

Research Article

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The Significance of Electrocardiographic Abnormalities and Serology for *T. cruzi* Infection

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Abstract

Background: Electrocardiography (EKG) is a basic complementary exam in the evaluation patients with Chagas disease (CD), where findings can precede symptoms and physical examination abnormalities.

Objective: To correlate positive Chagas serology and electrocardiographic abnormalities associated with chronic chagasic cardiomyopathy.

Method: We evaluated the correlation between results of an ELISA using crude antigen (TcLys) and recombinant antigen (TcF) with the presence of EKG disturbances, within a cohort of individuals with either epidemiologic risk or clinical symptoms suggestive of CD, sent to our Laboratory for Tropical Diseases for testing in state of Bahia, Brazil.

Results: 84 individuals had a positive ELISA using TcLys or TcF antigen. Overall, 49 patients (58.3%) were symptomatic with CD, 42 (85.7%) with isolated evidence of the cardiac form, one (2.0%) with megacolon and mixed (megavisera and cardiac) in six (12.2%). TcLys ELISA was positive in 45/49 (91.8%) and TcF in 42/49 (85.7%) of the symptomatic patients. The most common EKG abnormality, Complete Right Bundle Branch Block (CRBBB), was seen in 47/84 patients (56.0%) of the patients. Interestingly, in 11/47 (23.4%) of the patients with CRBBB, serologies were discordant. Conclusions: EKG plays a key role in the initial evaluation of patients with positive Chagas serology using crude or recombinant antigens. Individuals with positive serology should be carefully followed with periodical cardiac medical examination to early detect EKG abnormality compatible with CD.

Keywords: Chagas' disease; Serology; Electrocardiography

Introduction

Chagas Disease (CD), also referred to as American trypanosomiasis, remains a major Latin America public health problem. The estimated prevalence of CD in the Americas is between 18 - 24.7 million cases [1]. CD is caused by the infection of the protozoan parasite *Trypanosoma cruzi*, naturally transmitted by the triatomine reduviid bugs [2]. Blood transfusions are also responsible for a significant number of infected patients [3]. Recently, accidental ingestion of sugarcane and açai fruit juice contaminated with *T. cruzi* from infected triatomines crushed during the preparation of the juice has been associated with an outbreak of acute Chagas' disease [4-6]. The disease occurs in two stages. The acute phase occurs is typically either asymptomatic, or associated with fever and other mild symptoms. One of the classic manifestations of acute CD is Romaña's sign, which is swelling of the eye on one side of the face, usually at the bite site where insect feces was rubbed into the eye. The other nonspecific symptoms seen in acute CD include fatigue, fever, an enlarged liver or spleen, and swollen lymph glands. Rarely, rash, loss of appetite, diarrhea, and vomiting can occur. In general, symptoms last for 4-8 weeks and then resolve, even without treatment [7,8]. Nevertheless, occasionally, life-threatening myocarditis or meningoencephalitis can occur during the acute phase, particularly in young children and immunocompromised persons [7,9].

The majority of infected individuals remain asymptomatic, but an unknown number of initially asymptomatic individuals eventually develop symptoms during the chronic phase of disease after a silent period that may last several years. The pathology of chronic Chagas'disease can result in irreversible changes to the heart, the esophagus and the colon [10], and also may affect the peripheral nervous system [11]. An estimated 30% of those infected with CD develop cardiac symptoms which may lead to sudden death, while approximately 6% develop gastrointestinal pathology such as megavisera [8,10,12] and at least 3% present with peripheral nervous

involvement [11]. In patients who are immunocompromised, including persons with HIV/AIDS, Chagas disease can be fatal. In these patients, difficult to treat unifocal or multifocal chagomas (parasitic lesions) may develop in the brain, resulting in meningoencephalitis in 70% of these cases [13]. Even individuals who remain asymptomatic probably are infected and infectious for life, with low levels of the parasite in their blood and other tissues.

