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Cohort Profile: Longitudinal Study of Patients with Chronic Chagas Cardiomyopathy in Brazil (SaMi-Trop Project)

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Cohort Profile: Longitudinal Study of Patients with Chronic Chagas Cardiomyopathy in Brazil (SaMi-Trop Project)

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ABSTRACT

Purpose: We have established a prospective cohort of 1,959 patients with chronic Chagas cardiomyopathy (CCC) 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.

Participants: The study is being conducted in 21 cities of the northern part of Minas Gerais state in Brazil, and includes a follow up of two years. The baseline evaluation included collection of socio-demographic information, social determinants of health, health-related behaviors, comorbidities, medicines in use, history of previous treatment for Chagas Disease (ChD), symptoms, functional class, quality of life, blood sample collection and ECG. Patients were mostly female, aged 50-74 years, with low family income and educational level, with known ChD for >10 years; 46% presented with FC > I. Previous use of benznidazole was reported by 25.2% and permanent use of pacemaker by 6.2%. Almost half of the patients presented with high blood cholesterol and hypertension and one third of them had diabetes mellitus. NT-ProBNP level were >300 (pg/Ml) in 30% of the sample.

Finding to date: In The SaMi-Trop cohort, clinical and laboratory markers predictive of severe and progressive ChD were identified, as high NT-ProBNP levels, as well as symptoms of advanced heart failure. These results confirm the important residual morbidity of ChD in the remote areas, thus supporting political decisions that should prioritize in addition to epidemiological surveillance the medical treatment of CCC in the coming years. The SaMi-Trop represents a major challenge for focused research in neglected diseases, with knowledge that can be applied in primary health care.

Future plans: We will continue following this patients' cohort to provide relevant information about the development and progression of ChD disease in remotes areas, with social and economic inequities.

Registration: NCT02646943

INTRODUCTION

Chagas disease (ChD), caused by the protozoan parasite *Trypanosoma cruzi,* remains one of the most neglected diseases in the world, with 8-10 million infected people and only one marginally effective therapeutic option. The most important consequence of ChD is Chronic Chagas Cardiomyopathy (CCC), which occurs in 20 to 40% of infected persons [1-4], with an incidence rate of 1.85% per year. CCC 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[5]. Although complex prognostic scores are available, a simple, low-cost and easy-to-use prognostic model, suitable for the primary care setting, is lacking.

The lack of good 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 CCC are under recognized or marginally treated.

Seeking to contribute to the knowledge of ChD, a large cohort of CCC patients was established in Minas Gerais State (Brazil). This investigation 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 CCC patients and be useful in the clinical management of such patients.

COHORT DESCRIPTION

This is a prospective cohort study with at least two years of follow up, including one visit at baseline and another at 24 months. The cohort of CCC patients was established, using patients under the care of the Telehealth Network of Minas Gerais, a program designed to support primary care in Minas Gerais State (Brazil)[6]. In this program, all patients' electrocardiogram (ECG) and clinical data are sent to a central reading unit centre that also collects clinical data, such as history of ChD. Using this database, we selected 21 cities within a limited region in the Northern part of the State of Minas Gerais, in which the prevalence of CCC patients was expected to be high (Figure 1).

(Figure 1 here).

Patients were selected based on the ECG results performed in 2011-12 by the Telehealth Network, which from now on will be called index ECG. Only patients who fulfilled all of the following inclusion criteria were selected: A) self-reported ChD; B) presence of the following abnormalities on the index ECG[7]: possible old myocardial infarction (major Q wave abnormalities or minor Q waves abnormalities with ST segment or T-wave abnormalities), complete intraventricular block (right, left or unspecified), frequent supraventricular or ventricular premature beats, major isolated ST segment or T-wave abnormalities, atrial fibrillation or flutter or supraventricular tachycardia or other major arrhythmias, major atrioventricular conduction abnormalities or pacemaker use, or major QT prolongation (QT index>115%), left or right ventricular hypertrophy and; C) age of 19 years or more. The exclusion criteria included pregnancy or breast-feeding and any life threatening disease with an ominous prognosis that suggested a life expectancy of less than two years.

All patients will be followed for two years, until primary outcome or loss follow-up. The primary outcome is death and the secondary outcomes are changes in the ECG pattern and hospitalization due to cardiovascular complications. Ascertainment of the occurrence of deaths will be done using the National Mortality Information System (SIM) from the Ministry of Health.

Table 1 summarizes the types of data collected at baseline and at that will be collected the 2-year follow up visit. At baseline the patients were interviewed using a standardized questionnaire, had a blood sample collected and an ECG evaluation. The baseline visit was performed at Public Health Primary Care Units by previously trained staff. The data were collected electronically and sent to the data center at the University of São Paulo via a Web-based System. The blood sample was centrifuged and aliquoted within 24h after collection and stored at -20^0C until shipment to the central laboratory in São Paulo. A resting 12-lead ECG was recorded using an ECG PC machine (TEB, São Paulo, Brazil). The ECG recordings were sent electronically to the Telehealth system in order to be read by a trained cardiologist and the written report was subsequently returned to the patient's physician. For research purposes, ECGs were also automatically analyzed using The University of Glasgow ECG analysis program (release 28.5, issued on January 2014) and reviewed by trained cardiologists to ensure quality control. ECGs

will be classified using the Minnesota code criteria using variables derived from the median complex of the Glasgow University software measurement matrix [11].

SIM data will be used to ascertain patients' vital status after the follow-up period as well as the underlying causes of death, which are coded under the International Classification of Disease (ICD-10).

This cohort study is a component of a larger study to evaluate biomarkers of ChD sponsored by a grant from NIAID/NIH Neglected Tropical Disease Centre. It was approved by the Institutional Review Board, number 179.685/2012 (National Commission of Ethics in Research, CONEP).

(Table 1 here)

CHARACTERISTICS OF THE STUDY POPULATION

Of the 55.480 ECG performed in the 21 selected cities from 2011 to 2012, a total of 4,689 patients were eligible for the study and 2,157 were located and completed the baseline assessment in 2013-14. In comparison to the eligible group, the participants had a higher percentage of women (67.1% *versus* 59.9% p<0.01) and were younger (59.5 years *versus* 60.7 years, p<0.01). All participants were tested for the presence of anti-*Trypanosoma cruzi* antibodies using *chemiluminescent micro particle immunoassay*. Negative results were confirmed by two other EIA presenting different antigens. The final cohort consists of 1,959 (90.8%) confirmed to be seropositive (Figure 2).

(Figure 2 here)

Table 2 shows the main socio-demographic characteristics, socio-economic categories and self-perception of health of study participants in baseline. Most patients were female (67.5%), aged between 50 and 74 years (62.6%), sharing the same household with two other people or less (56.6%) and had a family monthly income of US$327.

The educational level was very low with 38.7% having between 1-4 years of school and 34.4% never having attended school. Cohort members self-reported their health status as average (57.7%) or good (25.8%).

(Table 2 here)

The majority of the patients self-reported that they have had ChD for over 10 years and that they had at least one family member with a history of ChD. Based on the New York Heart Association (NYHA) Functional Classification, 45.9% of the patients were classified as level II or more (i.e. have symptoms of heart failure). Among ChD patients in the cohort 6.2% reported permanent use of a pacemaker. Previous treatment for ChD was reported by 51.6% of ChD patients (25.2%), including 492 who reported previous treatment with benznidazole. The NT-ProBNP level was > 300 pg/mL in 30% of the sample (Table 3).

