The Prothrombotic State in Early Stages of Chronic Chagas' Disease

Ramón N. Herrera, Elba Díaz, Rossana Pérez, Sergio Chaín, Roque Sant-Yacumo, Eduardo Rodríguez, Jorge Blanchi, Alfredo Coviello, Julio Miotti, Isolina Flores, Fernando de la Serna, Juan Muntaner, Sofia Berman and Héctor Luciardi

Departamento de Hemostasia y Trombosis, Hospital Centro de Salud Zenón, J. Santillián, Facultad de Medicina, Universidad Nacional de Tucumán, Argentina.

Background. Thromboembolic complications are frequent in advanced Chagas' disease.

Objective. This study was designed to explore the presence of a prothrombotic state in the early stages of chronic Chagas' disease by evaluating serum markers of thrombosis and fibrinolysis.

Patients and method. Forty-two patients with chronic Chagas' disease (12 men and 30 women, 32.5 ± 6.7 years) were compared with 21 healthy volunteers (10 men and 11 women, 24.2 ± 5.6 years). The markers of thrombotic activation used were fragment 1 + 2, ATM complex, PDF/pdf, D-dimer, and \( \beta \)-thromboglobulin. Fibrinolysis was evaluated before and after venous occlusion, together with euglobulin lysis time, \( t \)-PA, and PAI-1 titers.

Results. The markers of thrombotic state were significantly higher in patients with chronic Chagas' disease than in controls: F1 + 2 (p < 0.0001), ATM (p < 0.0001), PDF/pdf (p < 0.05), and D dimer (p < 0.05). There was no significant difference in \( \beta \)-thromboglobulin (p = 0.06). Euglobulin lysis time, a global fibrinolytic marker, differed significantly (p < 0.0001) between patients with Chagas' disease and healthy volunteers. However, the more specific fibrinolytic markers \( t \)-PA and PAI-1 did not differ significantly between the two study groups.

Conclusions. Although there were no significant differences in fibrinolytic markers between patients with chronic Chagas' disease and healthy volunteers, the significant increase in thrombosis markers (F1 + 2, ATM complex, PDF/pdf, and D dimer) suggests the presence of a prothrombotic state in the early stages of chronic Chagas' disease.

Key words: Chagas' disease. Markers. Thrombosis. Fibrinolysis.

Estado protrombótico en estadios tempranos de la enfermedad de Chagas crónica

Introducción. Las complicaciones tromboembólicas son frecuentes en estadios avanzados del período crónico de la enfermedad de Chagas.

Objetivo. Estudiar, con marcadores de trombosis (trombóticos y fibrinolíticos), si existe un estado protrombótico en los estadios tempranos de la enfermedad de Chagas crónica.

Pacientes y método. Se estudió a 42 pacientes con enfermedad de Chagas crónica (12 varones y 30 mujeres) con una edad promedio de 32.5 ± 6.7 años, comparándolos con 21 voluntarios sanos (10 varones y 11 mujeres) con una edad promedio de 24.2 ± 5.6 años. Los marcadores de trombosis utilizados fueron: fragmento 1 + 2, complejo ATM, PDF/pdf, dimero D y \( \beta \)-tromboglobulina. Se evaluó la fibrinólisis pre y poscompresión con el tiempo de lisis de las euglobulinas, así como la dosificación de \( t \)-PA y PAI-1.

Resultados. En los marcadores de trombosis se observaron diferencias estadísticamente significativas entre pacientes con enfermedad de Chagas crónica y controles en las variables F1 + 2 (p < 0.0001), ATM (p < 0.0001), PDF/pdf (p < 0.05) y dimero D (p < 0.05). \( \beta \)-tromboglobulina no alcanzó significación estadística (p = 0.06). En cuanto a las variables fibrinolíticas, la diferencia fue estadísticamente significativa en el tiempo de lisis de las euglobulinas (p < 0.0001), tanto en condiciones basales como después de provocar estrés con oclusión venosa. En cambio, los valores de \( t \)-PA y PAI-1 en condiciones similares no pusieron de manifiesto diferencias estadísticamente significativas entre los grupos estudiados.

