A Population-Based Study of the Association between *Trypanosoma cruzi* Infection and Cognitive Impairment in Old Age (The Bambuí Study)

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Abstract

Background: Limited clinical data suggest that chronic *Trypanosoma cruzi* infection, which causes Chagas' disease (ChD), is associated with cognitive impairment. This study investigated this association in a large population-based sample of older adults. ***Methods:*** Participants in this cross-sectional study comprised 1,449 persons aged ≥60 years from a Brazilian endemic area (Bambuí). Cognitive functioning was ascertained by the Mini-Mental State Examination (MMSE), considering its score in percentiles [≤14 (<5th percentile), 15–22 (5th to <25th) and ≥23]. Hypothesized risk factors were *T. cruzi* infection, ChD-related electrocardiographic (ECG) abnormalities and use of digoxin medication. Potential confounders included depressive symptoms, smoking, stroke, hemoglobin, HDL cholesterol, blood glucose, systolic blood pressure, and use of psychoactive medication. ***Results:*** The prevalence of *T. cruzi* infection was 37.6%. There was a graded and independent association between infection and the MMSE score (adjusted odds ratios estimated by ordinal logistic regression = 1.99; 95% CI 1.43–2.76). No significant associations between the MMSE score and ECG abnormalities or digoxin medication use were found. ***Conclusions:*** This study provides for the first time epidemiological evidence of an association between *T. cruzi* infection and cognitive impairment which was not mediated by either ChD-related ECG abnormalities or digoxin medication use.

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Introduction

Chagas' disease (ChD), which is caused by the protozoan *Trypanosoma cruzi*, is transmitted by the bite of infected bloodsucking triatomine insects, blood transfusion, organ transplantation, contaminated food and transplacentally [1]. It is endemic in South and Central American countries, with about 8 million infected people [2] and 14,000 annual deaths, contributing with 667,000 years of life lost [3]. Heart involvement is the major feature [1, 4]. There is no effective treatment for the chronic phase of ChD and no immunoprophylaxis is available [5]. Since early 1990s, successful interventions have been undertaken to interrupt the ChD transmission in various regions of the Americas. The control strategy is mostly based on the control of domiciliated insect vectors (responsible for most of the transmission in the Southern

Cone of South America) and systematic screening of blood donors [2]. Brazil, Chile and Uruguay have been declared free of ChD transmission due to *Triatoma infestans*, the main domiciliated vector in these countries [2]. The complete eradication of *T. cruzi* infection is unlikely because the protozoan is largely spread in sylvatic ectopes all over the American Continent, and continuous transmission is assured via the sylvatic cycle [5].

As control interventions become more successful, the impact of *T. cruzi* is increasingly seen in older adults, through the operation of a cohort effect. Twenty years after the interruption of transmission of *T. cruzi* infection in an endemic area in Brazil (Bambuí), the infection was no longer seen in young people, but was highly prevalent in old ages [6]. Brazilian hospitalization and mortality statistics also show that a substantial proportion of the burden of *T. cruzi* infection affects older adults [7]. Rapid demographic ageing in Latin America will lead to increases in the number of older adults who are already infected by *T. cruzi*. Most studies of the natural history of *T. cruzi* infection have focused upon younger people [1, 4], and the consequences of *T. cruzi* infection in the elderly have received little attention.

Clinical data from Argentine suggest that chronic *T. cruzi* infection is associated with cognitive impairment. The neuropsychological performance of 45 chronic chagasic patients was compared with those of 26 controls matched by age, education and years of residence in an endemic area. ChD subjects showed lower Mini-Mental State Examination (MMSE) score, and poor orientation and attention. ChD was also associated with lower Wechsler Adult Intelligence Scale; digit symbol, picture completion, picture arrangement and object assembly were the most affected performance subtests. The authors concluded that the association between ChD and cognitive dysfunction was suggestive of white matter disease [8].

The association between cognitive impairment and ChD is biologically plausible. Congestive heart failure and thromboembolism are manifestations of severe ChD [1, 4, 9, 10], and there is some evidence for an association with cerebrovascular disease [11]. Autoimmunity is an established feature of ChD and the presence of autoantibodies against muscarinic receptors contributes to the pathogenesis of ChD cardiac dysautonomia, probably by desensitization and/or downregulation and progressive blockade of neurotransmitter receptors [12–14]. Furthermore, some studies have suggested that autoantibodies against muscarinic receptors might also be involved in the pathogenesis of Alzheimer's disease [15, 16].

