line 13 "... with an incidence rate of 1.85% ..." from which these data and how reliable are they?

R: This data was obtained from a 10-year retrospective cohort involving T. cruzi infected blood donors, the NIH REDS2 cohort (Sabino et al, 2013). In this study, incidence rates of Chagas cardiomyopathy were calculated by dividing the number of incident cases by the person-years of follow-up, calculated from the date of index donation until the date of clinical examination. The results showed a rate of progression to cardiomyopathy (1.85%/y) among persons infected with T. cruzi, but without cardiomyopathy at baseline.

This paper was published in Circulation. 2013 Mar 12; 127(10): 1105–1115, and it is available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3643805. We consider this source highly reliable, since it was a carefully conducted study, with a large sample, published in a first-line cardiology journal.

We changed the text in the present manuscript to include that the incidence rate was reported using person-years.

Is it the same rate of progression in patients with LV increase and dysfunction that that with only conduction abnormalities?

R: In the cited study (Sabino et al, 2013), the presence of Chagas cardiomyopathy was assessed by an expert panel of three cardiologists, considering data from several sources, as clinical questionnaire, laboratory, ECG, and echocardiogram. The expert panel review criteria of the REDS-II Chagas study protocol was detailed in the referenced article. Since the present study did not use this criteria, we choose not to include this data in the manuscript.

-Page 3, line 20. The ECG and chest radiography are possible and necessary in the first level of care. With these simple and none spends methods you can make the clinical classification of patients.

R: We answered this question above. In brief, in primary care facilities in remote rural areas of Brazil, chest radiography is not readily available. Chest X-ray is not a sensitive method for LV dysfunction. Our aim was not to classify patients using current protocols, but to propose a simple, easy to use predictive rule based in clinical evaluation, ECG and BNP levels.

-Page 3, line 27. Marginally treated or undertreated? It is not the same concept.

R: We agree with the reviewer and changed "marginally treated" to "undertreated".

- Page 4, line 10. Reported self-Chagas? Was reactive serology an inclusion criterion?

R: All patients that self-reported Chagas disease during the ECG realization in this 21 municipalities

were initially eligible to be evaluated in base line. However, the patient was included in the cohort after reactive serology. This information was clarified in the method session.

“All eligible participants were tested for the presence of anti-Trypanosoma cruzi antibodies using chemiluminescent micro particle immunoassay. Negative results were confirmed by two other enzyme immunoassay (EIA) presenting different antigens. The final cohort consists of patients confirmed to be seropositive.”

-Page 4, line 23. Some ECG findings are not clearly related to disease in a sero-positive individual, as the likely overload or hypertrophy and abnormal T waves.

R: These ECG abnormalities were used only as eligibility criteria and was based in a previous study of one of the authors in which these findings were grouped as “major abnormalities” (Ribeiro et al, 2015). However, the final cohort includes only patients with reactive serology for Chagas disease.

Ribeiro AL, Marcolino MS, Prineas RJ, Lima-Costa MF. Electrocardiographic abnormalities in elderly Chagas disease patients: 10-year follow-up of the Bambui Cohort Study of Aging. J Am Heart Assoc. 2014 Feb 7;3(1): e000632.

-Page 5, line 12. Why was separated from the main study?

R: Indeed, the cohort was not separated from the main study, but, as stated, is part of larger study on biomarkers in Chagas disease. To conduct this study, a NIAID/NIH Neglected Tropical Disease Centre was established and includes this cohort and other studies on biomarkers.

Page 7, lines 13-14. The pro-BNP is a marker of advanced disease and heart failure, and it is unclear its predictive power in early stages and as an independent predictor of progression.

R: In this section, “Findings to date”, page 7, lines 13-14, we stated that “Clinical and laboratory markers predictive of severe and progressive Chagas Disease were identified in SAMI-TROP cohort, as high NT-ProBNP levels, as well as symptoms of advanced heart failure.” We did not state that high pro-BNP levels will have predictive power in early stages or be an independent predictor of progression. However, previous studies have shown that BNP levels predict the risk of death in Chagas disease patients in a community, suggesting that it may have this role even in patients without such advanced disease (Lima-Costa et al., 2010). The argument of the reviewer – the uncertainty of the predictive value of BNP/proBNP values in earlier stages of cardiopathy – seems to be a good argument in favor of conducting the present study.

Lima-Costa MF, Cesar CC, Peixoto SV, Ribeiro AL. Plasma B-type natriuretic peptide as a predictor of mortality in community-dwelling older adults with Chagas disease: 10-year follow-up of the Bambui Cohort Study of Aging. Am J Epidemiol. 2010 Jul 15;172(2):190-6.