Cardiac involvement is characterized by an inflammatory and fibrosing cardiomyopathy with progressive failure of myocardial contractile function. Parasite persistence and the parasite-driven immune response are considered the main mechanisms that explain the pathogenesis of Chagas heart disease [14]. Chronic Chagas cardiomyopathy presents as three major conditions: cardiac dysrhythmia, heart failure and thromboembolism, all of which may coexist in the same individual [8,15]. Clinical findings vary extensively according to disease duration and the extent and site of cardiac lesions. Hence, some individuals may have a form of Chagas disease with preserved ventricular function, characterized only by arrhythmias and intraventricular or atrioventricular conduction disturbances [12,16]. It has been well established in the natural history of CD that Electrocardiography (EKG) disturbances precede symptoms and

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physical examination findings [12]. Right Bundle Branch Block (RBBB) is the most common EKG finding seen in CD, and thus may act as a predictor of more serious disease. Nevertheless, it remains a challenge in the management of CD to predict which individuals are at risk of developing disturbances associated with chronic forms of the disease. It is not well established whether positive serology or level of serum antibodies are related to the development of cardiac or digestive abnormalities.

Due to the low parasitemia during the chronic stage of the disease, parasitological tests are not routinely used. Therefore, serological tests based on the detection of antibodies to *T. cruzi* are the most useful diagnostic tool [17]. Most commercially available Enzyme Linked Immunosorbent Assays (ELISA) use antigens obtained from lysis of the epimastigote or trypomastigote forms of the parasite (TcLys). These tests have high sensitivity, but often fail to distinguish which antibodies are specific for *T. cruzi* [18,19]. In recent years, several recombinant antigens and synthetic peptides have been developed for the serologic diagnosis of CD [20–22]. TcF is a recombinant antigen composed of four antigens developed from the combination TcLo1.2 and the tripeptide 2/D/E, which consists of the peptides PEP-2, TcD and TcE. This polypeptide was previously studied in a group of patients with confirmed Chagas' disease from endemic areas in Brazil [23]. The polypeptide based ELISA demonstrated a high sensitivity and specificity when compared with indirect immunofluorescence tests and ELISA using crude lysate antigens [23,24].

The aim of this study was to determine the correlation between the results of ELISAs using crude antigen (TcLys) and recombinant antigen (TcF) with EKG abnormalities associated with chronic chagasic cardiomyopathy in a group of individuals from an endemic area of *T. cruzi* infection in Brazil.

Materials and Methods

Study design and sample definition

We conducted a retrospective cross-sectional study to identify electrocardiographic abnormalities associated with Chagas' disease in individuals that had performed ELISAs using *T. cruzi* crude and recombinant antigens. This study was carried out at the main University Hospital in Salvador, Brazil, Complexo Hospitalar Universitário Professor Edgard Santos (C-HUPES). This study was approved by the Ethical Research Committee of the Complexo Hospitalar Universitário Professor Edgard Santos under number 097/09.

The study population was comprised of individuals who were referred to the Laboratory of Tropical Diseases at C-HUPES for clinical findings or epidemiology that suggested Chagas' disease. These individuals were tested with both the standard of care TcLys-ELISA and the experimental TcF-ELISA during a period extending from June 2006 to October 2008.

Clinical data

For all individuals included in the study we collected complementary exams, clinical and demographic data from medical charts. EKG and barium enema radiographic studies were performed in the cardiology and gastroenterology department of C-HUPES, respectively. EKG abnormalities including: complete right bundle branch block (with or without left anterior fascicular block), left bundle branch block, 2nd and 3rd degree intraventricular conduction blocks, sinus node dysfunction, atrial fibrillation, ventricular extra systoles, electrically inactive area, primary T wave abnormalities, and heart rate under 40 bpm

were considered suggestive of Chagas cardiomyopathy, based on the Brazilian Consensus Statement on Chagas disease [10]. Other findings were considered as unspecific isolated abnormalities. Barium studies were used to identify the presence of megaesophagus and megacolon. In the absence of abnormal findings on these tests, individuals were classified as possible carriers of the indeterminate form of CD. The presence of other comorbidities was also evaluated.

ELISA testing

Antigen source: Epimastigotes forms of *Trypanosome cruzi* were obtained and lysate (TcLys) following a previous established protocol described elsewhere [23].

