CARDIOVASCULAR MEDICINE

Risk progression to chronic Chagas cardiomyopathy: influence of male sex and of parasitaemia detected by polymerase chain reaction

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Heart 2003;**89**:1186–1190

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Accepted 7 May 2003

Background: Polymerase chain reaction (PCR) allows detection of *Trypanosoma cruzi* in blood throughout the course of Chagas' disease.
Objective: To determine whether *T cruzi* DNA detected by PCR is associated with progression to chronic Chagas cardiomyopathy.
Design: Prospective cohort study.
Setting: A tertiary care centre in Argentina.
Patients: 56 consecutive patients with chronic *T cruzi* infection.
Methods: Clinical examination, ECG, and Doppler echocardiography were carried out at baseline and at the end of the follow up. Detection of *T cruzi* DNA by PCR amplifying a nuclear sequence was undertaken in all patients at baseline.
Main outcome measures: Progression was defined as death from chronic cardiomyopathy or the presence of a new ECG or left ventricular echocardiographic abnormality at the end of follow up.
Results: The 56 patients (21 male, 35 female; mean (SD) age, 56.0 (11.3) years) were followed for a mean 936.3 (244.39) days. Progression to cardiomyopathy was detected in 12 patients (21.4%). Three of these patients died after baseline evaluation. Univariate analysis showed that a positive PCR (relative risk 4.09, 95% confidence interval (CI) 1.60 to 9.85) and male sex (5.00, 95% CI 1.65 to 15.73) were associated with progression. Multivariable logistic regression indicated that both sex and PCR were independent variables affecting the outcome.
Conclusions: In a cohort of seropositive individuals, patients with *T cruzi* DNA detected by PCR and male patients were at higher risk of progression. These results highlight the importance of *T cruzi* in the pathophysiology of chronic cardiomyopathy.

Chagas' disease is caused by *Trypanosoma cruzi*, a flagellated protozoan parasite. It has a wide distribution in Central and South America and is endemic in 21 countries, with 16 to 18 million persons infected and 100 million people at risk.[1] After the acute infection, which is apparent in only 3% of cases, there ensues a longer chronic phase. During this stage approximately 60–70% of infected individuals remain in the indeterminate form of the disease in which there may be no clinical symptoms or signs, but in 2–5% annually there is evolution to clinically significant disease. Classically, Chagas' cardiomyopathy has been considered the clinical hallmark of the chronic period.[2]

A large number of parasites can be detected in the bloodstream during the acute phase. Later, through the indeterminate and chronic stages, parasites are either absent in blood smears or present in such low concentrations that they cannot be detected by methods such as xenodiagnosis or blood cultures.[3] However, polymerase chain reaction (PCR) can detect *T cruzi* with higher sensitivity during the chronic phase of the disease.[4 5] Studies investigating the prevalence of *T cruzi* DNA by PCR assay in chronic disease have been undertaken in several epidemiological settings. PCR detection varies widely from 3.8% to 100% in adult patients with chronic Chagas' infection.[3–10] In addition, the proportion of positive assays is highly variable when different endemic regions of the same country are studied and shows a strong correlation with xenodiagnosis.[11] However, the role of PCR determination in predicting progression of the disease is unknown.

In the current cohort study, we present a long term follow up of a well defined urban population with chronic *T cruzi* infection, assessing the relation between circulating parasites detected by PCR and the progression of chronic Chagas' cardiomyopathy.

METHODS

Patients and study protocol

We enrolled patients attending the cardiovascular clinic of the Hospital Privado Centro Médico de Córdoba with positive Chagas serological tests and who resided in the city of Córdoba, Argentina. In all patients, three different serological tests for detection of chronic *T cruzi* infection were done: indirect immunofluorescence assay (positive ⩾ 1:32 dilution; Biocientífica, Buenos Aires, Argentina), haemagglutination inhibition assay (positive ⩾ 1:28 dilution, Biochagas, Biocientífica, Buenos Aires), and enzyme linked immunosorbent assay (Abbott Labs, Abbott Park, Illinois, USA). Chronic Chagas' disease was defined by the presence of two or more positive serological determinations.

Informed consent for the study was obtained from each patient.