-Page 7, lines 36-40. You should mention the results of the BENEFIT study.

R: The text in these line already mentioned the BENEFIT in the reference (Morillo et al, 2015). A sentence was included with the study conclusions:

“In the recently released BENEFIT trial, [14], that included Brazilian patients, treatment with benznidazole did not significantly reduce cardiac clinical deterioration through 5 years of follow-up in Chagas cardiomyopathy.”

-Strengths and Limitations.

The study population already have advanced disease and old age, target populations should be people without heart disease and younger.

R: We do not consider this aspect a limitation of the present study. Chagas disease is mostly a disease of older ages in countries where the vectorial transmission was interrupted in the past. In order to understand how to manage the cardiac conditions on this population, it is important to conduct studies with this target population. There are several communities based studies of young patients, without cardiopathy, but few studies of advanced disease outside hospitals, what can turn this cohort unique and very useful to provide information for patients of the same age range and clinical condition. So, we added to the Limitations section:

“Because the focus was to find biomarkers related to the cardiac outcome, and given the budgetary limitations, no indeterminate form or negative controls were included in this cohort, and this will preclude the study of the early biomarkers of disease progression. However, Chagas disease is mostly a disease of adult and old ages in countries where the vectorial transmission was interrupted and this cohort provides a unique opportunity of recognizing predictors of higher risk using simple biomarkers in a community sample of Chagas cardiomyopathy patients.”

Another limitation mentioned above is the lack of clinical patient stratification.
Finally, another important limitation is the lack of baseline echocardiograms. Those patients with abnormal ECG have preserved or reduced systolic function, and this has a strong prognostic value.

R: We addressed the issue of clinical stratification in previous answers. We included as one of the limitations the absence of the echocardiogram, which could help in the clinical stratification of the patients.
“Another important limitation is the lack of baseline echocardiograms, which could help in the clinical stratification of patients. All this information is being collected in the second follow up visit.”

-Page 10. References.
The review of the literature is insufficient. Many indexed publications were omitted.

R: Indeed, since this a “Cohort Profile”, not a review article, we included only the references necessary to the understating of the study motivation and design, as well as the initial findings. However, we included new references in the revised version.

Reviewer: 2

Reviewer Name
Richard Schulz

Institution and Country
Departments of Pediatrics & Pharmacology
Mazankowski Alberta Heart Institute
University of Alberta
Edmonton, Alberta
Canada

Please state any competing interests or state ‘None declared’:
None to declare.

Please leave your comments for the authors below
The manuscript by Cardoso et al describes a longitudinal cohort study of nearly 2000 patients living across 21 municipalities in northern part of the state of Minas Gerais, Brazil living with Chagas' cardiomyopathy, also known more commonly as Chagas' disease. This is one of the most common causes of heart failure in Central and South America and is a neglected tropical disease primarily affects impoverished people which has no known cure.

The patients were found through a national telehealth program whereby electrocardiograms (ECGs) of patients self-reporting as having Chagas' disease were screened for abnormalities consistent with the 20-25% subgroup of persons infected with Trypanosoma cruzi (T. cruzi) that progress to heart failure. Approximately 6 million persons are infected with T. cruzi and several more million are at risk in endemic regions throughout Central and South America. In those susceptible patients infected with T. cruzi, over a extraordinary time course of 20-40 years they develop an auto-immune like cardiac inflammation which leads to cardiac dysrhythmias, heart failure and death. Finding biomarkers that could help to identify those who may progress to cardiac complications and a cure to prevent or treat Chagas disease are sorely needed. The results of the first ever trial in Chagas disease patients unfortunately showed that antiparasitic therapy with benznidazole in those with Chagas cardiomyopathy showed no benefit in preventing the progression to heart failure (ref. 14 in manuscript).

This important and useful study is a prospective cohort designed to evaluate whether a clinical prediction rule based on ECG and brain natriuretic peptide (BNP) levels in the blood could be useful in clinical practice in the prediction of those who may progress to severe heart outcomes. This paper outlines the study and the baseline socio-demographic, clinical information and co-morbidities of the cohort of 1959 patients and invites other researchers to participate in the study which will have a open access for two years following the end of the data collection in August 2018.