Conclusiones. En los resultados obtenidos se observa que no existe alteración de la fibrinólisis, pero el incremento significativo de los marcadores de trombosis (F1 + 2, complejo ATM, PDF/pdf y dimero D) sugeriría la existencia de un estado protrombótico en estadios tempranos de la enfermedad de Chagas crónica.


INTRODUCTION

Chagas’ disease is a histaminic, hematological, parasitic condition caused by the flagellate protozoan Trypanosoma cruzi, a blood parasite that dwells and...
reproduces in the tissues. This infection is transmitted by hemipter insects, hematophages of the sub-family triatomidae; in Argentina, the most frequently occurring during the nosogenic cycle is Triatoma infestans, which has adapted to and is ecologically established in human dwellings. As has been verified by the World Health Organization (WHO), Chagas’ disease is the most frequently occurring tropical disease in Latin America. Approximately 90 million people who live in endemic areas and coexist with vectors are exposed to the risk of developing Chagas’ disease, and it is estimated that 24.7 million people are infected with the T. cruzi parasite. Chagas’ disease occurs only in continental America and is widespread in Latin America. Its geographic distribution extends from latitude 40° north in the southern United States to latitude 45° south in Argentina and Chile. In Argentina, where the most frequently-occurring lesion of the viscera is cardiac in nature, it is estimated that approximately 3 million people are infected with the T. cruzi parasite. Chagas’ disease occurs only in continental America and is widespread in Latin America. Its geographic distribution extends from latitude 40° north in the southern United States to latitude 45° south in Argentina and Chile. In Argentina, where the most frequently-occurring lesion of the viscera is cardiac in nature, it is estimated that approximately 3 million people are infected with Chagas’, of whom 750,000 patients are estimated to experience significant cardiac changes during their lifetime, with an incidence of 60 new cases per year. In the advanced stages of the chronic phase of Chagas’ disease pulmonary thromboembolic complications are common and, over time, systemic complications occur due to paradoxical emboli which are produced by the detachment emboli from the thrombi formed centrally in dyskinetic areas or in aneurysms of the right cardiac cavities, or both, as well as from emboli that detach from thrombosis in the venous areas of the inferior vena cava causing considerable morbidity and mortality. In physiopathological terms, a series of factors have been implicated in the thrombotic process, classically summarized in the Virchow triad of: a) venous stasis and changes in blood flow; b) endothelial injury, and c) blood hypercoagulability.

The goal of our study was to identify—by means of markers of thrombosis and fibrinolysis—the presence of a prothrombotic state in patients who were in the early stages of developing chronic Chagas’ disease and in functional class Ia, Ib, and II (accordi Puigbo et al.’s classification published in 1992) and compared them to healthy volunteers.

### PATIENTS AND METHODS

Between March, 1996, and March, 2001, we studied 42 patients with chronic Chagas’ disease (12 men and 30 women) with an average age of 32.5 years ± 6.7 years, and compared with 21 healthy volunteers (10 men and 11 women) with an average age of 24.2 years ± 5.6 years.

The control group was selected by a random sample of student volunteers in their last year of medical school at the Universidad Nacional de Tucumán. The group underwent the same tests as the patients with Chagas’ disease. Patient and volunteer demographic data and a list of tests performed are given in Table 1.

As inclusion criteria, the patients had to have had positive results from 2 serological tests that detected IgG > 1:32 and be in functional class Ia, Ib, or II of the Puigbo classification (Table 2).