(Table 3 here)

As seen in Table 4, the prevalence of one or more self-reported comorbid conditions at baseline was high, including high serum cholesterol (40.1%), hypertension (36.0%), diabetes mellitus (10.1%), thyroid disorder (8.1%) and kidney disease (7.3%). leishmaniasis was reported by 22 patients (1.2%). Only 22.3% of patients reported having performed any physical activity during the prior week, 16.2% reported having drunk alcohol in the previous month, and 7.3% reported that they were current smokers. In terms of medications, 36.4% of patients reported the current use of one or two medicines, while 30.1% reported no current use of any. The most common medicines being used were diuretics (49.1%), angiotensin converting enzyme-ACE (28.6%), angiotensin receptor blockers-ARBs (28.4%), aspirin (26.2%) and amiodarone (22%).

Considering the fact that almost half of the patients were in functional class II or more, with 30% with BNP levels higher than 300 units/l, there is an overall low usage of the recommended drugs for heart failure, in particular of beta-blockers. This may explain at least in part the high frequency of cardiac symptoms reported: 63.5% had heartbeat racing, 62.5% had prolonged faintness or dizziness, 61.3% had an abnormal ECG, 61.0% had heart palpitations and 59.4% had shortness of breath at exercises. The

relative high frequency of amiodarone use may be related to the high frequency of cardiac arrhythmias in CCC, as well as the established practice, in Brazil, to prescribe amiodarone to prevent sudden death.

(Table 4 here)

FINDINGS TO DATE

Clinical and laboratory markers predictive of severe and progressive ChD were identified in SAMI-TROP cohort, as high NT-ProBNP levels, as well as symptoms of advanced heart failure. The NT-ProBNP level was > 300 pg/mL in 30% of the sample. High circulating levels of natriuretic peptides are related to the presence of left ventricular dysfunction[12] and higher risk of death[13].

Among ChD patients in the cohort, 6.2% reported permanent use of a pacemaker. This percentage is far below the 14.0% found in another recently published Brazilian cohort study of patients with ChD[14]. However, ChD is still a major cause of use of pacemakers and defibrillators in Brazil, even surpassing the indications due to coronary artery disease in some regions. The literature to pointed the under-use of this device in Brazil when compared to other countries[15], which is unfortunately what we expected to find in the remote regions represented in the current study.

In this large multicentre cohort, previous use of benznidazole was reported by one quarter of the patients. We well know that the persistence of T cruzi is directly related in the pathology of the chronic phase, but it remains to be proved that parasite load reduction by trypanocidal treatment leads concomitant attenuation of cardiomyopathy. Indeed, the intact parasites may coexist within the host tissue, even after trypanocidal treatment [2,14].

Results presented in this paper confirm the important residual morbidity of ChD in the remote areas, thus supporting political decisions that should prioritize in addition to epidemiological surveillance the medical treatment of CCC in the coming years. The SaMi-Trop cohort represents a major challenge for focused research in neglected diseases, with knowledge that can be applied in primary health care. The study has the potential to provide relevant information about the development and progression of ChD disease in remotes areas, with social and economic inequities.

STRENGTHS AND LIMITATIONS

The SaMi-Trop is one of the largest multicentre cohort study of ChD conducted in the world. It has the potential of identifying biomarkers that will be used to predict the risk of disease progression and death, as well as to permit comparative analysis with other similar cohorts. Most studies that evaluated biomarkers in ChD had a cross sectional design. The large number of patients included in this investigation is outstanding, especially in a rural and dispersed area. Our preliminary results confirmed the important residual morbidity of ChD in such remote areas and found that these patients are currently being under-treated. We hope that our findings will guide political decisions aiming at enhancing access to health care of ChD patients in the coming years.

Second, the SaMi-Trop cohort represents a major challenge for focused research in neglected diseases, with knowledge that can be applied in primary health care. The study has the potential to provide relevant information about the development and progression of ChD disease in remotes areas with social and economic inequalities. As pointed for Maguire[16], there is an urgent need for a new strategy for ChD treatment and studies for evaluation of results because the infected patients are aging, and time for them is running out.

One weakness of the study is that no data on the weight and height of patients or use of health services indicators were included at the baseline visit. Such information will be collected in the next follow-up visits. 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.

COLLABORATIONS

Collaborations in data analysis will be welcome through specific research proposals sent to individual SaMi-Trop investigators. Exchange of doctoral or postdoctoral fellows is very welcome.

The Dataset will be open access for two years at the end of the data collection process (August, 2018). In the meantime, applications to use the data should be made by

contacting the researchers of the SaMi-Trop cohort and filling up the application form. The questionnaires and interviewer guides of the baseline will also be available in electronic formats at http://www.ufsj.edu.br/tecnologiasemsaude_pesquisa/projetos.php

FUNDING

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COMPETING INTEREST STATEMENT

The authors have no financial relationships relevant to this article to disclose, nor any other type of conflict of interest.

CONTRIBUTORSHIP STATEMENT

The authors declare to have substantially contributed to conception and design or analysis and interpretation of data (Cardoso CS, Sabino EC, Oliveira CD, Ribeiro ALP); to have substantially contributed to drafting the article or revising it critically for important intellectual content; and have given the final approval of the version to be published (Cardoso CS, Sabino EC, Oliveira CD, Oliveira LC, Ferreira AM, Cunha-Neto E, Bierrenbach AL, Ferreira JE, Ana Haikal DS, Reingold AL, Ribeiro ALP).

REFERENCES

1.Bern C. Chagas' Disease. *N Engl J Med* 2015; 373:456-66.

2.Marin-Neto JA, Cunha-Neto E, Benedito CM et al. Pathogenesis of Chronic Chagas Heart Disease. *Circulation* 2007; 115:1109-1123.

3-Laranja FS, Dias E, Nobrega G et al. Chagas' disease: a clinical, epidemiologic and pathologic study. *Circulation* 1956; 14: 035–1059.

4.Sabino EC, Ribeiro AL, Salemi VM et al. National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study-II (REDS-II), International Component. Ten-year incidence of Chagas cardiomyopathy among asymptomatic Trypanosoma cruzi-seropositive former blood donors. *Circulation*. 2013 Mar 12;127(10):1105-15.

5.Nunes MC, Dones W, Morillo CA et al. Council on Chagas Disease of the Interamerican Society of Cardiology. Chagas disease: an overview of clinical and epidemiological aspects. *J Am Coll Cardiol* 2013; 27;62(9):767-76.

6.Alkmim MB, Figueira RM, Marcolino MS et al. Improving patient access to specialized health care: the Telehealth Network of Minas Gerais, Brazil. *Bull World Health Organ*. 2012 May 1;90(5):373-8.

7.Ribeiro AL, Marcolino MS, Prineas RJ et al. 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.

8.Goldman L, Hashimoto B, Cook EF et al. Comparative reproducibility and validity of systems for assessing cardiovascular functional class: advantages of a new specific activity scale. *Circulation* 1981;64(6):1227-34.

9.Fleck MPA, Louzada S, Xavier M et al. Aplicação da versão em português do instrumento WHOQOL-bref. *Rev Saude Publica* 2000;34(2):178-83.

10.Apolinario D, Braga RCOP, Magaldi RM et al. Short Assessment of Health Literacy for Portuguese-speaking Adults. *Rev Saúde Pública* 2012; 46 (4): 702-711.

11.Macfarlane PW, Latif S. Automated serial ECG comparison based on the Minnesota code. *J Electrocardiol* 1996; 29 Suppl:29-34.