Comments

Major
1. Although it may be common to refer to Chagas' cardiomyopathy as Chagas' disease, this is not clearly stated or defined. Does a patient self-reporting to have Chagas' disease actually have Chagas cardiomyopathy? Does T. cruzi seropositivity, plus self-reporting, equate to a diagnosis of Chagas cardiomyopathy? A clearly explanation of the study rationale in the abstract, introduction and study objectives would help, as it appears that the study was designed to provide a clinical prediction rule.

R: Thank you for your comments.
The patient self-reporting to have Chagas disease do not mean that patient have Chagas cardiomyopathy. We selected patients that self-reported to have Chagas disease with major ECG abnormalities and positive serology to recognize those Chagas cardiomyopathy.
Chagas cardiomyopathy was defined in the paper as suggested.
A clearly explanation of the study rationale was including in the abstract, introduction and study objectives. So, we added to the explanation in the follow section:

Abstract: “Purpose: We have established a prospective cohort of 1,959 patients with chronic Chagas cardiomyopathy to evaluate if a clinical prediction rule based on electrocardiogram (ECG), Brain Natriuretic Peptide (BNP) levels and other biomarkers can be useful in clinical practice. This paper outlines the study and baseline characteristics of the cohort participants.”
Introduction: “Chronic Chagas cardiomyopathy comprises a wide range of manifestations, including

heart failure, arrhythmias, heart blocks, sudden death, thromboembolism and stroke [Nunes et al, 2013]. Clinical presentation typically varies widely according to the degree of myocardial damage and most patients present a mild form of heart disease, frequently characterized only by the presence of asymptomatic abnormalities on the ECG or in other complimentary exams [Rocha et al, 2007]. The Brazilian Consensus of Chagas disease defines Chagas cardiomyopathy as the presence of typical ECG abnormalities in patients with a positive serologic test for T. cruzi infection [Consenso, 2005]. When heart failure and/or severe arrhythmias manifest, the prognosis is ominous, with high and premature mortality rates, typically in adult male patients [Rocha et al, 2003], but also in the elderly [Lima-Costa et al, 2010]. Indeed, when compared to patients with idiopathic cardiomyopathy, patients with chronic Chagas cardiomyopathy have poorer survival, irrespective of other clinical and echocardiographic parameters [Pereira et al, 2010]."
Seeking to contribute to the knowledge of Chagas Disease, a large cohort of chronic Chagas cardiomyopathy patients was established in Minas Gerais State (Brazil). This cohort aiming to develop a prognostic algorithm, based on simple ECG measurements in conjunction with clinical information and Brain Natriuretic Peptide (BNP) levels, that would be used to predict the risk of disease progression and death in chronic Chagas cardiomyopathy patients and be useful in the clinical management of such patients. This paper outlines the study and baseline characteristics of the cohort participants."

2. There is no explanation of the statistics used in the methods. It seems that more statistical comparisons of these data could be made. Why is it that the majority of the subjects (68%) are female? Does this correspond to known gender predictions of this disease and/or does this have perhaps reasons in the socio-economic conditions of Brazil, including migration of the poor for jobs and difficulties in follow-up within Brazil? Some analysis and discussion of this may be required.

R: The explanation of the analysis was included in methods session.
"In this paper it was performed a descriptive analysis of the baseline characteristics of the cohort participants using frequency and percentage distribution. SPSS version 19 (SPSS Inc., IBM, Armonk, NY) and Arcview, version 10.1 (Environmental Systems Research Institute Inc., http://www.esri.com/software/ arcview/) were used."

R: According to the suggestion of the reviewer, a paragraph with a discussion on the higher frequency of women among the participants was added to the discussion.

"Although T. cruzi infection does not have a sexual predilection (Kirchhoff 2014), studies show higher prevalence among women (Matos et al, 2014; Marcolino et al, 2015). This difference may be related to more often use of health services by women, even after controlling for restrictions in routine activities due to health reasons (Travassos et al, 2002), as well as greater availability to participate in scientific studies, especially those with longitudinal component, such as in this investigation."

3. Although there is a lack of predictive biomarkers for Chagas cardiomyopathy, some more background and references may be useful for comparison. In the first paragraph of the Introduction which 'complex prognostic scores' are available? Briefly discuss and cite. A recent biomarker study showing the potential utility of plasma matrix metalloproteinase-2 and -9 activities in a Colombian cohort as diagnostic markers in the progression to Chagas disease should be cited (Batista-Lopez et al. Am Heart J 2013 165:558).

