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

A L Basquiera, A Sembaj, A M Aguerri, M Omelianiu, S Guzmán, J Moreno Barral, T F Caeiro, R J Madoery, O A Salomone

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.

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 ≥ 1:28 dilution; Biochagas, 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
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. The sequence of the primers used was: O1, 5′-TGCTTGGAGGAATATTG-3′; O2, 5′-AGAGTACGGTGTACGT-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. 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.

### 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 χ²-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). 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

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Baseline status</th>
<th>Follow up status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ECG LVEF (%)</td>
<td>LVDd (mm)</td>
</tr>
<tr>
<td>1</td>
<td>61</td>
<td>M</td>
<td>SB, LAFB 72</td>
<td>52</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>M</td>
<td>RBBB 60</td>
<td>39</td>
</tr>
<tr>
<td>3†</td>
<td>55</td>
<td>M</td>
<td>NSST-TCs 62</td>
<td>54</td>
</tr>
<tr>
<td>4</td>
<td>59</td>
<td>M</td>
<td>RBBB, LAFB 63</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>M</td>
<td>Normal 58</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>55</td>
<td>F</td>
<td>RBBB 58</td>
<td>42</td>
</tr>
<tr>
<td>7</td>
<td>72</td>
<td>M</td>
<td>SB, FDAVB 77</td>
<td>52</td>
</tr>
<tr>
<td>8</td>
<td>58</td>
<td>M</td>
<td>RBBB, LAFB 29</td>
<td>60</td>
</tr>
<tr>
<td>9</td>
<td>39</td>
<td>M</td>
<td>Normal 63</td>
<td>45</td>
</tr>
<tr>
<td>10</td>
<td>55</td>
<td>F</td>
<td>LBBB 16</td>
<td>73</td>
</tr>
<tr>
<td>11</td>
<td>58</td>
<td>F</td>
<td>PM 20</td>
<td>65</td>
</tr>
<tr>
<td>12</td>
<td>74</td>
<td>M</td>
<td>NSST-TCs 19</td>
<td>82</td>
</tr>
</tbody>
</table>

*Patients who died are numbers 10, 11, and 12.
†An implantable defibrillator was placed in this patient.

FDABB, first degree atrioventricular block; F, female; RBBB, 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, pacemaker; RBBB, right bundle branch block; SB, sinus bradycardia.
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).

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.

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

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Total (n=56)</th>
<th>PCR positive (n=11)</th>
<th>PCR negative (n=45)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progression (%)</td>
<td>12 (21.4)</td>
<td>6 (54.55)</td>
<td>6 (13.33)</td>
<td>4.09 (1.6 to 9.85)</td>
</tr>
<tr>
<td>No progression (%)</td>
<td>44 (78.6)</td>
<td>5 (45.45)</td>
<td>39 (86.67)</td>
<td></td>
</tr>
</tbody>
</table>

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

### Table 4 Univariate analysis of baseline factors determining progression

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

*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.

### Table 5 Multivariate analysis of factors determining progression*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>6.28 (1.35 to 29.27)</td>
<td>0.0193</td>
</tr>
<tr>
<td>PCR pos</td>
<td>5.7 (1.16 to 27.89)</td>
<td>0.0318</td>
</tr>
</tbody>
</table>

*Logistic regression analysis. CI, confidence interval; PCR, polymerase chain reaction; pos, positive.
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. 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. 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. 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. 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 genetic and serological 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 identified the persistence of kinetoplast DNA (kDNA) of *T. cruzi* by an in situ PCR assay in murine models of chronic Chagas’ disease. Tissues were characterised by a diffuse inflammatory reaction and the distribution of *T. cruzi* kDNA amplification products in these tissues corresponded with the infiltrating 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. These findings were confirmed by in situ PCR assay in other studies with chronically infected patients.

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.

**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. 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. Thus parasitaemia may be explained by reinfestation, 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. Finally, reactivation has been shown in patients with an immunodeficiency state. 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**

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Cor triatriatum sinister presenting in the adult as mitral stenosis

R D Slight, O C Nzewi, R Sivaprakasam, P S Mankad

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.
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