Baseline evaluations included the following: clinical examination, 12 lead ECG at rest, colour Doppler transthoracic echocardiography, a three lead exercise ECG, and 24 hour ECG monitoring. Determination of parasitaemia by PCR assay was undertaken in all patients at baseline. During the follow up visit all evaluations were repeated except for exercise testing and 24 hour ECG monitoring. The latter was repeated only as part of the assessment of new symptoms. A subset of patients had another PCR determination at the end of follow up. For

Table 1 Baseline characteristics of the study population

Characteristic	All patients (n=56)
Age (years)	56.0 (11.3)
Range	19 to 84
Male	21 (37.5)
NYHA class	
I	45 (80.4)
II	9 (16.1)
III	2 (3.6)
IV	0 (0)
Abnormal ECGs	27 (48.2)
SB	8 (16.7)
RBBB + LAFB	6 (10.7)
PM	6 (10.7)
Only RBBB	5 (8.9)
IRBB	2 (3.6)
FDAVB	2 (3.6)
LBBB	1 (1.8)
Transthoracic echocardiography	
LVEF (%)	59.6 (14.2)
Range	16 to 80
LVDd (mm)	47.6 (94.2)
Range	26 to 82

Data are n (%) for sex, NYHA class, and ECG data, or mean (SD). FDAVB, first degree atrioventricular block; IRBBB, incomplete right bundle branch block; LAFB, left anterior fascicular block; LBBB, left bundle branch block; LVEF, left ventricular ejection fraction; LVDd, left ventricular diameter in diastole; NYHA, New York Heart Association; PM, pacemarker; RBBB, right bundle branch block; SB, sinus bradycardia.

each patient we recorded data on New York Heart Association (NYHA) functional class and hospital admissions for cardiovascular events during the follow up period.

The investigators who did the echocardiograms were blinded to the PCR results and those who did the PCR tests were blinded to the patients' clinical status.

Patients with ischaemic cardiomyopathy (detected by a stress test) were excluded. No patients with primary valvar disease, hypertrophic obstructive cardiomyopathy, pericardial disease, congenital heart disease, or a history of viral myocarditis or alcoholism were included.

PCR procedure

A highly repetitive nuclear DNA sequence was amplified. This sequence, named E13, is distributed over most of the chromosomes and constitutes about 7% of the total nuclear DNA.[12] The accuracy of this test has been demonstrated previously.[13]

Peripheral venous blood samples were drawn from each subject for detection of circulating *T cruzi* by the PCR test. Whole blood was immediately mixed with an equal volume of 6 M guanidine hydrochloride (0.2 M EDTA solution). In this solution, DNA remains undegraded for months.[14] The sequence of the primers used was: O1, 5′-TGGCTTGGA GGAGTTATTGT-3′; O2, 5′-AGGAGTGACGGTTGATCAGT-3′. Amplified products were subjected to electrophoresis in 1.6% agarose gel containing 0.5 μg/ml of ethidium bromide and photographed under ultraviolet light.

The positive PCR control was genomic DNA isolated from *T cruzi* epimastigotes of Tulahuen strain. All the samples were tested under the same conditions with human plasma β actin protein specific primers.

Outcome criteria

The primary outcome of the study was progression, defined as death from chronic cardiomyopathy, or the presence of a new ECG abnormality typical of chronic Chagas' disease, or new echocardiographic abnormalities at the end of follow up. ECG abnormalities included non-drug related sinus bradycardia (< 50 beats/min), first degree atrioventricular block, complete heart block, incomplete right bundle branch block, right bundle branch block, left anterior fascicular block, and the need for a permanent pacemaker.[15] A new echocardiographic finding was defined as a left ventricular ejection fraction (LVEF) of $< 50\%$, or a left ventricular diameter in diastole above the normal value, adjusted for sex and height using a previously validated classification.[16]

Statistical analysis

Continuous variables are presented as mean (SD), and categorical variables as numbers and percentages. All variables were dichotomised for univariate analysis and compared using χ^2 tests or Fisher's exact test as appropriate. Estimates of PCR effect and other variables determining outcome were expressed as relative risk for progression, with 95% confidence intervals (CI).[17] Variables considered significant on univariate analysis, with probability values of $p < 0.1$, were selected for testing in a multiple logistic regression model. A value of $p < 0.05$ was then considered significant. All statistical tests were two sided. All data were analysed using StatsDirect Statistical Software, version 2.2.3.