12.Ribeiro AL, dos Reis AM, Barros MV *et al*. Brain natriuretic peptide and left ventricular dysfunction in Chagas' disease. *Lancet* 2002; 10;360(9331):461-2.

13.Lima-Costa MF, Cesar CC, Peixoto SV et 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; 15;172(2):190-6.

14.Morillo CA, Marin□Neto JA, Avezum A *et al*. Randomized Trial of Benznidazole for Chronic Chagas'Cardiomyopathy. *N Engl J Med* 2015;373(14):1295-306.

15.Mond HG, Proclemer A. The 11th world survey of cardiac pacing and implantable cardioverter-defibrillators: calendar year 2009--a World Society of Arrhythmia's project. *Pacing Clin Electrophysiol* 2011;34(8):1013-27.

16.Maguire H. Treatment of Chagas' Disease - Time Is Running Out. *N Engl J Med* 2015; 373(14):1369-70.

Table 1. Measurements obtained at different phases of the SaMi-Trop study.

Phase	Measurements
Baseline: 2013- 2014	Questionnaires with socio-demographic information, social determinants of health, health-related behaviours (smoking, alcohol consumption and physical activity), self-reported comorbidities, medication use, history of previous treatment for ChD, signs and symptoms, functional class (Cardiovascular Functional Class Scale)[8] and quality of life (WHO-QOL-Bref)[9]. Electrocardiogram Blood collection: immunassays, PCR (Polymerase chain reaction) for T. cruzi, NT-ProBNP (Brain Natriuretic Peptide).
Follow-up: 2015-2016	Questionnaires with socio-demographic information, social determinants of health, health-related behaviours, self-reported comorbidities, medication use, signs and symptoms since baseline. Functional class and quality of life. Vital status, history of health service utilization including hospitalizations[a], health literacy (Short Assessment of Health Literacy) [10]. Electrocardiogram Echocardiogram Blood collection: NT-ProBNP

Table 2- Socio-demographic characteristics of SAMI-TROP cohort members (N= 1959)

Variables	N	%
Sex		
Female	1.323	67.5
Male	636	32.5
Age		
<50 years	499	25.6
50 - 74 years	1.223	62.6
> 74 years	231	11.8
Number of household members		
1-3	1.106	56.6
4-6	709	36.3
7-17	138	7.1
Family income		
> US$327	1.037	53.1
≤ US$327	916	46.9
Skin colour		
Mixed	1.144	58.6
White	426	21.8
Black	348	17.8
Others	32	1.8
Years of school		
1 to 4 year	862	44.2
Illiterate	670	34.4
5 to 8 years	320	16.4
Other	98	5.0
Marital status		
Married or living with partner	1.238	63.4
Widower	449	23.0
Single	176	9.0
Divorced	90	4.6
Self-perception of health		
Very good	57	2.9
Good	499	25.8
Average	1.116	57.7
Bad or very bad	264	13.6

Small differences in total N for each variable are due to missing values.
Others in Skin colours include Asians (27) and Native Americans (5)
Others in Educational level included: Elementary school (81) and Graduate school (17)
Dollar quotation from July/2013

Table 3. Distribution of patients according to self-reported Chagas Disease, cardiovascular functional class and ProBNP results in the SAMI-TROP study.

Variables	Valid N	N	%
Chagas disease self-reported	1.955		
Yes		1,870	95.6
No		64	3.3
No response		21	1.1
Duration of Chagas disease (years)	1.896		
> 10		1,179	62.2
1-10		695	36.6
< 1		22	1.2
Chagas disease in another family member	1.947		
Yes		1,384	71.1
No		384	19.7
Don't know		179	9.2
Previous treatment for Chagas disease	1.953		
Yes		1,008	51.6
No		873	44.7
Don't know		72	3.7
Previous use of Benznidazole medicine	1.955		
No		1,320	67.5
Yes		492	25.2
Don't know		143	7.3
NYHA functional classification	1.931		
I		1,059	54.8
II or more		872	45,2
ProBNP level	1.955		
<300		1,368	70.2
>=300		581	29.8

Table 4- Prevalence of comorbid conditions, selected behavioural characteristics, medications used, signs and symptoms and self-reported health in the SaMi-Trop cohort.

Variables	Valid N	N	%
Comorbid conditions	1.959		
High serum cholesterol		785	40.1
Hypertension		706	36.0
Diabetes mellitus		198	10.1
Thyroid disorder		159	8.1
Kidney disease		143	7.3
Leishmaniosis		22	1.2
Behavioural characteristics	1.945		
Physical activity last week (minimal 30 min)		434	22.3
Alcohol last month		318	16.2
Current smoking		143	7.3
Number of medicine in use	1.959		
0		589	30.1
1-2		714	36.4
3-4		538	27.5
≥ 5		118	6.0
Medicine in use (Yes)	1.940		
Diuretics		951	49.1
Angiotensin converting enzyme-ACE		553	28.6
Angiotensin receptor blockers-ARBs		550	28.4
Aspirin		507	26.2
Amiodarone		429	22.0
Carvedilol		380	19.6
Digoxin		140	7.2
β-blockers		140	7.2
Vasodilators		84	4.3
Warfarin		11	0.6
Signs and symptoms or self-reported conditions (Yes)	1.924		
Heartbeat racing or beating abnormally		1.222	63.5
Prolonged faintness or dizziness		1.203	62.5
Problems on electrocardiogram		1.180	61.3
Heart palpitations		1.174	61.0
Short of breath during physical exercises		1.143	59.4
Heartbeat racing at rest		1.015	52.8
Heartbeat not regular		902	46.9
Difficulty breathing when lying down		752	39.1
Unable to climb two flights of stairs		749	38.9
Awake during the night unable to breath		683	35.5
Trouble swallowing		599	31.1
Swelling or puffiness of the feet in the morning		502	26.1
No bowel movement for three or more days		478	24.8
Fainting or loss of consciousness		429	22.0
Visible neck veins when standing up or sitting		409	21.3
Pain when swallowing food		342	17.8
Pacemaker		110	6.2