In order to evaluate autonomic dysfunction of the cardiovascular system in chronic Chagas’ disease, we

<table>
<thead>
<tr>
<th>TABLE 1. Demographic data and tests results from patients with Chagas’ disease and from control individuals</th>
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</thead>
<tbody>
<tr>
<td><strong>Sex, age, test</strong></td>
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<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>Men</td>
</tr>
<tr>
<td>Women</td>
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<tr>
<td><strong>Age, mean±SD</strong></td>
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<tr>
<td><strong>Clinical evaluation</strong></td>
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<tr>
<td><strong>Functional class</strong></td>
</tr>
<tr>
<td>Ib</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
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<tr>
<td>Ib</td>
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<tr>
<td>II</td>
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<tr>
<td><strong>ECG</strong></td>
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<tr>
<td><strong>Radiography</strong></td>
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<tr>
<td><strong>Echocardiogram</strong></td>
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<tr>
<td>EF</td>
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<tr>
<td>WM</td>
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<td><strong>Denervation tests</strong></td>
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P<.05. NS indicates not significant; N, normal; A, abnormal; EF, left ventricular ejection fraction; WM, movement of the left ventricular posterior wall.
applied the denervation protocol used to study autonomic disturbances of the cardiovascular system in chronic Chagas’ disease, applying the reference values from Ewing,14 keeping in mind that, at the present time, in order to evaluate early dysautonomy with electrocardiographic methods, the modulated nonlinear technique can also be used, as it is more sensitive and specific for detecting incipient changes.15

We used the following exclusion criteria:

1. The presence of a deep venous thrombus diagnosed with bilateral radioisotope phlebography according to international criteria,16 and the presence of pulmonary emboli on abnormal perfusion ventilation gammography (V/Q) diagnosed according to conventional PIOPED criteria,17 performed with Spect (Elscint) gamma camera model SP×4.
2. The presence of deep venous insufficiency revealed by bilateral radioisotope phlebography according to international criteria.16
3. Doppler echocardiogram (Ving-Med 800) images consistent with intracavity thrombi.18
4. An abnormal coagulogram that showed: a) a platelet count <150.000/379 µL (Brecker and Cronkite direct method); b) a bleeding time >4 minutes 30 seconds (Ivy method); c) a partial activated thromboplastin time >50 seconds (Bell and Alton technique); d) a prothrombin time >120% (Quick method); e) a thrombin time >20 seconds (Dade Behring); f) fibrinogen (Clauss method, average of 3 tests), and g) petechiae analysis (conventional method with negative pressure).
5. The presence of other baseline disease treated or untreated with medication.
6. Abnormal routine laboratory results: a) complete hemogram with hematimetric indices (with the Coulter AcT-10 hematological counter); b) erythrosedimentation rate (Westergren method); c) urea (Fawcet and Scott method); d) creatinine (Hare procedure); e) TGO, TGP, and alkaline phosphatase (optimized kinetic method); f) glycemia, cholesterolemia, and triglyceridemia (enzyme methods), and g) a complete urine screen.
7. The presence of nonthrombophilic risk factors for venous thromboembolic disease: a) age greater than 40 years; b) the presence of varices according to CEAP classification; c) a history of venous thromboembolic disease; d) a body mass index >30 kg/m²; e) cancer; f) treatment of cancer; g) prolonged immobilization (longer than 4 days); h) myocardial infarct; i) heart failure; j) cerebrovascular accident; k) myeloproliferative syndromes; l) kidney disease; m) pregnancy or puerperium, and n) estrogen therapy (replacement or therapeutic).
8. Chronic atrial fibrillation or flutter.

In order to determine thrombin production in our study, we used F1+2 (Organon Teknika, B.V., The Netherlands F1+2, normal value [NV], 0.2 to 2.7 nmol/L). To evaluate the proteases and complexes formed by them with their inhibitors we determined the ATM complex20 (T/IXa/Xa/XIa-AT III; Asserachrom ATM-Stago, NV<20 ng/mL). To evaluate the degradation products derived from thrombin or fibrinogen or fibrinolysis, we determined the FDP/fdp by agglutination with latex particles (Stago, NV<4.3 U/mL) and D-dimer (Asserachrom DD-Stago, NV<500 U/mL). To evaluate plaque activation we determined the ß-thromboglobulin (Asserachrom ß-TG Stago, NV, 10 to 40 U/mL). The markers of thrombosis were grouped according to Yamamoto and Saito’s classification method.21 We used as an overall test, the euoglobulin lysis time (ELT), to evaluate fibrinolysis under baseline conditions and after inducing stress by venous occlusion (Kaulla method22). We considered NV to be up to 120 minutes under baseline conditions and ≤50 min after inducing stress with venous occlusion. To determine the tissue plasminogen activator (t-PA) and tissue plasminogen activator inhibitor 1