In the present study, we sought to investigate the association between chronic *T. cruzi* infection and cognitive impairment in a large, community-based sample of older adults living in an endemic area in Brazil.

Methods

Setting
The study was conducted in Bambuí city (15,000 inhabitants), which is situated in the Southeast of Brazil. Bambuí is one of the oldest known endemic areas for ChD. The first experiments using insecticides to control the disease were developed in this area and these successfully interrupted the transmission by 1970 [6], some decades earlier than for the rest of the country.

Study Population
This analysis was based upon the baseline survey of the Bambuí Health Aging Study, which is a community-based prospective cohort study of adverse health outcomes in older adults. A complete census was carried out in Bambuí city for enumeration and identification of inhabitants; 1,606 out of all 1,742 residents aged 60 or more years participated in the baseline study [17].

Cognitive Functioning
Cognitive functioning was ascertained using a Brazilian validated version of the MMSE [18, 19]. The MMSE score is influenced by the schooling level [20], and there is no consensual validated cut-off point to be used in individuals with low schooling level. Because low schooling level predominated in the study population, cognitive functioning was categorized by the percentile distribution of the MMSE score. Subjects with scores bellow the 5th percentile were considered as cases with a high probability of cognitive impairment and scores between the 5th and below the 25th percentile were considered as possible cases.

Measures Related to T. cruzi Infection
Infection with *T. cruzi* was assessed by two different methods run in parallel. Infection was defined by seropositivity in both indirect hemagglutination assay and enzyme-linked immunoabsorbent assay (Biolab-Mérieux and Abbott, respectively). The agreement (Cohen's kappa) between these examinations in the study population was 0.98 (95% CI 0.93–1.00).

Electrocardiographic Abnormalities
The 12-lead electrocardiographic (ECG) recordings were made on all participants (Hewlett Packard MI700A, USA). Reading was performed via computer algorithm and by an independent cardiologist, using a standardized method developed for ChD [21]. ECG abnormalities are, by definition, always present in Chagas' cardiomyopathy [22]. Right bundle branch block and left anterior hemiblock are the most frequent features. Other abnormalities can be seen but they are less common and less specific [1]. We first compared ECG abnormalities among infected and noninfected participants in the Bambuí sample (see Results for details). We classified the ECG abnormalities found to be positively and significantly associated (p < 0.05) with *T. cruzi* infection as major (typically related with severe cardiomyopathy) and minor

Table 1. Characteristics of the study participants, by *T. cruzi* infection

Characteristics	Total (n = 1,449)	Seropostives (n = 545)	Seronegatives (n = 904)	p value
Age, years	69.0 ± 7.1	69.5 ± 7.1	68.7 ± 7.2	0.055
Females, %	60.8	67.9	56.5	<0.001
Schooling level (<4 years), %	63.7	83.8	51.7	<0.001
Median MMSE score	26 (15, 30)	24 (12, 29)	27 (16, 30)	<0.001
Chagas' disease ECG abnormalities*, %				
Minor	13.6	16.6	11.8	
Major	18.0	34.6	8.0	<0.001
Use of digoxin medication, %	14.8	21.8	10.6	<0.001
Depressive symptoms (GHQ-12 score ≥5), %	37.9	45.8	33.2	<0.001
Currently smoking, %	17.9	17.1	18.5	0.498
Hemoglobin	14.5 ± 1.4	14.4 ± 1.4	14.5 ± 1.4	0.064
Stroke, %	3.7	5.2	2.9	0.027
Median fasting HDL cholesterol, mg/dl	47 (30, 76)	49 (30, 76)	46 (29, 76)	0.002
Median fasting blood glucose, mg/dl	99 (80, 167)	98 (79, 145)	100 (80, 180)	0.082
Systolic BP, mm Hg	137.3 ± 22.3	135.3 ± 22.7	138.5 ± 22.1	0.004
Current use of psychoactive medication (hypnotics and benzodiazepines), %	22.2	23.5	21.4	0.343

Values for age, hemoglobin and systolic BP are expressed as mean ± SD. Figures in parentheses indicate 5th and 95th percentiles. p value: Student's t test, Pearson's χ^2 test and Mann-Whitney two sample rank test for differences between means, frequencies and medians, respectively.