Microtiter plates (Nunc ImmunoModule Plates, maxiSorp surface, no. 473768, Roskilde, Denmark) were coated using 50 µl/well of the TcLys (1 µg/well) or TcF, (200 ng/well) antigens diluted in carbonate-bicarbonate buffer pH 9.6, 37°C and incubated overnight at 7° C. For plate blocking, 200 µl of blocking buffer (PBS 1X+1% skimmed milk powder was used) was added to each well and incubated for 2 hours, followed by five washes with wash buffer. Human sera were then diluted at a ratio of 1:100 in PBS+0.1% BSA+0.05% Tween 20 and distributed in 50 µl/well and incubated for at least 20 minutes at room temperature. Negative controls and serum of individuals were tested at minimum in duplicate. The plate was washed five times with washing buffer (PBS-T) and then peroxidase conjugate Protein A from *Staphylococcus aureus* - total IgG (Sigma P 8651, St. Louis, USA) diluted at a ratio of 1:20,000 in 1X PBS+0.1% BSA+0.05% Tween 20 was added and the plates were then incubated for 20 minutes at room temperature. The plate was again washed five times with PBS-T and 50 µl/well of substrate enzyme 3,3',5,5'-TetraMethylbenzidine (TMB) produced by Kierkegaard & Perry Laboratories Inc. Gaithersburg - USA, was added and the plate incubated for 15 minutes. Finally, 50 µl/well of 1N H₂SO₄ was added to stop the color reaction. The micro plate was read in spectrophotometer at 450nm filter.

Data analysis

The cut off value for a positive test was derived from the average of three optical densities from the negative controls plus three standard deviations. Negative controls were obtained from the serum bank of the Laboratory of Tropical Diseases and included healthy individuals with no clinical signs and negative epidemiology for Chagas' disease. For each ELISA experiment, a negative control in duplicate was used. Based on these results, an optical density greater than or equal to 1.4 was considered positive.

Results

We reviewed ELISA results and clinical data from the charts of 161 patients with epidemiology or clinical symptoms suggestive of Chagas' disease. A total of 77 patients were excluded due to missing clinical or complementary exams data in medical charts. The final study population included 84 individuals with positive serology for *T. cruzi* infection using either the TcLys or TcF antigens. The study population included 31 men and 53 women ranging in age from 21-85 years (mean 51.6 ± 13.7). Table 1 presents the frequency of EKG changes and other comorbidity in the studied population. As noted, the majority 47/84 (56%) had the most common EKG abnormality seen in patients with Chagas' disease, Complete Right Bundle Branch Block (CRBBB).

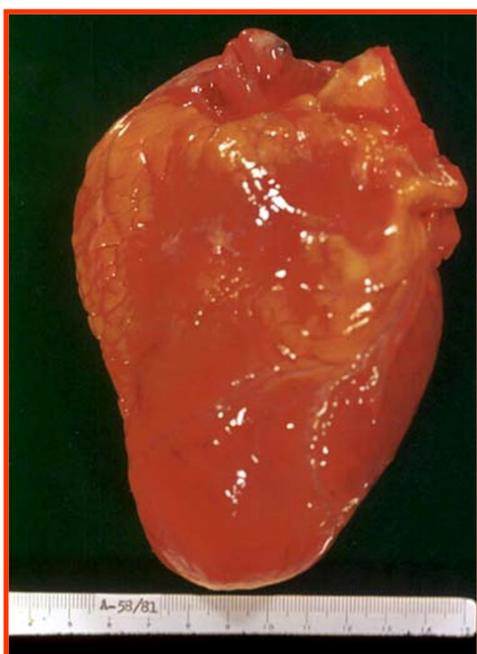
Forty-nine out of the 84 patients (58.3%) had a clinical manifestation compatible with one of the clinical forms of CD. Among them, the cardiac form was suspected in 48/49 (98%) including 47 with CRBBB

and one with isolated intraventricular block. Both the classic cardiac form, cordis bovis, (Figure 1) and megacolon were seen (Figure 2). No subjects had megaesophagus, (Figure 3). TcLys ELISA was positive in 38 (90.5%) and TcF in 35 (85.3%) of the patients with EKG abnormalities compatible with CD as shown in Table 2. Interestingly, in eleven patients in the group with suspected cardiac Chagas' disease, discordant serology results were documented between the TcLys and TcF antigens. Table 3 depicts the EKG type of abnormalities in each of these patients. Only one did not have CRBBB, but had Intraventricular Block (IVB), also compatible with CD. The other 35 patients in the studied population with serology positive either TcLys or TcF were asymptomatic, had a normal EKG and were considered to have an indeterminate form of CD.

Condition	n	%
Abnormality*		
Complete right bundle branch block	47	56
Intraventricular conduction blocks	08	8.8
Left bundle branch block	02	2.2
Ventricular extra systoles	01	1.1
Electrically inactive area	01	1.1
Unspecific isolated abnormalities	33	36.6
Comorbidity*		
Hypertension	37	41.1
Type 2 diabetes	07	7.7
Dyslipidemia	05	5.5
Osteoarthritis	03	3.3
Leishmaniasis	02	2.2
HCV	01	1.1
Other	18	19.8

* Patients could present more than one EKG abnormality or comorbidity

Table 1: Frequencies of EKG abnormalities and comorbidities in all 84 patients studied patients.