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### TABLE 2. Clinical classification of Chagas’ cardiopathy

<table>
<thead>
<tr>
<th>Phase</th>
<th>Symptoms</th>
<th>ECG</th>
<th>Cardiac size</th>
<th>Left ventricular ejection fraction</th>
<th>Left ventricular wall motility</th>
<th>Autonomic function</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>A</td>
<td>None</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>None</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>May be abnormal</td>
</tr>
<tr>
<td>II</td>
<td>Minimal</td>
<td></td>
<td>Normal</td>
<td>Normal</td>
<td>Segmental akinesia</td>
<td>May be abnormal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Conduction abnormalities or extrasystoles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Congestive heart failure</td>
<td>Pathological</td>
<td>Major</td>
<td>Reduced</td>
<td>Overall dysfunction</td>
<td>Usually abnormal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>O conduction abnormalities</td>
<td></td>
<td>Segmental abnormality of wall motility</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arrhythmia</td>
<td></td>
<td>Complex arrhythmia</td>
<td></td>
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</tbody>
</table>

Taken from Puigbo JJ, et al.13

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mean and SD. Nevertheless, for measurable characteristics, the significance of the differences between the patients with chronic Chagas’ disease and the control group were determined by means of the Mann-Whitney U test.

The fibrinolitic variables (ELT, t-PA, and PAI-1) are described as mean and SD, both under baseline conditions and after inducing stress by means of venous occlusion in both groups. The significance of the differences between the patients with chronic Chagas’ disease and the control individuals with regard to the effect of stress was also determined via the Mann-Whitney U test.

A value of $P<.05$ was considered statistically significant. For our calculations we used Arcus Quickstat Biomedical Research Solutions statistical package (Addison Wesley Longman, Cambridge, UK).

### RESULTS

The patients with Chagas’ disease in the study consisted of 30 women and 12 men, with an average age of 32.5 years ±6.7 years. According to the Puigbo classification, 31 patients were in functional class Ib (73.8% of patients; 23 women and 8 men) and 11 patients were in functional class II (26.2% of patients; 7 women and 4 men).

Upon studying autonomic dysfunction of the cardiovascular system, the response was abnormal to testing in 9 patients (21.4%), to 2 tests in 13 patients (31%), to 3 tests in 18 patients (42.9%), and to 4 tests in 2 patients (4.8%). The 5 tests were never all abnormal in the same patient.

The results obtained from analyzing the thrombotic variables are shown in Table 3. For the markers of thrombosis we observed statistically significant differences between patients with chronic Chagas’ disease and the control individuals for the variables F1+2 ($P<.0001$), ATM ($P<.0001$), FDP/fdp ($P<.05$), and D-dimer ($P<.05$). Differences in the ß-thromboglobulin variable did not reach statistical significance ($P=.06$).

When we evaluated fibrinolysis, upon analysis of the euoglobulin lysis time (ELT) as an overall test, the patients with Chagas’ disease were classified into 2 categories according to their response under baseline conditions and after inducing stress by means of venous occlusion. Of the 42 patients with Chagas’ disease studied, 27 (64.0%) had a normal response and 15 (36.0%) had an abnormal response. The ELT, t-PA, and PAI-1 values under baseline conditions and after inducing stress by means of venous occlusion, both in the patients with Chagas’ disease and in the control subjects, are shown in Table 4.

Upon analysis of these fibrinolitic variables, the differences were statistically significant for euoglobulin lysis time ($P<.0001$), both under baseline conditions

### Statistical analysis

Demographic data and the tests used to classify our study patients with chronic Chagas disease and our control individuals are described in terms of their frequency, with the exception of the variable of age, for which we used mean and standard deviation.