Minor ChD-related ECG abnormalities were left anterior hemiblock, supraventricular premature beats, incomplete right bundle branch block, and right ventricular overload.

Major ChD-related ECG abnormalities were right bundle branch block, ventricular premature beats, atrioventricular block, atrial fibrillation, and left posterior hemiblock.

[22]; those with other ECG abnormalities or without ECG abnormalities were considered as nonexposed.

Current Use of Digoxin

Of the potential drug treatments for cardiac failure, digoxin has been reported to be associated with cognitive impairment [23], and it is the most widely used drug among those with ChD in the study area. Use of digoxin was, therefore, considered in this analysis.

Other Measures

Other measures in this study were those previously reported to be associated with cognitive impairment. These measures were: depressive symptoms, stroke, smoking, systolic blood pressure (BP), fasting blood glucose, fasting HDL cholesterol, hemoglobin and use of psychoactive medications (hypnotics and benzodiazepines) [24–31]. Other characteristics, also available from the Bambuí Health Aging Study data set (body mass index, waist circumference, total and LDL cholesterol and blood urea nitrogen/creatinine), were not considered in this analysis because they showed colinearity with some of the above-mentioned measures (not shown).

Symptoms of depression were assessed by General Health Questionnaire 12, considering 4/5 as the cutoff point [32]. Information on lifetime history of stroke and current smoking were obtained by interview, as previously described [33]. BP measures were performed using standard protocols [34]; systolic BP was considered as the mean of the second and third out of three measurements. Hemoglobin, fasting blood glucose and fasting HDL cholesterol levels were determined with standardized methods.

Statistical Analysis

Unadjusted and adjusted odds ratios (OR) were estimated by ordinal logistic regression to assess the relation between chronic *T. cruzi* infection and the MMSE score. Gender, schooling, depressive symptoms, smoking, and stroke were used as categorical measures, and age, log HDL, log blood glucose, hemoglobin and systolic BP were used as continuous measures. The analyses were based on four models. First we estimated the crude association between chronic *T. cruzi* infection and the MMSE score, and then adjusted incrementally for (1) demographic variables (age, sex and schooling), which were a priori considered confounding variables in this study, (2) potential mediators of an association between *T. cruzi* infection and cognitive functioning (ChD-related ECG abnormalities and use of digoxin) and (3) other potential confounders. The proportional odds assumptions for all models were tested by using the Wald test. Because no effect modifier was noted, all analyses were for both genders with gender as a covariate. The STATA Software Package (version 10.0, College Station, Tex., USA) was used for all analyses.

Table 2. Results of the analyses of the association between *T. cruzi* infection, ECG abnormalities and current use of digoxin medication, and MMSE score

ChD-related measures	MMSE score			Unadjusted OR (95% CI)	Adjusted for age, sex and schooling OR (95% CI)	Adjusted for age, sex, schooling, and for *T. cruzi*, ECG or digoxin[1] OR (95% CI)	Adjusted for age, sex, schooling and mutually adjusted for *T. cruzi*, ECG or digoxin and for all other potential confounders[1, 2], OR (95% CI)
	>23	15–22 (5 to <25th percentile)	≤14 (<5th percentile)				
T. cruzi infection							
No	772	114	18	1.0	1.0	1.0	1.0
Yes	379	132	34	2.59 (2.00–3.36)	1.99 (1.49–2.62)	1.83 (1.34–2.48)	1.99 (1.43–2.76)
ECG abnormalities related to *T. cruzi* infection							
No	812	152	26	1.0	1.0	1.0	1.0
Minor	148	40	10	1.56 (1,09–2,23)	1.30 (0.89–1.90)	1.14 (0.77–1.69)	0.99 (0.65–1.53)
Major	189	55	17	1.78 (1.30–2.44)	1.46 (1.05–2.04)	1.11 (0.77–1.60)	1.03 (0.70–1.52)
Use of digoxin medication (vs. no)							
No	997	198	39	1.0	1.0	1.0	1.0
Yes	154	48	13	1.68 (1.22–2.33)	1.49 (1.05–2.12)	1.38 (0.97–1.98)	1.17 (0.78–1.73)

OR (95% CI) estimated by ordinal logistic regression. The p value of the Wald test of parallel lines assumption was 0.36 in the final adjusted model.