Megaesophagus	117	6.1

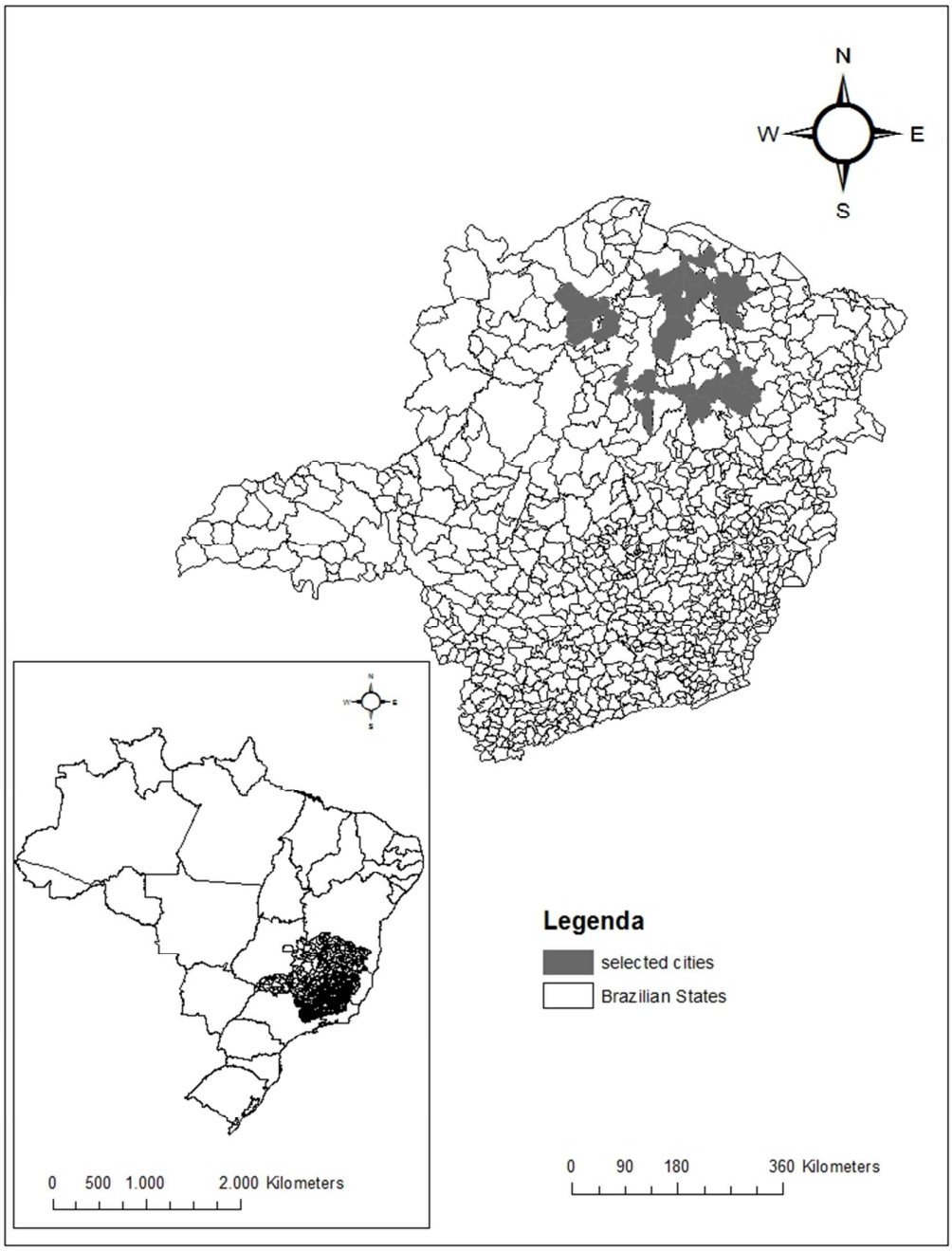

Figure 1. Geographical location of the 21 cities included in the SAMI-TROP project. Minas Gerais, Brazil.

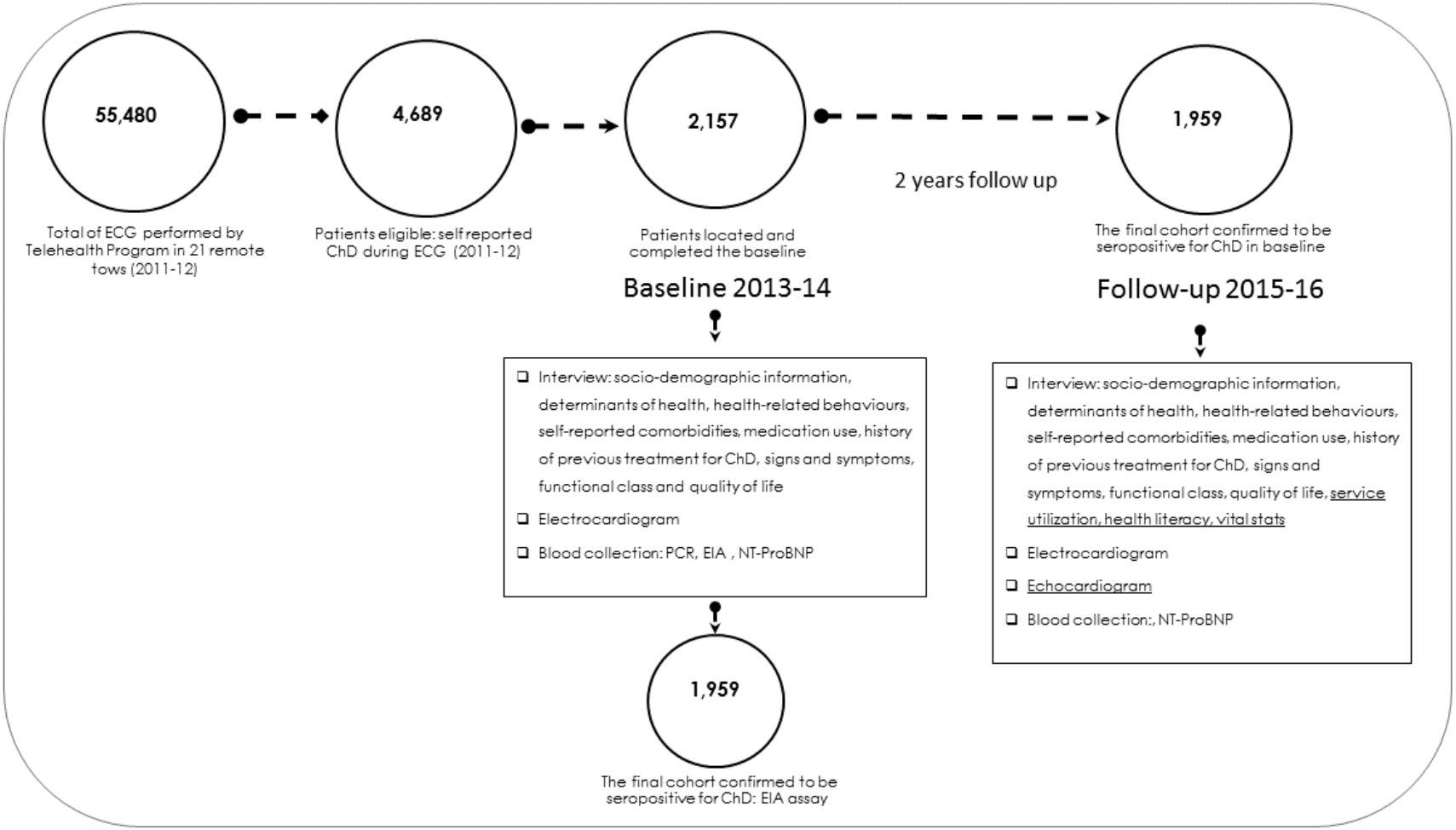

Figure 2. The SAMI-TROP project diagram

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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 participants.

Participants: The study is being conducted in 21 municipalities of the northern part of Minas Gerais state in Brazil, and includes a follow up of two years. The baseline evaluation included collection of socio-demographic information, social determinants of health, health-related behaviors, comorbidities, medicines in use, history of previous treatment for Chagas disease, functional class, quality of life, blood sample collection and ECG. Patients were mostly female, aged 50-74 years, with low family income and educational level, with known Chagas Disease for >10 years; 46% presented with FC > I. Previous use of benznidazole was reported by 25.2% and permanent use of pacemaker by 6.2%. Almost half of the patients presented with high blood cholesterol and hypertension and one third of them had diabetes mellitus. NT-ProBNP level were >300 (pg/Ml) in 30% of the sample.

Finding to date: Clinical and laboratory markers predictive of severe and progressive Chagas Disease were identified, as high NT-ProBNP levels, as well as symptoms of advanced heart failure. These results confirm the important residual morbidity of Chagas Disease in the remote areas, thus supporting political decisions that should prioritize in addition to epidemiological surveillance the medical treatment of chronic Chagas cardiomyopathy in the coming years. The SaMi-Trop represents a major challenge for focused research in neglected diseases, with knowledge that can be applied in primary health care.