The aneurismatic feature of tip of an enlarged heart from an autopsy of a chagas disease patient

Figure 1: Typical cardiomegaly (cordis bovis) seen in a patient with Chagas' disease.

Results of TcLys and TcF ELISA were compared among the groups of individuals. The mean optical density divided by the cut off in TcLys ELISA was 4.4 (± 3.0) in patients with the cardiac form, 3.4 (± 2.5) in those with digestive or mixed form and 2.2 (± 2.0) in those asymptomatic. In TcF ELISA, the groups with cardiac, digestive/mixed and asymptomatic forms presented mean of optical density / cut off of 3.3 (± 2.1), 3.0 (± 1.0) and 2.2 (± 2.0), respectively. Figure 1 shows the range of results in both ELISA tests.

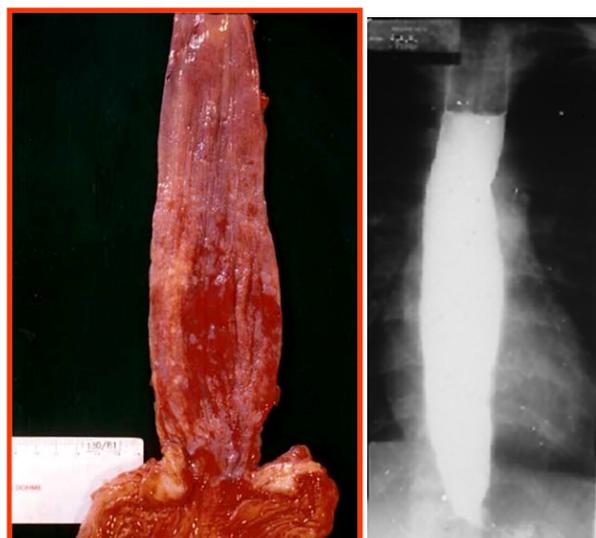
Discussion

EKG is a basic complementary exam in evaluation patients with Chagas disease and may be the only available diagnostic instrument in poverty-stricken areas, where there is often higher prevalence of *T. cruzi* infected patients. CRBBB is the most common EKG abnormality documented in patients with the cardiac form of Chagas' disease [12,25], and in our study, 56% of individuals had CRBBB isolated or combined



The left side is a megacolon feature of an autopsy of a patient with Chagas' disease. On the right side is the radiographic barium enema characteristic of megacolon disease.

Figure 2: The typical megacolon documented in a patient with Chagas' disease.



On the the left side, a megaesophagus is shown from an autopsy of a patient with CD. On the right side is the typical radiographic barium enema image

Figure 3: Megasophagus caused by Chagas' disease.

Chagas Disease Form	Number of patients	TcLys ELISA positive	TcF ELISA Positive	TcLys and TcF ELISA positive
Cardiac	42	38	35	31
Megaesophagus	0	0	0	0
Megacolon	1	1	1	1
Mixed*	6	6	6	6
Total	49	45	42	39

TcLys ELISA= indirect Enzyme immunosorbent assay with *T. cruzi* lysate antigen; TcF ELISA= indirect Enzyme immunosorbent assay with *T. cruzi* recombinant antigen; *Mixed forms: cardiac and megaesophagus 3 patients; cardiac and megacolon 2 patients; 1 patient with all three clinical CD forms

Table 2: Serologic tests to detect anti *T. cruzi* antibodies among patients with clinical forms of Chagas'Disease.

Patient	Age (years)	CRBBB	IVB	EIA	LBBB	VES	TcLys	TcF
# 1	32	Yes	yes	no	no	no	-	+
#2	66	No	yes	no	no	no	-	+
#3	76	Yes	No	no	no	no	-	+
#4	40	Yes	No	no	no	no	-	+
# 5	42	Yes	No	no	no	no	+	-
#6	57	yes	No	no	no	no	+	-
#7	67	yes	No	no	no	no	+	-
#8	55	yes	No	no	no	no	+	-
#9	52	yes	No	no	no	no	+	-
#10	39	yes	yes	no	no	no	+	-
#11	40	yes	No	no	no	no	+	-

CRBBB: complete right bundle branch block; IVB: intraventricular conduction blocks; EIA: electrically inactive area; LBBB: left bundle branch block; VES: ventricular extra systoles, TcLys ELISA= indirect Enzyme immunosorbent assay with *T. cruzi* lysate antigen; TcF ELISA= indirect Enzyme immunosorbent assay with *T. cruzi* recombinant antigen