[1] *T. cruzi* infection was adjusted by ECG abnormalities and digoxin use; ECG abnormalities were adjusted by infection and digoxin use; digoxin use was adjusted by infection and ECG abnormalities.

[2] Smoking, depressive symptoms, stroke, systolic BP, and current psychoactive medication. Other variables had no impact on the association between the MMSE score, *T. cruzi* infection, ECG abnormalities and use of digoxin medication.

Results

From the 1,606 cohort members, those included in the present analysis were the 1,449 baseline participants for whom cognitive status and serology for *T. cruzi* were determined (145 were excluded for refusals to perform blood tests and 12 because their serological tests were inconclusive for *T. cruzi* infection). Comparing those included in this analysis with those excluded, younger people (mean age = 69.0 ± 7.1 and 71.9 ± 9.1 years, respectively; p < 0.001), women (68.8 and 52.9%, respectively; p = 0.054), and those with less than 4 years of schooling (36.3 vs. 19.9%, respectively; p < 0.001) were overrepresented. The participants' mean age was 69.0 years (SD 7.1); 60.8% were women and the median number of complete years of schooling was 3.

The prevalence of *T. cruzi* infection was 37.6%, the overall prevalence of current digoxin use was 14.8% (21.8% among seropositive), and 18.0% had major ECG abnormalities associated with the *T. cruzi* infection (34.6% among seropositive). Further characteristics of the study population, and by *T. cruzi* infection, are shown in table 1.

Major ECG abnormalities found to be positively and significantly associated with *T. cruzi* infection were right bundle branch block (23.3% among seropositive), ventricular premature beats (6.3%), atrioventricular block (5.7%), atrial fibrillation (3.3%), and left posterior hemiblock (2.8%). Minor ChD-related ECG abnormalities included left anterior hemiblock (17.3%), supraventricular premature beats (3.3%), incomplete right bundle branch block (1.5%), and right ventricular overload (1.0%).

Table 2 shows unadjusted and adjusted OR for the associations between MMSE score and ChD related measures. *T. cruzi* infection was significantly associated with lower MMSE score, and this association remained after adjusting incrementally for age, gender and schooling, for ECG abnormalities and current use of digoxin medication, and for all other potential confounding variables (OR = 1.99; 95% CI 1.43–2.76). Regarding ECG abnormalities and current digoxin medication use, significant associations with lower MMSE score were found in the unadjusted analysis, but these associations did not remain significant after adjusting for confounders. Note that a Wald test for parallel lines was performed for all models (p value varied from 0.36 to 0.79 and it was 0.36

for the final model). These results indicate that all models, including the final model, do not violate the proportional odds/parallel lines assumption.

Discussion

Principal Findings

This study provides, for the first time, epidemiological evidence that *T. cruzi* infection is associated with cognitive impairment in community-dwelling older adults; the observed association persisted after careful controlling for potential confounders, and suggests graded effect. Our results also indicated that this association is not mediated either by ECG abnormalities related with ChD or use of digoxin medication.

Strengths and Limitations

Some limitations of this study merit discussion. Cognitive impairment was ascertained using the MMSE, which is strongly influenced by schooling level [20]. In this study, we used lower cut-off points than the recommended for populations living in higher income countries [18], and adjusted the association between MMSE score and *T. cruzi* infection for school level. Residual confounding is a possibility, given the concentration of ChD among the most economically and socially disadvantaged. Nevertheless, we were able to control for health-related indicators of inequality in the study population; smoking, depressive symptoms and stroke were independently associated with both low family income and low schooling level (not shown).

The diagnostic of chronic *T. cruzi* infection relies on serologic methods. Two tests based on different antigens or techniques in parallel are recommended to increase the accuracy of the diagnosis [35]. Reported individual sensitivity and specificity of indirect hemagglutination assay and ELISA (crude antigen), the serological tests used in this study, range from 92 to 100% and from 87 to 100%, respectively [36–39]. Therefore, whereas some misclassification on *T. cruzi* infection in this study is likely, this is likely to be nondirectional and thus should bias toward null results. Additionally, serological tests for *T. cruzi* fail to distinguish antibodies against this parasite and those against *Leishmani* sp, leading to false-positive results [36]. Cross-reactivity is unlikely in the present study because leishmaniasis is not endemic in the study area.