Future plans: We will continue following this patients' cohort to provide relevant information about the development and progression of Chagas Disease in remotes areas, with social and economic inequities.

Registration: NCT02646943

INTRODUCTION

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi,* remains one of the most neglected diseases in the world, with 8-10 million infected. The most important consequence of Chagas disease is chronic Chagas cardiomyopathy, which occurs in 20 to 40% of infected persons [1-4], with an incidence rate of 1.85% person-year [4].

Chronic Chagas cardiomyopathy comprises a wide range of manifestations, including heart failure, arrhythmias, heart blocks, sudden death, thromboembolism and stroke [5]. 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 [6]. 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 [7]. When heart failure and/or severe arrhythmias manifest, the prognosis is ominous, with high and premature mortality rates, typically in adult male patients [8], but also in the elderly [9]. Indeed, when compared to patients with idiopathic cardiomyopathy, patients with chronic Chagas cardiomyopathy have poorer survival, irrespective of other clinical and echocardiographic parameters [10].

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 [5]. Risk scores have been developed [11-13], including a validated one [11]. However, current risk scores rely on the availability of several diagnostic tests, including Holter monitoring, stress testing, echocardiographic examination and chest X-ray [11], or special exams, as signal averaged ECG [12,13]. 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 [14,15], 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.

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.

COHORT DESCRIPTION

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. The SaMi-Trop project is a prospective cohort study with at least two years of follow up, including one visit at baseline and another at 24 months. The cohort of chronic Chagas cardiomyopathy patients was established, using patients under the care of the Telehealth Network of Minas Gerais, a program designed to support primary care in Minas Gerais State, Brazil [16]. In this program, all patients' electrocardiogram (ECG) and clinical data are sent to a central reading unit centre that also collects clinical data, such as history of Chagas Disease. 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 chronic Chagas cardiomyopathy patients was expected to be high (Figure 1).

(Figure 1 here)

Eligible patients were selected based on the ECG results performed in 2011-12 by the Telehealth Network, which from now on will be called index ECG. Only patients who fulfilled all of the following inclusion criteria were selected: A) self-reported Chagas Disease; B) presence of the following abnormalities on the index ECG [17]: possible old myocardial infarction (major Q wave abnormalities or minor Q waves abnormalities with ST segment or T-wave abnormalities), complete intraventricular block (right, left or unspecified), frequent supraventricular or ventricular premature beats, major isolated

ST segment or T-wave abnormalities, atrial fibrillation or flutter or supraventricular tachycardia or other major arrhythmias, major atrioventricular conduction abnormalities or pacemaker use, or major QT prolongation (QT index>115%), left or right ventricular hypertrophy and; C) age of 19 years or more. The exclusion criteria included pregnancy or breast-feeding and any life threatening disease with an ominous prognosis that suggested a life expectancy of less than two years.

The simple size was calculated considering the minimal number of events per variable acceptable in a proportional hazards regression analysis of 10 events per variable [18], 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 was 2,000 subjects.

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. The patient distribution by municipalities and it distance from the reference center in health is presented in table 1.

Table 1. Distribution of patients including in SaMi_Trop cohort according to the municipality and distance to the reference center, Montes Claros (n=1,959).

Municipalities	Number	%	Distance (km)
São Francisco	325	16.6	163
Carbonita	202	10.3	203
Minas Novas	164	8.4	289
Janaúba	166	8.5	134
Turmalina	131	6.7	264
Bocaiúva	128	6.5	47
Chapada do Norte	122	6.2	295
Berilo	113	5.8	333
Porteirinha	71	3.6	170
Brasília de Minas	71	3.6	105
Fruta de Leite	68	3.5	186
Claros dos Poções	62	3.2	79,5
Verdelândia	69	3.5	173
Pai Pedro	56	2.9	185
Ubaí	54	2.8	153
Leme do Prado	42	2.1	273
Francisco Sá	38	1.9	52
Rio Pardo de Minas	28	1.4	276
Jenipapo de Minas	19	1.0	369
Francisco Badaró	16	0.8	347
Monte Azul	14	0.7	244
Total	**1,959**	**100.0**	**--**

The cohort will be followed for two years, until primary outcome or loss follow-up. The primary outcome is death and the secondary outcomes are changes in the ECG pattern and hospitalization due to cardiovascular complications. Ascertainment of the occurrence of deaths will be done using the National Mortality Information System (SIM) from the Ministry of Health.

Table 2 summarizes the types of data collected at baseline and at that will be collected the 2-year follow up visit. All eligible participants were recruited by the family health program team. The baseline visit was performed at Public Health Primary Care Units by previously trained staff. The patients were interviewed using a standardized questionnaire, had a blood sample collected and an ECG evaluation. The data were

collected electronically and sent to the data center at the University of São Paulo via a Web-based System.

Table 2. Measurements obtained at different phases of the SaMi-Trop study.

Phase	Measurements
Baseline: 2013- 2014	Questionnaires with socio-demographic information, social determinants of health, health-related behaviours (smoking, alcohol consumption and physical activity), self-reported comorbidities, medication use, history of previous treatment for Chagas Disease, signs and symptoms, functional class (Cardiovascular Functional Class Scale) [19] and quality of life (WHO-QOL-Bref) [20]. Electrocardiogram Blood collection: immunassays, PCR (Polymerase chain reaction) for T. cruzi, NT-ProBNP (Brain Natriuretic Peptide).
Follow-up: 2015-2016	Questionnaires with socio-demographic information, social determinants of health, health-related behaviours, self-reported comorbidities, medication use, signs and symptoms since baseline. Functional class and quality of life. Vital status, history of health service utilization including hospitalizations[a], health literacy (Short Assessment of Health Literacy) [21]. Electrocardiogram Echocardiogram Blood collection: NT-ProBNP

A resting 12-lead ECG was recorded using an ECG PC machine (TEB, São Paulo, Brazil). The ECG recordings were sent electronically to the Telehealth system in order to be read by a trained cardiologist and the written report was subsequently returned to the patient's physician. For research purposes, ECGs were also automatically analyzed using The University of Glasgow ECG analysis program (release 28.5, issued on January 2014) and reviewed by trained cardiologists to ensure quality control. ECGs will be classified using the Minnesota code criteria using variables derived from the median complex of the Glasgow University software measurement matrix [22].

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.

Brazilian Mortality Information (SIM) data will be used to ascertain patients' vital status after the follow-up period as well as the underlying causes of death, which are coded under the International Classification of Disease (ICD-10).

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.

This cohort study is a component of a larger study to evaluate biomarkers of Chagas Disease sponsored by a grant from NIAID/NIH Neglected Tropical Disease Centre. It was approved by the Institutional Review Board, number 179.685/2012 (National Commission of Ethics in Research, CONEP).

CHARACTERISTICS OF THE STUDY POPULATION

Of the 55.480 ECG performed in the 21 selected municipalities from 2011 to 2012, a total of 4,689 patients were eligible for the study and 2,157 were located and completed the baseline assessment in 2013-14. In comparison to the eligible group, the participants had a higher percentage of women (67.1% *versus* 59.9% p<0.01) and were younger (59.5 years *versus* 60.7 years, p<0.01). The final cohort consists of 1,959 (90.8%) participants confirmed to be seropositive for Chagas Disease (Figure 2).

(Figure 2).