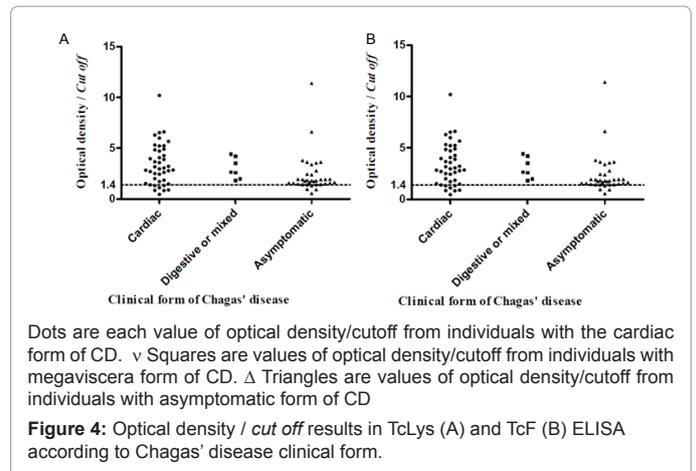
Table 3: EKG characteristics of patients with TcLys Elisa or TcF Elisa negative.

with other EKG disturbances. Intraventricular conduction block was the second most frequent electrocardiographic abnormality seen.

Serologic testing is considered the most practical standard method to screen for *T. cruzi* infection [26]. In our study, both crude and recombinant antigens showed more than 85% of positivity among individuals with EKG abnormalities suggestible of CD. In the presence of digestive symptoms, the combined use of both ELISAs showed higher number of positive results. While it is not well established how well clinical signs and symptoms can be used to predict chronic forms of the disease, it has been shown that the clinical diagnosis of cardiac Chagas' disease and EKG abnormalities are associated with *T. cruzi* seropositivity [27]. Our results showed higher level of antibodies in individuals with the cardiac form of CD comparing to asymptomatic patients (Figure 4).

Many years ago, a prospective study of CD in a highly endemic area in Brazil revealed that 20.2% of individuals with *T. cruzi* infection had EKG abnormalities [25]. At that time only crude lysate antigen preparation was available for the ELISA. Our results are compatible with a more recent study on cardiac abnormalities in patients with *T. cruzi* infection, which have shown that EKG disturbances are present in 92% of seropositive individuals [28]. This suggests that in conjunction with a crude lysate ELISA, EKG may be a useful adjunctive tool in the diagnosis of CD.

Several studies have shown that recombinant antigens are more specific in the detection of anti-*T. cruzi* antibodies than crude antigen preparations, but may also be less sensitive [20,29]. Evaluations of



Dots are each value of optical density/cutoff from individuals with the cardiac form of CD. v Squares are values of optical density/cutoff from individuals with megaviscera form of CD. Δ Triangles are values of optical density/cutoff from individuals with asymptomatic form of CD

Figure 4: Optical density / cut off results in TcLys (A) and TcF (B) ELISA according to Chagas' disease clinical form.

serodiagnostic tests using six different *T. cruzi* recombinant antigens have shown sensitivities varying from 4.2% to 97%. However, the use of combinations of recombinant peptides has improved the sensitivity of serodiagnostic tests to nearly 100% [30]. Nevertheless, in our study we found no significant differences between the positive rates of TcLys compared to the recombinants peptides used in the ELISA.

WHO recommends the use of three different serological tests for confirm the *T. cruzi* positive serology and only consider individuals who have a test positive on two or more tests to be a true positive serology, due to the critical the poor specificity of many available commercial serological tests for CD [17]. However, it is difficult to have access to all these diagnostic tools in many endemic areas of CD. A combination approach using serology and EKG, may be an alternative method to obtain a more reliable diagnosis [26].

In conclusion, our results demonstrate that EKG can play a key role in the initial evaluation of patients with positive serology using crude or recombinant antigens. It has been shown that the relative risk of a person with positive serology for CD compared to a seronegative individual for ventricular dysfunctions is 3.69, and if the person has any EKG abnormality at diagnosis, this risk is 2.72-fold higher [12]. Thus, not only do we recommend the use of EKG in the initial evaluation of subjects for CD, we also strongly recommend that individuals in endemic areas with serologic or EKG evidence of CD should be carefully followed with periodical cardiac medical examination, especially when a right bundle branch block is present.

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