RESULTS

Patients

Between 24 July 1997 and 31 December 1998, 75 patients with a serological diagnosis of Chagas' disease were enrolled. Seven

Table 2 Characteristics of patients with progression of Chagas' disease*

			Baseline status			Follow up status		
Patient	Age (years)	Sex	ECG	LVEF (%)	LVDd (mm)	ECG	LVEF (%)	LVDd (mm)
1	61	M	SB, LAFB	72	52	SB, LAFB, RBBB	58	52
2	57	M	RBBB	60	39	FDAVB, RBBB	72	51
3†	55	M	NSST-TCs	62	54	NSST-TCs, SB	60	64
4	59	M	RBBB, LAFB	63	41	SB, FDAVB, RBBB	60	43
5	19	M	Normal	58	50	IRBBB	47	51
6	55	F	RBBB	58	42	PM	60	43
7	72	M	SB, FDAVB	77	52	SB, FDAVB	40	45
8	58	M	RBBB, LAFB	29	60	PM	25	66
9	39	M	Normal	63	45	RBBB	52	44
10	55	F	LBBB	16	73	NE	NE	NE
11	58	F	PM	20	65	NE	NE	NE
12	74	M	NSST-TCs	19	82	NE	NE	NE

*Patients who died are numbers 10, 11, and 12.
†An implantable defibrillator was placed in this patient.
FDAVB, first degree atrioventricular block; F, female; IRBBB, incomplete right bundle branch block; LAFB, left anterior fascicular block; LBBB, left bundle branch block; LVDd, left ventricular diameter in diastole; LVEF, left ventricular ejection fraction; M, male; NE, not evaluated; NSST-TCs, non-specific ST-T wave changes; PM, pacemarker; RBBB, right bundle branch block; SB, sinus bradycardia.

Table 3 Progression to chronic chagasic cardiomyopathy in relation to parasitemia by PCR assay

Outcome	Total (n=56)	PCR positive (n=11)	PCR negative (n=45)	RR (95% CI)
Progression (%)	12 (21.4)	6 (54.55)	6 (13.33)	4.09 (1.6 to 9.85)
No progression (%)	44 (78.6)	5 (45.45)	39 (86.67)	

Data are n (%).
CI, confidence interval; PCR, polymerase chain reaction; RR, relative risk.

Table 4 Univariate analysis of baseline factors determining progression

	All patients			
Variable	Event (n=12)	No event (n=44)	Relative risk (95% CI)	p Value*
Age ⩾60 years	3	13	0.83 (0.27 to 2.39)	>0.9999
Male sex	9	12	5 (1.65 to 15.73)	0.0053
PCR positive	6	5	4.09 (1.6 to 9.85)	0.0083
Abnormal ECG	8	20	1.86 (0.68 to 5.32)	0.3316
RBBB	4	8	1.83 (0.65 to 4.62)	0.2626
LVEF <50%	4	6	2.3 (0.82 to 5.61)	0.1958
Abnornal LVDd	4	7	2.04 (0.72 to 5.08)	0.2239
Medical history				
Hypertension	1	14	0.24 (0.04 to 1.24)	0.1492
Hyperlipidaemia	1	14	0.24 (0.04 to 1.24)	0.1492
Current cigarette use	0	2	0 (infinity to 6.34)	>0.9999
Familial history of ischaemic cardiomyopathy	1	3	1.22 (0.18 to 7.5)	>0.9999
Diabetes mellitus	0	2	0 (infinity to 6.34)	>0.9999

*Fisher's exact test. CI, confidence interval; LVDd, left ventricular diameter in diastole; LVEF, left ventricular ejection fraction; PCR, polymerase chain reaction; RBBB, right bundle branch block.

patients did not complete the study protocol, one was excluded because of ischaemic cardiopathy, and one received a heart transplant immediately after enrolment and was withdrawn from the study.

Of the remaining 67 patients, 11 were lost to follow up. Of these, four were alive but unwilling to continue (two of those had a positive basal PCR determination), and seven were not available for follow up (all of those were negative by PCR assay).