Table 3 shows the main socio-demographic characteristics, socio-economic categories and self-perception of health of study participants in baseline. Most patients were female (67.5%), aged between 50 and 74 years (62.6%), sharing the same household with two other people or less (56.6%) and had a family monthly income of US$327. The educational level was very low with 38.7% having between 1-4 years of school and 34.4% never having attended school. Cohort members self-reported their health status as average (57.7%) or good (25.8%).

Table 3- Socio-demographic characteristics of SAMI-TROP cohort members (N= 1959)

Variables	N	%
Sex		
Female	1.323	67.5
Male	636	32.5
Age		
<50 years	499	25.6
50 - 74 years	1.223	62.6
> 74 years	231	11.8
Number of household members		
1-3	1.106	56.6
4-6	709	36.3
7-17	138	7.1
Family monthly income data		
> US$327	1.037	53.1
≤ US$327	916	46.9
Skin colour		
Mixed	1.144	58.6
White	426	21.8
Black	348	17.8
Others	32	1.8
Years of school		
1 to 4 year	862	44.2
Illiterate	670	34.4
5 to 8 years	320	16.4
Other	98	5.0
Marital status		
Married or living with partner	1.238	63.4
Widower	449	23.0
Single	176	9.0
Divorced	90	4.6
Self-perception of health		

Very good	57	2.9
Good	499	25.8
Average	1.116	57.7
Bad or very bad	264	13.6

Small differences in total N for each variable are due to missing values.
Others in Skin colours include Asians (27) and Native Americans (5)
Others in Educational level included: Elementary school (81) and Graduate school (17)
Dollar quotation from July/2013

The majority of the patients self-reported that they have had Chagas Disease for over 10 years and that they had at least one family member with a history of Chagas Disease. Based on the New York Heart Association (NYHA) Functional Classification, 45.9% of the patients were classified as level II or more (i.e. have symptoms of heart failure). Among Chagas Disease patients in the cohort 6.2% reported permanent use of a pacemaker. Previous treatment for Chagas Disease was reported by 51.6% of Chagas Disease patients (25.2%), including 492 who reported previous treatment with benznidazole. The NT-ProBNP level was > 300 pg/mL in 30% of the sample (Table 4).

Table 4. Distribution of patients according to self-reported Chagas Disease, cardiovascular functional class and ProBNP results in the SAMI-TROP study.

Variables	**Valid N**	**N**	**%**
Chagas disease self-reported	1.955		
Yes		1,870	95.6
No		64	3.3
No response		21	1.1
Duration of Chagas disease (years)	1.896		
> 10		1,179	62.2
1-10		695	36.6
< 1		22	1.2
Chagas disease in another family member	1.947		
Yes		1,384	71.1
No		384	19.7
Don't know		179	9.2
Previous treatment for Chagas disease	1.953		
Yes		1,008	51.6
No		873	44.7
Don't know		72	3.7
Previous use of Benznidazole medicine	1.955		
No		1,320	67.5
Yes		492	25.2
Don't know		143	7.3
NYHA functional classification	1.931		
I		1,059	54.8

II or more		872	45,2
ProBNP level	1.955		
<300		1,368	70.2
>=300		581	29.8

As seen in Table 5, the prevalence of one or more self-reported comorbid conditions at baseline was high, including high serum cholesterol (40.1%), hypertension (36.0%), diabetes mellitus (10.1%), thyroid disorder (8.1%) and kidney disease (7.3%). leishmaniasis was reported by 22 patients (1.2%). Only 22.3% of patients reported having performed any physical activity during the prior week, 16.2% reported having drunk alcohol in the previous month, and 7.3% reported that they were current smokers. In terms of medications, 36.4% of patients reported the current use of one or two medicines, while 30.1% reported no current use of any. The most common medicines being used were diuretics (49.1%), angiotensin converting enzyme-ACE (28.6%), angiotensin receptor blockers-ARBs (28.4%), aspirin (26.2%) and amiodarone (22%).

Considering the fact that almost half of the patients were in functional class II or more, with 30% with BNP levels higher than 300 units/l, there is an overall low usage of the recommended drugs for heart failure, in particular of beta-blockers. This may explain at least in part the high frequency of cardiac symptoms reported: 63.5% had heartbeat racing, 62.5% had prolonged faintness or dizziness, 61.3% had an abnormal ECG, 61.0% had heart palpitations and 59.4% had shortness of breath at exercises. The relative high frequency of amiodarone use may be related to the high frequency of cardiac arrhythmias in chronic Chagas cardiomyopathy, as well as the established practice, in Brazil, to prescribe amiodarone to prevent sudden death.

Table 5- Prevalence of comorbid conditions, selected behavioural characteristics, medications used, signs and symptoms and self-reported health in the SaMi-Trop cohort.

Variables	Valid N	N	%
Comorbid conditions	1.959		
High serum cholesterol		785	40.1
Hypertension		706	36.0
Diabetes mellitus		198	10.1
Thyroid disorder		159	8.1
Kidney disease		143	7.3
Leishmaniosis		22	1.2
Behavioural characteristics	1.945		
Physical activity last week (minimal 30 min)		434	22.3
Alcohol last month		318	16.2
Current smoking		143	7.3
Number of medicine in use	1.959		
0		589	30.1
1-2		714	36.4
3-4		538	27.5
≥ 5		118	6.0
Medicine in use (Yes)	1.940		
Diuretics		951	49.1
Angiotensin converting enzyme-ACE		553	28.6
Angiotensin receptor blockers-ARBs		550	28.4
Aspirin		507	26.2
Amiodarone		429	22.0
Carvedilol		380	19.6
Digoxin		140	7.2
β-blockers		140	7.2
Vasodilators		84	4.3
Warfarin		11	0.6
Signs and symptoms or self-reported conditions (Yes)	1.924		
Heartbeat racing or beating abnormally		1.222	63.5
Prolonged faintness or dizziness		1.203	62.5
Problems on electrocardiogram		1.180	61.3
Heart palpitations		1.174	61.0
Short of breath during physical exercises		1.143	59.4
Heartbeat racing at rest		1.015	52.8
Heartbeat not regular		902	46.9
Difficulty breathing when lying down		752	39.1
Unable to climb two flights of stairs		749	38.9
Awake during the night unable to breath		683	35.5
Trouble swallowing		599	31.1
Swelling or puffiness of the feet in the morning		502	26.1
No bowel movement for three or more days		478	24.8
Fainting or loss of consciousness		429	22.0
Visible neck veins when standing up or sitting		409	21.3
Pain when swallowing food		342	17.8
Pacemaker		110	6.2

Megaesophagus	117	6.1

FINDINGS TO DATE

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. The NT-ProBNP level was > 300 pg/mL in 30% of the sample. High circulating levels of natriuretic peptides are related to the presence of left ventricular dysfunction [23] and higher risk of death [24].

Among Chagas Disease patients in the cohort, 6.2% reported permanent use of a pacemaker. This percentage is far below the 14.0% found in another recently published Brazilian cohort study of patients with Chagas Disease [25]. However, Chagas Disease is still a major cause of use of pacemakers and defibrillators in Brazil, even surpassing the indications due to coronary artery disease in some regions. The literature to pointed the under-use of this device in Brazil when compared to other countries [26], which is unfortunately what we expected to find in the remote regions represented in the current.