Despite the cross-sectional nature of this study, reverse causality between ChD and low cognitive functioning is unlikely; transmission of *T. cruzi* was interrupted by the 1970s in the study area [6]. Information bias is also improbable, given that the interviewers were unaware of the participants' *T. cruzi* status, and the lab team was unaware of the MMSE score. Prevalence bias is a potential limitation, but it seems unlikely that *T. cruzi* infection would selectively increase the longevity of individuals with cognitive impairment, or vice versa.

Cognitive Functioning and T. cruzi *Infection: Underlying Mechanisms*

Three main mechanisms, acting individually or in combination, could explain the association found in this study. Cognitive impairment could be a consequence of (1) chagasic cardiomyopathy, (2) treatment for cardiomyopathy, and/or (3) direct central nervous system involvement.

First, cardiomyopathy is the most frequent consequence of *T. cruzi* chronic infection, with consequent heart failure [1, 4]. Cognitive dysfunction has been associated with heart failure [40] and commonly accompanies clinical syndromes associated with cerebrovascular diseases [41]. Cardiac diseases increase the risk for ischemic stroke [42]. Cerebral infarction has been found in autopsies of chagasic patients with heart failure [43], and there is some evidence that ischemic stroke is associated with ChD [11]. The association between cognitive impairment and chronic *T. cruzi* observed in this study could, therefore, be explained by cerebrovascular disease arising from chagasic cardiomyopathy. We sought to investigate this possibility by adjusting for ECG abnormalities related with *T. cruzi* infection and for self-reported stroke, and did not find any evidence for mediation; neither did ECG abnormalities modify the effect of CHD upon the MMSE score. The presence of ECG abnormalities is an imperfect proxy of the occurrence of heart failure in ChD, but severe heart failure is extremely unusual in the absence of typical ChD ECG abnormalities. Nevertheless, further studies are necessary to assess heart failure and cardiomyopathy influence on the development of cognitive impairment in ChD.

Second, medication used to treat the cardiac complications of ChD may have been implicated. Treatment in the chronic phase is based on easing symptoms of heart failure. Medications such as angiotensin-converting enzyme inhibitors, beta-blockers and digoxin are recommended [44]. Among those medications, only digoxin has been previously noted to be associated with cognitive impairment [23]. Despite digoxin no longer being considered to be a first line drug treatment [45], 22% of the study

participants infected with *T. cruzi* were taking the drug. Controlling for digoxin medication did not attenuate the effect of ChD upon the MMSE score, and the effect of ChD upon cognitive functioning remained robust and largely independent. There was therefore no evidence to support the hypothesis that digoxin use was an important intervening variable.

Third, more direct central nervous system effects might be implicated. There is some electrophysiological evidence of cerebral involvement in chronic ChD, which is not correlated with chagasic cardiomyopathy [46]. Also, autoimmune mechanisms may interfere with cholinergic transmission; there is an association between circulating antipeptide autoantibodies (anti-M2) in chagasic patients and the presence of dysautonomia [12–14]. Alzheimer's disease is characterized by a selective downregulation of M2 muscarinic acetylcholine receptors, associated with increased β-secretase expression and hence stimulus of the amyloidogenic processing pathway of the β-amyloid peptide. M2 receptor regulation has, moreover, been shown to have important effects on the expression of several genes and proteins with potential relevance to the etiopathology of Alzheimer's disease [15, 16]. These raise the possibility of a central nervous system mechanism, either through direct involvement or M2 muscarinic receptor autoantibodies.

Conclusions

Interest in the chronic effects of CHD has focused, understandably, on the prominent cardiac complications. Our findings indicate a significant and independent association between chronic *T. cruzi* infection and cognitive impairment in a population of Brazilian elderly. Further studies are warranted to confirm these observations, to elucidate whether the relationship observed between cognitive impairment and *T. cruzi* chronic infection is causal, and to investigate possible mechanisms.

Acknowledgment

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