Thus 56 patients remained eligible for the study (21 men and 35 women; mean (SD) age, 56.0 (11.3) years), with a mean follow up of 936.3 (244.39) days. Follow up examinations took place between 3 July 2000 and 10 October 2001. The baseline clinical and laboratory characteristics of the 56 patients are shown in table 1. Basal 24 hour ECG recordings showed abnormalities in 12 cases (21.4%). These were: non-sustained ventricular tachycardia (n = 6), paroxysmal supraventricular tachycardia (n = 6), ventricular premature complexes accounting for more than 1% of normal beat count (n = 2), accelerated idioventricular rhythm (n = 2), and atrial fibrillation (n = 2).

Parasitaemia detected by PCR

Eleven of the 56 patients had a positive PCR determination at baseline (19.6%). A subset of 48 patients had another PCR determination at the end of follow up, and nine of these became positive (mean time between the two determinations, 968.6 (216.9) days). Globally, parasitaemia by PCR assay was detected in 20 of the 56 patients at least once during the follow up period.

Progression of disease

Progression to Chagas' cardiomyopathy was observed in 12 of the 56 patients (21.4%). Three of these 12 patients died from chronic cardiomyopathy 147, 431, and 627 days after baseline evaluation, respectively, and were included in the final analysis. The PCR assay was positive in one of these patients. Of the nine surviving patients with Chagas' cardiomyopathy, six had a new ECG abnormality, one had a new echocardiographic abnormality, and two had both abnormalities (table 2).

Table 5 Multivariate analysis of factors determining progression*

Variable	Odds ratio (95% CI)	p Value
Male sex	6.28 (1.35 to 29.27)	0.0193
PCR pos	5.7 (1.16 to 27.89)	0.0318

*Logistic regression analysis.
CI, confidence interval; PCR, polymerase chain reaction; pos, positive.

Death or the development of a new cardiac abnormality was significantly more common in patients with parasitaemia (6/11, 55%) than in those without (6/45, 13%). The relative risk of progression was 4.09 (95% CI 1.6 to 9.85) (table 3).

We considered other variables that could affect the progression of chronic cardiomyopathy. In univariate analysis, with all variables dichotomised, both a positive PCR (relative risk 4.09, 95% CI 1.6 to 9.85) and male sex (relative risk 5.00, 95% CI 1.65 to 15.73) were associated with progression (table 4). The logistic regression model indicated that both sex and a positive PCR remained independent predictors of progression in this cohort of patients (table 5).

Additional analyses

Only 10 Holter recordings were repeated during the follow up period. These were done because of symptoms suggestive of arrhythmia. Changes with respect to baseline were found in three cases: two had premature ventricular complexes amounting to more than 1% of the normal beat count, and one patient with previous ventricular tachycardia had a normal record on the second occasion.

There were seven hospital admissions for cardiovascular events. These were: heart failure (n = 2), pacemaker implantation (n = 2), cardiac debrillator implantation (n = 1), paroxysmal atrial fibrillation (n = 1), and chest pain (n = 1). The two patients with admissions for heart failure had had previous admissions before being included in the study and died after the baseline evaluation. We detected only four patients with worsening of the NYHA functional class, two of whom had progression criteria. We did not consider hospital admission or functional class as progression criteria in themselves.

DISCUSSION

In his original description of the disease, Chagas postulated a direct invasion of the parasite as the mechanism responsible for chronic heart damage.[18] However, parasites were rarely isolated from blood or tissues of chronically infected patients and antimicrobial treatment against *T cruzi* was disappointing. Most investigators have since focused on alternative pathophysiological mechanisms.[19] For this reason, the primary importance of the parasites has largely been ignored in the clinical setting.

Principal findings

We observed parasitaemia, detected by PCR assay, in a substantial number of patients with chronic infection, and for the first time showed an association between the presence of parasitic DNA in the blood and progression of cardiac damage. From our observations, the persistence of *T cruzi* DNA may be a reliable predictor of progressive cardiac disease.

An unequivocal survival advantage for women after the development of heart failure was shown in the Framingham heart study for all causes of heart failure.[20] However, reports on sex differences in progressive Chagas' disease are controversial and previous studies have found either that it is more common in men or that it is unrelated to sex.[21–23] In our series there was a significantly higher risk of progression to chronic cardiomyopathy in men than in women. Furthermore, the greater proportion of women in our study population raised the statistical power for detecting sex differences in disease progression.