In this large multicentre cohort, previous use of benznidazole was reported by one quarter of the patients. We well know that the persistence of T cruzi is directly related in the pathology of the chronic phase, but it remains to be proved that parasite load reduction by trypanocidal treatment leads concomitant attenuation of cardiomyopathy [2, 25]. In the recently released BENEFIT trial [25], that included Brazilian patients, treatment with benznidazole did not significantly reduce cardiac clinical deterioration through 5 years of follow-up in Chagas cardiomyopathy.

Although *T. cruzi* infection does not have a sexual predilection [27], studies show higher prevalence among women [28,29]. 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 [30] as well as greater availability to participate in scientific studies, especially those with longitudinal component, such as in this investigation.

Results presented in this paper confirm the important residual morbidity of Chagas Disease in the remote areas, thus supporting political decisions that should prioritize in addition to epidemiological surveillance the medical treatment of chronic Chagas

cardiomyopathy in the coming years. The SaMi-Trop cohort represents a major challenge for focused research in neglected diseases, with knowledge that can be applied in primary health care. The study has the potential to provide relevant information about the development and progression of Chagas Disease disease in remotes areas, with social and economic inequities.

STRENGTHS AND LIMITATIONS

The SaMi-Trop is one of the largest multicentre cohort study of Chagas Disease conducted in the world. It has the potential of identifying biomarkers that will be used to predict the risk of disease progression and death, as well as to permit comparative analysis with other similar cohorts. Most studies that evaluated biomarkers in Chagas Disease had a cross sectional design. The large number of patients included in this investigation is outstanding, especially in a rural and dispersed area. Our preliminary results confirmed the important residual morbidity of Chagas Disease in such remote areas and found that these patients are currently being under-treated. We hope that our findings will guide political decisions aiming at enhancing access to health care of Chagas Disease patients in the coming years.

Second, the SaMi-Trop cohort represents a major challenge for focused research in neglected diseases, with knowledge that can be applied in primary health care. The study has the potential to provide relevant information about the development and progression of Chagas Disease disease in remotes areas with social and economic inequalities. As pointed for Maguire [31], there is an urgent need for a new strategy for Chagas Disease treatment and studies for evaluation of results because the infected patients are aging, and time for them is running out.

One weakness of the study is that no data on weight and height about the use of health services indicators were included at the baseline. 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. 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.

COLLABORATIONS

Collaborations in data analysis will be welcome through specific research proposals sent to individual SaMi-Trop investigators. Exchange of doctoral or postdoctoral fellows is very welcome.

The Dataset will be open access for two years at the end of the data collection process (August, 2018). In the meantime, applications to use the data should be made by contacting the researchers of the SaMi-Trop cohort and filling up the application form. The questionnaires and interviewer guides of the baseline will also be available in electronic formats at http://www.ufsj.edu.br/tecnologiasemsaude_pesquisa/projetos.php

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COMPETING INTEREST STATEMENT

The authors have no financial relationships relevant to this article to disclose, nor any other type of conflict of interest.

CONTRIBUTORSHIP STATEMENT

The authors declare to have substantially contributed to conception and design or analysis and interpretation of data (Cardoso CS, Sabino EC, Oliveira CD, Ribeiro ALP); to have substantially contributed to drafting the article or revising it critically for important intellectual content; and have given the final approval of the version to be published (Cardoso CS, Sabino EC, Oliveira CD, Oliveira LC, Ferreira AM, Cunha-Neto E, Bierrenbach AL, Ferreira JE, Ana Haikal DS, Reingold AL, Ribeiro ALP).

REFERENCES

1-Bern C. Chagas' Disease. *N Engl J Med* 2015; 373:456-66.

2-Marin-Neto JA, Cunha-Neto E, Benedito CM et al. Pathogenesis of Chronic Chagas Heart Disease. *Circulation* 2007; 115:1109-1123.

3-Laranja FS, Dias E, Nobrega G et al. Chagas' disease: a clinical, epidemiologic and pathologic study. *Circulation* 1956; 14: 035–1059.

4-Sabino EC, Ribeiro AL, Salemi VM et al. National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study-II (REDS-II), International Component. Ten-year incidence of Chagas cardiomyopathy among asymptomatic Trypanosoma cruzi-seropositive former blood donors. *Circulation* 2013;12;127(10):1105-15.

5-Nunes MC, Dones W, Morillo CA et al. Council on Chagas Disease of the Interamerican Society of Cardiology. Chagas disease: an overview of clinical and epidemiological aspects. *J Am Coll Cardiol* 2013; 27;62(9):767-76.

6-Rocha MO, Teixeira MM, Ribeiro AL. An update on the management of Chagas cardiomyopathy. *Expert Rev Anti Infect Ther* 2007; 5(4):727-743.

7-MS - Ministério da Saúde 2005. Consenso brasileiro em doença de Chagas. *Rev Soc Bras Med Trop* 2005; 38 (3): 1-29.

8-Rocha MO, Ribeiro AL, Teixeira MM. Clinical management of chronic Chagas cardiomyopathy. *Front Biosci.* 2003; 8:e44-e54.

9-Lima-Costa MF, Peixoto SV, Ribeiro AL. Chagas disease and mortality in old age as an emerging issue: 10 year follow-up of the Bambui population-based cohort study (Brazil). *Int J Cardiol.* 2010; 145(2):362-363.

10-Pereira N, Barbosa MM, Ribeiro AL *et al*. Predictors of Mortality in Patients with Dilated Cardiomyopathy: Relevance of Chagas Disease as an Etiological Factor. *Rev Esp Cardiol* 2010; 63(7):788-797.

11-Rassi A Jr, Rassi A, Little WC *et al.* Development and validation of arisk score for predicting death in Chagas' heart disease. *N Engl J Med*. 2006 Aug 24;355(8):799-808.

12-Ribeiro AL, Cavalvanti PS, Lombardi F *et al.* MO. Prognostic value of signal-averaged electrocardiogram in Chagas disease. *JCardiovasc Electrophysiol*. 2008;19(5):502-9.

13-Benchimol-Barbosa PR, Tura BR, Barbosa EC *et al*. Utility of a novelrisk score for prediction of ventricular tachycardia and cardiac death in chronicChagas disease - the SEARCH-RIO study. *Braz J Med Biol Res.* 2013; 46(11):974-984.

14-Bautista-López NL, Morillo CA, López-Jaramillo P *et al*. Matrix metalloproteinases 2 and 9 as diagnostic markers in the progression to Chagas cardiomyopathy. *Am Heart J*. 2013; 165:558–566.

15- Ferreira LR, Frade AF, Santos RH *et al*. MicroRNAs miR-1, miR-133a, miR-133b, miR-208a and miR-208b are dysregulated in Chronic Chagas disease Cardiomyopathy. *Int J Cardiol*. 2014; 175(3):409-17.

16-Alkmim MB, Figueira RM, Marcolino MS *et al*. Improving patient access to specialized health care: the Telehealth Network of Minas Gerais, Brazil. *Bull World Health Organ*. 2012 May 1;90(5):373-8

17-Ribeiro AL, Marcolino MS, Prineas RJ *et al*. 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.

18-Peduzzi P, Concato J, Feinstein AR *et al*. Importance of events per independent variable in proportional hazards regression analysis II. Accuracy and precision of regression estimates. *J Clin Epidemiol* 1995; 48(12):1503-1510.

19-Goldman L, Hashimoto B, Cook EF et al. Comparative reproducibility and validity of systems for assessing cardiovascular functional class: advantages of a new specific activity scale. *Circulation* 1981;64(6):1227-34.

20-Fleck MPA, Louzada S, Xavier M et al. Aplicação da versão em português do instrumento WHOQOL-bref. *Rev Saude Publica* 2000;34(2):178-83.