For the 5 thrombotic variables (F1+2, ATM, ß-thromboglobulin, FDP/fdp, and D-dimer) we calculated mean and SD.
and after inducing stress by means of venous occlusion. In contrast, the t-PA and PAI-1 values in similar conditions did not show statistically significant differences between the 2 groups studied.

**DISCUSSION**

During the natural course of Chagas’ disease, there are generally no thrombotic events in the early stages of the chronic phase, although these events often occur in the advanced symptomatic phases and usually occur in conjunction with the presence of segmental contractile changes, aneurysms and heart failure, and peripherally in conjunction with thrombogenic risk factors.

The markers for thrombosis are defined as the presence or increase, or both, of the plasma concentration of certain products derived from the activation of various systems that intervene in thrombogenesis. In our study, the significant increase in F1+2, the ATM complex, FDP/fdp, and D-dimer suggest the existence of a prothrombotic state in the early stages of the chronic phase of Chagas’ disease.

When we analyzed comprehensive fibrinolysis by means of euoglobulin lysis time (ELT) in the patients with chronic Chagas’ disease and compared them with the control group, the difference was statistically significant. Nevertheless, given than ELT is a comprehensive test in which other variables may intervene, we determined more specific markers for fibrinolysis: t-PA and PAI-1. The results of the t-PA and PAI-1 measurements at baseline and after inducing stress with venous occlusion suggest that the fibrinolysis would not be altered in the early stages of the chronic phase of Chagas’ disease.

The variable of age is a thrombogenic risk factor after age 40 years. Therefore, the difference in age found between the group of patients with chronic Chagas’ disease and the group of control individuals was discounted. As far as the predominance of the female sex in the sample population, we also discounted this as significant because sex per se is not an independent thrombogenic risk factor. Analysis of the inclusion and exclusion criteria applied to our study, limiting the size of our sample, leaves only congenital or acquired thrombophilia, or both, as probable thrombotic risk factors.

A hypercoagulable state is defined as the presence, in certain individuals, of thrombotic potentialities that activate the endothelium and the formative elements of the blood (principally the platelets) that favors plasma kinetics that lead to the formation of thrombin, which disturbs fibrinolitic activity and produces hemorheological changes with turbulence phenomena that predispose to thrombogenesis. The thrombotic risk factors are grouped, according to international consensus, into 3 large groups: a) general (age, obesity, immobilization, history of venous thromboembolic disease, varices, congenital and acquired thrombophilia, and other hematological changes; b) associated with surgical procedures (very high, high, medium, and low risk), and c) associated with medical conditions or events (cerebrovascular accident, gestation and puerperium, oral contraceptives, hormone replacement therapy, neoplasia, oncological therapy, myocardial infarction, heart failure, and arrhythmia).

The finding of a prothrombotic state would constitute, in our criteria, an independent thrombotic risk factor that should be included into the evaluation of the thromboembolic complications of Chagas’ disease. In patients with venous thromboembolic disease, 96% of patients present with 1 or more thrombotic risk factors, with the frequency of venous thromboembolic disease increased in patients with a higher number of risk factors accumulated by the patient is higher. There is also a «venous memory» that may be involved in the recurrence of the same event, since in the natural course of venous thromboembolic disease there is a relapse rate of 15% at 2 years, 30% at 4 years, and 70% at 8 years. The permanence of risk factors has an important role in thrombotic recurrences.

As Chagas’ disease develops with progressive organic deterioration, it is possible that the established prothrombotic state is perpetuated and even aggravated with the course of the disease. This prothrombotic state would constitute, in our opinion, an independent thrombotic risk factor, and it is likely that its presence creates the need to re-examine the prophylactic and treatment practices currently used to treat the thromboembolic complications associated with this disease.

**CONCLUSIONS**

The detection of statistically significant differences in the markers for thrombosis among patients with chronic Chagas’ disease and control subjects for the variables F1+2 (<0.0001), ATM (<0.0001), FDP/fdp (<0.05), and D-dimer (<0.05), shows the existence of a prothrombotic state in stages IB and II of the Puigbo classification of chronic Chagas’ disease.

**REFERENCES**