Possible mechanisms

The role of *T cruzi* in the pathophysiological mechanisms leading to chronic disease has been highlighted by new parasitological techniques, which have confirmed the importance of the parasite during the chronic stages of the disease. It has been shown that the presence of *T cruzi* is strongly correlated with an ongoing inflammatory process that is likely to lead to cell necrosis and fibrosis in chronic cardiomyopathy. Zhang and colleagues analysed the persistence of kinetoplast DNA (kDNA) of *T cruzi* by an in situ PCR assay in murine models of chronic Chagas' disease.[24] Tissues were characterised by a diffuse inflammatory reaction and the distribution of *T cruzi* kDNA amplification products in these tissues corresponded with the inflammatory infiltrates. In patients with chronic cardiomyopathy, Belloti and colleagues detected the presence of *T cruzi* antigens in myocardial biopsies from sites of inflammation observed on magnetic resonance imaging.[25] These findings were confirmed by in situ PCR assay in other studies with chronically infected patients.[26 27]

All these data support the view that the presence of parasites at sites of disease activity is the main stimulus to perpetuating inflammation, and this in turn promotes cardiac damage. Although a persistently high degree of parasitaemia detected by xenodiagnosis and PCR assay is more common among patients with Chagas cardiomyopathy, its importance as a predisposing factor for disease progression is unclear.[28 29] It has been shown in longitudinal studies that higher levels of specific antibodies are associated with progressive cardiomyopathy.[30] On the other hand, patients living in highly endemic areas have more ECG abnormalities than those in non-endemic regions, suggesting that reinfection may play a role in the variability and severity of the clinical course of Chagas' disease.[31–33]

Unanswered questions and future research

Nine patients had become PCR positive by the end of follow up. We cannot know from our study whether these patients previously had false negative results because of low test sensitivity or if they had recent parasitaemia. In the present study, we amplified a nuclear DNA sequence of *T cruzi*, but a greater sensitivity of PCR assay in chronic disease has been described using amplification of kDNA.[5] However, to date there have been no published trials comparing the two sequences in terms of the development of cardiomyopathy. It is known that *T cruzi* DNA is derived from recently released or dead parasites and not from chronic persistence of DNA from the original infection.[24] Thus parasitaemia may be explained by reinfection, as previously mentioned, by cyclic parasitaemia, or by reactivation. It is possible that disease progression in these patients may have been apparent later in the follow up, in view of the slow progression of the disease. A fluctuating pattern of parasitaemia identified by xenodiagnosis has been reported previously, and the existence of intermittent parasitaemias as an explanation for negative PCR tests in chronic chagasic patients has also been suggested.[34–38] Finally, reactivation has been shown in patients with an immunodeficiency state.[39] However, more frequent PCR determinations are necessary to prove this hypothesis.

Study limitations

We studied a limited number of patients and 2.5 years may not be sufficient time to detect a combined end point such as mortality and admissions for heart failure, because of the long asymptomatic period that precedes the development of heart failure. Likewise, to demonstrate changes in NYHA functional class would require a longer follow up in Chagas' disease. However, changes in this variable are rarely found in the absence of previous cardiomyopathy in Chagas' disease.

Clinical implications

Both seropositive men and patients with chronic *T cruzi* infection who have episodes of parasitaemia are at increased risk of disease progression and the development of typical Chagas cardiomyopathy. These subgroups of patients need close follow up.

ACKNOWLEDGEMENTS

We are indebted to Dr Antonio Blanco (Universidad Nacional de Córdoba) and Dr Aldo Tabares (Hospital Privado de Córdoba) for the critical review of a previous version of the manuscript. We also thank to reviewers for their useful contributions. This investigation was supported in part by a grant from Agencia Córdoba Ciencias and Secretaría de Ciencia y Tecnología (SECYT) of the Universidad Nacional de Córdoba, Argentina.

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Cor triatriatum sinister presenting in the adult as mitral stenosis

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Cor triatriatum sinister is a rare congenital defect in which the left atrium is divided by a fibromuscular membrane into two distinct chambers. Classically, patients present in infancy although in some cases they remain asymptomatic until adulthood. The clinical features on presentation can mimic those of mitral stenosis due to the obstructive properties of the membrane. Cor triatriatum sinister presented in this case in an adult as mitral stenosis. Factors that may be relevant in determining late presentation are also discussed.

(*Heart* 2003;**89**:e26) www.heartjnl.com/cgi/content/full/89/10/e26