21-Apolinario D, Braga RCOP, Magaldi RM et al. Short Assessment of Health Literacy for Portuguese-speaking Adults. *Rev Saúde Pública* 2012; 46 (4): 702-711.

22-Macfarlane PW, Latif S. Automated serial ECG comparison based on the Minnesota code. *J Electrocardiol* 1996; 29 Suppl:29-34.

23-Ribeiro AL, dos Reis AM, Barros MV *et al*. Brain natriuretic peptide and left ventricular dysfunction in Chagas' disease. *Lancet* 2002; 10;360(9331):461-2.

24-Lima-Costa MF, Cesar CC, Peixoto SV *et 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; 15;172(2):190-6.

25-Morillo CA, Marin‐Neto JA, Avezum A *et al*. Randomized Trial of Benznidazole for Chronic Chagas'Cardiomyopathy. *N Engl J Med* 2015;373(14):1295-306.

26-Mond HG, Proclemer A. The 11th world survey of cardiac pacing and implantable cardioverter-defibrillators: calendar year 2009--a World Society of Arrhythmia's project. *Pacing Clin Electrophysiol* 2011;34(8):1013-27.

27-Kirchhoff LV. Chagas Disease (American Trypanosomiasis): Background, Pathophysiology, Epidemiology. 2014. *Medscape reference. Drugs, Diseases & procedures*. Available: http://emedicine.medscape.com/article/214581-overview#a6

28-Matos CS, Santos JJE, Medeiros FAC *et al*. Current situation and perspectives regarding human Chagas disease in midwestern of the state of Minas Gerais, Brazil. *Mem. Inst. Oswaldo Cruz*. 2014; 109 (3): 374-378.

29-Marcolino MS, Palhares DM, Ferreira LR *et al*. Electrocardiogram and Chagas Disease A Large Population Database of Primary Care Patients. *Global Heart*. 2015; 10 (3): 167-172.

30-Travassos C, Viacava F, Pinheiro R *et al*. Utilização dos serviços de saúde no Brasil: gênero, características familiares e condição social. *Rev Panam Salud Publica*. 2002;11(5-6): 365-373.

31-Maguire JH. Treatment of Chagas' Disease - Time Is Running Out. *N Engl J Med* 2015; 373(14):1369-70.

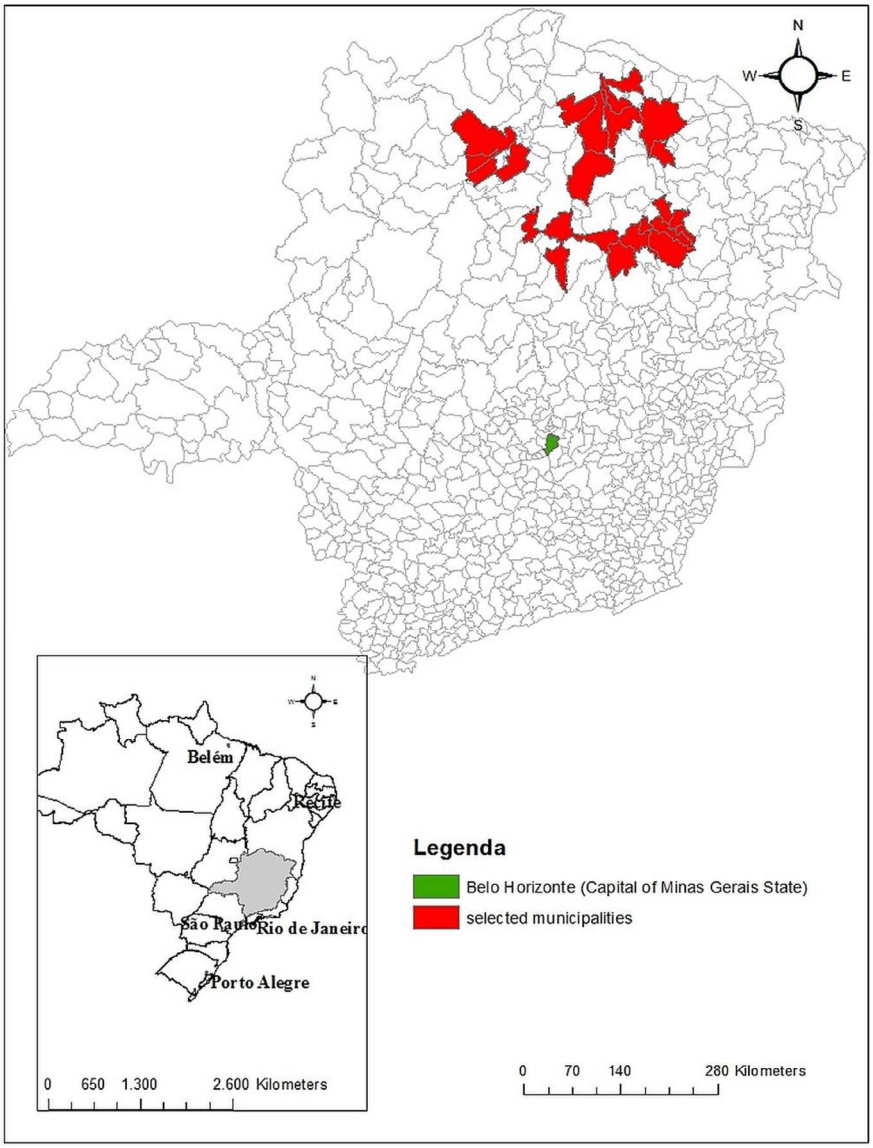

Figure 1. Geographical location of the 21 municipalities included in the SAMI-TROP project. Minas Gerais, Brazil.
90x116mm (300 x 300 DPI)

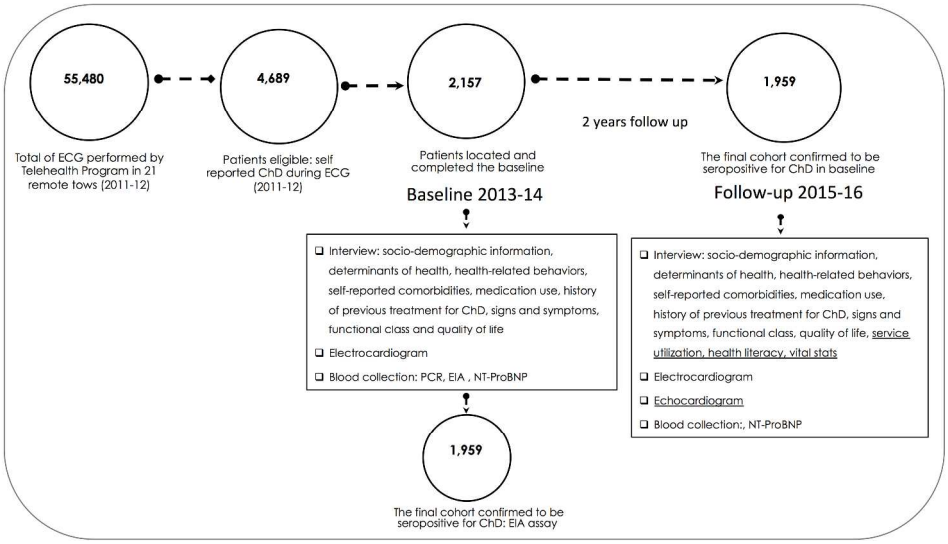

Figure 2. The SAMI-TROP project diagram
324x174mm (300 x 300 DPI)