R: The text was completely reformulated to explain what we understand as "complex prognostic scores", which are scores that rely on multiple diagnostic tests, generally not available in the primary care setting. A sentence on new biomarkers was also included. The corrected paragraph stands as follow:
"Chronic Chagas cardiomyopathy is a potentially lethal condition, but the severity of the disease varies widely and accurate stratification of the risk of disease progression and death remains an

unsolved challenge [Nunes et al, 2013]. Risk scores have been developed [Rassi et. al, 2006; Ribeiro et al, 2008; Benchimol-Barbosa et al, 2013], including a validated one [Rassi et al, 2006]. However, current risk scores rely on the availability of several diagnostic tests, including Holter monitoring, stress testing, echocardiographic examination and chest X-ray [Rassi et al, 2006], or special exams, as signal averaged ECG [Ribeiro et al, 2008; Benchimol-Barbosa et al, 2013]. These methods are not readily available in the rural endemic areas and have limited role in risk stratification in the primary care setting. Indeed, a simple, low-cost and easy-to-use prognostic model, suitable for the primary care setting, is lacking. Although some promising studies showing the potential value of some new biomarkers [Baptista-Lopes, 2013; Ferreira et al, 2014] the lack of validated and easily available biomarkers for active infection or clinical end-points are a problem for assessing the performance of new drugs or therapeutic interventions. In addition, the lack of health service structure, mainly in remote areas, with low levels of awareness among health care providers, cases of chronic Chagas cardiomyopathy are under-recognized and under-treated."

Minor

4. Please provide the current university department and business address of the corresponding author.

R: Included, as suggested.

4. I believe that the better term to describe the regions selected is 'municipality' or 'municipal district' and not 'city'. Please replace throughout the manuscript and in Fig 1 legend. In Fig. 1, for those less familiar with the geography of Brazil it would be useful to state in the figure legend that the insert is the entire country (mark the biggest population 5 cities on the map and label the dark area as 'State of Minais Gerais', with Belo Horizonte as its capital. In the larger map of Minas Gerais again it would be useful for reference to give at least the 5 largest cities overall. Please provide a table of the names of the 21 municipalities that were selected. It was also not obvious as to why exactly these 21 places were selected (what were the criteria)?

R:
1- City was replaced by municipality.
2- The figure 1 was reformulated.
3- It was included a table with the names of the 21 municipalities and the distance of the reference health center.
4- Criteria: "Using this database, we selected 21 municipalities within a limited region in the Northern part of the State of Minas Gerais, in which the prevalence of Chagas disease was expected to be high (Figure 1 and table 1)".
This explanation was added to the first paragraph in session Cohort Description

5. Please explain what kind of blood sample was taken (serum or plasma) and if the latter, what was the anticoagulant used. What were the conditions of centrifugation (time, g, temperature?). Were these samples aliquoted and kept at -20 C during shipment within Brazil?
R: "Blood was collected into serum-separating tubes, and allowed to clot at room temperature for 30 minutes. The serum was centrifuged at 1300g for 10 minutes at room temperature. Storage at -20ºC and shipped with dry ice to the central laboratory in São Paulo."

6. Many abbreviations are either unnecessary or undefined. Please reduce to a minimum for clarity. Those to be deleted include ChD and CCC. What are SIM and EIA on pg. 5? SAMI-TROP needs to be explained.

R: 1-The abbreviation was defined:

a) “SaMi-Trop: The São Paulo-Minas Gerais Tropical Medicine Research Center (SaMi-Trop) consists of a network of collaborating scientists in the States of Minas Gerais and São Paulo which has been established for the purpose of developing and conducting research projects on Chagas Disease”.
b) “EIA: enzyme immunoassay”.
c) “SIM: (Mortality information System)”.

2- Abbreviations ChD and CCC were supressed.

7. Note in Table 2 that this is monthly income data.

R: This information in table 2 was modified by: “Family monthly income data”.

8. Having cited the NEJM BENEFIT trial study, I think it would be useful to state more clearly in the discussion for the reader that this study was multi-national in scope (including Brazil) and showed that antiparasitic treatment in those with established Chagas cardiomyopathy has no treatment benefit.

R: It was including the information about Brazilians patients in the BENEFIT study.

9. On pg. 8 it should rather read as 'As stated by Maguire'. The first initial of this author is missing in the cited reference list.

R: This information was included.

VERSION 2 – REVIEW

REVIEWER	Richard Schulz Departments of Pediatrics & Pharmacology Mazankowski Alberta Heart Institute University of Alberta Edmonton, Alberta Canada
REVIEW RETURNED	24-Mar-2016

GENERAL COMMENTS	Thank you for your comments and revisions to the manuscript.